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Article

Construction of 2DE-patterns of plasma proteins: aspect of potential tumor markers

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Abstract: Cancer is a complex systemic disease that changes the entire proteome. The analysis of this transformation makes it possible to determine tumor markers, that is, the most characteristic biomacromolecules produced by tumor cells. Here, the question of finding ideal tumor markers, which should be sensitive, specific, and reliable, is an acute issue. Unfortunately, none of the tumor markers, even those used in the clinic, has all these characteristics. Despite this, many tumor markers have demonstrated excellent clinical relevance for monitoring the effectiveness of different treatments for cancer patients. The use of markers also aids in the early detection of cancer recurrence and prognosis. Therefore, the situation in this area can be improved in two ways – by attempting to find an ideal single tumor marker or generating panels of different markers. In both cases, proteomics certainly plays a major role. Human plasma is one of the most popular samples as it is commonly collected in the clinic and provides noninvasive, rapid analysis for any type of disease including cancer. Many efforts have been applied in searching for "ideal" tumor markers digging very deep plasma proteome. There is a line of evidence that the most abundant, so-called "classical plasma proteins", may be used to generate a tumor biomarker profile. To be comprehensive these profiles should have information not only about protein levels but proteoform distribution for each protein. Initially, the profile of these proteins in norm should be generated. Here, we present data about these profiles generated by two-dimensional electrophoresis with the following mass-spectrometry and immunodetection.

Keywords: plasma; biomarker; proteomics; 2DE; proteoform; pattern.

1. Introduction

In a broad sense, tumor biomarkers are components that are either produced directly or indirectly because of a tumor. Moreover, these biomarkers can be common cellular products that are overproduced by cancer cells or the products of genes that are expressed only during malignant transformation. Thus, a tumor marker that is present in significant quantities indicates the presence of cancer. The marker can be present inside the tumor or enter the bloodstream [1,2]**.** This point is fundamentally important, as it allows a non-invasive examination and treatment of patients with various malignant neoplasms. The list of biochemical tumor markers known today is large [2]. Although some of these biomarkers have been successfully used in treatment, none of them fully

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satisfy the so-called "ideal marker", which should be highly sensitive, specific, reliable with high predictive value, and correlate with the stages of tumor development [3].

Therefore, the search for new markers continues, where proteomics plays a central role since tumor biomarkers are mostly proteins. From a proteomic point of view, the search is based on a comparative analysis of proteomes. These proteomes are from body fluids (blood plasma, cerebrospinal fluid, saliva, urine, etc.) or tissues. Here, human plasma is one of the most popular clinical samples as it provides noninvasive, rapid analysis for any type of disease. A special human plasma proteome project (HPPP) project was initiated in 2002 (https://www.hupo.org/plasma-proteome-project). Now, this initiative has achieved great success in plasma protein analysis (http://plasmaproteomedatabase.org/index.html) [4,5]. One of the main advantages of using plasma samples is that only a minimally invasive assay such as a routine blood test analysis is required. To the greatest extent, this certainly concerns the hematopoietic organs (for instance, the major human plasma proteins are synthesized mostly in the liver), but also applies to other tissues, even the brain, which is separated by the blood-brain barrier. It is expected that the blood plasma proteome should reflect, to varying degrees, changes in cellular proteomes caused by diseases. In recent years, biomarker selection guidelines have been developed [6–10]. Here, the classical proteomic approaches are used: two-dimensional electrophoresis (2DE), immunodetection, and mass spectrometry (MS), which have many methodological options that allow highly productive analysis individually or together in different combinations. Electrophoretic separation of plasma proteins offers a valuable diagnostic tool, as well as a way to monitor clinical progress [11]. MS measures with high accuracy the masses of peptides obtained by specific hydrolysis of proteins and is very specific. This approach was applied for detecting ovarian cancer (OC) based on just MS-spectra [12]. In addition, MS-based proteomics can detect and quantify protein variants − proteoforms [13]. Ideally, MS-based proteomics can analyze a whole proteome [14–16]. A rapid, robust, and reproducible shotgun plasma proteomics workflow was developed to produce “plasma proteome profiles” [14,17].

Accordingly, there are several directions for proteomics to develop ideal oncomarkers. First, we can go deep − find highly specific proteoforms/oncomarkers secreted by a tumor in low abundancy. Second, go wide − select, and analyze a panel of multiple proteins/oncomarkers. Third, combine these approaches. There are already some examples of generation such panels [18]. This strategy can be applied to solid or liquid biopsy depending on the real situation. Here, the question arises about how to select these oncomarkers, as the concentration range of putative oncomarkers in plasma is very wide. The plasma proteome is the most complete version of the whole human proteome. In addition to the "classical plasma proteins", it contains tissue proteins plus numerous individual immunoglobulins [19,20]. In clinics, a lot of information about the health state is obtained by analysis of blood proteins. Accordingly, in diagnosis and therapeutic monitoring, human plasma proteome analysis is a promising solution. The major protein, albumin, accounts for ~50% of the mass of all proteins. Nine (IgG, apolipoprotein A1, apolipoprotein A2, transferrin, fibrinogen, haptoglobin, alpha1-antitrypsin, transthyretin) - 40%, other 12 make up the next 9%, and only 1% all the rest. Accordingly, it is a common practice to remove the most abundant proteins (deplete) before deep proteomics analysis of plasma [21].

2DE-analysis of human plasma proteins has a long history, where, possibly, input of L. Anderson & N.G. Anderson is most impressive [22–24]. There are many publications where the 2DE-image of plasma proteins was used as a specific profile for testing the cancer-related changes in the human body [25–29]. But if we are going to decipher the whole panel of plasma proteins as a

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combined tumor biomarker, we need to obtain reliable data about every protein in connection to its response during the malignancy process. Previously, we have started to collect information about proteoform profiles of different cellular proteins into a database "2DE-pattern" [30]. Here, as a next step in searching for specific oncomarkers, we have obtained similar profiles for the human plasma proteins. The "classical plasma proteins" were selected as they are detected most reliably.

2. Results

In our study, using classical 2DE, sectional 2DE, and semi-virtual 2DE in combination with liquid chromatography-electrospray ionization-tandem mass spectrometry (LC ESI-MS/MS) we have generated 2DE patterns for the most abundant plasma proteins. In Fig.1, these 2DE images of plasma proteins is presented. The 2DE patterns of more than 100 reliable and confidently detected sets (Supplementary Table 1 and Table 2) are presented in Supplementary Fig.1. We have also collected the data from literature about the possibilities of using these plasma proteins as cancer biomarkers (Table 1) [20,31]. More detailed information about these proteins is presented below.

2.1. ALPHA-1-ACID GLYCOPROTEIN 1 (A1AG1_HUMAN)

Alpha-1-acid glycoprotein 1 (AGP-1, orosomucoid-1, OMD 1) is synthesized primarily in the liver, secreted into the bloodstream, and functions as a transport protein. AGP 1 is also an acute-phase protein (APP), and its level is increased during inflammation and cancer. AGP 1 belongs to cancer-related genes and is an unfavorable prognostic marker in renal cancer [32]. It has been speculated that AGP-1 plays an important role in cancer, but its exact biological function is still confusing. In multiple types of cancer (sarcomas, Hodgkin's disease, hepatocellular carcinoma (HCC), gastric adenocarcinoma, ovarian cancer (OC), peritoneal carcinomatosis, breast cancer (BC), glioblastoma multiform (GBM), lung cancer) AGP-1 level in plasma is enhanced [33–35]. Furthermore, in some cases, the increased level of AGP-1 correlates with tumor progression. AGP-1 has been also identified as one of the potentially useful biomarkers for estimating the risk of all-cause mortality [36]. The glycosylated form of alpha-1-acid glycoprotein 1 is potential lung cancer and breast biomarker [37,38]. The level of alpha1-3 fucosylated glycoforms of AGP-1 is also increased in pancreatic cancer (PDAC) and could be potentially regarded as a PDAC biomarker [39]. Multifucosylated tetra-antennary structures of AGP-1 are significantly elevated in patients with colorectal cancer (CRC), whereas the single fucosylated derivative – is not [40].

The precursor polypeptide chain of AGP-1 has 201 AA, where AA 1-18 – the signal peptide, AA 19-201 – mature alpha-1-acid glycoprotein 1 (pI/Mw: 5.00/21560). 2DE pattern of AGP-1 represents a chain of spots in the pI-range from 3 to 5 (Suppl. Fig.1). This pattern is well-represented in the SWISS-2DPAGE (pI/Mw: 4.11-4.29/43-46000) [22]. Such a pattern is a result of heavy glycosylation (82 N-linked glycans at 6 sites), phosphorylation (2 sites), acetylation (2 sites), ubiquitylation (1 site) (https://www.uniprot.org/uniprot/P02763).

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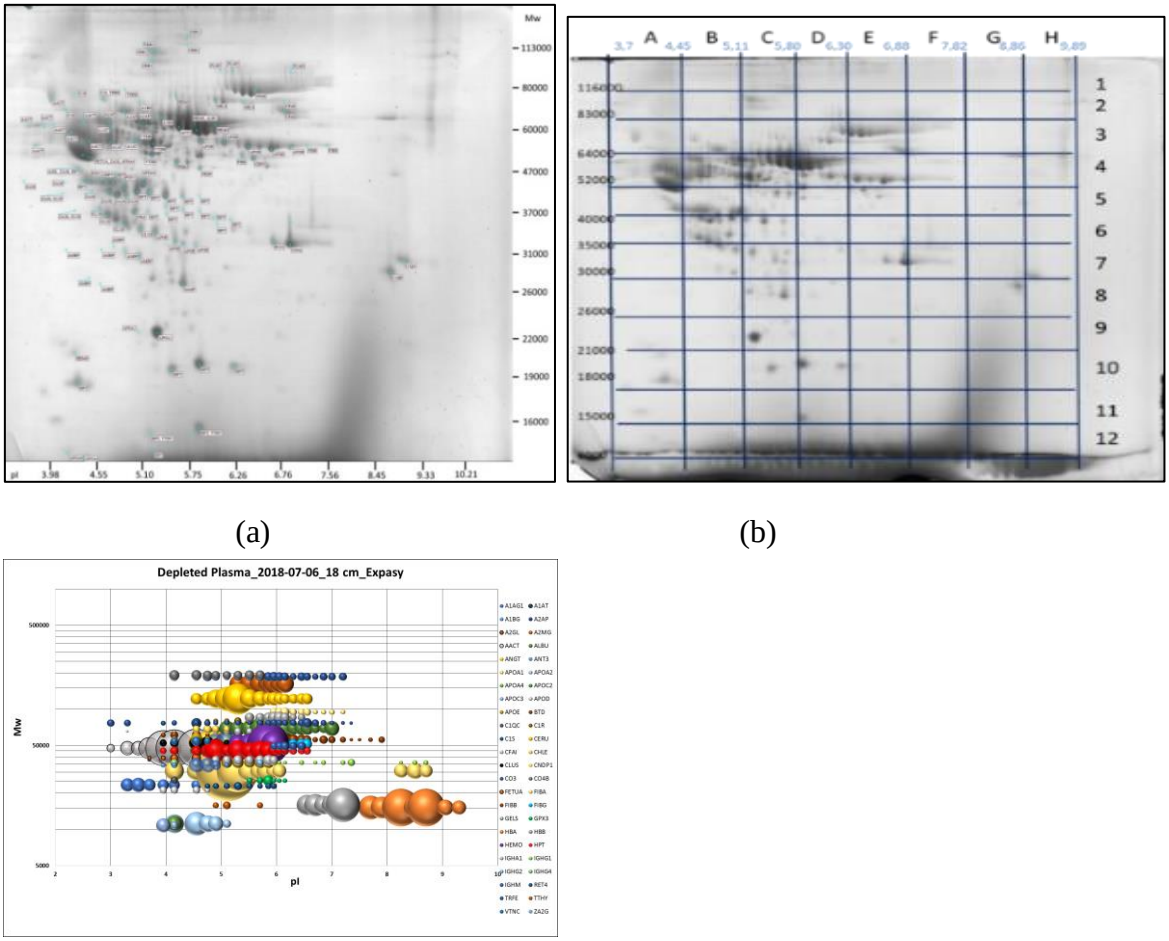

(a) (b)

(c)

Figure 1. 2DE-image of depleted plasma proteins taken for the sectional analysis [41]. (**a**) A classical annotated 2DE image of plasma proteins; (**b**) A sectional analysis of the gel presented in (**a**); (**c**) A semi-virtual 2DE of the major plasma proteins.

Table 1. The most abundant plasma proteins related to cancer.

Concentration (μg/ml) is presented according to [42] if otherwise not shown. Abundance (emPAI) was calculated according to data from semi-virtual 2DE (Suppl. Table 1).

N	UniProt	ID (Uniprot)	Uniprot name (*Gene*)	pI/Mw	μg/ml	em PAI	Cancer
2	P02763	A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 (*ORM1*)	5.11/21588	220	116.0	[33–40]
3	P19652	A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 (*ORM2*)	5.12/21651	220	75.0	[32,43]
1	P01009	A1AT_HUMAN	Alpha-1-antitrypsin (*SERPINA1*)	5.37/44325	350	38.0	[32,34,44–50]
4	P04217	A1BG_HUMAN	Alpha-1B glycoprotein (*A1BG*)	5.63/51922	50	44.0	[32,51–53]
5	P01023	A2MG_HUMAN	Alpha-2-Macroglobulin (*A2M*)	5.98/160810	220	112.0	[27,54–56]
6	P08697	A2AP_HUMAN	Alpha-2-antiplasmin (*SERPINF2*)	5.87/50451	12	36.0	[57,58]
7	P02750	A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein (*LRG1*)	5.66/34346	2.7	17.0	[59–61]
8	P01011	AACT_HUMAN	Alpha 1-antichymotrypsin (*SERPINA3*)	5.32/45266	110	151.0	[62–64]

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9	Q15848	ADIPO_HUMAN	Adiponectin (*ADIPOQ*)	5.46/24544	0.12	2.4	[65]
10	P43652	AFAM_HUMAN	Afamin (*AFM*)	5.58/66577	320	39.0	[66–70]
11	P02768	ALBU_HUMAN	Albumin (*ALBU*)	5.67 / 66472	1600	1207.0	[71–75]
12	P02760	AMBP_HUMAN	Protein AMBP (*AMBP*) Alpha-1-microglobulin Bikunin	5.76 / 37115 6.13 / 20847 4.89 / 15974	48	28.0	[56,76,77]
13	P01019	ANGT_HUMAN	Angiotensinogen (*AGT*)	5.60/ 49761	11	46.0	[37]
14	P01008	ANT3_HUMAN	Antithrombin-III (*SERPINC1*)	5.95 / 49039	60	155.0	[48,78,79]
15	P02647	APOA1_HUMAN	Apolipoprotein A-I (*APOAI*)	5.27 / 28079	310	354.0	[32,80–86]
16	P02652	APOA2_HUMAN	Apolipoprotein A-II (*APOA2*)	5.05 / 8708	750	285.0	[70,80,87–91]
17	P06727	APOA4_HUMAN	Apolipoprotein A-IV (*APOA4*)	5.18 / 43376	32	78.5	[68,82,92,93]
18	P04114	APOB_HUMAN	Apolipoprotein B-100 (*APOB*)	6.57/512858	33	21.0	[52,69,94]
19	P02654	APOC1_HUMAN	Apolipoprotein C1 (*APOC1*)	7.93 / 6631	77	8.3	[95,96]
20	P02655	APOC2_HUMAN	Apolipoprotein C-II (*APOC2*)	4.58 / 8204	240	6.1	[20,32]
21	P02656	APOC3_HUMAN	Apolipoprotein C-III (*APOC3*)	4.72 / 8765	170	6.1	[32,69,91,95]
22	P05090	APOD_HUMAN	Apolipoprotein D (*APOD*)	5.20 / 19303	82	16.8	[32,97]
23	P02649	APOE_HUMAN	Apolipoprotein E (*APOE*)	5.52 / 34237	14	52.2	[69,97–101]
24	Q13790	APOF_HUMAN	Apolipoprotein F (*APOF*)	4.40 / 17425	4.1	2.0	[102]
25	P02749	APOH_HUMAN	Beta-2-glycoprotein 1 (*APOH*)	8.37 / 36255	78	0.1	[103–107]
26	O95445	APOM_HUMAN	Apolipoprotein M (*APOM*)	5.66 / 21253	1.5	7.9	[108]
27	P02747	C1QC_HUMAN	Complement C1q subcomponent subunit C (*C1QC*)	8.33 / 22813	0.91	1.8	[109]
28	P00736	C1R_HUMAN	Complement C1r subcomponent (*C1R*)	5.76/ 78213	4.3	9.7	[110,111]
29	P09871	C1S_HUMAN	Complement C1s subcomponent (*C1S*)	4.85/74887	5.2	5.6	[112,113]
30	P05156	CFAI_HUMAN	Complement factor I (*CFI*)	7.38/63487	0.006	11.9	[49][114]
31	P00751	CFAB_HUMAN	Complement factor B (*CFB*)	6.66/83001	95[115]	1.14	[58,69,116,117]
32	P00746	CFAD_HUMAN	Complement factor D (*CFD*)	6.85/24405	2.9	7.1	[118]
33	P08603	CFAH_HUMAN	Complement factor H (*CFH*)	6.12/137053	57	0.24	[32,119–121]
34	P06681	CO2_HUMAN	Complement C2 (*C2*)	7.57/81085	35	20.5	[32]
35	P01024	CO3_HUMAN	Complement C3 (*C3*)	6.00/ 184951	260	31.1	[58,93,116,122–125]
36	P0C0L4	CO4A_HUMAN	Complement C4-A (*C4A*)	6.60/190534	63 [115	36.3	[52,124,125]
37	P0C0L5	CO4B_HUMAN	Complement C4-B (*C4B*)	6.83/ 190500	90	37.8	[124,125]
38	P01031	CO5_HUMAN	Complement C5 (*C5*)	6.07/186341	95	35.7	[69,126]
39	P13671	CO6_HUMAN	Complement C6 (*C6*)	6.17/102412	3.7	15.7	[32,52]
40	P10643	CO7_HUMAN	Complement C7 (*C7*)	6.09/91115	2.6	17	[69,127]
41	P02748	CO9_HUMAN	Complement component C9 (*C9*)	5.42/60979	5.2	11.8	[128–130]
42	P00915	CAH1_HUMAN	Carbonic anhydrase (*CA1*)	6.63/28739	0.59	2.5	[131,132]
43	P08185	CBG_HUMAN	Corticosteroid-binding globulin (*SERPINA6*)	5.64/42639	1.2	27.9	[133]
44	P15169	CBPN_HUMAN	Carboxypeptidase N catalytic chain (*CPN1*)	6.88/50034	0.72	6.4	[134]
45	P08571	CD14_HUMAN	Monocyte differentiation antigen CD14 (*CD14*) urinary form	5.58/37215	0.42	4.5	[135,136]
46	P00450	CERU_HUMAN	Ceruloplasmin (*CP*)	5.41/120085	86	86.7	[34,54,137–142]
47	P06276	CHLE_HUMAN	Cholinesterase (*BCHE*)	6.33/65084	0.17	2.97	[143]
48	P10909	CLUS_HUMAN	Clusterin (*CLU*)	5.89/50063	25	29.9	[32,52,58,69,144–149]
49	Q96KN2	CNDP1_HUMAN	Beta-Ala-His dipeptidase (*CNDP1*)	5.08/53864	0.23	2.7	[150–153]
50	P22792	CPN2_HUMAN	Carboxypeptidase N subunit 2 (*CPN2*)	5.54/58227	2	6.1	[32]
51	P02741	CRP_HUMAN	C-reactive protein (*CRP*) C-reactive protein(1-205)	5.28/23047 5.28/22950	0.26	1.0	[154,155]

52	Q16610	ECM1_HUMAN	Extracellular matrix protein 1 (*ECM1*)	6.19/58812	0.77	9.6	[156–158]
53	P23142	FBLN1_HUMAN	Fibulin-1 (*FBLN1*)	5.03/74291	0.62	11.8	[159–163]
54	O75636	FCN3_HUMAN	Ficolin-3 *(FCN3*)	6.22/30354	1	11.8	[164–168]
55	P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein (*AHSG*)	4.53/30238	82	30.6	[169,170]
56	Q9UGM5	FETUB_HUMAN	Fetuin-B *(FETUB)*	6.52/40488	0.27	1.8	[171]
57	P02671	FIBA_HUMAN	Fibrinogen alpha chain (*FGA*)	5.79/91359	0.13	10.9	[32,69,172,173]
58	P02675	FIBB_HUMAN	Fibrinogen beta chain (*FGB)*	7.95/50763	130	62.5	[32,173,174]
59	P02679	FIBG_HUMAN	Fibrinogen gamma chain (*FGG*)	5.24/48483	98	39.2	[32,69,175–178]
60	P02751	FINC_HUMAN	Fibronectin (*FN1*)	5.25/269259	20	14.1	[48,94,179–182]
61	P06396	GELS_HUMAN	Plasma gelsolin (*GSN*)	5.72 / 82959	16	23.4	[166,183,184]
62	P22352	GPX3_HUMAN	Glutathione peroxidase 3 (*GPX3*)	7.85 / 23464	10	11.7	[185]
63	P69905	HBA_HUMAN	Hemoglobin subunit alpha (*HBA1*)	8.73/ 15126	41	1129	[54]
64	P68871	HBB_HUMAN	Hemoglobin subunit beta (*HBB*)	6.81/ 15867	30	847.0	[54,186]
65	P02790	HEMO_HUMAN	Hemopexin (*HPX*)	6.43 / 49295	180	165.0	[177,187–189]
66	P05546	HEP2_HUMAN	Heparin Cofactor 2 (*SERPIND1*)	6.26/ 54960	4.3	43.0	[58,189–192]
67	P00738	HPT_HUMAN	Haptoglobin (Zonulin) (*HP*) haptoglobin alpha 1 chain haptoglobin alpha 2 chain haptoglobin beta chain	6.13/ 43349 5.23 / 9355 5.57 / 15946 6.32 / 27265	210	323.0	[166,193–199]
68	P00739	HPTR_HUMAN	Haptoglobin-related protein (*HPR)*	6.63/ 39030	41 [200]	105.0	[201]
69	P04196	HRG_HUMAN	Histidine-rich glycoprotein (*HRG*)	7.03/ 57660	35	24.0	[202,203]
70	P05155	IC1_HUMAN	Plasma protease C1 inhibitor (*SERPING1*)	5.97 / 52843	12	9.4	[204,205]
71	P19827	ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1 (*ITIH1*)	6.33/71415	24	25.0	[29,206–210]
72	Q06033	ITIH3_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H3 (*ITIH3*)	5.01/69360	2	7.7	[207]
73	Q14624	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 (*ITIH4*)	5.92/70586	42	41.6	[29,207]
74	P29622	KAIN_HUMAN	Kallistatin (*SERPINA4*)	7.88/46355	1.1	81.8	[211]
75	P01042	KNG1_HUMAN	Kininogen 1 (*KNG1*)	6.23/ 69897	28	7.7	[212–214]
76	P04180	LCAT_HUMAN	Phosphatidylcholine-sterol acyltransferase (*LCAT*)	5.71/47084	0.22	1.8	[171]
77	P51884	LUM_HUMAN	Lumican *(LUM)*	6.17/36661	4	6.4	[166,215–217]
78	P11226	MBL2_HUMAN	Mannose-binding protein C (*MBL2*)	5.40/24021	0.07	6.4	[171,218,219]
79	P36955	PEDF_HUMAN	Pigment epithelium-derived factor (*SERPINF1*)	5.90/44388	7.2	14.5	[220]
80	Q96PD5	PGRP2_HUMAN	N-acetylmuramoyl-L-alanine amidase (*PGLYRP2*)	7.64/59980	14	4.0	[171,221,222]
81	P80108	PHLD_HUMAN	Phosphatidylinositol-glycan-specific phospholipase D (*GPLD1*)	5.78/89811	4	3.7	[32,223,224]
82	P00747	PLMN_HUMAN	Plasminogen (*PLG*) Plasmin heavy chain A Angiostatin Plasmin heavy chain A, short form Plasmin light chain	7.08 / 88432 6.79/ 63245 8.30/ 44053 7.44/ 54341 7.67/ 25205	25	81.0	[225,226]
83	P27169	PON1_HUMAN	Serum paraoxonase/arylesterase 1 (*PON1*)	5.08 / 39600	7.7	43.4	[79,227–232]
84	P27918	PROP_HUMAN	Properdin (*CFP*)	8.33/ 48494	0.33	1.7	[233]

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85	P07225	PROS_HUMAN	Vitamin K-dependent protein S (*PROS1*)	5.17 / 70645	1.7	7.7	[234]
86	P02753	RET4_HUMAN	Plasma retinol-binding protein 4 (*PRBP*)	5.27 / 21072	580	39.3	[235–237]
87	P0DJI8	SAA1_HUMAN	Serum amyloid A-1 (*SAA1*)	5.89/ 11683	7.4	4.3	[32,34,238–240]
88	P02743	SAMP_HUMAN	Serum amyloid P-component (*APCS*)	6.12/ 23259	8.7	39.5	[241]
89	P04278	SHBG_HUMAN	Sex hormone-binding globulin (*SHBG*)	5.83 / 40468	0.26	5.8	[242,243]
90	P05109	S10A8_HUMAN	Protein S100-A8 (*S100A8*)	6.50/ 10835	0.27	0.9	[244–246]
91	P06702	S10A9_HUMAN	Protein S100-A9 (*S100A9*)	5.71/13242	1.9	2.6	[244,247]
93	P05452	TETN_HUMAN	Tetranectin (*CLEC3B*)	5.80 / 20139	58	31.5	[32,248,249]
94	P05543	THBG_HUMAN	Thyroxine-binding globulin (*SERPINA7*)	5.76 / 44102	1.3	12.5	[250,251]
95	P00734	THRB_HUMAN	Prothrombin *(F2)*	5.23 / 65308	27	24.8	[252,253]
96	P02787	TRFE_HUMAN	Serotransferrin (*TF*)	6.70 / 75195	360	41.8	[25,254]
97	P02766	TTHY_HUMAN	Transthyretin (*TTR*)	5.31 / 13761	770	23.9	[32,95,177,255–257]
98	P02774	VTDB_HUMAN	Vitamin D-binding protein (*GC*)	5.16 / 51197	57	181.4	[32,258,259]
99	P04004	VTNC_HUMAN	Vitronectin (*VTN*)	5.47 / 52278	35	22.7	[25,32,52,260]
100	P25311	ZA2G_HUMAN	Zinc-alpha2-glycoprotein (*AZGP1*)	5.58 / 32145	31	31.5	[261–264]

2.2. ALPHA-1-ACID GLYCOPROTEIN 2 (A1AG2_HUMAN)

Alpha-1-acid glycoprotein 2 (AGP 2, orosomucoid-2, OMD 2) is a key acute phase plasma protein (APP) secreted by hepatocytes. AGP 2 plays important role in anti-inflammation, immunomodulation, and drug delivery. AGP 2 is an unfavorable prognostic marker in renal cancer [32]. It was shown that plasma ORM2 level could be an independent prognostic factor in patients with stage II of CRC [43]. The precursor polypeptide chain of AGP-2 has 201 AA, where AA 1-18 – the signal peptide, AA 19-201 – mature alpha-1-acid glycoprotein 2 (pI/Mw: 5.12/21651). 2DE pattern of AGP-2 is very similar to AGP-1 (Suppl. Fig.1) and in SWISS-2DPAGE is overlapped with AGP-1 pattern [22]. AGP-2 can be glycosylated (99 N-linked glycans at 7 sites) and acetylated (1 site). (https://www.uniprot.org/uniprot/P19652).

2.3. ALPHA-1-ANTITRYPSIN (A1AT_HUMAN)

Alpha-1-antitrypsin (alpha-1 protease inhibitor, serpin A1) is a serine protease inhibitor of the serpin superfamily. This protein is produced not only in the liver but in many other tissues (bone marrow, lymphoid tissue, gut, etc.). Its concentration in human serum can be strongly increased during inflammation, infection, injury, or increased estrogen level [265]. The opposite situation can be observed in chronic liver or lung diseases, where dysregulation of alpha-1-antitrypsin is observed [266,267]. An increased level of serpin A1 in plasma was observed in the case of various tumors (non-small-cell lung cancer (NSCLC), PDAC, prostate cancer (PC), cervix, ovary, larynx, head and neck tumor, CRC, and other carcinomas) [34,44–47]. But according to "The Human Protein Atlas" [32] it is a favorable prognostic marker in BC. Also, it was shown the higher levels of α-1-antitrypsin in non-relapsing Hodgkin lymphoma (HL) compare to relapsing HL [48]. As far as posttranslational modification (PTMs) and proteoforms, different glycosylated patterns of serpin A1 were observed in lung cancer and PDAC patients [49,50]. Serpin A1 has 3 known splice-isoforms and 8 potential isoforms that are computationally mapped. Additionally, differential glycosylation produces several proteoforms. The canonical precursor polypeptide chain of serpin A1 has 418 AA, where AA 1-24 – the signal peptide, AA 25-418 – mature alpha-1-antitrypsin (theoretical pI/Mw: 5.37/44325) that cleaved into the short peptide from AAT (pI/Mw: 9.40/5068). 2DE-pattern of Serpin A1 represents a

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chain of spots in the pI-range 4.5-5.1 (Suppl. Fig.1) [268] that is a result of multiple N-linked glycosylations (112 N-linked glycans at 5 sites, 5 O-linked glycans at 6 sites), phosphorylation (13 sites), and acetylation (17 sites) (https://www.phosphosite.org/) [269]. Accordingly, in SWISS-2DPAGE 22 spots of serpin A1 are present (pI/Mw: 4.87-5.10/48-108000) [22].

2.4. ALPHA-1B GLYCOPROTEIN (A1BG_HUMAN)

Alpha-1B glycoprotein (alpha-1-B glycoprotein) is a glycoprotein of unknown function. The protein shows sequence similarity to some immunoglobulins. It is produced by multiple cell types, mostly hepatocytes, in response to growth hormone. Its level is enhanced in plasma in the case of NSCLC [51], CRC [52], and cervical intraepithelial neoplasia [53]. But it is considered a favorable prognostic marker in liver cancer [32]. Its precursor polypeptide chain has 495 AA, where AA 1-21 – the signal peptide, AA 22-495 – alpha-1-B glycoprotein (pI/Mw: 5.63/51922). There is one isoform variant that utilizes M123 as an alternative start site. 2DE-pattern of alpha-1-B glycoprotein represents a long chain of spots in the pI-range 4-6 and Mw~54000 (Suppl. Fig.1) that is a result of heavy glycosylation (24 N-linked glycans at 4 sites, 2 O-linked glycans at 1 site) and phosphorylation (1 site) (https://glyconnect.expasy.org/browser/proteins/780). In SWISS-2DPAGE, alpha-1-B glycoprotein is represented as a chain of 6 spots (pI/Mw: 4.99-5.25 / 73-76000) [22].

2.5. ALPHA-2-ANTIPLASMIN (A2AP_HUMAN)

Alpha-2-antiplasmin (alpha-2-AP, alpha-2-plasmin inhibitor, serpin F2) produced mainly in the liver is a serine protease inhibitor that is a key regulator of plasmin-mediated proteolysis of coagulation factors and other molecules. The increased risk or poor outcome in cardiovascular diseases exists in the case of high α2AP levels, as it can cause venous thrombosis, pulmonary embolism, arterial thrombosis, and ischemic stroke [270]. A high level of alpha-2-antiplasmin is also a predictor of poor prognosis in patients with lung cancer [57], or B-cell acute lymphoblastic leukemia (B-ALL) [58]. Its precursor polypeptide chain has 491 AA, where AA 1-27 – the signal peptide, AA 28-39 – propeptide, and AA 40-491 (pI/Mw: 5.87/50451) – alpha-2-antiplasmin. 2DE pattern of α2AP represents a chain of spots in the pI-range 4-6 with Mw~50000 (Suppl. Fig.1) that is a result of glycosylation (4 N-linked glycans at 1 site, 3 O-linked glycans at 4 sites), phosphorylation (7 sites), and ubiquitylation (2 sites) (https://glygen.org/protein/P08697#glycosylation). In SWISS-2DPAGE, alpha-2-antiplasmin is represented as a chain of 7 spots (pI/Mw: 4.87-5.17 / 66-74000).

2.6. LEUCINE-RICH ALPHA-2-GLYCOPROTEIN (A2GL_HUMAN)

Leucine-rich alpha-2-glycoprotein (LRG1) is a liver-produced APP that is involved in promoting neovascularization and is differentially expressed in many kinds of diseases. LRG1 level is also increased in plasma of CRC patients and may be a useful biomarker [59,60]. A high level of LRG1 is also unfavorable in metastatic prostate cancer [61]. LRG1 precursor polypeptide chain has 347 AA, where AA 1-35 – the signal peptide, AA 36-347 (pI/Mw: 5.66/34346) – mature LRG1. 2DE pattern of LRG1 represents a chain of spots in the pI-range 3.5-5.0 with Mw ~40000-50000 (Suppl. Fig.1). This pattern is well-represented in the SWISS-2DPAGE https://world-2dpage.expasy.org/ and has a characteristic for multiple glycosylation profile, where acidic spots have higher Mw [22]. LRG1 has at least 6 sites of glycosylation: one is O-GalNAc and 5 – N-GlcNAc (https://www.uniprot.org/uniprot/P02750).

2.7. ALPHA-2-MACROGLOBULIN (A2MG_HUMAN)

Alpha-2-macroglobulin (alpha-2-M) is a liver-produced glycoprotein. It is a key member of the alpha macroglobulin superfamily and has many functions, where the best known is the inhibition of

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proteases by a unique 'trapping' mechanism [271]. According to "The human protein atlas" alpha-2-macroglobulin belongs to cancer-related genes [32]. There is data that the alpha-2-M level in plasma is increased in the case of PDAC or OC [54] but decreased in the case of BC [27,55] or NSCLC [56]. Its precursor polypeptide chain has 1474 AA, where AA 1-23 – the signal peptide, AA 24-1474 (pI/Mw: 5.98/160810) – alpha-2-M. 2DE pattern of alpha-2-M represents a chain of heavy Mw spots (mostly in the pI-range 5.8-6.3) (Suppl. Fig.1). This pattern is also well-represented in the SWISS-2DPAGE https://world-2dpage.expasy.org/ [22]. Alpha-2-M has 8 sites of O-GalNAc and 8 sites of N-GlcNAc (https://glygen.org/protein/P01023#glycosylation).

2.8. ALPHA-1-ANTICHYMOTRYPSIN (AACT_HUMAN)

Alpha 1-antichymotrypsin (ACT, alpha globulin glycoprotein) is a liver-produced APP of the serpin superfamily. It inhibits the activity of cathepsin G and chymases and protects tissues from damage caused by these proteases [272]. Clinically, its level is elevated in inflammatory conditions, ulcerative colitis, burn injuries, and some malignancies. The PSA-ACT complex level is considered a biomarker for PC [62]. But deficiency of ACT relates to liver disease, and in the case of BC, it is a prognostic favorable marker. Decreased ACT level was associated with poor survival in patients with liver cancer, increased ACT level was associated with shorter survival in patients with PDAC, and decreased ACT expression was associated with shorter survival in patients with early lung cancer [63]. Based on quantitative proteomics data it was suggested that the GlcNAcylated ACT will be a promising clinical biomarker in the diagnosis of early NSCLC [64]. ACT precursor polypeptide chain has 423 AA, where AA 1-23 – the signal peptide, AA 24-423 – mature alpha-1-antichymotrypsin (pI/Mw: 5.32/45266) that is cleaved to alpha-1-antichymotrypsin His-Pro-less (pI/Mw: 5.26/45032). 2DE pattern of ACT represents a chain of spots in the pI-range 4.0-5.0 and Mw 50-60000 (Suppl. Fig.1). This pattern is also well-represented in the SWISS-2DPAGE, where 2 chains (20 spots) of both ACT forms are presented [22]. ACT has 7 sites of N-GlcNAc and 4 sites of O-GalNAc (https://glygen.org/protein/P01011#glycosylation).

2.9. ADIPONECTIN (ADIPO_HUMAN)

Adiponectin (ADPN, ACRP30, adipocyte, gelatin-binding protein) is an adipokine protein that affects several metabolic processes and is mainly known for its insulin-sensitizing and anti-inflammatory effects. Adiponectin is produced by adipose, though other tissues produce it as well. In the case of OC, the ADPN level declines [65]. Its precursor polypeptide chain has 244 AA, where AA 1-18 – the signal peptide, AA 19-244 – mature adiponectin (pI/Mw: 5.46/24544). 2DE pattern of adiponectin represents a chain of spots in the pI-range 5.0-5.5 and Mw 26000 (Suppl. Fig.1). There are 6 sites of O-linked glycosylation and 2 sites of phosphorylation in adiponectin (https://glygen.org/protein/Q15848#glycosylation).

2.10. AFAMIN (AFAM_HUMAN)

Afamin (alpha-albumin, alpha-alb), a vitamin E-binding glycoprotein, is the fourth member of the human albumin gene family including albumin, a-fetoprotein, and vitamin D-binding protein [273]. It is mainly produced in the liver and secreted into the bloodstream. The serum level of afamin is reduced in patients with OC and is associated with therapy response and survival rate in advanced OC. Accordingly, afamin is considered a potential marker of OC, HCC, GC, and possibly other cancers [66–70]. The precursor polypeptide chain of afamin has 599 AA, where AA 1-21 – the signal peptide, AA 22-599 – mature afamin (pI/Mw: 5.58/66577). 2DE pattern of adiponectin represents a

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chain of spots in the pI-range 4.5-6.0 and Mw ~70000 (Suppl. Fig.1). There are 6 sites of N-linked glycosylation in afamin, and more than 90% of the glycans are sialylated (https://glygen.org/protein/P43652#glycosylation).

2.11. ALBUMIN (ALBU_HUMAN)

Albumin is the major protein in human blood with a concentration in plasma of 35–50 mg/mL [274]. It is involved in diverse physiological and pathological processes: the regulation of osmotic pressure, transport of endogenous molecules (hormones, fatty acids, metabolites, etc.), and exogenous drugs. Classically, the level of albumin was considered a reflection of the nutritional status of patients. However, this view has changed, as it was found that many factors, including inflammation, also affect the albumin level. Usually, cancer patients, especially with CRC, have a high prevalence of hypoalbuminemia. Here, a correlation between the low serum albumin level with increased HCC aggressiveness exists [70]. A similar correlation was detected in many other cancers (breast, lung, gastric, colorectal, pancreatic) [71–75]. Albumin is translated in the liver as a precursor polypeptide with 609 AA, where AA 1-18 – the signal peptide, AA 19-24 – propeptide, and AA 25-609 – mature albumin. The theoretical physicochemical parameters (pI/Mw) of the processed mature canonical form of albumin are 5.67/66472. 2DE pattern of albumin represents a chain of spots in the pI-range 5.5-6.5 and Mw ~70000 (Suppl. Fig.1). Its pattern is also well-represented in the SWISS-2DPAGE [22]. Albumin can be modified by N-linked glycans at one site, 7 O-linked glycans at 11 sites, phosphorylated at multiple sites (at least 15), and acetylated (1 site) (https://glygen.org/protein/P02768#glycosylation).

2.12. PROTEIN AMBP (AMBP_HUMAN)

Protein AMBP, a precursor expressed in hepatocytes and other cells, is cleaved by endopeptidase into alpha-1-microglobulin that has immunoregulatory properties and inter-alpha-trypsin inhibitor light chain/bikunin that is a Kunitz-type proteinase inhibitor. AMBP is a favorable prognostic marker in endometrial cancer (Protein Atlas version 21.0). The expression of AMBP could be an independent predictive factor of cervical lymph node metastasis and a prognostic factor of overall survival, and it is involved in cell invasion and metastasis in cervical lymph nodes in oral squamous cell carcinoma (OSCC) [62]. Its enhanced plasma level is associated with BC [76] and lung cancer [56]. In the case of the acute phase and cancer, the concentrations of bikunin in plasma and urine are increased [77]. AMBP is translated as a polypeptide chain with 352 AA (pI/Mw: 5.95/38999), where AA 1-19 – the signal peptide, AA 20-352 – alpha-1-microglobulin/bikunin precursor (pI/Mw: 5.76/37115) that is further cleaved into 2 chains: AA 20-203 – alpha-1-microglobulin (pI/Mw: 6.13 / 20847) and AA 206-352 – inter-alpha-trypsin inhibitor light chain/bikunin (pI/Mw: 4.89/15974). 2DE pattern of AMBP represents two groups of spots in the pI-range 4.0-6.5: alpha-1-microglobulin with Mw ~26000 and inter-alpha-trypsin inhibitor light chain/bikunin that is assembled in a high Mw complex by a chondroitin-like glycosaminoglycan (GAG) cross-linking with Mw 120000 (Suppl. Fig.1). In the SWISS-2DPAGE, AMBP is represented only by the chain of 3 alpha-1-microglobulin spots. AMBP can be glycosylated (31 N-Linked glycans at 2 sites, 7 O-Linked glycans at 3 sites), phosphorylated (3 sites), and acetylated (1 site) (https://www.phosphosite.org/).

2.13. ANGIOTENSINOGEN (ANGT_HUMAN)

Angiotensinogen (serpin A8) is produced in the liver and cleaved by renin in response to lowered blood pressure. The resulting product, angiotensin I, is then cleaved by the angiotensin-

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converting enzyme (ACE) to generate the physiologically active angiotensin II. The protein is involved in maintaining blood pressure, body fluid, electrolyte homeostasis, and the pathogenesis of essential hypertension and preeclampsia. It was shown the high levels of angiotensinogen (α-1-antitrypsin, antithrombin III, as well) in non-relapsing Hodgkin lymphoma (HL) in relapsing HL [37]. Its precursor polypeptide chain has 476 AA, where AA 1-24 – the signal peptide, AA 25-476 – mature angiotensinogen (pI/Mw: 5.60/49761) that is cleaved into 8 chains (angiotensin-1, angiotensin 1-9, angiotensin-2, angiotensin 1-7, angiotensin 1-5, angiotensin 1-4, angiotensin-3, angiotensin-4). 2DE pattern of angiotensinogen represents a chain of spots in the pI-range 4.0-6.4 and Mw~50000 (Suppl. Fig.1). In the SWISS-2DPAGE, angiotensinogen is represented by one spot (pI/Mw: 5.07/58973). It was reported 20 N-linked glycans at 3 sites and 1 O-linked glycan (1 site) (https://glygen.org/protein/P01019#glycosylation).

2.14. ANTITHROMBIN III (ANT3_HUMAN)

Antithrombin-III (ATIII, serpin C1) is a serine protease inhibitor that is synthesized in the liver and regulates the blood coagulation cascade. ATIII inhibits thrombin, matriptase-3/TMPRSS7, as well as factors IXa, Xa, and XIa. This inhibitory activity is increased in the presence of heparin. Clinically, a reduced level of ATIII is an indicator of hypercoagulability. A high level of ATIII is associated with an increased risk of hemorrhagic complications and a reduced risk of thromboembolic complications. This situation was observed in patients with bladder carcinoma [78]. There is data that ATIII together with PON1 has the potential to serve as effective biomarkers for distinguishing AFP-negative HCC from cirrhosis [79]. Also, there is a higher level of ATIII in non-relapsing HL compared to relapsing HL [48]. ATIII precursor polypeptide chain has 464 AA, where AA 1-32 – the signal peptide, AA 33-464 – mature antithrombin-III (pI/Mw: 5.95/49039). 2DE pattern of ATIII represents a chain of 8 spots in the pI-range 4.5-6.0 and Mw~ 50000 (Suppl. Fig.1). In the SWISS-2DPAGE, only 2 spots are presented (pI/Mw: 5.20/58973 and 5.27/58653) for ATIII [22]. The protein can be glycosylated (24 N-linked glycans at 4 sites), 2 O-linked glycans at 1 site), phosphorylated (9 sites), and ubiquitinated (1 site) (https://www.phosphosite.org/).

2.15. APOLIPOPROTEIN A-I (APOA1_HUMAN)

Apolipoprotein A-I (apoA-I) is a major protein component of high-density lipoproteins (HDL) in plasma. It is a multifunctional protein involved in cholesterol traffic and regulation of inflammatory and immune responses. ApoA-I is an unfavorable prognostic marker in renal cancer and favorable in liver cancer [32]. ApoA-I expression is inversely associated with BC risk and tumor size [97]. Alterations of apoA-I during the development and progression of various types of cancer suggest that serum apoA-I level may represent a useful biomarker for estimation of cancer risk, diagnosis, and prognosis [80,81]. Expression levels of apoA-I in plasma were decreased in the oral cancer group [82]. Also, decreased level of apoA-I predicts a poor prognosis of patients with de novo myelodysplastic syndromes [83], NSCLC [84], and nasopharyngeal carcinoma (NPC) [85]. Also, a high level of serum apoA-I is a favorable prognostic factor for overall survival in esophageal squamous cell carcinoma [86]. ApoA-I precursor polypeptide chain has 266 AA, where AA 1-18 – the signal peptide, AA 19-267 – proapolipoprotein A-I, AA 25-267 – mature apoA-I (pI/Mw: 5.27/28079), AA 25-266 – truncated apoA-I (pI/Mw: 5.27/27950). 2DE pattern of apoA-I represents a chain of spots in the pI-range 4.5-6.5 and Mw~26000 (Suppl. Fig.1). In the SWISS-2DPAGE, 9 spots are presented (chain of 5 spots pI/Mw: 4.99-5.48/~23000 and 4 spots with Mw ~8000-9000) [22]. The protein can be

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heavily phosphorylated (13 sites), acetylated (13 sites), ubiquitinated (7 sites), succinylated (3 sites), or glycosylated (2 sites) (https://www.phosphosite.org/).

2.16. APOLIPOPROTEIN A-II (APOA2_HUMAN)

Apolipoprotein A-II (apoA-II, apolipoprotein A2) is the second most abundant protein of the HDL. It stabilizes HDL structure by its association with lipids and affects HDL metabolism. The level of apoA-II was dramatically reduced in the serum of patients with GC and MM [70,87] but increased in HCC and PC [88,89]. ApoA-II is highly overexpressed in the cerebrospinal fluid of patients with pediatric tumors [90]. Expression of apoA-II including its hemi-truncated form was significantly reduced in pancreatic cancer and apoA-II might be used as an early diagnostic marker and a risk factor for it [80,91]. ApoA-II precursor polypeptide chain has 100 AA, where AA 1-18 – the signal peptide, AA 19-100 – proapolipoprotein A-2 (6.51/9304), AA 24-100 – apoA-II (pI/Mw: 5.05/8708), AA 24-99 – truncated apo-A-II (pI/Mw: 5.05/8580). 2DE pattern of apoA-II represents a chain of spots in the pI-range 4.5-6.0 and Mw~9000 (Suppl. Fig.1). In the SWISS-2DPAGE, 2 spots are presented (pI/Mw: 4.74/12520 and 4.71/7250) [22]. ApoA-II can be glycosylated (3 O-linked glycans at 3 sites), phosphorylated (7 sites), acetylated (1 site), succinylated (1 site).

2.17. APOLIPOPROTEIN A-IV (APOA4_HUMAN)

Apolipoprotein A-IV (apoA-IV) is synthesized in the small intestine and has multiple physiologic functions (lipid absorption and metabolism, anti-atherosclerosis, thrombosis, glucose homeostasis…) [275]**.** The level of apoA-IV in HCC tissues is dramatically reduced compared to normal controls [92]**.** The plasma level of apoA-IV was also reduced in patients with OC [68]**,** oral cancer [82], or thyroid carcinoma [93]**.** ApoA-IV precursor polypeptide chain has 396 AA, where AA 1-20 – the signal peptide, AA 21-396 – apoA-IV (pI/Mw: 5.18/43375). 2DE pattern of apoA-IV represents a chain of spots in the pI-range 4.5-6.0 and Mw~40000 (Suppl. Fig.1). In the SWISS-2DPAGE, 6 spots are presented (pI/Mw: 5.05-5.10/~43000 (3 spots), 5.11/21945, and 4.87-4.97/9-10000(3 spots)). ApoA-IV can be glycosylated (1 O-linked glycan at 1 site), phosphorylated (7 sites), acetylated (9 site), ubiquitinated (1 site) (https://www.phosphosite.org/).

2.18. APOLIPOPROTEIN B-100 (APOB_HUMAN)

Apolipoprotein B-100 (apo B-100) is involved in the transport of cholesterol. There is considerable evidence that the concentration of apo B-100 is a superior indicator of vascular/heart disease [276]. The level of apo B-100 is enhanced in the plasma of patients with CRC and HCC [52,69]**.** Also, abnormal glycosylation changes of apo B-100 were detected in the case of OC [94]**.** Its precursor polypeptide chain has 4563 AA, where AA 1-27 – the signal peptide, AA 28-4563 – apo B-100 (pI/Mw: 6.6/512853) that can be cleaved into apolipoprotein B-48 (AA 28-2179, pI/Mw: 7.99/240836). 2DE pattern of apo B-100 represents a long chain of spots in the pI-range 3.5-7.5 and heavy Mw > 120000 (Suppl. Fig.1). Apo B-100 can be glycosylated (28 sites, 82 N-linked glycans at 16 sites, 1 O-linked glycan at 7 sites), phosphorylated (43 sites), acetylated (4 sites), ubiquitinated (8 sites) (https://www.phosphosite.org/).

2.19. APOLIPOPROTEIN C-I (APOC1_HUMAN)

Apolipoprotein C1 (apo-CI) is an inhibitor of lipoprotein binding to the low-density lipoproteins (LDL) receptors. Apo-CI binds free fatty acids and reduces their intracellular esterification. Apo-CI is a favorable prognostic marker in liver cancer [32]. Apo-CI might be a potential prognostic and diagnostic marker for GC [95,96]. It was shown that the apo-CI level in serum and tissues of GC patients was much higher than in healthy control. Besides, apo-CI level was associated with clinical

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characteristics of GC patients [96]. Apo-CI precursor polypeptide chain has 83 AA, where AA 1-26 – the signal peptide, AA 27-83 – mature apo-CI (pI/Mw: 7.93/6631), AA 29-83 – truncated apo-CI (pI/Mw: 8.24/6432). 2DE pattern of apo-CI represents a chain of spots in the pI-range 7.8-8.5 and Mw~6000 (Suppl. Fig.1). This protein can be acetylated (3 sites) and ubiquitinated (4 sites) (https://www.phosphosite.org/).

2.20. APOLIPOPROTEIN C-II (APOC2_HUMAN)

Apolipoprotein C-II (apo-CII, apolipoprotein C20) plays an important role in lipoprotein metabolism as an activator of lipoprotein lipase [277]. Apo C-II level is enriched in liver cancer [32]. Its precursor polypeptide chain has 101 AA, where AA 1-24 – the signal peptide, AA 25-101 – proapolipoprotein C-II, and AA 29-101 – mature Apo-CII (pI/Mw: 4.58/8204). 2DE pattern of apo-CII represents a chain of spots in the pI-range 4.8-5.2 and Mw~8000 (Suppl. Fig.1). In the SWISS-2DPAGE, 2 spots for apo-CII are presented (pI/Mw: 4.51/9976 and 4.58/9248). This protein can be acetylated (5 sites), ubiquitinated (2 sites) (https://www.phosphosite.org/) and glycosylated (3 O-linked glycans at 4 sites) (https://www.glygen.org/protein/P02655).

2.21. APOLIPOPROTEIN C-III (APOC3_HUMAN)

Apolipoprotein C-III (apoC-III, apolipoprotein C3) is a component of triglyceride-rich VLDL and HDL in plasma. ApoC-III plays a multifaceted role in triglyceride homeostasis. ApoC-III is an unfavorable prognostic marker in renal cancer and favorable in liver cancer [32]**.** A level of apoC-III is enhanced in the plasma of patients with HCC [69]**.** A level of apoC-III and its unglycosylated form was significantly decreased in plasma of patients with PDAC or GC [91,95]**.** ApoC-III precursor polypeptide chain has 99 AA, where AA 1-20 – the signal peptide, AA 21-99 – mature apoC-III (pI/Mw: 4.72/8765). 2DE pattern of apoC-III represents a chain of spots in the pI-range 3.8-6.1 and Mw ~9000 (Suppl. Fig.1). In the SWISS-2DPAGE, only one spot for apoC-III is presented pI/Mw: 4.63/8528). This protein can be phosphorylated (7 sites) acetylated (1 site), ubiquitinated (1 site) (https://www.phosphosite.org/) and glycosylated (4 O-linked glycans at 1 site) (https://www.glygen.org/protein/P02655).

2.22. APOLIPOPROTEIN D (APOD_HUMAN)

Apolipoprotein D (apoD) is produced by numerous tissues, mostly by adrenal glands and kidney. On HDL, apoD plays a role in stabilizing LCAT and increasing cholesterol esterification. ApoD is able to transport a variety of ligands in a number of different contexts. ApoD is an unfavorable prognostic marker in thyroid cancer and stomach cancer, but favorable in BC [32]. ApoD expression is positively associated with a risk of BC [97]. ApoD precursor polypeptide chain has 189 AA, where AA 1-20 – the signal peptide, AA 21-189– apoD (pI/Mw: 5.20 / 19303). 2DE pattern of apoD represents an unusual set of spots in the pI-range 3.5-6.5 and Mw from ~15000 to ~26000 and 80000 (Suppl. Fig.1). In the SWISS-2DPAGE, a cluster of 12 spots for apoD is presented (pI/Mw: 4.44-4.78/27-32000). ApoD can be heavily glycosylated (115 N-linked glycans at 2 sites, 1 O-linked glycan at1 site), and phosphorylated (1 site) (https://www.uniprot.org/uniprotkb/P05090/entry).

2.23. APOLIPOPROTEIN E (APOE_HUMAN)

Apolipoprotein E (apoE) plays a key role in cell proliferation, angiogenesis, tumorigenesis, and metastasis. ApoE overexpression was associated with aggressive biological behaviors and poor prognosis in a variety of tumors [98]**.** An elevated level of apoE in PDAC mediates immune suppression and high serum apoE level correlates with poor patient survival [99]**.** ApoE expression shows a positive association with the degree of BC malignancy [97]**.** The level of apoE is enhanced in

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plasma of patients with HCC, BC, and NPC. Moreover, the increased apoE level may act here as a potential biomarker for diagnosis [69,100,101]**.** ApoE precursor polypeptide chain has 317 AA, where AA 1-18 – the signal peptide, AA 19-317– apoE (pI/Mw: 5.52/34237). 2DE pattern of apoE represents a chain of spots in the pI-range 4.5-6.5 and Mw ~35000 (Suppl. Fig.1). In the SWISS-2DPAGE, a chain of 3 spots (pI/Mw: 5.24-5.49/34-35320). ApoE can be glycosylated (6 O-linked glycans at 6 sites), phosphorylated (9 sites), acetylated (1 site), and ubiquitinated (5 sites) (https://www.phosphosite.org/).

2.24. APOLIPOPROTEIN F (APOF_HUMAN)

Apolipoprotein F (apoF, lipid transfer inhibitor protein, LTIP) is a less abundant apolipoprotein that is expressed by the liver and associates with LDL. It inhibits cholesteryl ester transfer protein activity and appears to be an important regulator of cholesterol transport. It is also associated to a lesser degree with VLDL, apoA-I and apoA-II. ApoF expression is down-regulated in HCC, which is associated with low recurrence-free survival rate. ApoF may serve as a tumor suppressor in HCC and be a potential application for the treatment of this disease. Decreased expression of apoF is associated with poor prognosis in HCC [102]**.** Its precursor polypeptide chain has 326 AA, where AA 1-35 – the signal peptide, AA 36-164 – propeptide (pI/Mw: 9.51/14093), and AA 165-326 – apoF (pI/Mw: 4.40/17425). 2DE pattern of apo-F represents a set of spots in the pI-range 3.5-4.2 and Mw from ~15000 to ~32000 (Suppl. Fig.1). ApoF can be glycosylated (16 N-linked glycans at 3 sites, 6 O-linked glycans at 5 sites), phosphorylated (1 site), ubiquitinated (2 sites) (https://www.phosphosite.org/).

2.25. BETA-2-GLYCOPROTEIN 1 (APOH_HUMAN)

Beta-2-glycoprotein 1 (APC inhibitor, activated protein C-binding protein, apolipoprotein H, apo-H) is a unique protein with a key role in homeostasis, hemostasis, and immunity. Apo-H is a favorable prognostic marker in liver cancer and unfavorable in renal cancer. It is highly overexpressed in hepatitis B-related HCC tissue [103], in the urine of renal carcinoma patients [104], and leukemia [105]. An elevated level of apo-H in serum samples from patients with early-stage NSCLC was reported [106]. Apo-H, α-1-acid glycoprotein 2 (ORM2), and complement C3 (C3) could be used for the discrimination between HCC patients and healthy people. A novel glycoprotein biomarker panel [apo-H, ORM2, C3, and α-fetoprotein (AFP)] has proven to outperform AFP, the known HCC serum biomarker, alone [107]. Apo-H precursor polypeptide chain has 345 AA, where AA 1-19 – the signal peptide, AA 20-345 – apo-H (pI/Mw: 8.37/36255). 2DE pattern of apo-H represents a chain of spots in the pI-range 6.2-8.4 and Mw ~52000 (Suppl. Fig.1), which is much higher than the theoretical one because of heavy glycosylation (85 N-linked annotations at 4 sites and 3 O-linked annotations at 3 sites) (https://glygen.org/protein/P02749#glycosylation).

2.26. APOLIPOPROTEIN M (APOM_HUMAN)

Apolipoprotein M (apoM) is produced primarily in the liver and kidneys. It is involved in lipid metabolism. Both ApoM mRNA and ApoM protein levels in the HCC tissues were significantly lower than in its adjacent tissues. However, the plasma apoM levels were higher in the HCC patients than in normal subjects [108]. ApoM polypeptide chain has 188 AA (pI/Mw: 5.66/21253), where AA 1-22 – the signal peptide that is not cleaved. 2DE pattern of apoM represents a chain of spots in the pI-range 4.5-6.5 and Mw~22000 (Suppl. Fig.1). There are 13 N-linked annotations at 1 site (N135) in apoM (https://glygen.org/protein/O95445#glycosylation).

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2.27. COMPLEMENT SYSTEM

The complement system includes proteins that cooperatively respond to infection through an inflammatory cascade. Moreover, many complement proteins are proteases that are activated by other proteases. That is, a trigger enzyme cascade occurs, where one complement enzyme, activated by cleavage of its precursor zymogen, cleaves the substrate, which in turn is another zymogen of the complex, to an active enzyme that cleaves the next zymogen, etc. Thus, little activation at the beginning cascade is enhanced after each reaction and leads to a large response. The results of several studies suggest that changes in the complement system can not only promote an anti-tumor response but on the contrary influence tumor development through proliferation, survival, angiogenesis, and invasiveness [278,279]. The presence of many complement components with different functions makes the study of this system very difficult [280]. In any case, it is becoming clear that complement activation stimulates carcinogenesis and protects against immune destruction, although it has long been believed that the complement system helps the body identify and eliminate transformed cells. Moreover, the complement is activated by different mechanisms in the case of different types of cancer, and the results of activation may be different for different types of cancer or over time for the same tumor [281–283].

2.27.1. C1R (C1R_HUMAN)

Complement C1r subcomponent (complement component 1 subcomponent r) is a serine protease that in combination with C1q and C1s forms C1, the first component of the classical pathway of the complement system. Complement C1r subcomponent like (C1RL) was found to be a potential prognostic marker in HCC [110] and renal cell cancer [111]. Its precursor polypeptide chain has 705 AA, where AA 1-17 – the signal peptide, AA 18-705 – complement C1r subcomponent (pI/Mw: 5.76/78213) that is cleaved into complement C1r subcomponent heavy chain (AA 18-463, pI/Mw: 6.10/51136) and complement C1r subcomponent light chain (AA 464-705, pI/Mw: 5.34/27096). 2DE pattern represents only a chain of spots of complement C1r subcomponent in the pI-range 4.5-6.2 and Mw ~80000 (Suppl. Fig.1) that corresponds to only a complement C1r subcomponent. The cleaved heavy and light chains were not detected. There are 25 N-linked glycosylation annotations at 4 sites and 1 phosphorylation site in the complement C1r subcomponent (https://glygen.org/protein/P00736#glycosylation).

2.27.2. C1S (C1S_HUMAN)

Complement C1s subcomponent (C1s, C1 esterase, complement component 1 subcomponent s) is a serine protease that combines with C1q and C1r to form C1, the first component of the classical pathway of the complement system. Overexpression of C1s by tumor cells could be a new escape mechanism to promote tumor progression [112]. The concentration of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1 in serum and tumor tissue may be an important biomarker in determining the diagnosis and prognosis in non-small cell lung cancer patients [113]. C1s precursor polypeptide chain has 688 AA, where AA 1-15 – the signal peptide, AA 16-688 – C1s (Mw: 4.85/74887) that is cleaved into complement C1s subcomponent heavy chain (AA 16-437, pI/Mw: 4.56/47254) and complement C1s subcomponent light chain (AA 438-688, pI/Mw: 7.0/27652). 2DE-pattern represents a chain of 5 spots of C1s in the pI-range from 4.0 to 4.9 and Mw ~80000 (Suppl. Fig.1). The cleaved heavy and light chains were not detected. There are 7 N-linked glycans at 2 sites (https://glygen.org/protein/P09871#glycosylation).

2.27.3. COMPLEMENT C1qC (C1QC_HUMAN)

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Complement C1q subcomponent subunit C associates with the proenzymes C1r and C1s to yield C1, the first component of the serum complement system. High levels of complement protein C1q in pulmonary fibrosis and NSCLC are associated with poor prognosis [109]. Its precursor polypeptide chain has 245 AA, where AA 1-28 – the signal peptide, AA 29-245 – complement C1q subcomponent subunit C (Mw: 8.33/22813). 2DE pattern of C1q represents a long horizontal chain of spots in the pI-range 3.0-9.5 with Mw~23000 and a vertical chain of heavy complexes (Mw 23000 and up) with pI~9.0 (Suppl. Fig.1). It was reported only one O-linked glycosylation of C1q (https://glygen.org/protein/P02747#glycosylation).

2.27.4. COMPLEMENT FACTOR I (CFAI_HUMAN)

Complement factor I (C3B/C4B inactivator) is a trypsin-like serine protease that plays an essential role in regulating the immune response by controlling all complement pathways. The level of complement factor I is enhanced in the plasma of patients with HCC [49]. In opposite, it is downgraded in GC sera compared to normal sera [114]**.** Its precursor polypeptide chain has 583 AA, where AA 1-18 – the signal peptide, AA 19-583 (pI/Mw: 7.38/63487) – a complement factor I that is cleaved to a complement factor I heavy chain (pI/Mw: 6.69/35316) and a complement factor I light chain (pI/Mw: 6.24/27592). 2DE pattern of the complement factor I represents the chains of many spots in the pI-range 4.5-6.8 from Mw~64000 (complement factor I) to Mw~30000 (the complement factor I heavy and light chains) (Suppl. Fig.1). In the SWISS-2DPAGE, the complement factor I is represented only by one spot (pI/Mw: 5.03/37900). There are 57 N-linked glycosylation annotations at 6 sites for the complement factor I (https://glygen.org/protein/P05156#glycosylation).

2.27.5. COMPLEMENT FACTOR B (CFAB_HUMAN)

The complement factor B (C3/C5 convertase, glycine-rich beta glycoprotein, PBF2, properdin factor B) is a part of the alternate pathway of the complement system is cleaved by factor D into 2 fragments: Ba and Bb. Bb, a serine protease, then combines with complement factor 3b to generate the C3 or C5 convertase. It has also been implicated in the proliferation and differentiation of preactivated B-lymphocytes, rapid spreading of peripheral blood monocytes, stimulation of lymphocyte blastogenesis, and lysis of erythrocytes. There is data about high up-regulation of CFB expression in cutaneous squamous cell carcinoma (cSCC) cells [116]. Also, the CFB could be a prognostic marker for THCA (thyroid carcinoma) and its expression influences the infiltration of immune cells [117]. CFB plasma level is enhanced in the case of HCC and B-cell acute lymphoblastic leukemia (B-ALL) [58,69]. Its precursor polypeptide chain has 764 AA, where AA 1-20 – the signal peptide, AA 21-764 – complement factor B (Mw: 6.66/83001) that is cleaved into complement factor B Ba fragment (pI/Mw: 5.97/25983) and complement factor B Bb fragment (pI/Mw: 7.27/57036). 2DE pattern of the complement factor B represents the chains of spots in the pI-range 4.5- 6.8 with Mw ~90000 (Suppl. Fig.1). The cleaved heavy and light chains were not detected. In the SWISS-2DPAGE, the complement factor B is represented by a chain of 6 spots (pI 5.88-6.28, Mw ~100000). There are 19 N-linked glycans (4 sites), and 3 O-linked glycans (3 sites) in the complement factor B (https://glygen.org/protein/P00751#glycosylation).

2.27.6. COMPLEMENT FACTOR D (CFAD_HUMAN)

Complement factor D (CFD) cleaves factor B when the latter is complexed with factor C3b, activating the C3bbb complex, which then becomes the C3 convertase of the alternate pathway. The levels of CFD (plasma and tissue) were significantly downregulated in patients with ductal carcinoma in-situ (DCIS) and invasive BC when compared to controls [118]. Its precursor polypeptide

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chain has 253 AA, where AA 1-20 – the signal peptide, AA21-25 – propeptide, AA 26-253 – complement factor D (Mw: 6.85/24405). 2DE pattern of the complement factor D represents 2 spots (pI ~8.0, Mw 25000) (Suppl. Fig.1). The protein can be phosphorylated (2 sites), glycosylated (2 sites), ubiquitinated (2 sites), methylated (1 site) (https://www.phosphosite.org).

2.27.7. COMPLEMENT FACTOR H (CFAH_HUMAN)

Complement factor H (H factor 1) is a central complement control protein. This glycoprotein has an essential role in the regulation of complement activation, restricting this innate defense mechanism to microbial infections. Alternate transcriptional splice variants, encoding different isoforms, have been characterized. The complement factor H belongs to cancer-related genes and is an unfavorable prognostic marker in renal cancer [32]. It is a progression marker and potential therapeutic target for many cancers including cutaneous squamous cell carcinoma (cSCC) of skin, lung adenocarcinoma, or bladder cancer [119–121]. Its precursor polypeptide chain has 1065 AA, where AA 1-18 – the signal peptide, AA 19-1231 – complement factor H (pI/Mw: 6.12/137053). 2DE pattern of the complement factor D represents a long chain of spots in the pI-range 5.5-7 with Mw ~140000 (Suppl. Fig.1). It was reported 62 N-linked glycans in 9 sites in the complement factor D (https://glygen.org/protein/P08603#glycosylation).

2.27.8 COMPLEMENT C2 (CO2_HUMAN)

The complement C2 (C3/C5 convertase) is part of the classical pathway of the complement system, acting as a multi-domain serine protease. The deficiency of C2 has been associated with several diseases including certain autoimmune diseases such as systemic lupus erythematosus [284]. The complement C2 is an unfavorable prognostic marker in renal cancer [32]. Its precursor polypeptide chain has 752 AA, where AA 1-20 – the signal peptide, AA 21-752 – complement C2 (pI/Mw: 7.57/81085) that is cleaved by factor C1 into the following 2 chains: complement C2b fragment (AA 21–243, pI/Mw: 8.63/23683) and complement C2a fragment (AA 244-752, pI/Mw: 6.46/57420). 2DE pattern of the complement C2 represents a chain of spots in the pI-range 6-7 with Mw~80000 (Suppl. Fig.1). It was reported 33 N-linked glycosylations at 9 sites and one phosphorylation (S266) of the complement C2 (https://glygen.org/protein/P06681#glycosylation).

2.27.9. COMPLEMENT C3 (CO3_HUMAN)

Complement C3 plays a central role in the activation of the complement system. Its activation is required for both classical and alternative complement activation pathways. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that form the mature protein, which is further processed to generate numerous peptide products. The C3a peptide, also known as the C3a anaphylatoxin, modulates inflammation and possesses antimicrobial activity. Complement C3 is an unfavorable prognostic marker in renal cancer and favorable in liver cancer. There is data about high up-regulation of C3 and CFB expression in cSCC cells [116], thyroid carcinoma [93], PDAC [122,123], MM [124,125], and B-cell acute lymphoblastic leukemia (B-ALL) [58]. Its precursor polypeptide chain has 1663 AA, where AA 1-22 – the signal peptide, AA 23-1663 – complement C3 (Mw: 6.00/184951) that is further processed in different fragments: complement C3 beta chain (pI/Mw: 6.82/71317), C3-beta-c (pI/Mw: 6.77/10531), complement C3 alpha chain (pI/Mw: 5.55/113028), C3a anaphylatoxin (pI/Mw: 9.69/9095), acylation stimulating protein (pI/Mw: 9.54/8938), complement C3b alpha' chain (pI/Mw: 5.18/103951), complement C3c alpha' chain fragment 1 (pI/Mw: 6.89/23590), complement C3dg fragment (pI/Mw: 5.00/38905), complement C3g

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fragment (pI/Mw: 3.98/5137), complement C3d fragment (pI/Mw: 5.89/33787), complement C3f fragment (pI/Mw: 10.83/2021), complement C3c alpha' chain fragment 2 (pI/Mw: 4.79/39488). 2DE pattern of the complement C2 represents a cluster of spots in the pI-range 3.5-7.5 and Mw from ~30000 to 180000 (Suppl. Fig.1). In the SWISS-2DPAGE, there are the complement C3 beta chain (5 spots with pI 6.81-6.98, Mw ~71000) and the complement C3dg fragment (a spot with pI 4.84 and Mw 40915). There are 50 N-linked glycans at 4 sites, 2 O-linked glycans at 2 sites, and 12 phosphorylation sites (https://glygen.org/protein/P01024#glycosylation).

2.27.10. COMPLEMENT C4-A (CO4A_HUMAN)

Complement C4-A (acidic complement C4), is a non-enzymatic component of C3 and C5 convertases and thus essential for the propagation of the classical complement pathway. It covalently binds to immunoglobulins and immune complexes (IC) and enhances the solubilization of immune aggregates and the clearance of IC through CR1 on erythrocytes. C4A isotype is responsible for effective binding to form amide bonds with immune aggregates or protein antigens, while C4B isotype catalyzes the transacylation of the thioester carbonyl group to form ester bonds with carbohydrate antigens. Derived from proteolytic degradation of complement C4, C4a anaphylatoxin is a mediator of the local inflammatory process. It induces the contraction of smooth muscle, increases vascular permeability, and causes histamine release from mast cells and basophilic leukocytes. The level of complement C4-A is enhanced in plasma of patients with HCC [52] and MM [124,125]. Its precursor polypeptide chain has 1744 AA, where AA 1-19 – the signal peptide, AA 20-1744 – complement C4-A (pI/Mw: 6.60/190534) that is cleaved into the following 6 chains: complement C4 beta chain (pI/Mw: 8.69/71679), complement C4-A alpha chain (pI/Mw: 5.33/84197), C4a anaphylatoxin (pI/Mw: 9.63/8764), C4b-A (pI/Mw: 4.99/75451), C4d-A (pI/Mw: 4.90/40613), complement C4 gamma chain (pI/Mw: 6.37/33074). 2DE pattern of the complement C4-A represents a wide cluster of spots in the pI-range 3.0-10.0 and Mw from ~25000 to 190000 (Suppl. Fig.1). There are 35 N-linked glycans (4 sites), 6 O-linked glycans (4 sites), and 3 phosphoserine sites (https://glygen.org/protein/P0C0L4#glycosylation).

2.27.11. COMPLEMENT C4-B (C4B) (CO4B_HUMAN)

Complement C4-B (basic complement C4) is a non-enzymatic component of the C3 and C5 convertases and thus essential for the propagation of the classical complement pathway. Complement C4-B covalently binds to immunoglobulins and immune complexes and enhances the solubilization of immune aggregates and the clearance of IC through CR1 on erythrocytes. C4A isotype is responsible for effective binding to form amide bonds with immune aggregates or protein antigens, while C4B isotype catalyzes the transacylation of the thioester carbonyl group to form ester bonds with carbohydrate antigens. The level of complement C4-A is enhanced in the plasma of patients with MM [124,125]. Its precursor polypeptide chain has 1744 AA, where 1-19 – the signal peptide, 20-1744 – complement C4-B (pI/Mw: 6.83/190500) that is cleaved into the following 6 chains: complement C4 beta chain (pI/Mw: 8.69/71679), complement C4-B alpha chain (pI/Mw: 5.51/84163), C4a anaphylatoxin (pI/Mw: 9.63/8764), C4b-B (pI/Mw: 5.12/75417), C4d-B (pI/Mw: 5.19/40549), complement C4 gamma chain (pI/Mw: 6.37/ 33074). 2DE pattern of the complement C4-B represents a wide cluster of spots in the pI-range 3.0-10.0 and Mw from ~35000 to 190000 (Suppl. Fig.1). In the SWISS-2DPAGE, only complement C4 gamma chain (2 spots with pI/Mw: 6.41/31942 and 6.54/31735) was detected. It was reported 34 N-linked glycans (4 sites) and 1 O-linked glycan (1 site) in the complement C4-B (https://glygen.org/protein/P0C0L5#glycosylation).

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2.27.12. COMPLEMENT C5 (CO5_HUMAN)

Complement C5 (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 4) is activated by a C5 convertase and initiates the assembly of the late complement components, C5-C9, into the membrane attack complex. C5b has a transient binding site for C6. The C5b-C6 complex is the foundation upon which the lytic complex is assembled. Complement C5 plasma level is enhanced in the case of HCC and NSCLC [69,126]. Its precursor polypeptide chain has 1676 AA, where 1-18 – the signal peptide, 19-1676 – complement C4-B that is cleaved into 4 chains: complement C5 beta chain (AA 19 – 673, pI/Mw: 5.01/73292), complement C5 alpha chain (AA 678 – 1676, pI/Mw: 7.82/112501), C5a anaphylatoxin (AA 678 – 751, pI/Mw: 8.93/8274), complement C5 alpha' chain (AA 752 – 1676, pI/Mw: 6.88/104246). 2DE pattern of the complement C4-B represents a wide cluster of spots in the pI-range 5.0-6.8 and Mw ~70000-190000 (Suppl. Fig.1). It was reported 8 N-linked glycans at 3 sites (https://glygen.org/protein/P01031#glycosylation).

2.27.13. COMPLEMENT C6 (CO6_HUMAN)

Complement C6 is a constituent of the membrane attack complex that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. Complement C6 belongs to cancer-related genes and is a favorable prognostic marker in renal cancer [32]. The level of complement C4-A is enhanced in the plasma of patients with HCC [52]. Its precursor polypeptide chain has 934 AA, where AA 1-21 – the signal peptide, AA 22-934 – complement C6 (pI/Mw: 6.17/102412). 2DE pattern of the complement C6 represents a chain of spots in the pI-range 4.0-6.5 and Mw~100000 (Suppl. Fig.1). It was reported 6 C-linked annotations at 6 sites, 12 N-linked annotations at 4 sites, 2 O-linked annotations at 2 sites) (https://glygen.org/protein/P13671#glycosylation) and 5 sites of phosphorylation (https://www.phosphosite.org/).

2.27.14. COMPLEMENT C7 (CO7_HUMAN)

Complement C7 is a constituent of the membrane attack complex (MAC) that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. C7 serves as a membrane anchor. Complement C7 plasma level is enhanced in the case of HCC and GC [69,127]. Its precursor polypeptide chain has 934 AA, where AA 1-22 – the signal peptide, AA 23-843 – complement C7 (Mw: 6.09/ 91115). 2DE pattern of the complement C7 represents a chain of spots in the pI-range 4.5-6.5 and Mw ~100000 (Suppl. Fig.1). It was reported 3 N-linked glycans at 2 sites and 2 O-linked glycans at 1 site (https://glygen.org/protein/P10643#glycosylation).

2.27.15. COMPLEMENT C9 (CO9_HUMAN)

Complement component C9 is the pore-forming subunit of the MAC. The interactions of cancer cells with components of the complement system are highly complex, leading to an outcome that is either favorable or detrimental to cancer cells. The complement system may differently affect cancer, ranging from the promotion of cancer growth and metastasis, on the one hand, to antibody-based cancer eradication, on the other [128]. Complement C9 is patented as a diagnostic marker capable of detecting metastasis and prognosis of cancer [129]. The level of C9 protein was significantly higher in GC compared with the norm [130]. Specific glycosylated forms of serum complement C9 were significantly different between esophageal adenocarcinoma (EAC) and benign groups [129]. The precursor polypeptide chain of complement C9 has 559 AA, where AA 1-21 – the signal peptide, AA 22-559 – complement C9 (pI/Mw: 5.42/60979) that is cleaved into 2 chains: complement component

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C9a (AA 22-265, pI/Mw: 4.59/27825) and complement component C9b (AA 266-559, pI/Mw: 8.63/33171). 2DE pattern of the complement C9 represents a chain of spots in the pI-range 4.5-5.5 and Mw~60000 (Suppl. Fig.1). The protein can be glycosylated (10 N-linked glycans at 2 sites, 4 O-linked glycans at 5 sites), phosphorylated (10 sites), acetylated (1 site), ubiquitinated (3 sites) (https://www.phosphosite.org/).

2.28. CARBONIC ANHYDRASE I (CAH1_HUMAN)

Carbonate dehydratase I (carbonic anhydrase B, CAB, carbonic anhydrase I) is a member of a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. CAB is a potential biomarker for PC as its plasma levels in PC patients were significantly higher than those in healthy controls [131]. In opposite, the abundances of CAB in plasma is generally downregulated in GC samples and further decreased in metastatic samples [132]. Its precursor polypeptide chain has 261 AA, where the initiator methionine is removed. AA 2-261 – CAB (pI/Mw: 6.63/28739). 2DE pattern of CAB represents a chain of spots in the pI-range 5-7 and Mw~28000 (Suppl. Fig.1). It was reported glycosylation (2 O-linked at 2 sites) (https://glygen.org/protein/P00915#glycosylation), phosphorylation (11 sites), acetylation (5 sites), ubiquitylation (2 sites) (https://www.phosphosite.org/).

2.29. CORTICOSTEROID-BINDING GLOBULIN (CBG_HUMAN)

Corticosteroid-binding globulin (CBG, serpin A6, transcortin, serpin peptidase inhibitor) is a transport protein. It is produced in the liver, binds sex steroids with high affinity and specificity, and plays a critical role in regulating levels of glucocorticoids in plasma and their access to target cells. CBG levels are increased two- to three-fold in pregnancy and also are raised with estrogen therapy but they are lower in Cushing's syndrome and disease states associated with increased protein loss or decreased synthesis [133]. CBG precursor polypeptide chain has 405 AA, where AA 1-22 – the signal peptide and AA 23-405 – CBG (pI/Mw: 5.64/42639). 2DE pattern of CBG represents a chain of spots in the pI-range 3.7-5.1 and Mw ~50000 (Suppl. Fig.1). It was reported 48 N-linked glycosylations at 6 sites and 2 O-linked glycosylations at 1 site (https://glygen.org/protein/P08185#glycosylation).

2.30. CARBOXYPEPTIDASE N CATALYTIC CHAIN (CBPN_HUMAN)

Carboxypeptidase N catalytic chain (CBPN, CPN1, arginine carboxypeptidase, lysine carboxypeptidase, plasma carboxypeptidase B) is synthesized in the liver, secreted into the blood, and protects the body from potent vasoactive and inflammatory peptides containing C-terminal R or K (kinins or anaphylatoxins), which are released into the bloodstream. CBPN is a potential biomarker complementing CA15-3 for BC and can be used as a tumor marker to diagnose and evaluate invasion and metastasis. The combined detection of CBPN and CA15-3 is more accurate and has a certain value in clinical application [134]. Its precursor polypeptide chain has 458 AA, where AA 1-20 – the signal peptide and AA 21-458 – CBPN (pI/Mw: 6.88/50034). 2DE pattern of CBPN represents a chain of spots in the pI-range 4.5-7 and Mw~50000 (Suppl. Fig.1). It was reported 32 N-linked glycans at 5 sites and 2 O-linked glycans at 1 site https://glygen.org/protein/P08185#glycosylation.

2.31. MONOCYTE DIFFERENTIATION ANTIGEN CD14 (CD14_HUMAN)

Monocyte differentiation antigen CD14 (myeloid cell-specific leucine-rich glycoprotein, CD antigen, CD14) is a part of the innate immune system. It helps to detect bacteria in the body by binding lipopolysaccharide (LPS), a pathogen-associated molecular pattern (PAMP). Its level is significantly elevated in patients with sarcoidosis and is related to the severity of this disease [135].

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The reverse trend was observed in the case of HCC [136]. Its precursor polypeptide chain has 367 AA, where AA 1-19 – the signal peptide, AA 20-367 (pI/Mw: 5.58/37215) – CD14, urinary form, AA 20-345 – CD14, membrane-bound form (pI/Mw: 5.44/35159). 2DE pattern of CD14 represents a chain of spots in the pI-range 4.5-5.8 and Mw~40000 (Suppl. Fig.1). It was reported 26 N-linked glycans at 2 sites and 4 O-linked glycans at 3 sites (https://glygen.org/protein/P08571#glycosylation).

2.32. CERULOPLASMIN (CERU_HUMAN)

Ceruloplasmin (ferroxidase) is a liver-produced glycoprotein that carries more than 95% of the total copper in healthy human plasma [285]. The rest is accounted for by macroglobulins. Ceruloplasmin exhibits a copper-dependent oxidase activity, therefore assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferric state. Serum ceruloplasmin was significantly elevated in advanced stages of solid malignant tumors and has characteristics of good diagnostic markers [137]. It is an unfavorable prognostic marker in renal cancer, HCC, BC, cervical cancer, bile duct cancer, leukoplakia, OC, and squamous cell carcinoma [34,54,138,139] Levels of ceruloplasmin in the ascites fluids were elevated in case of OC compared with other gynecological diseases [140]. Also, ceruloplasmin from normal plasma has four N-glycans, and no LacdiNAc structure that was found only in the case of OC [141]. An increased level of another glycan, sialyl-Lewis X, was detected in the serum of PDAC patients [142]. A precursor polypeptide chain of ceruloplasmin has 1065 AA, where AA 1-19 – the signal peptide, AA 20-1065 – ceruloplasmin (pI/Mw: 5.41/120085). 2DE pattern of ceruloplasmin represents a chain of spots in the pI-range 4.0-6.2 and Mw~120000 (Suppl. Fig.1). In the SWISS-2DPAGE, 3 chains of 27 spots with pI 4.96-5.24 and Mw~120-160000 are present. There are 237 N-linked annotations at 8 sites, 10 O-linked annotations at 7 sites of glycosylation, and 3 sites of phosphorylation for ceruloplasmin (https://glygen.org/protein/P00450#glycosylation).

2.33. CHOLINESTERASE (CHLE_HUMAN)

Cholinesterase (acylcholine acylhydrolase, butyrylcholine esterase, choline esterase II, pseudocholinesterase) is an esterase with broad substrate specificity that is involved in the inactivation of the neurotransmitter acetylcholine. Serum cholinesterase level was significantly lower in the GC group than in the benign gastric disease group [143]. Also, NSCLC patients with a higher level of serum cholinesterase were observed a better prognosis in survival [286]. Its precursor polypeptide chain has 602 AA, where AA 1-28 – the signal peptide and AA 29-602 – cholinesterase (pI/Mw: 6.33/65084). 2DE pattern of cholinesterase represents a chain of 5 spots in the pI-range 4.5-5.2 and Mw~65000 (Suppl. Fig.1). There are 34 N-linked annotations at 12 sites, one O-linked annotation for glycosylation, and phosphorylation at S226 for cholinesterase (https://glygen.org/protein/P06276#glycosylation).

2.34. CLUSTERIN (CLUS_HUMAN)

Clusterin (apolipoprotein J1 (Apo-J), complement cytolysis inhibitor1(CLI1), complement-associated protein SP-40, sulfated glycoprotein 21) is a ubiquitous multifunctional glycoprotein [287]. Through these functions, clusterin is involved in many diseases related to oxidative stress (neurodegeneration, inflammatory diseases, cancers, etc.) [144–146]. Clusterin is a molecular chaperone and has several isoforms that have been differentially implicated in pro- or antiapoptotic processes. Isoform 1 (canonical) functions as an extracellular chaperone that prevents the aggregation of proteins and the formation of amyloid fibrils. Clusterin is a favorable prognostic marker in thyroid

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cancer [32]. Differential glycoform abundance of plasma proteins may be a useful source of biomarkers for the clinical course and prognosis of clear cell renal cell carcinoma (ccRCC) [147]. The level of clusterin is enhanced in plasma of patients with BC, CRC, HCC, and B-cell acute lymphoblastic leukemia (B-ALL) [52,58,69,148,149]. Clusterin precursor polypeptide chain has 449 AA, where AA 1-22 – the signal peptide, AA 23-449 – clusterin (pI/Mw: 5.89/50063) that is cleaved to clusterin beta chain (AA 23-227, pI/Mw: 6.27/24197), and clusterin alpha chain (AA 228-449, pI/Mw: 5.65/25883). 2DE pattern of ceruloplasmin represents a chain of 18 spots in the pI-range 4.5-6.5 and Mw~35000 (Suppl. Fig.1). In the SWISS-2DPAGE, 17 spots with pI 4.73-5.07 and Mw~35-39000 are shown. Clusterin is heavyly glycosylated (149 N-linked glycans at 6 sites, O-linked glycan at 1 site) and phosphorylated (4 sites) (https://glygen.org/protein/P10909#glycosylation).

2.35. BETA-ALA-HIS DIPEPTIDASE (CNDP1_HUMAN)

Beta-Ala-His dipeptidase (CNDP dipeptidase 1, carnosine dipeptidase 1, glutamate carboxypeptidase-like protein 2, serum carnosinase) catalyzes the peptide bond hydrolysis in Xaa-His dipeptides, displaying the highest activity toward carnosine (beta-alanyl-L-histidine) and anserine (beta-alanyl-3-methyl-histidine). It is expressed mainly in the brain and liver. Beta-Ala-His dipeptidase levels in plasma increase with age up to ~15 years [288]. Alterations of its activity are associated with several pathological conditions, such as neurological disorders, chronic diseases, and cancer [150]. Its reduced levels were observed in plasma specimens of patients with GBM, GC, and metastatic prostate cancer [151–153]. The dipeptidase precursor polypeptide chain has 507 AA, where AA 1-26 – the signal peptide, AA 27-507– beta-Ala-His dipeptidase (pI/Mw: 5.08/53864). 2DE pattern of beta-Ala-His dipeptidase represents 2 spots around pI-5.0 and Mw~54000 (Suppl. Fig.1). It was reported 18 N-linked glycans at 1 site, 1 O-linked glycan at 2 sites, and phosphorylation at S219 (https://glygen.org/protein/Q96KN2#glycosylation).

2.36. CARBOXYPEPTIDASE N SUBUNIT 2 (CPN2_HUMAN)

Carboxypeptidase N subunit 2 (carboxypeptidase N 83 kDa chain, carboxypeptidase N large subunit) is produced mainly in the liver and secreted into the bloodstream. The carboxypeptidase N subunit 2 belongs to favorable prognostic markers in liver cancer and unfavorable markers in renal cancer [32]. Its precursor polypeptide chain has 545 AA, where AA 1-21 – the signal peptide, AA 22-545 – carboxypeptidase N subunit 2 (pI/Mw: 5.54/58227). 2DE pattern of carboxypeptidase N subunit 2 represents 9 spots with pI-3.5-5.5 and Mw~65000 (Suppl. Fig.1). It is known 10 N-linked glycans at 3 sites in carboxypeptidase N subunit 2 (https://www.glygen.org/protein/P22792).

2.37. C-REACTIVE PROTEIN (CRP_HUMAN)

C-reactive protein (CRP) displays several functions associated with host defense: it promotes agglutination, bacterial capsular swelling, phagocytosis, and complement fixation through its calcium-dependent binding to phosphorylcholine. It can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells. CRP belongs to cancer-related genes and is an unfavorable prognostic marker in renal cancer. Elevated CRP levels (> 10 μg/ml) are associated with active cancer disease. Significantly, elevated CRP levels (above 50–100 μg/ml) are associated with advanced cancer, metastasis, and poor response prognosis [154,155]. The precursor polypeptide chain of CRP has 224 AA, where AA 1-18 – the signal peptide, AA 19-224 – CRP (pI/Mw: 5.28/23047). 2DE pattern of CRP represents a single spot (pI/Mw: 5.2/24000) (Suppl. Fig.1). In the SWISS-2DPAGE, a similar situation exists – a single spot (pI/Mw: 5.12/23760). So far, it was reported

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only one PTM (a pyroglutamic acid, Q19) for CRP (https://www.uniprot.org/uniprotkb/P02741/entry).

2.38. EXTRACELLULAR MATRIX PROTEIN I (ECM1_HUMAN)

Extracellular matrix protein 1 (ECM1, secretory component p85) is a glycoprotein secreted by different tissues and involved in cell proliferation, angiogenesis, and differentiation. It is also involved in endochondral bone formation as a negative regulator of bone mineralization. Its high level is correlated with invasiveness and poor prognosis in different cancers (BC, GC, cholangiocarcinoma, laryngeal carcinoma). In particular, high ECM1 level in serum of BC patients is associated with recurrence of tumor [156]. ECM1 is a potential plasma protein biomarker for the detection and progression of primary ESCC [157]. It is an unfavorable prognostic marker in renal cancer, urothelial cancer, and PDAC [36]. In opposite, ECM1 level in plasma of OC patients was lower than in healthy control [158]**.** Its precursor polypeptide chain has 540 AA, where AA 1-19 – the signal peptide, AA 20-540 – ECM1 (pI/Mw: 6.19/58812). 2DE pattern of ECM1 represents a spot (pI/Mw: 6.0/60000) (Suppl. Fig.1). It was reported 21 N-linked glycans at 4 sites, 3 O-linked glycans at 6 sites (https://www.glygen.org/protein/Q16610).

2.39. FIBULIN-1 (FBLN1_HUMAN)

Fibulin-1 (FIBL-1) is a component of the extracellular matrix (ECM). It mediates platelet adhesion via binding fibrinogen. Plasma fibulin-1 level is significantly increased in plasma of patients with HCC compared with those in healthy controls, individuals with chronic hepatitis B, and patients with HBV-induced liver cirrhosis [159]. A similar situation was observed in the case of CRC and malignant mesothelioma [160–162]. Fibulin-1 and cathepsin F (CTSF) levels were specifically upregulated in sera and tissues of patients with NSCLC with brain metastasis (BM) compared with NSCLC without BM and primary brain tumor. The combined diagnostic performance of CTSF and FBLN-1 was superior to their ones [163]. Its precursor polypeptide chain has 703 AA, where AA 1-29 – the signal peptide, AA 30-703 – fibulin-1 (pI/Mw: 5.03/74291). 2DE pattern of FIBL-1 represents a chain of 4 spots (pI/Mw: 4.5-5.2/75000) (Suppl. Fig.1). It was reported 10 N-linked glycans at 2 sites, one O-linked glycan, and one phosphorylation at S147 (https://www.glygen.org/protein/P23142).

2.40. FICOLIN-3 (FCN3_HUMAN)

Ficolin-3 (collagen/fibrinogen domain-containing lectin 3 p35, collagen/fibrinogen domain-containing protein 3, Hakata antigen) – is a lectin involved in the activation of the anti-microbial and anti-cancer pathway. There are three ficolins (1,2,3), and they may be considered biomarkers of acute myeloid leukemia (AML): low ficolin-1 as well as high ficolin-2 and ficolin-3 seem to be associated with AML and differentiate patients not only from healthy controls but also from some other hematological malignancies (at least multiple myeloma and lymphoma) [164]. Serum ficolin-3 level could be an independent prognostic marker for survival in patients with esophageal cancer (EC) [165]. Elevated plasma levels of ficolin-3 have been reported in lung cancer, OC, and MM [166–168]. Ficolin-3 precursor polypeptide chain has 299 AA, where AA 1-23 – the signal peptide, AA 24-299 – ficolin (pI/Mw 6.22/30354). 2DE-pattern of ficolin-3 represents a chain of 5 spots (pI/Mw: 5.8-6.5/30000) (Suppl. Fig.1). It was reported 5 N-linked annotation(s) at 1 site (https://www.glygen.org/protein/O75636).

2.41. ALPHA-FETOPROTEIN (FETA_HUMAN)

Alpha-fetoprotein (AFP, alpha-1-fetoprotein, alpha-fetoglobulin, alpha fetal protein), is a major fetal serum globulin [289]. The fetal liver can produce AFP at a rate of 30 mg/day. Its plasma level is high either in malignant tumors or during intrauterine or early postnatal development. The AFP gene is almost completely repressed in a mature fetus, which leads to the protein's disappearance [290,291]. AFP blood concentration in normal adults is below 40 ng/ml. Serum AFP has long been

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used as a diagnostic marker for HCC. A level above 500 ng/ml indicates malignant neoplasm (HCC), except in pregnancy [291]. AFP can be heavily glycosylated (N251) and phosphorylated (S111, S115, S117, S344, S444, S445). There are several proteoforms of AFP that contain different sugars. According to their binding ability to the lectin lens agglutinin (LCA), it is known at least three glycoforms (AFP-L1, AFP-L2, and AFP-L3). AFP-L1 is the main glycoform in various benign liver diseases. In contrast, AFP-L3 is present only in the serum of patients with HCC [292]. Accordingly, a method of measuring total and glycosylated AFP was developed to improve the efficacy of AFP as HCC diagnostic marker, [293,294]. AFP precursor polypeptide chain has 609 AA, where AA 1-18 – the signal peptide (cleaved after processing) and AA 19-609 – alpha-fetoprotein (pI/Mw: 5.53/66478) [295]. It was reported 61 N-linked annotations at 3 sites, 1 O-linked annotation at 1 site for glycosylation, and 7 sites of phosphorylation (https://www.glygen.org/protein/P02771).

2.42. ALPHA-2-HS-GLYCOPROTEIN (FETUA_HUMAN)

Alpha-2-HS-glycoprotein (fetuin-A, alpha-2-Z-globulin, Ba-alpha-2-glycoprotein) is a serum glycoprotein synthesized by hepatocytes, secreted in plasma, and selectively concentrated in bone matrix. Fetuin-A is a protein of the fetuin cysteine protease inhibitor family, that is negatively involved in the acute phase response. The plasma level of fetuin is 5-fold higher in the fetus than in adults [296]**.** It is involved in several processes, including endocytosis, brain development, diabetes, formation of bone tissue, kidney disease, and cancer [169,170]**.** The fetuin-A auto-antibodies often can be detected in the tumor patient's plasma [170]. Also, there is a possibility that the modified forms of fetuin-A such as fucosylated forms could become a more reliable tumor biomarker. Its precursor polypeptide chain has 367 AA, where AA 1-18 – the signal peptide, AA 19-367 – alpha-2-HS-glycoprotein that is cleaved to chain A (AA 19-300, pI/Mw: 4.53/30238) and chain B (AA 341-367, pI/Mw: 10.86/2740). 2DE-pattern of fetuin-A represents a set of proteoforms (pI/Mw: 3.7-6.3/~40000-up) (Suppl. Fig.1). In the SWISS-2DPAGE, 15 spots (pI/Mw: 4.56-4.77/52-58000) are shown. The protein is heavily glycosylated (126 N-linked annotations at 2 sites, 43 O-linked annotations at 14 sites and phosphorylated (https://www.glygen.org/protein/P02765).

2.43. FETUIN-B (FETUB_HUMAN)

Fetuin-B (16G2, fetuin-like protein IRL685, Gugu) is a protease inhibitor required for egg fertilization. Fetuin-B is a second protein of the fetuin family that is produced in the liver and testis. The higher level of fetuin-B in plasma is correlated with a favorable outcome in the case of CRC [171]. Its precursor polypeptide chain has 382 AA, where AA 1-15 – the signal peptide, AA 16-382 – fetuin-B (pI/Mw: 6.52/40488). 2DE pattern of fetuin-B represents a chain of proteoforms (pI/Mw: 5.0-6.3/50000-up) (Suppl. Fig.1). The protein is heavily glycosylated (26 N-linked annotations at 3 sites, 8 O-linked annotation(s) at 6 sites) and phosphorylated (https://www.glygen.org/protein/Q9UGM5).

2.44. FIBRINOGEN ALPHA CHAIN (FIBA_HUMAN)

Fibrinogen is a glycoprotein composed of two trimers (fibrinogen alpha (FBA), fibrinogen beta (FBB), and fibrinogen gamma (GBG)). It is secreted in the liver and is involved in the formation of blood clots to help stop bleeding. This process is triggered by its cleavage, which leads to the formation of the insoluble fibrin matrix. Fibrin deposition is also associated with infection and the immune response. It was proposed that "high levels of serum fibrinogen may be associated with increased fibrinogen deposits in tumor tissue and serve as an extracellular matrix for tumor cell adhesion or migration, which may lead to tumor metastasis, and promote tumor neovascularization

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and angiogenesis, enhance adhesion and invasion" [172]. FGA plasma level is enhanced in HCC [69]. What is important, using 2DE it was shown that FGA has 8 proteoforms, which levels behaved differently during OC progression [173]. FGA is an unfavorable prognostic marker in renal cancer and favorable in liver cancer [32]. A precursor polypeptide chain of FBA has 491 AA, where AA 1-19 – the signal peptide, AA 20-35 – fibrinopeptide A (pI/Mw: 3.92/1537), and AA 36-866 -- mature FBA (pI/Mw: 5.79/91359). 2DE-pattern of FBA represents several sets of chains with pI 5.0-7.5 (Mw~30-35000, Mw~64-83000, Mw~110000 and up) (Suppl. Fig.1). In the SWISS-2DPAGE, a double chain of 19 spots (pI/Mw: 6.65-7.78/63-67000) is presented [22]. The protein can be heavily glycosylated (12 N-linked annotations at 3 sites, 43 O-linked annotations at 34 sites) and phosphorylated (https://www.glygen.org/protein/P02671).

2.45. FIBRINOGEN BETA CHAIN (FIBB_HUMAN)

Fibrinogen beta together with FGA and FGG polymerizes to form an insoluble fibrin matrix. It was proposed that FGB could play a key role as a diagnostic and therapeutic biomarker for hepatic metastatic CRC [174]. In the case of OC, FIBB is over-expressed together with other APPs (orosomucoid-1 (A1AG1), FIBA, FIBG, HPT) [173]. FBB chain is a prognostic unfavorable marker in renal cancer [32]. FBB precursor polypeptide chain has 491 AA, where AA 1-30 – the signal peptide, AA 31-44 – fibrinopeptide B (pI/Mw: 4.14/1570), and AA 45-491 – mature FBB chain (pI/Mw: 7.95/50763). 2DE-pattern of FBB represents a chain of spots (pI/Mw: 5.5-8.5/~52000) (Suppl. Fig.1). In the SWISS-2DPAGE, a chain of 4 spots (pI/Mw: 6.1-6.55/55-56000) is presented [22]. The protein can be glycosylated (52 N-linked annotations at 4 sites and 5 O-linked annotations at 3 sites) (https://www.glygen.org/protein/P02675).

2.46. FIBRINOGEN GAMMA CHAIN (FIBG_HUMAN)

Together with FGA and FGB, FGG forms an insoluble fibrin matrix. In addition, it functions during the early stages of wound repair, stabilizes the lesion, and guide cell migration during re-epithelialization. FGG is an unfavorable prognostic marker in renal cancer [32]. FGG plasma level is enhanced in the case of HCC [69,175]. The serum FGG levels in patients with castration-resistant prostate cancer (CRPC) were significantly higher than in those with localized PC [176]. Using 2D-DIGE of pleural effusions from lung adenocarcinoma and benign inflammatory disease (pneumonia and tuberculosis) it was shown that FGG is up-regulated in cancer samples [177]. An elevated level of crosslinked fibrinogen gamma-chain dimer in plasma may correlate with tumor-associated fibrin deposition [178]. FGG precursor polypeptide chain has 453 AA, where AA 1-26 – the signal peptide, AA 27-453 –- mature FGG (pI/Mw: 5.24/48483). 2DE-pattern of FGG represents a chain of spots with pI 4.5-7 (Mw~50000) (Suppl. Fig.1). In the SWISS-2DPAGE, 3 chains of 13 spots (pI/Mw: 5.07-5.65/44-51000) are presented [22]. The protein is glycosylated (39 N-linked annotations at 4 sites, 1 O-linked annotation at 1 site) and phosphorylated at S68 (https://www.glygen.org/protein/P02679).

2.47. FIBRONECTIN (FINC_HUMAN)

Soluble plasma fibronectin (FN, Cold-insoluble globulin, CIG) is a major protein component of blood plasma (300 μg/ml) and is produced by hepatocytes. Fibronectin binds cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. The enhanced plasma level of fibronectin may be used as a diagnostic marker in patients with GC [179]. FN levels were significantly elevated at all stages of BC and returned to normal after tumor removal [180]. Plasma FN has been proposed as a promising tool for cancer screening based on preclinical and clinical data. Altered

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fibronectin expression, degradation, and organization have been associated with many pathologies, including cancer, arthritis, and fibrosis [48,181,182]. The abnormal glycosylation changes of fibronectin were detected in the case of OC [94]. FN precursor polypeptide chain has 2477 AA, where AA 1-31 – the signal peptide, AA 32-2477– FN (pI/Mw: 5.25/269259) that is cleaved into the following 4 chains: anastellin (AA 627-702), Ugl-Y1 (AA 723-911), Ugl-Y2 (AA 723-903), Ugl-Y3 (AA 723- ?). 2DE-pattern of fibronectin represents a chain of spots with pI 4.5-6.7 (Mw~112000 up) (Suppl. Fig.1). The protein is heavily glycosylated (265 N-linked annotations at 13 sites, 37 O-linked annotations at 25 sites) and phosphorylated (https://www.glygen.org/protein/P02751).

2.48. PLASMA GELSOLIN (GELS_HUMAN)

Plasma gelsolin (AGEL, actin-depolymerizing factor, ADF, brevin) is a highly conserved plasma protein. Its role is to keep inflammation localized to the site of injury and to boost the body's ability to clear pathogens. Its extraordinary structural conservation reflects its critical regulatory role in multiple essential functions [297]**.** Its roles include the breakdown of filamentous actin released from dead cells, activation of macrophages, and localization of the inflammatory response. Substantial decreases in plasma levels are observed in acute and chronic infection and injury in both animal models and in humans. Supplementation therapies with recombinant human gelsolin have been shown effective in more than 20 animal models. Actin is the most abundant cellular protein, and its release into extracellular fluid and circulation following cellular injury from disease or injury leads to increased blood viscosity, hindered microcirculation, and activation of platelets [297]**.** Hemodialysis patients with low levels of gelsolin and high levels of actin in blood had markedly higher mortality [185]**.** Circulating gelsolin levels were significantly lower in patients with head and neck cancer (HNC) or LC compare to healthy controls [166,183]**.** Moreover, circulating gelsolin outperformed other candidate biomarkers as an independent diagnostic biomarker of HNC in both sensitivity and specificity [183]**.** Proteomics provided data about increased levels of gelsolin in metastatic versus non-metastatic patients. This data was supported by the correlation of gelsolin levels expressed and secreted by CRC cells and their enhanced migration capabilities *in vitro*. Accordingly, plasma gelsolin could be a biomarker of metastasis status in CRC patients [184]**.** Its precursor polypeptide chain has 782 AA, where AA 1-27 – the signal peptide, AA 28-782 – gelsolin (pI/Mw: 5.72/82959). 2DE-pattern of gelsolin represents a chain of spots with pI 4.5-6.5 (Mw~83000) (Suppl. Fig.1). The protein can be heavily phosphorylated (25 sites), acetylated (12 sites), ubiquitinated (10 sites) (https://www.phosphosite.org).

2.49. GLUTATHION PEROXIDASE 3 (GPX3_HUMAN)

Glutathione peroxidase 3 (GPx-3, GSHPx-3, extracellular glutathione peroxidase, plasma glutathione peroxidase) protects cells and enzymes from oxidative damage, by catalyzing the reduction of hydrogen peroxide, lipid peroxides, and organic hydroperoxide, by glutathione. Patients with CRC had higher levels of oxidative stress and antioxidant activity including glutathione peroxidase compared to healthy controls. After surgical resection, a significant decline in oxidative stress biomarkers is observed [213]**.** Its precursor polypeptide chain has 226 AA, where AA 1-20 – the signal peptide, AA 21-226 –- mature glutathione peroxidase 3 chain (pI/Mw: 7.85/23464). 2DE-pattern of this protein represents a chain of spots with pI 4.9-6.9 (Mw~25000) (Suppl. Fig.1). It's phosphorylated (4 sites) and acetylated (3 sites) (https://www.phosphosite.org/).

2.50. HEMOGLOBIN SUBUNIT ALPHA (HBA_HUMAN)

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Hemoglobin subunit alpha (alpha-globin, hemoglobin alpha chain) is involved in oxygen transport from the lung to the various peripheral tissues. Hemoglobin α/β (14.4 kDa was overexpressed in the OC sera [54]. Hemoglobin subunit alpha precursor polypeptide chain has 124 AA, where the initiator methionine is removed (pI/Mw: 8.73/15126). 2DE-pattern of this protein represents a chain of spots with pI 7.5-9 (Mw~15000) (Suppl. Fig.1). In the SWISS-2DPAGE, 2 spots (pI/Mw: 9.2/11000, 8.9/11000) are presented. The protein is glycosylated (3 O-linked annotations at 3 sites), glycated (6 sites) (https://www.glygen.org/protein/P69905), phosphorylated (17 sites), acetylated (4 sites), and ubiquitinated (8 sites) (https://www.phosphosite.org/).

2.51. HEMOGLOBIN SUBUNIT BETA (HBB_HUMAN)

Hemoglobin subunit beta (beta-globin, hemoglobin beta chain) is involved in oxygen transport from the lung to the various peripheral tissues. The available information shows that there is more than one reason to pay attention to Hb levels in cancer patients: increasing Hb not only corrects anemia and thereby improves physical functioning and quality of life but also may improve clinical outcomes [186]. Hemoglobin subunit beta precursor polypeptide chain has 127 AA, where the initiator methionine is removed (pI/Mw: 6.81/15867). It can be further cleaved into the following 2 peptides: LVV-hemorphin-7 (AA 33-42, pI/Mw: 8.75/1309) and spinorphin (AA 33-39, pI/Mw: 5.52/877). 2DE-pattern of this protein represents a chain of spots with pI 6.5-6.9 (Mw~15000) (Suppl. Fig.1). In the SWISS-2DPAGE, 2 spots (pI/Mw: 7/15000, 6.9/15000) are presented. The protein is glycosylated (4 O-linked annotations at 4 sites), glycated (6 sites), and phosphorylated (14 sites) (https://www.glygen.org/protein/P68871).

2.52. HEMOPEXIN (HEMO_HUMAN)

Hemopexin (beta-1B-glycoprotein) binds heme and transports it to the liver for breakdown and iron recovery, after which the free hemopexin returns to circulation. According to "The human protein atlas", hemopexin is a favorable prognostic marker in liver cancer. Hemopexin has emerged in a series of works looking for possible diagnostic markers for different kinds of tumors [187]. Using 2D-DIGE of pleural effusions from lung adenocarcinoma and benign inflammatory disease (pneumonia and tuberculosis) it was shown that hemopexin was up-regulated in cancer samples [177]. It was observed the appearance of fucosylated Hx in the sera of HCC patients [187,188]. Hemopexin is among the genes that were most significantly correlated with the differences in proteomic profiles of normal and BC tissues [189]. Its precursor polypeptide chain has 462 AA, where AA 1-23 – the signal peptide, AA 24-462 – mature hemopexin (pI/Mw: 6.43/49295). 2DE-pattern of this protein represents a chain of spots with pI 5-6.9 (Mw~50000) (Suppl. Fig.1). In the SWISS-2DPAGE, a chain of 5 spots (pI 5.25-5.59 /Mw ~72-77000) and 2 spots (4.48/19274, 4.56/18289) are presented. The protein is glycosylated (184 N-linked annotations at 6 sites, 21 O-linked annotations at 6 sites (https://www.glygen.org/protein/P02790).

2.53. HEPARIN COFACTOR 2 (HEP2_HUMAN)

Heparin cofactor 2 (heparin cofactor II, HC-II, protease inhibitor leuserpin-2, HLS2, serpin D1) is a thrombin inhibitor activated by the glycosaminoglycans, heparin, or dermatan sulfate. Heparin cofactor II enhances cell motility and promotes metastasis in NSCLC [190]. Serpin D1 level is significantly elevated in epithelial OC [191]. The levels of plasma serpin D1 were significantly higher in the case of BC or B-Cell acute lymphoblastic leukemia (B-ALL) compared to the control groups. Moreover, the relative level showed a significant positive correlation with cancer grade, stage, and

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presence of metastasis [58,191]. But plasma levels of serpin D1 in the PC patients were below normal [192]. The precursor polypeptide chain of serpin D1 canonical isoform has 499AA, where AA 1-19 – the signal peptide, AA 20-499 – serpin D1 (Mw: 6.26/54960). 2DE-pattern of this protein represents a chain of spots with pI 4.9-6.5 (Mw~55000) (Suppl. Fig.1). The protein is glycosylated (39 N-linked annotations at 3 sites, 13 O-linked annotations at 9 sites) and phosphorylated at S37 (https://www.glygen.org/protein/P05546).

2.54. HAPTOGLOBIN (HPT_HUMAN)

Haptoglobin (Hp) captures free plasma hemoglobin to allow the recycling of heme iron and to prevent kidney damage. Haptoglobin also acts as an antioxidant, has antibacterial activity, and plays a role in modulating many aspects of the acute phase response. Hemoglobin/haptoglobin complexes are rapidly cleared by the macrophage CD163 scavenger receptor expressed on the surface of liver Kupfer cells through an endocytic lysosomal degradation pathway. The human Hp gene is polymorphic and has three structural alleles that control the synthesis of three major phenotypes of haptoglobin: homozygous Hp1-1 and Hp2-2, and heterozygous Hp2-1, determined by a combination of allelic variants that are inherited. Accordingly, haptoglobin has two isoforms. The precursor polypeptide chain of haptoglobin canonical isoform 1 has 406 AA, where AA 1-18 – the signal peptide, AA 19-406 – pro-haptoglobin that is further cleaved into the following 2 chains: haptoglobin alpha 2 chain (AA 19-160, pI/Mw: 5.57/15946) and haptoglobin beta-chain (AA 162-406, pI/Mw: 6.32/27265). The sequence of isoform 2 is characterized by the absence of AA 38-96 (haptoglobin alpha 1 chain, pI/Mw: 5.23/9355). The concentration in normal human serum of haptoglobin beta chain is 6–40 μmol/L, haptoglobin alpha 1 chain or haptoglobin alpha 2 chain – 0–40 μmol/L [20]. A variant is possible here, when the canonical form encoded by the Hp2-2 allele does not undergo proteolytic processing, remains full-length, and functions as a zonulin protein. Proteomic analysis showed that the zonulin protein is identical to Pre-Hp2 [298]. Zonulin is involved in the permeability of tight junctions between cells in the digestive tract [299]. According to "The human protein atlas" haptoglobin belongs to cancer-related genes and is an unfavorable prognostic marker in renal cancer. An elevated level of serum Hp was identified as a prognostic marker in multiple types of solid tumors, which is correlated with poor prognosis [193]. In patients with OC or lung cancer, a significant increase in the level of Hp in plasma was found in comparison with the control [166,194]. Moreover, the increased amount of fucosylated forms of the α-chain of haptoglobin in ascitic fluids and tumor tissues of patients with OC confirms their potential as biomarkers of disease progression [195–198]. There is also a possibility that a non-processed form of haptoglobin (zonulin) can be a biomarker of GBM [199]. 2DE pattern of Hp represents ~16 spots of beta-chain with pI 4.8-6.0 (Mw~40000) and 3 spots of alpha 2 chain (Fig.3). In the SWISS-2DPAGE, a chain of 19 spots (pI 4.88-5.86 /Mw ~40000, beta chain), 3 spots (pI 5.68-6.37/ Mw ~17000, alpha 2 chain), and 2 spots (pI 5.13-5.37/ Mw ~12000, alpha 1 chain) are presented. Hp is heavyly glycosylated (351 N-linked annotations at 4 sites, 1 O-linked annotations at 1 site) (https://www.glygen.org/protein/P00738).

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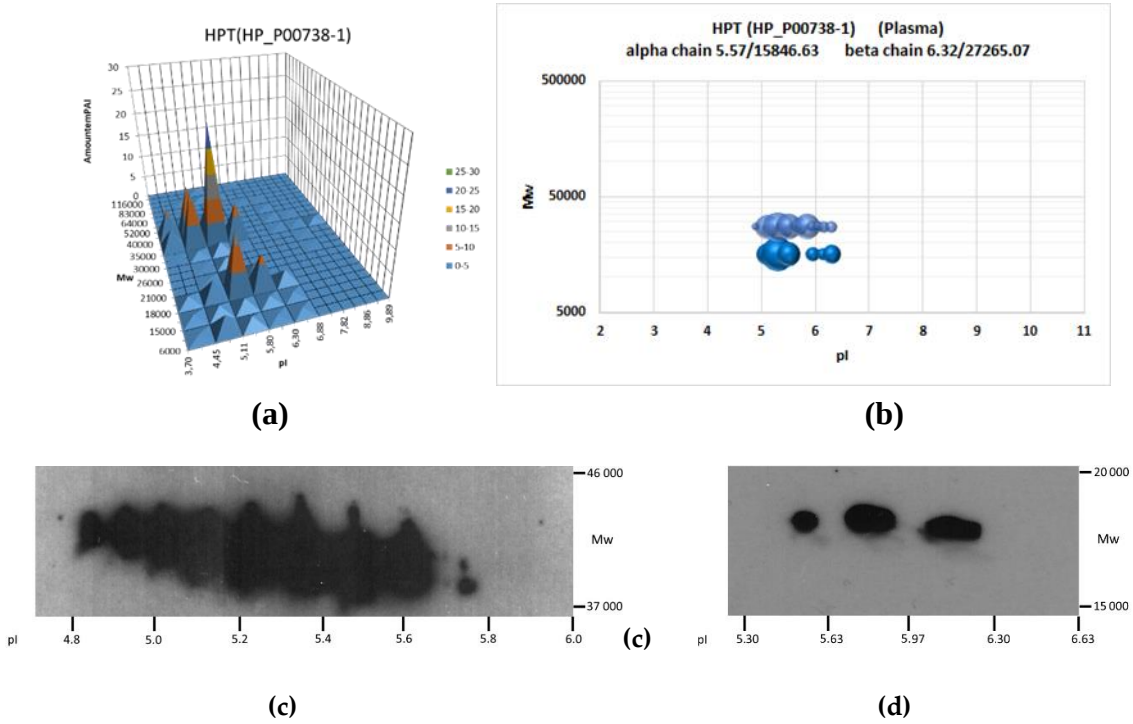

Figure 2. 2DE-patterns of haptoglobin alpha- and beta-chains. (a) A sectional analysis of alpha- and beta-chains. (b) A semi-virtual 2DE alpha- and beta-chains (c) 2DE-Western of beta chain. (d) 2DE-Western of alpha2 chain.

2.55. HAPTOGLOBIN-RELATED PROTEIN (HPTR_HUMAN)

Haptoglobin-related protein (Hpr) mediates human innate immune protection against many species of African trypanosomes. Similar to haptoglobin, it binds hemoglobin with high affinity and may contribute to the clearance of cell-free hemoglobin. Serum Hpr levels in the malignant lymphoma patients were significantly higher than in the control group. Also, the Hpr levels were correlated with advances in the disease [201]. Its polypeptide chain has 348 AA (pI/Mw: 6.63/39030), where AA 1-18 – the signal peptide that is not cleaved. 2DE pattern of this protein represents a chain of spots with pI 4.6-6.5 (Mw~40000) (Suppl. Fig.1). The protein is N-linked glycosylated (5 sites), acetylated (1 site), and ubiquitinated (2 sites) (https://www.phosphosite.org/).

2.56. HISTIDINE-RICH GLYCOPROTEIN (HRG_HUMAN)

Histidine-rich glycoprotein (HRG, histidine-proline-rich glycoprotein, HPRG) is a liver-produced glycoprotein but could also be synthesized by monocytes, macrophages, and megakaryocytes [300]**.** Through binding multiple ligands, HRG has many functions in immunity, cell adhesion, angiogenesis, and thrombosis. Many of these functions are involved in tumor progression and antitumor response [301]**.** For instance, its serum and expression levels were positively related to the poor risk in acute lymphoblastic leukemia (ALL) patients, and HRG might be a diagnostic biomarker, with high sensitivity and specificity [202]**.** But it was shown that a decreased HRG level occurs in advanced LC and is associated with the disease stage and hypofibrinolysis [203]**.** HRG precursor polypeptide chain has 525 AA, where AA 1-18 – the signal peptide and AA 19-525 – histidine-rich glycoprotein (pI/Mw: 7.03/57660). 2DE-pattern of this protein represents a chain of spots with pI 4.5-7.8 (Mw~64000) and spots around pI/Mw: 5.5/53000 (Suppl. Fig.1). In the SWISS-

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2DPAGE, only a single spot (pI/Mw: 5.3/ 53000) is present. The protein is glycosylated (44 N-linked glycans at 4 sites, 4 O-linked glycans at 3 sites) (https://www.glygen.org/protein/P04196).

2.57. PLASMA PROTEASE C1 INHIBITOR (IC1_HUMAN)

Plasma protease C1 inhibitor (C1-inh, C1 esterase inhibitor) is a protease inhibitor belonging to the serpin superfamily that circulates in the blood at a level of around 0.25 g/L [302]. The level rises ~2-fold during inflammation, as it is an APP. Its main function is the inhibition of the complement system to prevent spontaneous activation but also as the major regulator of the contact system [303]. In the study of patients with various solid cancers, it was shown a high pre-operative C1-inh level. What is more, this situation was observed in patients with early cancer recurrence [204]. But in another study, it was shown that the levels of C1-inh were significantly lowered in BC patients compared to those of the controls [205]. C1-inh precursor polypeptide chain has 500 AA, where AA 1-22 – the signal peptide, AA 23-500 – plasma protease C1 inhibitor (pI/Mw: 5.97/52843). 2DE-pattern of this protein represents a long chain of spots with pI 3.2-5.2 (Mw~64000). The protein is heavily glycosylated (107 N-linked annotations at 8 sites, 33 O-linked annotations at 21 sites) (https://www.glygen.org/protein/P05155).

2.58. INTER-ALPHA-TRYPSIN INHIBITOR HEAVY CHAINS (ITIH1, ITIH2, ITIH3, ITIH4, ITIH5)

The inter-alpha (globulin) inhibitor (ITI) family (more commonly called the family of inter-alpha-trypsin inhibitors) is composed of serine protease inhibitors that are assembled from two precursor proteins: a light chain and either one or two heavy chains [304]. While there is only one type of light chain, there are different homologous heavy chains (ITIHs), to date consisting of five members Inter-alpha-trypsin inhibitors (ITI) are a family of plasma protease inhibitors, assembled from a light chain – bikunin, encoded by AMBP – and five homologous heavy chains (encoded by ITIH1, ITIH2, ITIH3, ITIH4, and ITIH5), contributing to extracellular matrix stability by covalent linkage to hyaluronan. So far, ITIH molecules have been shown to play a particularly important role in inflammation and carcinogenesis [206]. ITIH levels are downregulated in human solid tumors, including breast, colon, and lung cancer [206]. IHC score assessment showed a dramatic reduction in ITIH3 expression and, conversely, upregulation of ITIH4 in colorectal carcinoma specimens relative to adjacent normal colorectal tissues. Circulating ITIH3 and ITIH4 levels are associated with carcinogenesis in CRC, supporting their potential diagnostic utility as surrogate biomarkers for CRC detection [207]. ITIH1 had a substantially decreased level in HCC compared with corresponding normal tissue, and its regulation adversely impacted the patient outcome. Moreover, ITIH1 level was consistently declining during the progression of HCC [208]. Plasma ITIH3 is downregulated while ITIH4 is upregulated in CRC patients, similar to the situation in CRC tissues [207]. But in the case of GC patients, levels of ITIH3 [209] and ITIH4 [210] were higher than in the control group. ITIH4 plasma level in endometrial cancer samples was also higher compared to the control subjects [29]. ITIH1 precursor polypeptide chain has 911 AA, where AA 1-27 – the signal peptide, AA 28-34 (pI/Mw: 9.75/661) – propeptide, AA 673-911 (pI/Mw: 6.11/26489) – propeptide, and AA 35-672 – inter-alpha-trypsin inhibitor heavy chain H1 (pI/Mw: 6.33/71415). Similarly are processed other ITHs. In our experiments, 2DE-patterns of these proteins are presented by the chains of the precursor proteoforms and the mature ITIH1 (Suppl. Figure 1). The proteins are heavily glycosylated, phosphorylated, acetylated, and ubiquitinated (https://www.phosphosite.org/).

2.*59. KALLISTATIN (KAIN_HUMAN)*

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Kallistatin (kallikrein inhibitor, peptidase inhibitor 4, PI-4, serpin A4) is an inhibitor of amidolytic and kininogenase activities of tissue kallikrein. Kallistatin is mainly secreted in the liver and protects organs and cells against inflammation, fibrosis, and oxidative stress. The patients with HCC had lower serum kallistatin levels than normal controls, but significantly higher than patients with liver cirrhosis. So, kallistatin can be used as an indicator of hepatic health status, not HCC [211]. Its precursor polypeptide chain has 427 AA, where AA 1-20 – the signal peptide, AA 21-427 – kallistatin (pI/Mw: 7.88/46355). In our experiments, the 2DE pattern of this protein is presented as a cluster of proteoforms around pI/Mw: 6-7/40-120000 (Suppl. Fig.1). The protein is glycosylated (31 N-linked annotations at 4 sites) and phosphorylated (thttps://www.glygen.org/protein/P29622, https://www.phosphosite.org/.

2.60. KININOGEN 1 (KNG1_HUMAN)

Kininogen-1 (alpha-2-thiol proteinase inhibitor, Fitzgerald factor, Williams-Fitzgerald-Flaujeac factor) is a precursor protein to high-molecular-weight kininogen (HMWK), low-molecular-weight kininogen (LMWK), and bradykinin. This protein is a potential plasma biomarker for the early detection of advanced colorectal adenoma and CRC [212,213]**.** Kininogen-1 levels in plasma, bronchoalveolar lavage fluid, and urine were significantly higher in lung squamous cell carcinoma (LSCC) patients than in controls [214]**.** Its precursor polypeptide chain has 644 AA, where AA 1-18 – the signal peptide, AA 19-644 – kininogen-1 that is cleaved into 6 chains: kininogen-1 heavy chain (AA 19-380, pI/Mw: 5.47/40625), T-kinin (Ile-Ser-Bradykinin) (AA 376-389, pI/Mw: 12.01/1633), bradykinin (Kallidin I) (AA 381-389, pI/Mw: 12.01/ 1188), lysyl-bradykinin (Kallidin II) (AA 380-389, pI/Mw: 12.00/1060), kininogen-1 light chain (AA 390-644, pI/Mw: 6.36/ 28248), low molecular weight growth-promoting factor (AA 431-434, pI/Mw: 5.24/528). 2DE-pattern of this protein represents multiple spots (pI 3.5-8.5, Mw~35-64000) (Suppl. Fig.1). In the SWISS-2DPAGE, only a single spot (pI/Mw: 6.48/7490) is present. The protein can be heavily glycosylated (159 N-linked annotations at 6 sites, 65 O-linked annotations at 26 sites) (https://www.glygen.org/protein/P01042), phosphorylated, acetylated, and ubiquitinated (https://www.phosphosite.org/).

2.61. PHOSPHATIDYLCHOLINE-STEROL ACYLTRANSFERASE (LCAT_HUMAN)

Phosphatidylcholine-sterol acyltransferase (1-alkyl-2-acetylglycerophosphocholine esterase, lecithin-cholesterol acyltransferase, LCAT, phospholipid-cholesterol acyltransferase) is a central enzyme in the extracellular metabolism of plasma lipoproteins. It is synthesized mainly in the liver and secreted into plasma where it converts cholesterol and phosphatidylcholines (lecithins) to cholesteryl esters and lysophosphatidylcholines on the surface of the high and low-density lipoproteins. High plasma levels of LCAT were correlated with a favorable outcome for patients with CRC [171]**.** Its precursor polypeptide chain has 440 AA, where AA 1-24 – the signal peptide, AA 25-440 – phosphatidylcholine-sterol acyltransferase (pI/Mw: 5.71/47084). 2DE-pattern of this protein represents 3 spots (pI 4.0-4.8, Mw~50000) (Suppl. Fig.1). The protein is glycosylated (22 N-linked annotations at 5 sites, 3 O-linked annotations at 3 sites) (https://www.glygen.org/protein/P04180), phosphorylated, and ubiquitinated (https://www.phosphosite.org/).

2.62. LUMICAN (LUM_HUMAN)

Lumican (keratan sulfate proteoglycan lumican, KSPG lumican) is an extracellular matrix protein that is expressed in various cancer tissues and has a positive or negative correlation with tumor progression [215]**.** Lumican is an important mediator of tumorigenesis and cancer progression involving the cellular functions of proliferation, motility, apoptosis, autophagy, and angiogenesis

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regulation. The behavior of lumican in the plasma of healthy control, chronic pancreatitis, and cancer correlates well with what has been observed in tissue proteomics study, i.e. it is up-regulated in PDAC [216]. Serum levels of lumican were significantly elevated in patients with urothelial carcinoma of the bladder [217]. Using DIGE-PAGE analysis with the following mass spectrometric protein identification, it was revealed that lumican, as well as several classical plasma proteins and the tissue-leakage proteins (catalase, clusterin, ficolin, gelsolin, tetranectin, triosephosphate isomerase, and vitronectin) were upregulated in plasma of lung cancer patients [166]. Lumican precursor polypeptide chain has 338 AA, where AA 1-18 – the signal peptide, AA 19-338 – lumican (pI/Mw: 6.17/36661). 2DE pattern of this protein represents chains of spots (pI 4.5-6.5, Mw~52-83000) (Suppl. Fig.1). The protein has multiple PTMs: (97 N-linked glycans at 4 sites, 2 O-linked glycans at 3 sites, phosphorylation (11 sites), acetylation (8 sites) (https://www.uniprot.org/uniprotkb/P51884/entry).

2.63. MANNOSE-BINDING PROTEIN C (MBL2_HUMAN)

Mannose-binding protein C (MBP-C, collectin-1, MBP1, mannan-binding protein, mannose-binding lectin) is involved in innate immune defense. It binds mannose, fucose, and N-acetylglucosamine of different microorganisms and activates the lectin complement pathway. It binds to late apoptotic cells, as well as to apoptotic blebs and necrotic cells, but not to early apoptotic cells, facilitating their uptake by macrophages, which can bind DNA. Proteomics analysis of serum protein profiling in PDAC patients by DIGE with the following mass spectrometry allowed detection of increased levels of MBP-C. This data was confirmed by Western blot [218]. A high plasma level of MBP-C is a marker of poor survival for stage III CRC patients [171]. Using ELISA technique it was revealed that the MBP-C protein was significantly reduced in HCC cases [219]. MBP-C precursor polypeptide chain has 248 AA, where AA 1-20 – the signal peptide, AA 21-248 – MBP-C (pI/Mw: 5.40/24021). 2DE-pattern of this protein represents just a single spot (pI/Mw: 5.3/26000) (Suppl. Fig.1).

2.64. PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF_HUMAN)

Pigment epithelium-derived factor (PEDF, EPC-1, serpin F1) is a neurotrophic protein that induces extensive neuronal differentiation in retinoblastoma cells. It is a potent inhibitor of angiogenesis. As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity. It is widely expressed in human tissues, but its expression decreases with age. The PEDF level in plasma was significantly higher in patients with prostate cancer than in control. Statistical analysis confirmed that PEDF was positively associated with pathological grading, it was proposed PEDF as a potential biomarker that would mirror the level of tumor aggressiveness and allow risk stratification of patients with prostate cancer [220]. The physiological PEDF serum concentration in healthy people, however, remains controversial – from 4 ng/ml to 15 μg/ml [305]. PEDF precursor polypeptide chain has 418 AA, where AA 1-19 – the signal peptide, AA 20-418 – PEDF (pI/Mw: 5.90/44388). 2DE-pattern of this protein represents a chain of spots (pI 4.5-6.5, Mw~40-52000). (Suppl. Fig.1). The protein is glycosylated (10 N-linked glycans at 1 site, 2 O-linked glycans at 5 sites) phosphorylated (10 sites), acetylated, and methylated (https://www.uniprot.org/uniprotkb/P36955/entry).

2.65. N-ACETYLMURAMOYL-L-ALANINE AMIDASE (PGRP2_HUMAN)

N-acetylmuramoyl-L-alanine amidase (peptidoglycan recognition protein 2, peptidoglycan recognition protein long, PGRP-L) plays a scavenger role by digesting biologically active

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peptidoglycan (PGN) into biologically inactive fragments. PGRP-L is a pattern recognition receptor that is specifically expressed in the liver and implicated in the regulation of innate immunity and immunosurveillance. PGRP-L was down-regulated in plasma of patients with HCC or CRC, which was linked with poor prognosis in patients [171,221,222]. PGRP-L precursor polypeptide chain has 576 AA, where AA 1-21 – the signal peptide, AA 22-576 – PGRP-L (pI/Mw: 7.64/59980). 2DE-pattern of this protein represents a chain of spots (pI 5.5-6.8, Mw~52-64000). (Suppl. Fig.1). The protein is glycosylated (12 N-linked glycans at 3 sites, 4 O-linked glycans at 7 sites) and phosphorylated (4 sites) (https://www.uniprot.org/uniprotkb/Q96PD5/entry).

2.66. PHOSPHATIDYLINOSITOL-GLYCAN-SPECIFIC PHOSPHOLIPASE D (PHLD_HUMAN)

Phosphatidylinositol-glycan-specific phospholipase D (PI-G PLD, glycoprotein phospholipase D, glycosyl-phosphatidylinositol-specific phospholipase D, GPI-PLD, GPI-specific phospholipase D) hydrolyzes the inositol phosphate linkage in proteins anchored by phosphatidylinositol glycans (GPI-anchor) thus releasing these proteins from the membrane. It is produced in many tissues and is a favorable prognostic marker in liver cancer [32]**.** Enhanced levels and activity of PI-G PLD have been detected in many cancers, including colon, breast, gastric, thyroid, brain, kidney, and uterine smooth muscle compare to adjacent non-tumor tissues [223]. But it was reported that the level of PI-G PLD in serum is decreased in HCC patients [224]. PI-G PLD precursor polypeptide chain has 840 AA, where AA 1-23 – the signal peptide, AA 24-840 – PI-G PLD (pI/Mw: 5.78/89811). 2DE-pattern of this protein represents a chain of spots (pI 4.2-5.6, Mw~83-116000). (Suppl. Fig.1). The protein is glycosylated (10 sites, 22 N-linked glycans at 4 sites), phosphorylated (5 sites), and acetylated (https://www.phosphosite.org/).

2.67. PLASMINOGEN (PLMN_HUMAN)

Plasminogen is a serine protease that circulates in blood plasma as an inactive zymogen and is converted to the active protease, plasmin, by several activators such as tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), kallikrein, and factor XII (Hageman factor). Its precursor polypeptide chain has 810 AA, where AA 1-19 – the signal peptide, AA 20-810 – plasminogen (pI/Mw: 7.08/88432). Plasminogen is cleaved into 5 chains: plasmin heavy chain A, activation peptide, angiostatin, plasmin heavy chain A (short form), and plasmin light chain B. The conversion of plasminogen to plasmin involves the cleavage of the peptide bond between R561 and V562. Plasmin cleavage also releases the angiostatin, which inhibits angiogenesis. Plasmin degrades many blood plasma proteins, including fibronectin, thrombospondin, laminin, von Willebrand factor, and is involved in the degradation of extracellular matrices, cell migration, inflammation, wound healing, oncogenesis, metastasis, myogenesis, muscle regeneration, neurite outgrowth, and fibrinolysis. Components of the plasminogen-plasmin system are found in most tumors and their expression not only signifies their function but also carries a prognostic value [225,226]. Plasminogen belongs to cancer-related genes and is a favorable prognostic marker in renal cancer. 2DE-pattern of this protein represents 2 chains of multiple spots (pI 3.3-4.1, Mw~83-116000) and (pI 6.7-8.5, Mw~83-116000) (Suppl. Fig.1). In the SWISS-2DPAGE, a single chain (7 spots) is present (pI 6.32-6.49, Mw~112-116000). The protein is glycosylated (54 N-linked glycans at 4 sites, 12 O-linked glycans at 12 sites) and phosphorylated (15 sites) (https://www.uniprot.org/uniprotkb/P00747/entry).

2.68. PARAOXONASE (PON1_HUMAN)

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Serum paraoxonase/arylesterase 1 (PON1, homocysteine thiolactonase, serum aryldialkylphosphatase 1) is secreted mainly by the liver, although this protein is found in almost all tissues. It protects humans from the acute and chronic harmful effects of organophosphorus compounds. Its plasma concentration is influenced by inflammation and the levels of serum oxidised-LDL [306]. PON1 has been shown to decrease the level of systemic oxidative stress, which is thought to contribute to cancer development [227]. A fucosylated PON1 is a potential biomarker of small cell lung cancer [228]. PON1 can serve as a diagnostic biomarker for CRC and raise the sensitivity and specificity when incorporated with traditional tumor biomarkers [229–231]. PON1 together with ATIIII could be a biomarker to distinguish AFP-negative HCC from cirrhosis [79]. Serum PON levels were lower in the patients with GC than in controls [232]. PON1 precursor polypeptide chain has 355 AA, where initiator methionine is removed (pI/Mw: 5.08/39600). 2DE-pattern of this protein represents a cluster of spots (pI 4.0-5.5, Mw~35-52000) (Suppl. Fig.1). In the SWISS-2DPAGE, two spots are presented (pI/Mw: 4.84/45937 and 4.93/43391). The protein is glycosylated (30 N-linked glycans at 3 sites), phosphorylated (3 sites), and acetylated (1 site) (https://www.uniprot.org/uniprotkb/P27169/entry).

2.69. PROPERDIN (PROP_HUMAN)

Properdin (complement factor P) is a positive regulator of the alternate pathway (AP) of complement. A reduced level of plasma properdin was the most frequent abnormality in chronic lymphatic leukemia [233]**.** Its precursor polypeptide chain has 469 AA, where AA 1-27 – the signal peptide, AA 28-469 – histidine-rich glycoprotein (pI/Mw: 8.33/48494). 2DE-pattern of this protein represents 2 spots (pI/Mw: 8.5/52000 and 8.7/52000) (Suppl. Fig.1). The protein is glycosylated (15 C-linked annotations at 15 sites, 2 N-linked annotations at 1 site, 4 O-linked annotations at 4 sites).

2.70. VITAMIN K-DEPENDENT PROTEIN S (PROS_HUMAN)

Vitamin K-dependent protein S is an anticoagulant plasma protein; it is a cofactor to activated protein C in the degradation of coagulation factors Va and VIIIa. It helps to prevent coagulation and stimulates fibrinolysis. Plasma levels of free PROS1 were lower in the case of HCC in comparison with control [234]. Its precursor polypeptide chain has 676 AA, where AA 1-24 – the signal peptide, AA 25- 41 – propeptide (pI/Mw: 12.31/2058), and AA 42-676– vitamin K-dependent protein S (pI/Mw: 5.17/70645). 2DE-pattern of this protein represents a chain of spots (pI 3.5-4.5, Mw~64-83000) (Suppl. Fig.1). The protein is glycosylated (5 N-linked annotations at 3 sites, 4 O-linked annotations at 4 sites), phosphorylated (8 sites) (https://www.uniprot.org/uniprotkb/P07225/entry).

2.71. PLASMA RETINOL-BINDING PROTEIN (RET4_HUMAN)

Plasma retinol-binding protein 4 (plasma retinol-binding protein, PRBP) is synthesized in the liver and mediates retinol transport in blood [242]. PRBP is a sensitive marker of under-nutrition and the monitoring of its serum concentration allows the monitoring of nutritional status. Decreased PRBP plasma levels are also observed in the event of hypovitaminosis A, tubular nephropathies, hepatocellular insufficiency, or severe or acute inflammation. An increased level of PRBP is correlated with glomerular renal failure, type 2 diabetes, or steatosis. Immunoassays for serum levels of PRBP are useful in the detection of liver disease, protein-calorie malnutrition, and vitamin A deficiency. In addition, because vitamin A is important in the maintenance of differentiation and rate of proliferation of epithelial tissue, the determination of PRBP levels is important in the mediation of anti-tumor effects [235]. There is data indicating that serum RBP4 level was positively related to the risk of NSCLC or BC [236,237]**.** PRBP precursor polypeptide chain has 201 AA, where AA 1-18 – the

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signal peptide, AA 19-201 – plasma retinol-binding protein 4 (pI/Mw: 5.27/21072) that is cleaved into the following 4 chains: AA 19-200, AA 19-199, AA 19-197, AA 19-194. 2DE-pattern of this protein represents a cluster of spots (pI 5.0-6.0, Mw~18-26000) (Suppl. Fig.1). In the SWISS-2DPAGE, three spots are presented (pI~5.0, Mw~20000). The protein can be phosphorylated and methylated (https://www.phosphosite.org/).

2.72. SERUM AMYLOID A (SAA1_HUMAN)

Serum amyloid A (amyloid protein A, SAA), a major liver-released APP, is highly upregulated in case of chronic inflammatory diseases including atherosclerosis, Crohn's disease, rheumatoid arthritis, and Alzheimer's disease [307]**.** SAA also plays an important role in the metabolism of cholesterol. This protein is involved in carcinogenesis and neoplastic diseases and may be a potential biomarker for certain tumors (GC, CRC, NSCLC, MM, renal cancer, neuroblastoma) [34,239]. Elevated SAA levels accompany neoplastic processes, and poor prognosis correlates with the level of SAA [238,239]. Subgroup analysis confirmed an elevated level of SAA as a strong prognostic marker in patients with different solid tumors [240]. It is an unfavorable prognostic marker in renal cancer [32].

SAA preproprotein (122 AA) is processed to generate the mature protein, where AA 1-18 – the signal peptide, AA 19-122 – serum amyloid A-1 protein (Mw: 5.89/11683) that itself is cleaved into 6 chains: amyloid protein A (19 – 94), serum amyloid protein A – (20-122), (20–121), (20–120), (21–122) (22–119). 2DE-pattern of this protein represents two spots (pI/Mw: ~5.6/12000 and ~5.8/12000) (Suppl. Fig.1). This protein is phosphorylated (5 sites) (https://www.phosphosite.org/).

2.73. SERUM AMYLOID P (SAMP_HUMAN)

Serum amyloid P-component (SAP, 9.5S alpha-1-glycoprotein) regulates several aspects of the innate immune system. Patients with BC have significantly increased serum concentrations of SAP. Moreover, in these patients, SAP levels correlated with the severity of the disease. Patients with carcinoma of the colon, however, did not differ from healthy individuals in the serum level of SAP [241]**.** SAP precursor polypeptide chain has 223 AA, where AA 1-19 – the signal peptide, AA 20-223 – serum amyloid P-component (pI/Mw: 6.12/23259) that is cleaved to AA 20-222 (pI/Mw: 12/23159). 2DE-pattern of this protein represents a cluster of spots (pI/Mw: ~4.5-6.1/21-35000) (Suppl. Fig.1). This protein is phosphorylated (7 sites), acetylated (3 sites), ubiquitinated (2 sites) (https://www.phosphosite.org/), and glycosylated (14 N-linked glycans at 1 site, 1 O-linked glycan) (https://www.glygen.org/protein/P02743).

2.74. SEX HORMONE-BINDING GLOBULIN (SHBG_HUMAN)

Sex hormone-binding globulin (SHBG, sex steroid-binding protein, SBP, testis-specific androgen-binding protein, ABP) functions as an androgen transport protein, but may also be involved in receptor-mediated processes. It regulates the plasma metabolic clearance rate of steroid hormones by controlling their plasma concentration. Label-free quantification analysis of the MS data found that plasma SHBG was significantly overexpressed in GC patients. A Western blot assay confirmed this data [243]. SHBG level was associated with the extraprostatic extension of a PC tumor [308]. The precursor polypeptide chain of SHBG has 402 AA, where AA 1-29 – the signal peptide, AA 30-402 – sex hormone-binding globulin (pI/Mw: 5.83/40468). 2DE-pattern of this protein represents a chain of spots (pI/Mw: ~5.0-6.0/35-52000) (Suppl. Fig.1). This protein is phosphorylated (4 sites) (https://www.phosphosite.org/) and glycosylated (12 N-linked glycans at 3 sites, 6 O-linked glycans at 1 site).

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2.75. S100A8 (CALPOTECTIN) (S10A8_HUMAN)

Calprotectin (S100A8/A9), is a heterodimer of the two calcium-binding proteins S100A8 and S100A9. It was originally discovered as an immunogenic protein expressed and secreted by neutrophils. Subsequently, it has emerged as an important pro-inflammatory mediator in acute and chronic inflammation. More recently, increased plasma S100A8 and S100A9 levels were also detected in various human cancers [244–246].

Protein S100-A8 has many alternative names: calgranulin-A, calprotectin L1L subunit, cystic fibrosis antigen, CFAG, leukocyte L1 complex light chain, migration inhibitory factor-related protein 8, MRP-8, p8, S100 calcium-binding protein A8, urinary stone protein band. Its polypeptide chain has 93 AA (pI/Mw: 6.50/ 10835). 2DE-pattern of this protein represents one spot (pI/Mw: ~6.8/10000) (Suppl. Fig.1). But this protein can be heavyly phosphorylated (9 sites), acetylated (https://www.phosphosite.org/), and glycosylated (1 O-linked glycan) (https://www.uniprot.org/).

2.76. S100A9 (CALPOTECTIN) (S10A9_HUMAN)

Protein S100-A9 (calgranulin-B, calprotectin L1H subunit, leukocyte L1 complex heavy chain, migration inhibitory factor-related protein, MRP-141, p141, S100 calcium-binding protein A9) is up-regulated in numerous cancer types including breast cancer (invasive ductal carcinoma), colitis-associated colon cancer, HCC, GC, pulmonary adenocarcinoma, CRC, breast apocrine carcinomas, NSCLC, and squamous cervical cancer [244,247]. The polypeptide chain of S100-A9 has 114 AA (pI/Mw: 5.71/13242). 2DE-pattern of S100-A9 represents one spot (pI/Mw: ~6.0/10000) (Suppl. Fig.1). This protein can be phosphorylated (5 sites), acetylated (3 sites), methylated (https://www.phosphosite.org/), and glycosylated (1 O-linked glycan) (https://www.uniprot.org/).

2.77. TETRANECTIN (TETN_HUMAN)

Tetranectin (TN; TNA; C-type lectin domain family 3, plasminogen kringle 4-binding protein) is expressed practically in all tissues, secreted in blood, binds to plasminogen, binds to isolated kringle 4, and may be involved in the packaging of molecules destined for exocytosis. Tetranectin is a favorable prognostic marker in liver cancer, head and neck cancer, and PDAC [32]. It could be an independent prognostic factor in OC [248]. Tetranectin was significantly under-expressed in both serum and saliva of metastatic OSCC compared to primary OSCC, and it was proposed that tetranectin may serve as a potential biomarker for metastatic oral squamous cell carcinoma (OSCC) [249]. The precursor polypeptide chain of TN has 202 AA, where AA 1-21 – the signal peptide, AA 22-202 – tetranectin (pI/Mw: 5.80/20139). 2DE-pattern of this protein represents a chain of spots (pI/Mw: ~5-6.5/21-26000) (Suppl. Fig.1). This protein can be glycosylated (1 O-linked annotation(s) at 1 site) (https://www.uniprot.org/uniprotkb/P05452/entry).

2.78. THYROXINE-BINDING GLOBULIN (THBG_HUMAN)

Thyroxine-binding globulin (TBG, serpin A7, T4-binding globulin) is a major thyroid hormone transport protein in plasma. In the clinic, TBG test is used to assess thyroid problems (hypothyroidism or hyperthyroidism), as well as other conditions (liver disease, or pregnancy, for instance). The elevated level of TBG is a sensitive, although nonspecific marker of liver tumors, especially in cases of metastasis to the liver [250]. But TBG is not a valuable tumor marker for the early diagnosis or follow-up of HCC [251]. TBG precursor polypeptide chain has 415 AA, where AA 1-20 – the signal peptide, AA 21-415 – thyroxine-binding globulin (pI/Mw: 5.76/44102). 2DE-pattern of this protein represents a chain of spots (pI/Mw: ~5-5.5/52-64000) (Suppl. Fig.1). This protein can be glycosylated (17 N-linked glycans at 3 sites, 1 O-linked glycan) and phosphorylated (https://www.phosphosite.org/).

2.79. PROTHROMBIN (THRB_HUMAN)

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Prothrombin (coagulation factor II) is cleaved to form thrombin in the clotting process. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions. It functions in blood homeostasis, inflammation, and wound healing. Prothrombin induced by vitamin K absence-II (PIVKA-II), also known as Des-gamma-carboxy prothrombin (DCP), is an abnormal protein produced in HCC. DCP is a mixture of prothrombin proteoforms having various numbers of Glu residues (1-10) instead of Gla [252]. PIVKA-II offered a complementary approach for HCC early detection. Compared to traditional diagnostic approaches, the combination of PIVKA-II and other biomarkers had better performance. Third, PIVKA-II was an indicator for the assessment of response to treatment in HCC [253]. Prothrombin precursor polypeptide chain has 622 AA, where AA 1-24 – the signal peptide, AA 25-43 – propeptide (pI/Mw: 12.48/2304), and AA 44-622 – prothrombin (pI/Mw: 5.23/65308) that cleaved into 4 chains: activation peptide fragment 1 (AA 44-198, pI/Mw: 5.01/17376), activation peptide fragment 2 (AA 199-327, pI/Mw: 4.25/14157), thrombin light chain (AA 328-363, pI/Mw: 4.65/4091), thrombin heavy chain (AA 364-622, pI/Mw: 8.88/29738). 2DE-pattern of this protein represents a chain of spots (pI/Mw: 5-6.5/64-83000) (Suppl. Fig.1). In the SWISS-2DPAGE, there is a chain of 5 spots (pI/Mw: 4.95-5.05/80000). This protein can be glycosylated (2 N-linked glycans at 5 sites, 1 O-linked glycan at 6 sites), phosphorylated (9 sites), acetylated (1 site), ubiquitinated (4 sites) (https://www.uniprot.org/uniprotkb/P00734/entry).

2.80. SEROTRANSFERRIN (TRFE_HUMAN)

Serotransferrin (transferrin, beta-1 metal-binding globulin, siderophilin) is a glycoprotein that is mainly produced in the liver but also in tissues, including the brain, as well. The function of this protein is to transport iron and be involved in the stimulation of cell proliferation. Transferrin is also involved in the removal of certain organic matter and allergens from serum. Transferrin that is not bound to iron is called "apotransferrin". Transferrin is APP. But in contrast to other APPs, e.g., C-reactive protein, which is increased in the acute phase it is decreased in inflammation, cancers, and certain diseases [254]. So, it is considered “a negative” APP. Also, the negative APPs are albumin, transthyretin, transcortin, and retinol-binding protein [254]. Changes in proteoform pattern of serotransferrin in plasma were observed in the case of adenocarcinoma (ADC) and SCC [25]. A comparative analysis of plasma samples obtained from lung cancer patients before chemotherapy versus after the second cycle of chemotherapy was performed using 2D-DIGE [25]. Analyses of gels revealed significant changes in proteins and/or their proteoforms between control patients and lung cancer patients, both before and after the second cycle of chemotherapy. Most of these proteins were related to inflammation, including APPs such as forms of haptoglobin and transferrin, complement component C3, and clusterin [25]. The variable expression of APPs can potentially be used for profiling lung cancer. The greatest changes observed after chemotherapy were in transferrin and serotransferrin, which likely reflect disturbances in iron turnover after chemotherapy-induced anemia. Serotransferrin precursor polypeptide chain has 698 AA, where sequence AA 1-19 – the signal peptide that is cleaved after processing and AA 20-698 – the serotransferrin (pI/Mw: 6.70/75195). 2DE-pattern of this protein represents a cluster of spots (pI/Mw: ~5.7-7.0/64-83000) (Suppl. Fig.1). In the SWISS-2DPAGE, there are 3 chains of 22 spots (pI/Mw: 6.14-6.64/76-87000) for serotransferrin. This protein can be glycosylated (145 N-linked glycans at 6 sites, 6 O-linked glycans at 2 sites), phosphorylated (21 sites), acetylated (10 sites), ubiquitinated (7 sites) (https://www.phosphosite.org/).

2.81. TRANSTHYRETIN (TTHY_HUMAN)

Transthyretin (ATTR, prealbumin, TBPA) is a carrier protein that transports thyroid hormones and retinol (vitamin A) in the cerebrospinal fluid and plasma. Transthyretin is enriched in liver cancer and pancreatic cancer [32]. There are many publications having proof that ATTR can be a potential biomarker for lung cancer, GC, or OC [95,255–257]. Using 2D-DIGE analysis proteins of pleural effusions from lung adenocarcinoma and benign inflammatory disease (pneumonia and tuberculosis) it was shown that ATTR is up-regulated in cancer samples [177]. ATTR precursor polypeptide chain has 147 AA, where AA 1-20 – the signal peptide, AA 21-147 – mature transthyretin

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(pI/Mw: 5.31/13761). 2DE pattern of this protein represents a chain of spots (pI/Mw: 4.8-5.7/15-18000) (Suppl. Fig.1). In the SWISS-2DPAGE, there are a chain of 3 spots (pI/Mw: 5.02-5.52/13800) and a spot (pI/Mw:5.52/35391) for transthyretin. This protein can be glycosylated (1 N-linked annotation), phosphorylated (6 sites), acetylated (2 sites), or ubiquitinated (4 sites) (https://www.phosphosite.org/).

2.82. VITAMIN D-BINDING PROTEIN (VTDB_HUMAN)

Vitamin D-binding protein (Gc-globulin, group-specific component, DBP, VDB) is a multifunctional protein mainly involved in the metabolism of vitamin D. Almost all DBP is produced in the liver. DBP is a very polymorphic protein with different alleles having impact on its functions [309]. Many diseases including cancer have been associated with variants of DBP [258,259]. DBP is an unfavorable prognostic marker in renal cancer and favorable in liver cancer [32]. DBP precursor polypeptide chain has 474 AA, where AA 1-16 – the signal peptide, AA 17-474 – vitamin D-binding protein (pI/Mw: 5.16/51197). 2DE pattern of this protein represents a cluster of spots (pI/Mw: 4.5-5.7/40-52000) (Suppl. Fig.1). In the SWISS-2DPAGE, there are 2 spots (pI/Mw: 5.16/53772 and 5.24/53918) for vitamin D-binding protein. This protein can be glycosylated (1 N-Linked glycan at 1 site, 1 O-Linked glycan), phosphorylated (12 sites), acetylated (1 site), or ubiquitinated (1 site) (https://www.phosphosite.org/).

2.83. VITRONECTIN (VTNC_HUMAN)

Vitronectin (VN, S-protein) is involved in the regulation of the coagulation pathway, wound healing, and tissue remodeling. Differential expression of this protein can promote either cell adhesion or migration as it links cells to the extracellular matrix using a variety of ligands. VN can also promote extracellular matrix degradation and thus plays a role in tumorigenesis. It is an unfavorable prognostic marker in renal cancer and favorable in liver cancer [32]. Expression of VN is amplified in BC and the serum VN level could be an early marker for BC survival [260] Significant differences between adenocarcinoma (ADC) and SCC patients were also revealed, suggesting use of the plasma VN as a potential marker of SCC [25]. The level of VN is also enhanced in the plasma of patients with CRC [52]. VN precursor polypeptide chain has 478 AA, where AA 1-19 – the signal peptide, AA 20-478 – vitronectin (pI/Mw: 5.47/52278) that is cleaved into the following 3 chains: AA 20-398 – vitronectin V65 subunit (pI/Mw: 5.54/43030), AA 20- 63 – vitronectin V10 subunit (pI/Mw: 4.51/5011), AA 399-478 – somatomedin-B (pI/Mw: 4.51/5011). 2DE-pattern of this protein represents a cluster of spots (pI/Mw: 5.02-5.52/52-116000) (Suppl. Fig.1). In the SWISS-2DPAGE, there is only one spot (pI/Mw: 4.58/9248) for vitronectin. VN can be glycosylated (1 N-linked annotation), phosphorylated (20 sites), acetylated (1 site), ubiquitinated (1 site) (https://www.phosphosite.org/).

2.84. ZINC-ALPHA-2-GLYCOPROTEIN (ZA2G_HUMAN)

Zinc-alpha-2-glycoprotein (Zn-alpha-2-GP, Zn-alpha-2-glycoprotein) induces lipid degradation in adipocytes and the extensive fat losses associated with some advanced cancers. It is considered a marker for many carcinomas and is highly expressed in the case of cancer cachexia [261]. Zinc alpha-2-glycoprotein may serve as a potential marker for PC [262]. The diagnostic value of serum zinc-alpha-2-glycoprotein in PC, BC, and CRC has been reported [263,264]. Its precursor polypeptide chain has 298 AA, where AA 1-20 – the signal peptide, AA 21-298 – Zn-alpha-2-GP (pI/Mw: 5.58/32145). 2DE-pattern of this protein represents a cluster of spots (pI/Mw: 4.2-5.0/35-40000) (Suppl. Fig.1). In the SWISS-2DPAGE, there is a chain of 4 spots (pI/Mw:4.8-4.97/40-42000) for zinc-alpha-2-glycoprotein. This protein can be glycosylated (108 N-Linked glycans at 3 sites, 1 O-Linked glycan at 1 site), phosphorylated (1 site), and ubiquitinated (5 sites) (https://www.phosphosite.org/).

3. Discussion

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Tumorigenesis leads to multiple variations in the human plasma proteome that can be dynamic and alterable during the progress of deceases. Practically all major, so-called "classical", plasma proteins are changing abundances or PTMs. The majority of these proteins are secreted by the liver, so it could be anticipated to see these changes only in the case of liver cancer. But they can be observed with other tumors as well as cancer induces disturbances in the blood homeostasis that is supported by "classical plasma proteins". It follows that it is possible to search the specific/unspecific ways of tumor prediction not only through the detection of products of the tumor but also by analyzing the changes in "classic plasma proteins". It is relevant to mention that the plasma analysis by a very different approach – differential scanning calorimetry (DSC) can give us a hint. Typically, DSC is used to determine the partial heat capacity of macromolecules as a function of temperature, from which their structural stability during thermal denaturation can be assessed. The method is very sensitive and allows precise determination of thermally induced conformational transitions of proteins present in plasma. There are already quite a few publications showing that DSC can be used to distinguish between normal and cancerous plasma samples [310,311]. Moreover, the data obtained by this method can be reproduced using major plasma proteins.

It follows that there is a possibility to build the test systems based on these major ("classical") proteins. What is important, many examples of such systems were introduced already. For example, the relationship between inflammation and clinical outcome is described using the Modified Glasgow Prognostic Scale (mGPS), which includes levels of C-reactive protein (CRP) and albumin [312]. The combination of elevated CRP (>10 mg/L) and decreased albumin (<35 g/L) corresponds to higher mGPS, which correlates with systemic inflammation and poor outcome of cancer therapy [313]. The OVA1 test uses the other major plasma proteins. OVA1 is an FDA-approved blood test that measures the levels of five proteins (CA125, transferrin, transthyretin, apolipoprotein A1, and beta-2 microglobulin) to detect ovarian cancer risk in women. Here, a sophisticated mathematical formula (multivariate index assay) is used to evaluate and combine the levels of these proteins in plasma producing an ovarian cancer risk score. Using this approach, OVA1 can detect early-stage ovarian cancer with 98% specificity. The OVERA (second-generation or OVA2) assesses a woman's malignancy risk using combined results from the following five proteins: apolipoprotein A1, human epididymis protein 4 (HE4), CA-125 II, follicle-stimulating hormone (FSH), and transferrin (Vermillion Inc. OVA1 Products. Updated 2020. Available at: https://vermillion.com/ova-products). The observation of enhanced levels of clusterin, ITIH4, antithrombin-III, and C1RL in sera of endometrial cancer patients allowed to build a mathematical model to detect cancer samples [29]. Accordingly, by the selection of the appropriate panels (proteomics signatures) of the plasma oncomarkers, it is possible to detect/monitor different types of cancers. The main point is to select the correct set of oncomarkers and develop an algorithm that will take into account all possible changes of these oncomarkers (level, PTMs etc.) that are related to cancer. This selection should be meticulously performed based on oncomarker behavior in plasma, not in tissue. We have performed a search for publications having information (level, PTMs) about "classical" plasma proteins in case of malignant processes in the human body (Table 1). As levels of some oncomarkers behave differently in different cancers (rise or fall) the test could specifically detect the type of cancer. Apolipoproteins are a good example here. SAA1 and CRP are APPs that are routinely measured in the clinic. The level of apoA-1 is reduced in many cancers but increased in some [80] The decreased level of apoA-I in plasma is observed in case of de novo myelodysplastic syndromes [83], NSCLC [84], nasopharyngeal carcinoma (NPC) [85], esophageal squamous cell carcinoma [86], BC [75], but it is increased in SCLC, HCC and bladder cancer [80]. The level of apoA-II is dramatically reduced in the serum of patients with gastric cancer and multiple myeloma [70,87] but increased in HCC and prostate cancer [88,89]. A similar situation can be observed for other apolipoproteins [80]. Another aspect that should be considered is an observation of specific proteoforms produced by PTMs and associated with cancer. So far, there are not so many examples of them, but progress in proteomics methods should improve the situation [314,315]. Proteomics is generating and analyzing a big volume of data and exactly fits the situation with multiple variations of plasma proteome during cancer development and progression. Here, high-throughput, quantitative mass-spectrometry is the

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best choice. There is already a good example of the possibility to use it in the clinic [14]. Geyer et al introduced a rapid and robust ''plasma proteome profiling'' LC-MS/MS pipeline. Their single-run shotgun proteomics workflow enables quantitative analysis of hundreds of plasma proteins just from 1 μl of plasma [14].

4. Materials and Methods

4.1. Plasma

Human plasma was from LLC Biobank "ILSbio" (USA). Depletion of serum albumin and immunoglobulins IgG was carried out according to Agilent Multiple Affinity Removal System (MARS) protocol ("Agilent Technologies", USA) [316,317].

4.2. 2DE

The detailed process was described previously [316]. In short, 10 μl of plasma (0.5 mg of protein) were mixed with 20 μl of lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 2% (v/v) ampholytes, pH 3-10, protease inhibitor cocktail) and then with 100 μl of rehydrating buffer (7 M urea, 2 M thiourea, 2 % CHAPS, 0.3% DTT, 0.5% IPG (v/v) buffer, pH 3-11 NL, 0.001% bromophenol blue). Immobiline DryStrip 3-11 NL (7 cm) was passively rehydrated by this solution for 4h at 4°C. IEF was run on Hoefer™ IEF100 (Thermo Fisher Scientific, USA). After IEF, strips were incubated 10 min in the equilibration solution (50 mM Tris, pH 8.8, 6 M urea, 2% SDS, 30% (v/v) glycerol, 1% DTT), following in the same solution with 5% IAM instead of DTT. The strips were sealed with a hot solution of 0.5% agarose prepared in electrode buffer (25mM Tris, pH 8.3, 200 mM glycine, and 0.1% SDS) on top of the polyacrylamide gel (14%), and run in the second direction [316]. Gels stained by Coomassie Blue R350 were scanned by ImageScanner III and analyzed using Image Master 2D Platinum 7.0. For the sectional 2DE analysis, this gel was cut into 96 sections with determined coordinates. Each section (~0.7 cm^2) was shredded and treated with trypsin. Tryptic peptides were eluted from the gel by extraction solution (5% (v/v) ACN, 5% (v/v) formic acid) and dried in Speed Vac. In the case of a semi-virtual 2DE, the 18-cm Immobiline DryStrip 3-11 NL was cut into 36 equal sections after IEF. For complete reduction, 300 μL of 3 mM DTT and 100 mM ammonium bicarbonate were added to each section and incubated at 50°C for 15 minutes. For alkylation, 20 μl of 100 mM IAM were added and samples were incubated in the dark at r.t. for 15 minutes. The peptides were eluted with 60% acetonitrile and 0.1% TFA and dried in Speed Vac.

4.3. ESI LC-MS/MS analysis.

A detailed procedures was described previously [316][317]. Peptides were dissolved in 5% (v/v) formic acid. Tandem mass spectrometry analysis was conducted in duplicate on an Orbitrap Q-Exactive mass spectrometer ("Thermo Scientific," USA). The data were analyzed by Mascot "2.4.1" (Matrix Science) or SearchGui [318] using the following parameters: enzyme – trypsin; maximum of missed cleavage sites – 2; fixed modifications – carbaidomethylation of cysteine; variable modifications – oxidation of methionine, phosphorylation of serine, threonine, tryptophan, acetylation of lysine; the precursor mass error – 10 ppm; the product mass error – 0.01 Da. As a protein sequence database, UniProt (October 2014) was used.

Only 100% confident results of protein identification were selected. Two unique peptides per protein were required for all protein identifications. Exponentially modified PAI (emPAI), the exponential form of protein abundance index (PAI) defined as the number of identified peptides divided by the number of theoretically observable tryptic peptides for each protein, was used to estimate protein abundance [319].

4.4. Immunostaining (Western blotting)

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Proteins were transferred (2 h, 28 V) from the gel onto PVDF membrane (Hybond P, 0.2 μm) using two sheets of thick paper (Bio-Rad, USA), saturated with 48 mM Tris, 39 mM glycine, 0,037% SDS, 20% ethanol. The membrane was treated following a protocol of Blue Dry Western [36] and treated with antibodies [21]. Primary antibodies were mouse monoclonal anti-Hp (C8, sc-376893, or F8, sc-390962, from Santa Cruz Biotechnology, USA) in dilution 1/25 (80 ng/ml in TBS [25 mM Tris (pH 7.5) and 150 mM NaCl containing 3% [w/v] BSA) or rabbit polyclonal anti-Hp (MBS177476, MyBioSource, San Diego, CA, USA). Secondary goat anti-mouse immunoglobulins G labeled by horseradish peroxidase (NA931V, "GE Healthcare") were used in TBS containing 3% [w/v] nonfat dry milk (1/5,000 dilution). The reaction was developed using ECL (Western Lightning Ultra, "PerkinElmer" USA) and X-ray film (Amersham Hyper film ECL).

5. Conclusions

For now, proteomics is collecting big data about the human plasma proteome http://plasmaproteomedatabase.org/index.html [320]. This data includes many proteome parameters: its dynamic, different protein presence, abundance, modifications, variations, etc. In the case of cancer, proteome performs multiple perturbations, where all its components are involved through changes in their levels and modifications. Here, the plasma proteome works as a united entity that executes and reflects the processes in the human body. Accordingly, the profiling of plasma proteomes is a promising and powerful approach to following these processes. This profiling could combine hundreds of already known plasma biomarkers and has a very promising future in biomedicine as could disclose information about any abnormal situation in a human body including cancer. There is a big chance that MS-based proteomics will become a part of the routine medical technique [14][321]. In addition to the usual MS analysis of proteins/proteoforms this technique should include special processing programs allowing to conclude about the human body state based on these variations in protein/proteoform signatures/profiles (level, PTMs, etc.). In our work, we have collected information about the connection of cancers with levels of "classical plasma proteins" and generated their proteoform profiles (Table 1, Suppl. Figure 1). As a next step, similar profiles representing protein perturbations in plasma produced in the case of different cancers should be generated. Also, based on this information different test systems can be developed.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/xxx/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

MS	Mass spectrometry
ESI LC-MS/MS	Liquid chromatography-electrospray ionization-tandem mass spectrometry
GBM	Glioblastoma multiform
2DE	Two-dimensional gel electrophoresis
emPAI	Exponentially modified protein abundance index
HCC	Hepatocellular carcinoma
CRC	Colorectal cancer
NSCLC	Non-small cell lung cancer

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SCLC	Small cell lung carcinoma
HDL	High-density lipoproteins
cSCC	Cutaneous squamous cell carcinoma
BC	Breast cancer
OC	Ovarian cancer
PDAC	Pancreatic cancer
OSCC	Oral squamous cell carcinoma
GC	Gastric cancer
MM	Multiple myeloma
PC	Prostate cancer

References

1. Bhatt, A. N.; Mathur, R.; Farooque, A.; Verma, A.; Dwarakanath, B. S. Cancer biomarkers - Current perspectives. *Indian J. Med. Res.* **2010**, *132*, 129–149.

2. Polanski, M.; Anderson, N. L. A List of Candidate Cancer Biomarkers for Targeted Proteomics. *Biomark. Insights* **2006**, *1*, 117727190600100.

3. Malati, T. Tumour markers: An overview. *Indian J. Clin. Biochem.* **2007**, *22*, 17–31.

4. Paik YK, Kim H, L. E. et al Overview and introduction to clinical proteomics. *Methods Mol Biol* **2008**, 1–31.

5. Nanjappa, V.; Thomas, J. K.; Marimuthu, A.; Muthusamy, B.; Radhakrishnan, A.; Sharma, R.; Ahmad Khan, A.; Balakrishnan, L.; Sahasrabuddhe, N. A.; Kumar, S.; Jhaveri, B. N.; Sheth, K. V.; Kumar Khatana, R.; Shaw, P. G.; Srikanth, S. M.; Mathur, P. P.; Shankar, S.; Nagaraja, D.; Christopher, R.; Mathivanan, S.; Raju, R.; Sirdeshmukh, R.; Chatterjee, A.; Simpson, R. J.; Harsha, H. C.; Pandey, A.; Prasad, T. S. K. Plasma Proteome Database as a resource for proteomics research: 2014 update. *Nucleic Acids Res.* **2014**, *42*.

6. Luque-Garcia JL, N. T. Sample preparation for serum/plasma profiling and biomarker identification by mass spectrometry. *J Chromatogr* **2007**, *1153*, 259–276.

7. Paulovich AG, Whiteaker JR, Hoofnagle AN, W. P. The interface between biomarker discovery and clinical validation: The tar pit of the protein biomarker pipeline. *Proteomics Clin Appl.* **2008**, *2*, 1386–1402.

8. Omenn, G. S.; States, D. J.; Adamski, M.; Blackwell, T. W.; Menon, R.; Hermjakob, H.; Apweiler, R.; Haab, B. B.; Simpson, R. J.; Eddes, J. S.; Kapp, E. A.; Moritz, R. L.; Chan, D. W.; Rai, A. J.; Admon, A.; Aebersold, R.; Eng, J.; Hancock, W. S.; Hefta, S. A.; Meyer, H.; Paik, Y. K.; Yoo, J. S.; Ping, P.; Pounds, J.; Adkins, J.; Qian, X.; Wang, R.; Wasinger, V.; Wu, C. Y.; Zhao, X.; Zeng, R.; Archakov, A.; Tsugita, A.; Beer, I.; Pandey, A.; Pisano, M.; Andrews, P.; Tammen, H.; Speicher, D. W.; Hanash, S. M. Overview of the HUPO Plasma Proteome Project: Results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database. *Proteomics* **2005**, *5*, 3226–3245.

9. Surinova S, Schiess R, Hüttenhain R, Cerciello F, Wollscheid B, A. R. On the development of plasma protein biomarkers. *J Proteome Res.* **2011**, *10*, 5–16.

10. Parker CE, B. C. Mass spectrometry based biomarker discovery, verification, and validation--quality assurance and control of protein biomarker assays. *Mol Oncol* **2014**, *8*, 840–58.

11. Hochstrasser DF, Sanchez JC, A. R. Proteomics and its trends facing nature's complexity. *Proteomics* **2002**, *2*, 807–812.

12. Petricoin, E. F.; Ardekani, A. M.; Hitt, B. A.; Levine, P. J.; Fusaro, V. A.; Steinberg, S. M.; Mills, G. B.; Simone, C.; Fishman, D. A.; Kohn, E. C.; Liotta, L. A. Use of proteomic patterns in serum to identify

ovarian cancer. *Lancet (London, England)* **2002**, *359*, 572–577.
13. Schiess R, Wollscheid B, A. R. Targeted proteomic strategy for clinical biomarker discovery. *Mol Oncol.* **2009**, *3*, 33–44.
14. Geyer, P. E.; Kulak, N. A.; Pichler, G.; Holdt, L. M.; Teupser, D.; Mann, M. Plasma Proteome Profiling to Assess Human Health and Disease. *Cell Syst.* **2016**, *2*, 185–195.
15. Geyer, P. E.; Holdt, L. M.; Teupser, D.; Mann, M. Revisiting biomarker discovery by plasma proteomics. *Mol. Syst. Biol.* **2017**, *13*, 942.
16. Farrah, T.; Deutsch, E. W.; Omenn, G. S.; Campbell, D. S.; Sun, Z.; Bletz, J. A.; Mallick, P.; Katz, J. E.; Malmström, J.; Ossola, R.; Watts, J. D.; Lin, B.; Zhang, H.; Moritz, R. L.; Aebersold, R. A high-confidence human plasma proteome reference set with estimated concentrations in PeptideAtlas. *Mol. Cell. Proteomics* **2011**, *10*, 1–14.
17. Cox, J.; Hein, M. Y.; Luber, C. A.; Paron, I.; Nagaraj, N.; Mann, M. Accurate Proteome-wide Label-free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ. *Mol. Cell. Proteomics* **2014**, *13*, 2513–2526.
18. Ray, S.; Patel, S. K.; Kumar, V.; Damahe, J.; Srivastava, S. Differential expression of serum/plasma proteins in various infectious diseases: specific or nonspecific signatures. *Proteomics. Clin. Appl.* **2014**, *8*, 53–72.
19. Anderson, N. L.; Anderson, N. G. The Human Plasma Proteome: History, Character, and Diagnostic Prospects. *Mol. Cell. Proteomics* **2002**, *1*, 845–867.
20. Hortin, G. L.; Sviridov, D.; Anderson, N. L. High-abundance polypeptides of the human plasma proteome comprising the top 4 logs of polypeptide abundance. *Clin. Chem.* **2008**, *54*, 1608–1616.
21. Silva-Costa, L. C.; Garcia-Rosa, S.; Smith, B. J.; Baldasso, P. A.; Steiner, J.; Martins-de-Souza, D. Blood plasma high abundant protein depletion unintentionally carries over 100 proteins. *Sep. Sci. PLUS* **2019**, *2*, 449–456.
22. Anderson, L.; Anderson, N. G. High resolution two-dimensional electrophoresis of human plasma proteins. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 5421–5.
23. Anderson NG, A. N. The Human Protein Index. *Clin. Chem.* **1982**, *28*, 739–748.
24. Anderson, N. L.; Anderson, N. G. The human plasma proteome: history, character, and diagnostic prospects. *Mol. Cell. Proteomics* **2002**, *1*, 845–867.
25. Ciereszko A, Dietrich MA, Słowińska M, Nynca J, Ciborowski M, et al. Identification of protein changes in the blood plasma of lung cancer patients subjected to chemotherapy using a 2D-DIGE approach. *PLoS One* **2019**, *14*.
26. Kondo, T. Cancer biomarker development and two-dimensional difference gel electrophoresis (2D-DIGE). *Biochim. Biophys. Acta* **2019**, *1867*, 2–8.
27. Opstal-van Winden, A. W. J.; Krop, E. J. M.; Kåredal, M. H.; Gast, M.-C. W.; Lindh, C. H.; Jeppsson, M. C.; Jönsson, B. A. G.; Grobbee, D. E.; Peeters, P. H. M.; Beijnen, J. H.; van Gils, C. H.; Vermeulen, R. C. H. Searching for early breast cancer biomarkers by serum protein profiling of pre-diagnostic serum; a nested case-control study. *BMC Cancer* **2011**, *11*, 381.
28. Looi, M. L.; Karsani, S. A.; Rahman, M. A.; Dali, A. Z. H. M.; Ali, S. A. M.; Ngah, W. Z. W.; Yusof, Y. A. M. Plasma proteome analysis of cervical intraepithelial neoplasia and cervical squamous cell carcinoma. *J. Biosci.* **2009**, *34*, 917–925.
29. Ura, B.; Biffi, S.; Monasta, L.; Arrigoni, G.; Battisti, I.; Di Lorenzo, G.; Romano, F.; Aloisio, M.; Celsi, F.; Addobbati, R.; Valle, F.; Rampazzo, E.; Brucale, M.; Ridolfi, A.; Licastro, D.; Ricci, G. Two Dimensional-Difference in Gel Electrophoresis (2D-DIGE) Proteomic Approach for the Identification

of Biomarkers in Endometrial Cancer Serum. *Cancers (Basel).* **2021**, *13*.
30. Naryzhny, S.; Klopov, N.; Ronzhina, N.; Zorina, E.; Zgoda, V.; Kleyst, O.; Belyakova, N.; Legina, O. A database for inventory of proteoform profiles: "2DE-pattern." *Electrophoresis* **2020**, *41*, 1118–1124.
31. Ahmed, F. E. Sample preparation and fractionation for proteome analysis and cancer biomarker discovery by mass spectrometry. *J. Sep. Sci.* **2009**, *32*, 771–798.
32. Sanders S The Human Protein Atlas_A 20-year journey_online_sml. *AAA Sci.* **2020**, *Nov*, 1–144.
33. Abramson, F. P.; Jenkins, J.; Ostchega, Y. Effects of cancer and its treatments on plasma concentration of alpha 1-acid glycoprotein and propranolol binding. *Clin. Pharmacol. Ther.* **1982**, *32*, 659–663.
34. Janciauskiene, S.; Wrenger, S.; Günzel, S.; Gründing, A. R.; Golpon, H.; Welte, T. Potential Roles of Acute Phase Proteins in Cancer: Why Do Cancer Cells Produce or Take Up Exogenous Acute Phase Protein Alpha1-Antitrypsin? . *Front. Oncol.* 2021, *11*.
35. Elpek, G. O. Orosomucoid in liver diseases. *World J. Gastroenterol.* 2021, *27*, 7739–7747.
36. Fischer, K.; Kettunen, J.; Würtz, P.; Haller, T.; Havulinna, A. S.; Kangas, A. J.; Soininen, P.; Esko, T.; Tammesoo, M.-L.; Mägi, R.; Smit, S.; Palotie, A.; Ripatti, S.; Salomaa, V.; Ala-Korpela, M.; Perola, M.; Metspalu, A. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. *PLoS Med.* **2014**, *11*, e1001606.
37. Ayyub, A.; Saleem, M.; Fatima, I.; Tariq, A.; Hashmi, N.; Musharraf, S. G. Glycosylated Alpha-1-acid glycoprotein 1 as a potential lung cancer serum biomarker. *Int. J. Biochem. Cell Biol.* **2016**, *70*, 68–75.
38. Jabir, R. S.; Ho, G. F.; Annuar, M. A. B. A.; Stanslas, J. Plasma alpha-1-acid glycoprotein as a potential predictive biomarker for non-haematological adverse events of docetaxel in breast cancer patients. *Biomarkers Biochem. Indic. Expo. response, susceptibility to Chem.* **2018**, *23*, 142–146.
39. Balmaña, M.; Giménez, E.; Puerta, A.; Llop, E.; Figueras, J.; Fort, E.; Sanz-Nebot, V.; de Bolós, C.; Rizzi, A.; Barrabés, S.; de Frutos, M.; Peracaula, R. Increased α1-3 fucosylation of α-1-acid glycoprotein (AGP) in pancreatic cancer. *J. Proteomics* **2016**, *132*, 144–154.
40. Tanabe, K.; Kitagawa, K.; Kojima, N.; Iijima, S. Multifucosylated Alpha-1-acid Glycoprotein as a Novel Marker for Hepatocellular Carcinoma. *J. Proteome Res.* **2016**, *15*, 2935–2944.
41. Sanchez, J. -C; Appel, R. D.; Golaz, O.; Pasquali, C.; Ravier, F.; Bairoch, A.; Hochstrasser, D. F. Inside SWISS-2DPAGE database. *Electrophoresis* **1995**.
42. Farrah, T.; Deutsch, E. W.; Omenn, G. S.; Campbell, D. S.; Sun, Z.; Bletz, J. A.; Mallick, P.; Katz, J. E.; Malmström, J.; Ossola, R.; Watts, J. D.; Lin, B.; Zhang, H.; Moritz, R. L.; Aebersold, R. A high-confidence human plasma proteome reference set with estimated concentrations in PeptideAtlas. *Mol. Cell. Proteomics* **2011**, *10*, M110.006353.
43. Gao, F.; Zhang, X.; Whang, S.; Zheng, C. Prognostic impact of plasma ORM2 levels in patients with stage II colorectal cancer. *Ann. Clin. Lab. Sci.* **2014**, *44*, 388–393.
44. Ercetin, E.; Richtmann, S.; Delgado, B. M.; Gomez-Mariano, G.; Wrenger, S.; Korenbaum, E.; Liu, B.; DeLuca, D.; Kühnel, M. P.; Jonigk, D.; Yuskaeva, K.; Warth, A.; Muley, T.; Winter, H.; Meister, M.; Welte, T.; Janciauskiene, S.; Schneider, M. A. Clinical Significance of SERPINA1 Gene and Its Encoded Alpha1-antitrypsin Protein in NSCLC. *Cancers (Basel).* **2019**, *11*.
45. Pérez-Holanda, S.; Blanco, I.; Menéndez, M.; Rodrigo, L. Serum concentration of alpha-1 antitrypsin is significantly higher in colorectal cancer patients than in healthy controls. *BMC Cancer* **2014**, *14*, 355.
46. Brkic, S.; Salkic, A.; Nuhbegovic, S.; Brkic, F.; Umihanic, S. Influence of surgical therapy on serum

values of alpha 1-antitrypsin in patients with head and neck tumors. *Med. Arh.* **2011**, *65*, 99–101.
47. Jaberie, H.; Hosseini, S. V.; Naghibalhossaini, F. Evaluation of Alpha 1-Antitrypsin for the Early Diagnosis of Colorectal Cancer. *Pathol. Oncol. Res.* **2020**, *26*, 1165–1173.
48. Repetto, O.; De Re, V.; Mussolin, L.; Tedeschi, M.; Elia, C.; Bianchi, M.; Buffardi, S.; Sala, A.; Burnelli, R.; Mascarin, M. Proteomic Profiles and Biological Processes of Relapsed vs. Non-Relapsed Pediatric Hodgkin Lymphoma. *Int. J. Mol. Sci.* **2020**, *21*.
49. Liang, Y.; Ma, T.; Thakur, A.; Yu, H.; Gao, L.; Shi, P.; Li, X.; Ren, H.; Jia, L.; Zhang, S.; Li, Z.; Chen, M. Differentially expressed glycosylated patterns of α-1-antitrypsin as serum biomarkers for the diagnosis of lung cancer. *Glycobiology* **2015**, *25*, 331–340.
50. Wu, C.-C.; Lu, Y.-T.; Yeh, T.-S.; Chan, Y.-H.; Dash, S.; Yu, J.-S. Identification of Fucosylated SERPINA1 as a Novel Plasma Marker for Pancreatic Cancer Using Lectin Affinity Capture Coupled with iTRAQ-Based Quantitative Glycoproteomics. *Int. J. Mol. Sci.* **2021**, *22*.
51. Liu, Y.; Luo, X.; Hu, H.; Wang, R.; Sun, Y.; Zeng, R.; Chen, H. Integrative proteomics and tissue microarray profiling indicate the association between overexpressed serum proteins and non-small cell lung cancer. *PLoS One* **2012**, *7*, e51748.
52. Kopylov, A. T.; Stepanov, A. A.; Malsagova, K. A.; Soni, D.; Kushlinsky, N. E.; Enikeev, D. V; Potoldykova, N. V; Lisitsa, A. V; Kaysheva, A. L. Revelation of Proteomic Indicators for Colorectal Cancer in Initial Stages of Development. *Molecules* **2020**, *25*.
53. Canales, N. A. G.; Marina, V. M.; Castro, J. S.; Jiménez, A. A.; Mendoza-Hernández, G.; McCARRON, E. L.; Roman, M. B.; Castro-Romero, J. I. A1BG and C3 are overexpressed in patients with cervical intraepithelial neoplasia III. *Oncol. Lett.* **2014**, *8*, 939–947.
54. Kim, J. H.; Kim, Y.-W.; Kim, I.-W.; Park, D. C.; Kim, Y. W.; Lee, K.-H.; Jang, C. K.; Ahn, W. S. Identification of candidate biomarkers using the Experion™ automated electrophoresis system in serum samples from ovarian cancer patients. *Int. J. Oncol.* **2013**, *42*, 1257–1262.
55. Gaziev, U. M.; Kit, O. I.; Frantsiyants, E. M.; Kozlova, L.; Mezentsev, S. S. Alpha-2-macroglobulin as the marker for pancreatic cancer process direction. *J. Clin. Oncol.* **2016**, *34*, e15686–e15686.
56. Boccellino, M.; Pinto, F.; Ieluzzi, V.; Giovane, A.; Quagliuolo, L.; Fariello, C.; Coppola, M.; Carlucci, A.; Santini, M.; Ferati, K.; Bexheti-Ferati, A.; Giordano, A.; Di Domenico, M. Proteomics analysis of human serum of patients with non-small-cell lung cancer reveals proteins as diagnostic biomarker candidates. *J. Cell. Physiol.* **2019**, *234*, 23798–23806.
57. Taguchi, O.; Gabazza, E. C.; Yoshida, M.; Yamakami, T.; Kobayashi, H.; Shima, T. High plasma level of plasmin-alpha 2-plasmin inhibitor complex is predictor of poor prognosis in patients with lung cancer. *Clin. Chim. Acta.* **1996**, *244*, 69–81.
58. Cavalcante, M. de S.; Torres-Romero, J. C.; Lobo, M. D. P.; Moreno, F. B. M. B.; Bezerra, L. P.; Lima, D. S.; Matos, J. C.; Moreira, R. de A.; Monteiro-Moreira, A. C. de O. A panel of glycoproteins as candidate biomarkers for early diagnosis and treatment evaluation of B-cell acute lymphoblastic leukemia. *Biomark. Res.* **2016**, *4*, 1.
59. Zhang, Q.; Huang, R.; Tang, Q.; Yu, Y.; Huang, Q.; Chen, Y.; Wang, G.; Wang, X. Leucine-rich alpha-2-glycoprotein-1 is up-regulated in colorectal cancer and is a tumor promoter. *Onco. Targets. Ther.* **2018**, *11*, 2745–2752.
60. Ivancic, M. M.; Megna, B. W.; Sverchkov, Y.; Craven, M.; Reichelderfer, M.; Pickhardt, P. J.; Sussman, M. R.; Kennedy, G. D. Noninvasive Detection of Colorectal Carcinomas Using Serum Protein Biomarkers. *J. Surg. Res.* **2020**, *246*, 160–169.
61. Guldvik, I. J.; Zuber, V.; Braadland, P. R.; Grytli, H. H.; Ramberg, H.; Lilleby, W.; Thiede, B.;

Zucknick, M.; Saatcioglu, F.; Gislefoss, R.; Kvåle, R.; George, A.; Grönberg, H.; Wiklund, F.; Neal, D. E.; Gnanapragasam, V. J.; Taskén, K. A.; Mills, I. G. Identification and Validation of Leucine-rich α-2-glycoprotein 1 as a Noninvasive Biomarker for Improved Precision in Prostate Cancer Risk Stratification. *Eur. Urol. Open Sci.* **2020**, *21*, 51–60.

62. Stenman, U. H.; Leinonen, J.; Alfthan, H.; Rannikko, S.; Tuhkanen, K.; Alfthan, O. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res.* **1991**, *51*, 222–226.

63. Jin, Y.; Wang, W.; Wang, Q.; Zhang, Y.; Zahid, K. R.; Raza, U.; Gong, Y. Alpha-1-antichymotrypsin as a novel biomarker for diagnosis, prognosis, and therapy prediction in human diseases. *Cancer Cell Int.* **2022**, *22*, 156.

64. Jin, Y.; Wang, J.; Ye, X.; Su, Y.; Yu, G.; Yang, Q.; Liu, W.; Yu, W.; Cai, J.; Chen, X.; Liang, Y.; Chen, Y.; Wong, B. H. C.; Fu, X.; Sun, H. Identification of GlcNAcylated alpha-1-antichymotrypsin as an early biomarker in human non-small-cell lung cancer by quantitative proteomic analysis with two lectins. *Br. J. Cancer* **2016**, *114*, 532–544.

65. Li, H.; Sun, L.; Chen, L.; Kang, Z.; Hao, G.; Bai, F. Effects of adiponectin, plasma D-dimer, inflammation and tumor markers on clinical characteristics and prognosis of patients with ovarian cancer. *J. Med. Biochem.* **2022**, *41*, 71–78.

66. Dieplinger, H.; Dieplinger, B. Afamin — A pleiotropic glycoprotein involved in various disease states. *Clin. Chim. Acta* **2015**, *446*, 105–110.

67. Melmer, A.; Fineder, L.; Lamina, C.; Kollerits, B.; Dieplinger, B.; Braicu, I.; Sehouli, J.; Cadron, I.; Vergote, I.; Mahner, S.; Zeimet, A. G.; Castillo-Tong, D. C.; Ebenbichler, C. F.; Zeillinger, R.; Dieplinger, H. Plasma concentrations of the vitamin E-binding protein afamin are associated with overall and progression-free survival and platinum sensitivity in serous ovarian cancer--a study by the OVCAD consortium. *Gynecol. Oncol.* **2013**, *128*, 38–43.

68. Dieplinger, H.; Ankerst, D. P.; Burges, A.; Lenhard, M.; Lingenhel, A.; Fineder, L.; Buchner, H.; Stieber, P. Afamin and apolipoprotein A-IV: novel protein markers for ovarian cancer. *Cancer Epidemiol. biomarkers Prev. a Publ. Am. Assoc. Cancer Res. cosponsored by Am. Soc. Prev. Oncol.* **2009**, *18*, 1127–1133.

69. Chang, T.-T.; Ho, C.-H. Plasma proteome atlas for differentiating tumor stage and post-surgical prognosis of hepatocellular carcinoma and cholangiocarcinoma. *PLoS One* **2020**, *15*, e0238251.

70. Humphries, J. M.; Penno, M. A. S.; Weiland, F.; Klingler-Hoffmann, M.; Zuber, A.; Boussioutas, A.; Ernst, M.; Hoffmann, P. Identification and validation of novel candidate protein biomarkers for the detection of human gastric cancer. *Biochim. Biophys. Acta* **2014**, *1844*, 1051–1058.

71. Oñate-Ocaña, L. F.; Aiello-Crocifoglio, V.; Gallardo-Rincón, D.; Herrera-Goepfert, R.; Brom-Valladares, R.; Carrillo, J. F.; Cervera, E.; Mohar-Betancourt, A. low serum albumin with increased long-term mortality in various malignancies, including breast, lung, gastric, colorectal, and pancreatic cancer. *Ann. Surg. Oncol.* **2007**, *14*, 381–389.

72. Lis, C. G.; Grutsch, J. F.; Vashi, P. G.; Lammersfeld, C. A. Is serum albumin an independent predictor of survival in patients with breast cancer? *JPEN. J. Parenter. Enteral Nutr.* **2003**, *27*, 10–15.

73. Boonpipattanapong, T.; Chewatanakornkul, S. Preoperative carcinoembryonic antigen and albumin in predicting survival in patients with colon and rectal carcinomas. *J. Clin. Gastroenterol.* **2006**, *40*, 592–595.

74. Suh, B.; Park, S.; Shin, D. W.; Yun, J. M.; Keam, B.; Yang, H.-K.; Ahn, E.; Lee, H.; Park, J. H.; Cho,

B. Low albumin-to-globulin ratio associated with cancer incidence and mortality in generally healthy adults. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2014**, *25*, 2260–2266.

75. Fujii, T.; Tokuda, S.; Nakazawa, Y.; Kurozumi, S.; Obayashi, S.; Yajima, R.; Shirabe, K. Implications of Low Serum Albumin as a Prognostic Factor of Long-term Outcomes in Patients With Breast Cancer. *In Vivo* **2020**, *34*, 2033–2036.

76. Cohen, A.; Wang, E.; Chisholm, K. A.; Kostyleva, R.; O'Connor-McCourt, M.; Pinto, D. M. A mass spectrometry-based plasma protein panel targeting the tumor microenvironment in patients with breast cancer. *J. Proteomics* **2013**, *81*, 135–147.

77. Pugia, M. J.; Valdes, R.; Jortani, S. A. Bikunin (Urinary Trypsin Inhibitor): Structure, Biological Relevance, And Measurement. In; Advances in Clinical Chemistry; Elsevier, 2007; Vol. 44, pp. 223–245.

78. Lu, Z.; Wang, F.; Liang, M. SerpinC1/Antithrombin III in kidney-related diseases. *Clin. Sci.* **2017**, *131*, 823–831.

79. Cao, X.; Cao, Z.; Ou, C.; Zhang, L.; Chen, Y.; Li, Y.; Zhu, B.; Shu, H. Combination of serum paraoxonase/arylesterase 1 and antithrombin-III is a promising non-invasion biomarker for discrimination of AFP-negative HCC versus liver cirrhosis patients. *Clin. Res. Hepatol. Gastroenterol.* **2021**, *45*, 101583.

80. Ren, L.; Yi, J.; Li, W.; Zheng, X.; Liu, J.; Wang, J.; Du, G. Apolipoproteins and cancer. *Cancer Med.* **2019**, *8*, 7032–7043.

81. Georgila K, Vyrla D, D. E. Apolipoprotein A-I (ApoA-I), Immunity, Inflammation and Cancer. *Cancers (Basel).* **2019**, *11*, 1097.

82. Chang, S.-C.; Lin, W.-L.; Chang, Y.-F.; Lee, C.-T.; Wu, J.-S.; Hsu, P.-H.; Chang, C.-F. Glycoproteomic identification of novel plasma biomarkers for oral cancer. *J. Food Drug Anal.* **2019**, *27*, 483–493.

83. Shi, C.; Gong, S.; Wu, A.; Yang, S.; Zou, D.; Zhang, Y.; Wu, N.; Ma, C.; Shi, S.; Chen, Y.; Wu, Y.; Zheng, X.; Huang, Z.; Ding, J.; Ouyang, G.; Mu, Q. Decreased serum apolipoprotein A1 level predicts poor prognosis of patients with de novo myelodysplastic syndromes. *BMC Cancer* **2022**, *22*, 127.

84. Cheng, T.; Dai, X.; Zhou, D.-L.; Lv, Y.; Miao, L.-Y. Correlation of apolipoprotein A-I kinetics with survival and response to first-line platinum-based chemotherapy in advanced non-small cell lung cancer. *Med. Oncol.* **2015**, *32*, 407.

85. Chang, H.; Wei, J.-W.; Chen, K.; Zhang, S.; Han, F.; Lu, L.-X.; Xiao, W.-W.; Gao, Y.-H. Apolipoprotein A-I Is a Prognosticator of Nasopharyngeal Carcinoma in the Era of Intensity-modulated Radiotherapy. *J. Cancer* **2018**, *9*, 702–710.

86. Wang, X.-P.; Li, X.-H.; Zhang, L.; Lin, J.-H.; Huang, H.; Kang, T.; Mao, M.-J.; Chen, H.; Zheng, X. High level of serum apolipoprotein A-I is a favorable prognostic factor for overall survival in esophageal squamous cell carcinoma. *BMC Cancer* **2016**, *16*, 516.

87. Hachem, H.; Favre, G.; Ghalim, N.; Puchois, P.; Fruchart, J. C.; Soula, G. Quantitative abnormalities of lipoprotein particles in multiple myeloma. *J. Clin. Chem. Clin. Biochem. Zeitschrift fur Klin. Chemie und Klin. Biochem.* **1987**, *25*, 675–679.

88. Liu, Y.; Sogawa, K.; Sunaga, M.; Umemura, H.; Satoh, M.; Kazami, T.; Yoshikawa, M.; Tomonaga, T.; Yokosuka, O.; Nomura, F. Increased concentrations of apo A-I and apo A-II fragments in the serum of patients with hepatocellular carcinoma by magnetic beads-assisted MALDI-TOF mass spectrometry. *Am. J. Clin. Pathol.* **2014**, *141*, 52–61.

89. Malik G, Ward MD, Gupta SK, Trosset MW, Grizzle WE, Adam BL, Diaz JI, S. O. Serum levels of

an isoform of apolipoprotein A-II as a potential marker for prostate cancer. *Cancer Res.* **2005**, *11*, 1073–85.

90. de Bont, J. M.; den Boer, M. L.; Reddingius, R. E.; Jansen, J.; Passier, M.; van Schaik, R. H. N.; Kros, J. M.; Sillevis Smitt, P. A. E.; Luider, T. H.; Pieters, R. Identification of apolipoprotein A-II in cerebrospinal fluid of pediatric brain tumor patients by protein expression profiling. *Clin. Chem.* **2006**, *52*, 1501–1509.

91. Honda, K.; Okusaka, T.; Felix, K.; Nakamori, S.; Sata, N.; Nagai, H.; Ioka, T.; Tsuchida, A.; Shimahara, T.; Shimahara, M.; Yasunami, Y.; Kuwabara, H.; Sakuma, T.; Otsuka, Y.; Ota, N.; Shitashige, M.; Kosuge, T.; Büchler, M. W.; Yamada, T. Altered plasma apolipoprotein modifications in patients with pancreatic cancer: protein characterization and multi-institutional validation. *PLoS One* **2012**, *7*, e46908.

92. Bharali, D.; Banerjee, B. D.; Bharadwaj, M.; Husain, S. A.; Kar, P. Expression analysis of apolipoproteins AI & AIV in hepatocellular carcinoma: A protein-based hepatocellular carcinoma-associated study. *Indian J. Med. Res.* **2018**, *147*, 361–368.

93. Farrokhi Yekta, R.; Arefi Oskouie, A.; Rezaei Tavirani, M.; Mohajeri-Tehrani, M. R.; Soroush, A. R. Decreased apolipoprotein A4 and increased complement component 3 as potential markers for papillary thyroid carcinoma: A proteomic study. *Int. J. Biol. Markers* **2018**, *33*, 455–462.

94. Li, B.; An, H. J.; Kirmiz, C.; Lebrilla, C. B.; Lam, K. S.; Miyamoto, S. Glycoproteomic analyses of ovarian cancer cell lines and sera from ovarian cancer patients show distinct glycosylation changes in individual proteins. *J. Proteome Res.* **2008**, *7*, 3776–3788.

95. Wang, M.; Wang, J.; Jiang, H. Diagnostic value of apolipoprotein C-I, transthyretin and apolipoprotein C-III in gastric cancer. *Oncol Lett* **2019**, *17*, 3227–3232.

96. Yi, J.; Ren, L.; Wu, J.; Li, W.; Zheng, X.; Du, G.; Wang, J. Apolipoprotein C1 (APOC1) as a novel diagnostic and prognostic biomarker for gastric cancer. *Ann. Transl. Med.* **2019**, *7*, 380.

97. Zhou, Y.; Luo, G. Apolipoproteins, as the carrier proteins for lipids, are involved in the development of breast cancer. *Clin. Transl. Oncol. Off. Publ. Fed. Spanish Oncol. Soc. Natl. Cancer Inst. Mex.* **2020**, *22*, 1952–1962.

98. Zhao, Z.; Zou, S.; Guan, X.; Wang, M.; Jiang, Z.; Liu, Z.; Li, C.; Lin, H.; Liu, X.; Yang, R.; Gao, Y.; Wang, X. Apolipoprotein E Overexpression Is Associated With Tumor Progression and Poor Survival in Colorectal Cancer. *Front. Genet.* **2018**, *9*, 650.

99. Kemp, S. B.; Carpenter, E. S.; Steele, N. G.; Donahue, K. L.; Nwosu, Z. C.; Pacheco, A.; Velez-Delgado, A.; Menjivar, R. E.; Lima, F.; The, S.; Espinoza, C. E.; Brown, K.; Long, D.; Lyssiotis, C. A.; Rao, A.; Zhang, Y.; Pasca di Magliano, M.; Crawford, H. C. Apolipoprotein E Promotes Immune Suppression in Pancreatic Cancer through NF-κB-Mediated Production of CXCL1. *Cancer Res.* **2021**, *81*, 4305–4318.

100. Xue, Y.; Huang, S.; Huang, J.; Li, S.; Zhang, C.; Zhou, X. Identification of Apolipoprotein E as a Potential Diagnostic Biomarker of Nasopharyngeal Carcinoma. *Cancer Manag. Res.* **2020**, *12*, 8943–8950.

101. Xu, X.; Wan, J.; Yuan, L.; Ba, J.; Feng, P.; Long, W.; Huang, H.; Liu, P.; Cai, Y.; Liu, M.; Luo, J.; Li, L. Serum levels of apolipoprotein E correlates with disease progression and poor prognosis in breast cancer. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* **2016**.

102. Wang, Y.-B.; Zhou, B.-X.; Ling, Y.-B.; Xiong, Z.-Y.; Li, R.-X.; Zhong, Y.-S.; Xu, M.-X.; Lu, Y.; Liang, H.; Chen, G.-H.; Yao, Z.-C.; Deng, M.-H. Decreased expression of ApoF associates with poor prognosis in human hepatocellular carcinoma. *Gastroenterol. Rep.* **2019**, *7*, 354–360.

103. Jing, X.; Piao, Y.-F.; Liu, Y.; Gao, P.-J. Beta2-GPI: a novel factor in the development of hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* **2010**, *136*, 1671–1680.
104. Mandili, G.; Notarpietro, A.; Khadjavi, A.; Allasia, M.; Battaglia, A.; Lucatello, B.; Frea, B.; Turrini, F.; Novelli, F.; Giribaldi, G.; Destefanis, P. Beta-2-glycoprotein-1 and alpha-1-antitrypsin as urinary markers of renal cancer in von Hippel-Lindau patients. *Biomarkers Biochem. Indic. Expo. response, susceptibility to Chem.* **2018**, *23*, 123–130.
105. Lee, S.-W.; Kim, I. J.; Jeong, B. Y.; Choi, M.-H.; Kim, J. Y.; Kwon, K.-H.; Lee, J. W.; Yu, A.; Shin, M.-G. Use of MDLC-DIGE and LC-MS/MS to identify serum biomarkers for complete remission in patients with acute myeloid leukemia. *Electrophoresis* **2012**, *33*, 1863–1872.
106. Pietrowska, M.; Jelonek, K.; Michalak, M.; Roś, M.; Rodziewicz, P.; Chmielewska, K.; Polański, K.; Polańska, J.; Gdowicz-Kłosok, A.; Giglok, M.; Suwiński, R.; Tarnawski, R.; Dziadziuszko, R.; Rzyman, W.; Widłak, P. Identification of serum proteome components associated with progression of non-small cell lung cancer. *Acta Biochim. Pol.* **2014**, *61*, 325–331.
107. Cao, W.-Q.; Jiang, B.-Y.; Huang, J.-M.; Zhang, L.; Liu, M.-Q.; Yao, J.; Wu, M.-X.; Zhang, L.-J.; Kong, S.-Y.; Wang, Y.; Yang, P.-Y. Straightforward and Highly Efficient Strategy for Hepatocellular Carcinoma Glycoprotein Biomarker Discovery Using a Nonglycopeptide-Based Mass Spectrometry Pipeline. *Anal. Chem.* **2019**, *91*, 12435–12443.
108. Jiang, J.; Wu, C.; Luo, G.; Zheng, L.; Chen, L.; Zhang, X.; Xu, N. Expression of apolipoprotein M in human hepatocellular carcinoma tissues. *Acta Histochem.* **2011**, *113*, 53–57.
109. Kou, W.; Li, B.; Shi, Y.; Zhao, Y.; Yu, Q.; Zhuang, J.; Xu, Y.; Peng, W. High complement protein C1q levels in pulmonary fibrosis and non-small cell lung cancer associated with poor prognosis. *BMC Cancer* **2022**, *22*, 110.
110. Xu, B.; Lv, W.; Li, X.; Zhang, L.; Lin, J. Prognostic genes of hepatocellular carcinoma based on gene coexpression network analysis. *J. Cell. Biochem.* **2019**.
111. Chinello, C.; Cazzaniga, M.; De Sio, G.; Smith, A. J.; Grasso, A.; Rocco, B.; Signorini, S.; Grasso, M.; Bosari, S.; Zoppis, I.; Mauri, G.; Magni, F. Tumor size, stage and grade alterations of urinary peptidome in RCC. *J. Transl. Med.* **2015**, *13*, 332.
112. Daugan, M. V; Revel, M.; Russick, J.; Dragon-Durey, M.-A.; Gaboriaud, C.; Robe-Rybkine, T.; Poillerat, V.; Grunenwald, A.; Lacroix, G.; Bougouin, A.; Meylan, M.; Verkarre, V.; Oudard, S. M.; Mejean, A.; Vano, Y. A.; Perkins, G.; Validire, P.; Cathelineau, X.; Sanchez-Salas, R.; Damotte, D.; Fremeaux-Bacchi, V.; Cremer, I.; Sautès-Fridman, C.; Fridman, W. H.; Roumenina, L. T. Complement C1s and C4d as Prognostic Biomarkers in Renal Cancer: Emergence of Noncanonical Functions of C1s. *Cancer Immunol. Res.* **2021**, *9*, 891–908.
113. Gürz, S.; Çelik, B.; Menteşe, A.; Us Altay, D. Diagnostic value of signal peptide-Complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1 on serum and tissue samples in non-small cell lung cancer. *Turk gogus kalp damar cerrahisi Derg.* **2018**, *26*, 246–253.
114. Liu, W.; Liu, B.; Xin, L.; Zhang, Y.; Chen, X.; Zhu, Z.; Lin, Y. Down-regulated expression of complement factor I: a potential suppressive protein for gastric cancer identified by serum proteome analysis. *Clin. Chim. Acta.* **2007**, *377*, 119–126.
115. Chambers, A. G.; Percy, A. J.; Yang, J.; Camenzind, A. G.; Borchers, C. H. Multiplexed quantitation of endogenous proteins in dried blood spots by multiple reaction monitoring-mass spectrometry. *Mol. Cell. Proteomics* **2013**, *12*, 781–791.
116. Riihilä, P.; Nissinen, L.; Farshchian, M.; Kallajoki, M.; Kivisaari, A.; Meri, S.; Grénman, R.; Peltonen, S.; Peltonen, J.; Pihlajaniemi, T.; Heljasvaara, R.; Kähäri, V.-M. Complement Component C3

and Complement Factor B Promote Growth of Cutaneous Squamous Cell Carcinoma. *Am. J. Pathol.* **2017**, *187*, 1186–1197.

117. Wu, P.; Shi, J.; Sun, W.; Zhang, H. The prognostic value of plasma complement factor B (CFB) in thyroid carcinoma. *Bioengineered* **2021**, *12*, 12854–12866.

118. Rajkumar, T.; Amritha, S.; Sridevi, V.; Gopal, G.; Sabitha, K.; Shirley, S.; Swaminathan, R. Identification and validation of plasma biomarkers for diagnosis of breast cancer in South Asian women. *Sci. Rep.* **2022**, *12*, 100.

119. Yang, Y.; Wan, C.; Li, H.; Zhu, H.; La, Y.; Xi, Z.; Chen, Y.; Jiang, L.; Feng, G.; He, L. Altered levels of acute phase proteins in the plasma of patients with schizophrenia. *Anal. Chem.* **2006**, *78*, 3571–3576.

120. Cui, T.; Chen, Y.; Knösel, T.; Yang, L.; Zöller, K.; Galler, K.; Berndt, A.; Mihlan, M.; Zipfel, P. F.; Petersen, I. Human complement factor H is a novel diagnostic marker for lung adenocarcinoma. *Int. J. Oncol.* **2011**, *39*, 161–168.

121. Cheng, Z.-Z.; Corey, M. J.; Pärepalo, M.; Majno, S.; Hellwage, J.; Zipfel, P. F.; Kinders, R. J.; Raitanen, M.; Meri, S.; Jokiranta, T. S. Complement factor H as a marker for detection of bladder cancer. *Clin. Chem.* **2005**, *51 5*, 856–863.

122. Suzuki, R.; Okubo, Y.; Takagi, T.; Sugimoto, M.; Sato, Y.; Irie, H.; Nakamura, J.; Takasumi, M.; Kato, T.; Hashimoto, M.; Kobashi, R.; Hikichi, T.; Ohira, H. The Complement C3a-C3a Receptor Axis Regulates Epithelial-to-Mesenchymal Transition by Activating the ERK Pathway in Pancreatic Ductal Adenocarcinoma. *Anticancer Res.* **2022**, *42*, 1207–1215.

123. Huang, Y.; Chen, F.; Zhang, L.; Lv, Q.; Yan, J.; Cui, W. MALDI-TOF-MS Analysis in the Discovery and Identification of the Serum Peptide Pattern of Pancreatic Ductal Adenocarcinoma. *Lab. Med.* **2021**, *52*, 558–566.

124. Li, C.-J.; Xia, H.-L.; Wu, Y.-M.; Ding, G.; Xu, D.-D. [Significance of Detecting Serum Complement C3 and C4 in Patients with Multiple Myeloma]. *Zhongguo shi yan xue ye xue za zhi* **2019**, *27*, 472—476.

125. Jiang, F.; Liu, H.; Liu, Z.; Song, J.; Li, L.; Ding, K.; Ren, Y.; Peng, F.; Shao, Z.; Fu, R. High serum levels of complements C3 and C4 as novel markers for myeloma bone disease. *Ann. Hematol.* 2017, *96*, 331–333.

126. Corrales, L.; Ajona, D.; Rafail, S.; Lasarte, J. J.; Riezu-Boj, J. I.; Lambris, J. D.; Rouzaut, A.; Pajares, M. J.; Montuenga, L. M.; Pio, R. Anaphylatoxin C5a creates a favorable microenvironment for lung cancer progression. *J. Immunol.* **2012**, *189*, 4674–4683.

127. Wang, S.; Hu, W.; Xie, Y.; Wu, H.; Jia, Z.; Zhang, Z.; Zhang, X. Functional genetic variants in complement component 7 confer susceptibility to gastric cancer. *PeerJ* **2022**, *10*, e12816.

128. Fishelson, Z.; Kirschfink, M. Complement C5b-9 and Cancer: Mechanisms of Cell Damage, Cancer Counteractions, and Approaches for Intervention. *Front. Immunol.* **2019**, *10*.

129. Joshi, V.; Shah, A.; Brown, I.; Winterford, C.; Hill, M. Complement component C9 as a new biomarker for esophageal adenocarcinoma. *J. Clin. Oncol.* **2017**, *35*, 19.

130. Chong, P.-K.; Lee, H.; Loh, M. C. S.; Choong, L.-Y.; Lin, Q.; So, J. B. Y.; Lim, K. H.; Soo, R. A.; Yong, W. P.; Chan, S. P.; Smoot, D. T.; Ashktorab, H.; Yeoh, K. G.; Lim, Y. P. Upregulation of plasma C9 protein in gastric cancer patients. *Proteomics* **2010**, *10*, 3210–3221.

131. Takakura, M.; Yokomizo, A.; Tanaka, Y.; Kobayashi, M.; Jung, G.; Banno, M.; Sakuma, T.; Imada, K.; Oda, Y.; Kamita, M.; Honda, K.; Yamada, T.; Naito, S.; Ono, M. Carbonic anhydrase I as a new plasma biomarker for prostate cancer. *ISRN Oncol.* **2012**, *2012*, 768190.

132. Abramowicz, A.; Wojakowska, A.; Gdowicz-Klosok, A.; Polanska, J.; Rodziewicz, P.; Polanowski, P.; Namysl-Kaletka, A.; Pietrowska, M.; Wydmanski, J.; Widlak, P. Identification of

serum proteome signatures of locally advanced and metastatic gastric cancer: a pilot study. *J. Transl. Med.* **2015**, *13*, 304.

133. Wood, P. J. 41 - Disorders of the Adrenal Cortex. In *Scientific Foundations of Biochemistry in Clinical Practice (Second Edition)*; Williams, D. L.; Marks, V., Eds.; Butterworth-Heinemann, 1994; pp. 681–706.

134. Cui, R.; Wang, C.; Zhao, Q.; Wang, Y.; Li, Y. Serum Carboxypeptidase N1 Serves as a Potential Biomarker Complementing CA15-3 for Breast Cancer. *Anticancer. Agents Med. Chem.* **2020**, *20*, 2053–2065.

135. Pforte, A.; Schiessler, A.; Gais, P.; Beer, B.; Ehlers, M.; Schütt, C.; Ziegler-Heitbrock, H. W. Expression of CD14 correlates with lung function impairment in pulmonary sarcoidosis. *Chest* **1994**, *105*, 349–354.

136. Guo, J.; Jing, R.; Zhong, J.-H.; Dong, X.; Li, Y.-X.; Liu, Y.-K.; Huang, T.-R.; Zhang, C.-Y. Identification of CD14 as a potential biomarker of hepatocellular carcinoma using iTRAQ quantitative proteomics. *Oncotarget* **2017**, *8*, 62011–62028.

137. Senra Varela, A.; Lopez Saez, J. J.; Quintela Senra, D. Serum ceruloplasmin as a diagnostic marker of cancer. *Cancer Lett.* **1997**, *121*, 139–145.

138. Yin, H.; Lin, Z.; Nie, S.; Wu, J.; Tan, Z.; Zhu, J.; Dai, J.; Feng, Z.; Marrero, J.; Lubman, D. M. Mass-selected site-specific core-fucosylation of ceruloplasmin in alcohol-related hepatocellular carcinoma. *J. Proteome Res.* **2014**, *13*, 2887–2896.

139. Mampilly, M. O.; Ravindran, N.; Parambil, M. S.; Nilesh, K.; Jayagopalan, P.; Dhamali, D. Assessment of Serum Selenium and Ceruloplasmin in Potentially Malignant Disorders and Oral Cancer. *J. Pharm. Bioallied Sci.* **2021**, *13*, S989–S992.

140. Sogabe, M.; Nozaki, H.; Tanaka, N.; Kubota, T.; Kaji, H.; Kuno, A.; Togayachi, A.; Gotoh, M.; Nakanishi, H.; Nakanishi, T.; Mikami, M.; Suzuki, N.; Kiguchi, K.; Ikehara, Y.; Narimatsu, H. Novel glycobiomarker for ovarian cancer that detects clear cell carcinoma. *J. Proteome Res.* **2014**, *13*, 1624–1635.

141. Harazono, A.; Kawasaki, N.; Itoh, S.; Hashii, N.; Ishii-Watabe, A.; Kawanishi, T.; Hayakawa, T. Site-specific N-glycosylation analysis of human plasma ceruloplasmin using liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Biochem.* **2006**, *348*, 259–268.

142. Balmaña, M.; Sarrats, A.; Llop, E.; Barrabés, S.; Saldova, R.; Ferri, M. J.; Figueras, J.; Fort, E.; de Llorens, R.; Rudd, P. M.; Peracaula, R. Identification of potential pancreatic cancer serum markers: Increased sialyl-Lewis X on ceruloplasmin. *Clin. Chim. Acta.* **2015**, *442*, 56–62.

143. Gao, H.; Wan, Y.; Fan, X.; Dong, Y.; Ji, X.; Song, W. The Role of Cholinesterase in Differential Diagnosis between Gastric Cancer and Benign Gastric Diseases. *Clin. Lab.* **2021**, *67*.

144. Lin, C.-C.; Tsai, P.; Sun, H.-Y.; Hsu, M.-C.; Lee, J.-C.; Wu, I.-C.; Tsao, C.-W.; Chang, T.-T.; Young, K.-C. Apolipoprotein J, a glucose-upregulated molecular chaperone, stabilizes core and NS5A to promote infectious hepatitis C virus virion production. *J. Hepatol.* **2014**, *61*, 984–993.

145. Koltai, T. Clusterin: a key player in cancer chemoresistance and its inhibition. *Onco. Targets. Ther.* **2014**, *7*, 447–456.

146. Sansanwal, P.; Li, L.; Sarwal, M. M. Inhibition of intracellular clusterin attenuates cell death in nephropathic cystinosis. *J. Am. Soc. Nephrol.* **2015**, *26*, 612–625.

147. Serie, D.; Myers, A. A.; Haehn, D. A.; Parker, A. S.; Bajalia, E.; Gonzalez, G.; Li, Q.; Wong, M. Y.; Moser, K.; Zhou, B.; Thiel, D. D. NOVEL PLASMA GLYCOPROTEIN BIOMARKERS PREDICT PROGRESSION-FREE SURVIVAL IN SURGICALLY RESECTED CLEAR CELL RENAL CELL CARCINOMA. *medRxiv* **2022**.

148. Chen, Q.-F.; Chang, L.; Su, Q.; Zhao, Y.; Kong, B. Clinical importance of serum secreted clusterin in predicting invasive breast cancer and treatment responses. *Bioengineered* **2021**, *12*, 278–285.
149. Rasmy, H. S.; Mohammed, H. A.; Mohammed, E. S.; Ahmed, A. S. M.; Isaac, A. Serum clusterin as a promising diagnostic and prognostic marker for hepatocellular carcinoma after locoregional treatment. *Egypt. J. Immunol.* **2022**, *29*, 26–40.
150. Bellia, F.; Vecchio, G.; Rizzarelli, E. Carnosinases, their substrates and diseases. *Molecules* 2014, *19*, 2299–2329.
151. Gautam, P.; Nair, S. C.; Gupta, M. K.; Sharma, R.; Polisetty, R. V.; Uppin, M. S.; Sundaram, C.; Puligopu, A. K.; Ankathi, P.; Purohit, A. K.; Chandak, G. R.; Harsha, H. C.; Sirdeshmukh, R. Proteins with altered levels in plasma from glioblastoma patients as revealed by iTRAQ-based quantitative proteomic analysis. *PLoS One* **2012**, *7*, e46153.
152. Arner, P.; Henjes, F.; Schwenk, J. M.; Darmanis, S.; Dahlman, I.; Iresjö, B.-M.; Naredi, P.; Agustsson, T.; Lundholm, K.; Nilsson, P.; Rydén, M. Circulating carnosine dipeptidase 1 associates with weight loss and poor prognosis in gastrointestinal cancer. *PLoS One* **2015**, *10*, e0123566.
153. Schwenk, J. M.; Igel, U.; Neiman, M.; Langen, H.; Becker, C.; Bjartell, A.; Ponten, F.; Wiklund, F.; Grönberg, H.; Nilsson, P.; Uhlen, M. Toward Next Generation Plasma Profiling via Heat-induced Epitope Retrieval and Array-based Assays*. *Mol. Cell. Proteomics* **2010**, *9*, 2497–2507.
154. Hart, P. C.; Rajab, I. M.; Alebraheem, M.; Potempa, L. A. C-Reactive Protein and Cancer-Diagnostic and Therapeutic Insights. *Front. Immunol.* **2020**, *11*, 595835.
155. Bruserud, Ø.; Aarstad, H. H.; Tvedt, T. H. A. Combined C-Reactive Protein and Novel Inflammatory Parameters as a Predictor in Cancer-What Can We Learn from the Hematological Experience? *Cancers (Basel).* **2020**, *12*.
156. Lee, K.; Nam, K.; Oh, S.; Lim, J.; Kim, R. K.; Shim, D.; Choi, J.; Lee, S.-J.; Yu, J.-H.; Lee, J. W.; Ahn, S. H.; Shin, I. ECM1 regulates tumor metastasis and CSC-like property through stabilization of β-catenin. *Oncogene* **2015**, *34*, 6055–6065.
157. Wang, X.; Peng, Y.; Xie, M.; Gao, Z.; Yin, L.; Pu, Y.; Liu, R. Identification of extracellular matrix protein 1 as a potential plasma biomarker of ESCC by proteomic analysis using iTRAQ and 2D-LC-MS/MS. *Proteomics. Clin. Appl.* **2017**, *11*.
158. Cheng, Y.; Liu, C.; Zhang, N.; Wang, S.; Zhang, Z. Proteomics Analysis for Finding Serum Markers of Ovarian Cancer. *Biomed Res. Int.* **2014**, *2014*, 179040.
159. Gong, J.; Jie, Y.; Xiao, C.; Zhou, W.; Li, X.; Chen, Y.; Wu, Y.; Cao, J.; Zhang, Q.; Gao, Z.; Hu, B.; Chong, Y. Increased Expression of Fibulin-1 Is Associated With Hepatocellular Carcinoma Progression by Regulating the Notch Signaling Pathway. *Front. cell Dev. Biol.* **2020**, *8*, 478.
160. Watany, M. M.; Elmashad, N. M.; Badawi, R.; Hawash, N. Serum FBLN1 and STK31 as biomarkers of colorectal cancer and their ability to noninvasively differentiate colorectal cancer from benign polyps. *Clin. Chim. Acta.* **2018**, *483*, 151–155.
161. Xia, H.; Feng, L.; Lin, L.; Jiang, Z.; Chen, J.; Shi, W.; Ying, S.; Yu, M.; Ju, L.; Zhu, L.; Shi, L.; Zhang, X.; Lou, J. Exploration of identifying novel serum biomarkers for malignant mesothelioma using iTRAQ combined with 2D-LC-MS/MS. *Environ. Res.* **2021**, *193*, 110467.
162. Zhu, J.; Chen, R.; Mo, L.; Tang, H.; Kuang, Y.; Fei, W.; He, C.; Hu, X. Expression of fibulin-1 predicted good prognosis in patients with colorectal cancer. *Am. J. Transl. Res.* **2015**, *7*, 339–347.
163. Wei, S.; Liu, W.; Xu, M.; Qin, H.; Liu, C.; Zhang, R.; Zhou, S.; Li, E.; Liu, Z.; Wang, Q. Cathepsin F and Fibulin-1 as novel diagnostic biomarkers for brain metastasis of non-small cell lung cancer. *Br. J. Cancer* **2022**.

164. Sokołowska, A.; Świerzko, A. S.; Gajek, G.; Gołos, A.; Michalski, M.; Nowicki, M.; Szala-Poździej, A.; Wolska-Washer, A.; Brzezińska, O.; Wierzbowska, A.; Jamroziak, K.; Kowalski, M. L.; Thiel, S.; Matsushita, M.; Jensenius, J. C.; Cedzyński, M. Associations of ficolins and mannose-binding lectin with acute myeloid leukaemia in adults. *Sci. Rep.* **2020**, *10*, 10561.

165. Li, Q.; Lin, Y. Evaluation of Ficolin-3 as a Potential Prognostic Serum Biomarker in Chinese Patients with Esophageal Cancer. *Genet. Test. Mol. Biomarkers* **2019**, *23*, 565–572.

166. Okano, T.; Kondo, T.; Kakisaka, T.; Fujii, K.; Yamada, M.; Kato, H.; Nishimura, T.; Gemma, A.; Kudoh, S.; Hirohashi, S. Plasma proteomics of lung cancer by a linkage of multi-dimensional liquid chromatography and two-dimensional difference gel electrophoresis. *Proteomics* **2006**, *6*, 3938–3948.

167. Andersen, J. D.; Boylan, K. L. M.; Xue, F. S.; Anderson, L. B.; Witthuhn, B. A.; Markowski, T. W.; Higgins, L.; Skubitz, A. P. N. Identification of candidate biomarkers in ovarian cancer serum by depletion of highly abundant proteins and differential in-gel electrophoresis. *Electrophoresis* **2010**, *31*, 599–610.

168. Greco, M.; Mitri, M. De; Chiriacò, F.; Leo, G.; Brienza, E.; Maffia, M. Serum proteomic profile of cutaneous malignant melanoma and relation to cancer progression: association to tumor derived alpha-N-acetylgalactosaminidase activity. *Cancer Lett.* **2009**, *283*, 222–229.

169. Stone, L. Fetuin-A—a marker for metastatic disease? *Nat. Rev. Urol.* **2015**, *12*, 178.

170. Ochieng, J.; Nangami, G.; Sakwe, A.; Moye, C.; Alvarez, J.; Whalen, D.; Thomas, P.; Lammers, P. Impact of Fetuin-A (AHSG) on Tumor Progression and Type 2 Diabetes. *Int. J. Mol. Sci.* **2018**, *19*.

171. Holm, M.; Joenväärä, S.; Saraswat, M.; Mustonen, H.; Tohmola, T.; Ristimäki, A.; Renkonen, R.; Haglund, C. Identification of several plasma proteins whose levels in colorectal cancer patients differ depending on outcome. *FASEB bioAdvances* **2019**, *1*, 723–730.

172. Zhang, X.; Long, Q. Elevated serum plasma fibrinogen is associated with advanced tumor stage and poor survival in hepatocellular carcinoma patients. *Medicine (Baltimore).* **2017**, *96*, e6694.

173. Bailey, M. J.; Shield-Artin, K. L.; Oliva, K.; Ayhan, M.; Reisman, S.; Rice, G. E. Stage-specific analysis of plasma protein profiles in ovarian cancer: Difference in-gel electrophoresis analysis of pooled clinical samples. *J. Carcinog.* **2013**, *12*, 10.

174. Yang, W.; Shi, J.; Zhou, Y.; Liu, T.; Li, J.; Hong, F.; Zhang, K.; Liu, N. Co-expression Network Analysis Identified Key Proteins in Association With Hepatic Metastatic Colorectal Cancer. *Proteomics. Clin. Appl.* **2019**, *13*, e1900017.

175. Zhu, W. L.; Fan, B. L.; Liu, D. L.; Zhu, W. X. Abnormal expression of fibrinogen gamma (FGG) and plasma level of fibrinogen in patients with hepatocellular carcinoma. *Anticancer Res.* **2009**, *29*, 2531–2534.

176. Peng, H. H.; Wang, J. N.; Xiao, L. F.; Yan, M.; Chen, S. P.; Wang, L.; Yang, K. Elevated Serum FGG Levels Prognosticate and Promote the Disease Progression in Prostate Cancer. *Front. Genet.* **2021**, *12*.

177. Wang, Z.; Wang, C.; Huang, X.; Shen, Y.; Shen, J.; Ying, K. Differential proteome profiling of pleural effusions from lung cancer and benign inflammatory disease patients. *Biochim. Biophys. Acta* **2012**, *1824*, 692–700.

178. Gerner, C.; Steinkellner, W.; Holzmann, K.; Gsur, A.; Grimm, R.; Ensinger, C.; Obrist, P.; Sauermann, G. Elevated plasma levels of crosslinked fibrinogen gamma-chain dimer indicate cancer-related fibrin deposition and fibrinolysis. *Thromb. Haemost.* **2001**, *85*, 494–501.

179. Tas, F.; Bilgin, E.; Karabulut, S.; Tastekin, D.; Duranyildiz, D. Levels of serum fibronectin as a biomarker in gastric cancer patients: Correlation with clinical diagnosis and outcome. *Mol. Clin.*

Oncol. **2016**, *4*, 655–659.

180. Moon, P.-G.; Lee, J.-E.; Cho, Y.-E.; Lee, S. J.; Chae, Y. S.; Jung, J. H.; Kim, I.-S.; Park, H. Y.; Baek, M.-C. Fibronectin on circulating extracellular vesicles as a liquid biopsy to detect breast cancer. *Oncotarget* **2016**, *7*, 40189–40199.

181. Lin, T.-C.; Yang, C.-H.; Cheng, L.-H.; Chang, W.-T.; Lin, Y.-R.; Cheng, H.-C. Fibronectin in Cancer: Friend or Foe. *Cells* **2019**, *9*.

182. Chai, Y. D.; Zhang, L.; Yang, Y.; Su, T.; Charugundla, P.; Ai, J.; Messadi, D.; Wong, D. T.; Hu, S. Discovery of potential serum protein biomarkers for lymph node metastasis in oral cancer. *Head Neck* **2016**, *38*, 118–125.

183. Chiu, C.-T.; Wang, P.-W.; Asare-Werehene, M.; Tsang, B. K.; Shieh, D.-B. Circulating Plasma Gelsolin: A Predictor of Favorable Clinical Outcomes in Head and Neck Cancer and Sensitive Biomarker for Early Disease Diagnosis Combined with Soluble Fas Ligand. *Cancers (Basel).* **2020**, *12*.

184. Tsai, M.-H.; Wu, C.-C.; Peng, P.-H.; Liang, Y.; Hsiao, Y.-C.; Chien, K.-Y.; Chen, J.-T.; Lin, S.-J.; Tang, R.-P.; Hsieh, L.-L.; Yu, J.-S. Identification of secretory gelsolin as a plasma biomarker associated with distant organ metastasis of colorectal cancer. *J. Mol. Med. (Berl).* **2012**, *90*, 187–200.

185. Lee, P.-S.; Sampath, K.; Karumanchi, S. A.; Tamez, H.; Bhan, I.; Isakova, T.; Gutierrez, O. M.; Wolf, M.; Chang, Y.; Stossel, T. P.; Thadhani, R. Plasma gelsolin and circulating actin correlate with hemodialysis mortality. *J. Am. Soc. Nephrol.* **2009**, *20*, 1140–1148.

186. Van Belle, S. J.-P. What is the value of hemoglobin as a prognostic and predictive factor in cancer? *Eur. J. Cancer Suppl.* **2004**, *2*, 11–19.

187. Fiorito, V.; Tolosano, E. y observed the appearance of fucosylated Hx in the sera of HCC patients when compared to those of normal individuals. *Int. J. Mol. Sci.* **2022**, *23*.

188. Comunale, M. A.; Lowman, M.; Long, R. E.; Krakover, J.; Philip, R.; Seeholzer, S.; Evans, A. A.; Hann, H.-W. L.; Block, T. M.; Mehta, A. S. Proteomic analysis of serum associated fucosylated glycoproteins in the development of primary hepatocellular carcinoma. *J. Proteome Res.* **2006**, *5*, 308–315.

189. Cine, N.; Baykal Tarik, A.; Sunnetci, D.; Canturk, Z.; Serhatli, M.; Savli, H. Identification of ApoA1, HPX and POTEE genes by omic analysis in breast cancer. *Oncol Rep* **2014**, *32*, 1078–1086.

190. Liao, W.-Y.; Ho, C.-C.; Hou, H.-H.; Hsu, T.-H.; Tsai, M.-F.; Chen, K.-Y.; Chen, H.-Y.; Lee, Y.-C.; Yu, C.-J.; Lee, C.-H.; Yang, P.-C. Heparin co-factor II enhances cell motility and promotes metastasis in non-small cell lung cancer. *J. Pathol.* **2015**, *235*, 50–64.

191. Namini, N. M.; Abdollahi, A.; Movahedi, M.; Razavi, A. E.; Saghiri, & R. Association of SERPIND1 expression with grade, stage and presence of metastasis in breast cancer. *Indian J. Biochem. Biophys.* **2021**, *58*, 71–82.

192. Beecken, W.-D.; Bentas, W.; Engels, K.; Glienke, W.; Urbschat, A.; Jonas, D.; Binder, J.; Scharrer, I. Reduced plasma levels of coagulation factors in relation to prostate cancer. *Prostate* **2002**, *53*, 160–167.

193. Naryzhny, S. N.; Legina, O. K. Haptoglobin as a biomarker. *Biomeditsinskaya Khimiya* **2021**, *67*, 105–118.

194. Zhao, C.; Annamalai, L.; Guo, C.; Kothandaraman, N.; Koh, S. C. L.; Zhang, H.; Biswas, A.; Choolani, M. Circulating haptoglobin is an independent prognostic factor in the sera of patients with epithelial ovarian cancer. *Neoplasia* **2007**, *9*, 1–7.

195. Garibay-Cerdenares, O. L.; Hernández-Ramírez, V. I.; Osorio-Trujillo, J. C.; Hernández-Ortíz, M.; Gallardo-Rincón, D.; Cantú De León, D.; Encarnación-Guevara, S.; Villegas-Pineda, J. C.; Talamás-

Rohana, P. Proteomic identification of fucosylated haptoglobin alpha isoforms in ascitic fluids and its localization in ovarian carcinoma tissues from Mexican patients. *J. Ovarian Res.* **2014**, *7*.

196. Ye B., Cramer D.W., Skates S.J., Gygi S.P., Pratomo V., Fu L., Horick N.K., Licklider L.J., Schorge J.O., Berkowitz R.S., M. S. C. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. *Clin. Cancer Res* **2003**, *9*, 2904–2911.

197. Zhu, J.; Lin, Z.; Wu, J.; Yin, H.; Dai, J.; Feng, Z.; Marrero, J.; Lubman, D. M. Analysis of serum haptoglobin fucosylation in hepatocellular carcinoma and liver cirrhosis of different etiologies. *J. Proteome Res.* **2014**, *13*, 2986–2997.

198. Wu, J.; Zhu, J.; Yin, H.; Buckanovich, R. J.; Lubman, D. M. Analysis of glycan variation on glycoproteins from serum by the reverse lectin-based ELISA assay. *J. Proteome Res.* **2014**, *13*, 2197–2204.

199. Naryzhny, S.; Ronzhina, N.; Zorina, E.; Kabachenko, F.; Zavialova, M.; Zgoda, V.; Klopov, N.; Legina, O.; Pantina, R. Evaluation of haptoglobin and its proteoforms as glioblastoma markers. *Int. J. Mol. Sci.* **2021**, *22*, 1–12.

200. Nielsen, M. J.; Petersen, S. V.; Jacobsen, C.; Oxvig, C.; Rees, D.; Møller, H. J.; Moestrup, S. K. Haptoglobin-related protein is a high-affinity hemoglobin-binding plasma protein. *Blood* **2006**.

201. Epelbaum, R.; Shalitin, C.; Segal, R.; Valansi, C.; Arselan, I.; Faraggi, D.; Leviov, M.; Ben-Shahar, M.; Haim, N. Haptoglobin-related protein as a serum marker in malignant lymphoma. *Pathol. Oncol. Res.* **1998**, *4*, 271–276.

202. Ahmed, M. , Almogbel, E. , Khirry, I. , Hassan, S. , Salem, T. and Saeed, A. Diagnostic and Prognostic Significance of Histidine-Rich Glycoprotein in Acute Lymphoblastic Leukemia. *Open J. Blood Dis.* **2017**, *7*, 16–28.

203. Winiarska, A.; Zareba, L.; Krolczyk, G.; Czyzewicz, G.; Zabczyk, M.; Undas, A. Decreased Levels of Histidine-Rich Glycoprotein in Advanced Lung Cancer: Association with Prothrombotic Alterations. *Dis. Markers* **2019**, *2019*, 8170759.

204. Göransson, J.; Jonsson, S.; ke Lasson Pre-operative plasma levels of C-reactive protein, albumin and various plasma protease inhibitors for the pre-operative assessment of operability and recurrence in cancer surgery. *Eur. J. Surg. Oncol.* **1996**, *22*, 607–617.

205. Lee, C.-S.; Taib, N. A. M.; Ashrafzadeh, A.; Fadzli, F.; Harun, F.; Rahmat, K.; Hoong, S. M.; Abdul-Rahman, P. S.; Hashim, O. H. Unmasking Heavily O-Glycosylated Serum Proteins Using Perchloric Acid: Identification of Serum Proteoglycan 4 and Protease C1 Inhibitor as Molecular Indicators for Screening of Breast Cancer. *PLoS One* **2016**, *11*, e0149551.

206. Hamm, A.; Veeck, J.; Bektas, N.; Wild, P. J.; Hartmann, A.; Heindrichs, U.; Kristiansen, G.; Werbowetski-Ogilvie, T.; Del Maestro, R.; Knuechel, R.; Dahl, E. Frequent expression loss of Inter-alpha-trypsin inhibitor heavy chain (ITIH) genes in multiple human solid tumors: a systematic expression analysis. *BMC Cancer* **2008**, *8*, 25.

207. Jiang, X.; Bai, X.-Y.; Li, B.; Li, Y.; Xia, K.; Wang, M.; Li, S.; Wu, H. Plasma Inter-Alpha-Trypsin Inhibitor Heavy Chains H3 and H4 Serve as Novel Diagnostic Biomarkers in Human Colorectal Cancer. *Dis. Markers* **2019**, *2019*, 5069614.

208. Chang, Q.-H.; Mao, T.; Tao, Y.; Dong, T.; Tang, X.-X.; Ge, G.-H.; Xu, Z.-J. Pan-cancer analysis identifies ITIH1 as a novel prognostic indicator for hepatocellular carcinoma. *Aging (Albany. NY).* **2021**, *13*, 11096–11119.

209. Chong, P. K.; Lee, H.; Zhou, J.; Liu, S.-C.; Loh, M. C. S.; Wang, T. T.; Chan, S. P.; Smoot, D. T.;

Ashktorab, H.; So, J. B. Y.; Lim, K. H.; Yeoh, K. G.; Lim, Y. P. ITIH3 is a potential biomarker for early detection of gastric cancer. *J. Proteome Res.* **2010**, *9*, 3671–3679.

210. Sun, Y.; Jin, J.; Jing, H.; Lu, Y.; Zhu, Q.; Shu, C.; Zhang, Q.; Jing, D. ITIH4 is a novel serum biomarker for early gastric cancer diagnosis. *Clin. Chim. Acta.* **2021**, *523*, 365–373.

211. Cheng, Z.; Lv, Y.; Pang, S.; Bai, R.; Wang, M.; Lin, S.; Xu, T.; Spalding, D.; Habib, N.; Xu, R. Kallistatin, a new and reliable biomarker for the diagnosis of liver cirrhosis. *Acta Pharm. Sin. B* **2015**, *5*, 194–200.

212. Wang, J.; Wang, X.; Lin, S.; Chen, C.; Wang, C.; Ma, Q.; Jiang, B. Identification of kininogen-1 as a serum biomarker for the early detection of advanced colorectal adenoma and colorectal cancer. *PLoS One* **2013**, *8*, e70519.

213. Yu, J.; Huang, Y.; Lin, C.; Li, X.; Fang, X.; Zhong, C.; Yuan, Y.; Zheng, S. Identification of Kininogen 1 as a Serum Protein Marker of Colorectal Adenoma in Patients with a Family History of Colorectal Cancer. *J Cancer* **2018**, *9*, 540–547.

214. Wang, W.; Wang, S.; Zhang, M. Evaluation of kininogen 1, osteopontin and α-1-antitrypsin in plasma, bronchoalveolar lavage fluid and urine for lung squamous cell carcinoma diagnosis. *Oncol Lett* **2020**, *19*, 2785–2792.

215. Giatagana, E.-M.; Berdiaki, A.; Tsatsakis, A.; Tzanakakis, G. N.; Nikitovic, D. Lumican in Carcinogenesis—Revisited. *Biomolecules* **2021**, *11*.

216. Pan, S.; Chen, R.; Brand, R. E.; Hawley, S.; Tamura, Y.; Gafken, P. R.; Milless, B. P.; Goodlett, D. R.; Rush, J.; Brentnall, T. A. Multiplex targeted proteomic assay for biomarker detection in plasma: a pancreatic cancer biomarker case study. *J. Proteome Res.* **2012**, *11*, 1937–1948.

217. Appunni, S.; Anand, V.; Khandelwal, M.; Seth, A.; Mathur, S.; Sharma, A. Altered expression of small leucine-rich proteoglycans (Decorin, Biglycan and Lumican): Plausible diagnostic marker in urothelial carcinoma of bladder. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* **2017**, *39*, 1010428317699112.

218. Rong, Y.; Jin, D.; Hou, C.; Hu, J.; Wu, W.; Ni, X.; Wang, D.; Lou, W. Proteomics analysis of serum protein profiling in pancreatic cancer patients by DIGE: up-regulation of mannose-binding lectin 2 and myosin light chain kinase 2. *BMC Gastroenterol.* **2010**, *10*, 68.

219. Su, C.; Lin, Y.; Cai, L.; Mao, Q.; Niu, J. Association between mannose-binding lectin variants, haplotypes and risk of hepatocellular carcinoma: A case-control study. *Sci. Rep.* **2016**, *6*, 32147.

220. Ide, H.; Yamagishi, S.-I.; Lu, Y.; Sakamaki, K.; Nakajima, A.; Horiuchi, A.; Kitamura, K.; Hisasue, S.-I.; Muto, S.; Yamaguchi, R.; Horie, S. Circulating pigment epithelium-derived factor (PEDF) is associated with pathological grade of prostate cancer. *Anticancer Res.* **2015**, *35*, 1703–1708.

221. Yang, Z.; Feng, J.; Xiao, L.; Chen, X.; Yao, Y.; Li, Y.; Tang, Y.; Zhang, S.; Lu, M.; Qian, Y.; Wu, H.; Shi, M. Tumor-Derived Peptidoglycan Recognition Protein 2 Predicts Survival and Antitumor Immune Responses in Hepatocellular Carcinoma. *Hepatology* **2020**, *71*, 1626–1642.

222. Holm, M.; Saraswat, M.; Joenväärä, S.; Ristimäki, A.; Haglund, C.; Renkonen, R. Colorectal cancer patients with different C-reactive protein levels and 5-year survival times can be differentiated with quantitative serum proteomics. *PLoS One* **2018**, *13*, e0195354.

223. Kang, D. W.; Choi, K.-Y.; Min, D. S. Functional Regulation of Phospholipase D Expression in Cancer and Inflammation*. *J. Biol. Chem.* **2014**, *289*, 22575–22582.

224. Tang, J.-H.; He, W.-J.; Huang, H.; Tan, C.-C.; Duan, Q.; Wang, K.-J.; Yuan, X.-Y.; Zhu, X.-J. Important roles of glycosylphosphatidylinositol (GPI)-specific phospholipase D and some GPI-anchored proteins in the pathogenesis of hepatocellular carcinoma. *Clin. Biochem.* **2009**, *42*, 400–407.

225. Kwaan, H. C.; McMahon, B. The role of plasminogen-plasmin system in cancer. *Cancer Treat. Res.* **2009**, *148*, 43–66.

226. Bharadwaj, A. G.; Holloway, R. W.; Miller, V. A.; Waisman, D. M. Plasmin and Plasminogen System in the Tumor Microenvironment: Implications for Cancer Diagnosis, Prognosis, and Therapy. *Cancers (Basel).* **2021**, *13*.

227. Vecka, M.; Jáchymová, M.; Vávrová, L.; Kodydková, J.; Macášek, J.; Urbánek, M.; Krechler, T.; Slabý, A.; Dušková, J.; Muravská, A.; Zák, A. Paraoxonase-1 (PON1) status in pancreatic cancer: relation to clinical parameters. *Folia Biol. (Praha).* **2012**, *58*, 231–237.

228. Aldonza, M. B. D.; Son, Y. S.; Sung, H.-J.; Ahn, J. M.; Choi, Y.-J.; Kim, Y.-I.; Cho, S.; Cho, J.-Y. Paraoxonase-1 (PON1) induces metastatic potential and apoptosis escape via its antioxidative function in lung cancer cells. *Oncotarget* **2017**, *8*, 42817–42835.

229. Zhang, J.; Yang, X.; Wei, L.; Tan, H.; Chen, J.; Li, W.; Chan, K.; Su, Y.; Zhao, L.; Hu, S.; Zhong, S.; Xiao, Y.; Liu, H. Improved diagnostic value by combining plasma PON1 level with tumor biomarkers in Colorectal Cancer patients. *J Cancer* **2020**, *11*, 6491–6496.

230. Ahn, S. B.; Sharma, S.; Mohamedali, A.; Mahboob, S.; Redmond, W. J.; Pascovici, D.; Wu, J. X.; Zaw, T.; Adhikari, S.; Vaibhav, V.; Nice, E. C.; Baker, M. S. Potential early clinical stage colorectal cancer diagnosis using a proteomics blood test panel. *Clin. Proteomics* **2019**, *16*, 34.

231. Afsar, C. U.; Gunaldı, M.; Okuturlar, Y.; Gedikbası, A.; Tiken, E. E.; Kahraman, S.; Karaca, F.; Ercolak, V.; Karabulut, M. Paraoxonase-1 and arylesterase activities in patients with colorectal cancer. *Int. J. Clin. Exp. Med.* **2015**, *8*, 21599–21604.

232. Akçay, M. N.; Yilmaz, I.; Polat, M. F.; Akçay, G. Serum paraoxonase levels in gastric cancer. *Hepatogastroenterology.* **2003**, *50 Suppl 2*, cclxxiii–cclxxv.

233. Schlesinger, M.; Broman, I.; Lugassy, G. The complement system is defective in chronic lymphatic leukemia patients and in their healthy relatives. *Leukemia* **1996**, *10*, 1509–1513.

234. Uehara, S.; Fukuzawa, Y.; Matuyama, T.; Gotoh, K. Role of Tyro 3 , Axl , and Mer Receptors and Their Ligands (Gas 6 , and Protein S) in Patients with Hepatocellular Carcinoma. In; 2017.

235. Bushue, N.; Wan, Y.-J. Y. Retinoid pathway and cancer therapeutics. *Adv. Drug Deliv. Rev.* **2010**, *62*, 1285–1298.

236. Hu, X.; Huang, W.; Wang, F.; Dai, Y.; Hu, X.; Yue, D.; Wang, S. Serum levels of retinol-binding protein 4 and the risk of non-small cell lung cancer: A case-control study. *Medicine (Baltimore).* **2020**, *99*, e21254.

237. Jiao, C.; Cui, L.; Ma, A.; Li, N.; Si, H. Elevated Serum Levels of Retinol-Binding Protein 4 Are Associated with Breast Cancer Risk: A Case-Control Study. *PLoS One* **2016**, *11*, e0167498.

238. Biaoxue, R.; Hua, L.; Wenlong, G.; Shuanying, Y. Increased serum amyloid A as potential diagnostic marker for lung cancer: a meta-analysis based on nine studies. *BMC Cancer* **2016**, *16*, 836.

239. Malle, E.; Sodin-Semrl, S.; Kovacevic, A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. *Cell. Mol. Life Sci.* **2009**, *66*, 9–26.

240. Lin, H.; Tan, G.; Liu, Y.; Lin, S. The prognostic value of serum amyloid A in solid tumors: a meta-analysis. *Cancer Cell Int.* **2019**, *19*, 62.

241. Levo, Y.; Wollner, S.; Treves, A. J. Serum amyloid P-component levels in patients with malignancy. *Scand. J. Immunol.* **1986**, *24*, 147–151.

242. De Nunzio, C.; Lombardo, R.; Albisinni, S.; Gacci, M.; Tubaro, A. Serum levels of sex hormone binding globulin (SHBG) are not predictive of prostate cancer diagnosis and aggressiveness: results from an Italian biopsy cohort. *Int. Braz J Urol* **2013**, *39*, 793–799.

243. Cheng, C.-W.; Chang, C.-C.; Patria, Y. N.; Chang, R.-T.; Liu, Y.-R.; Li, F.-A.; Shih, H.-M.; Lin, C.-Y. Sex hormone-binding globulin (SHBG) is a potential early diagnostic biomarker for gastric cancer. *Cancer Med.* **2018**, *7*, 64–74.

244. Gebhardt, C.; Németh, J.; Angel, P.; Hess, J. S100A8 and S100A9 in inflammation and cancer. *Biochem. Pharmacol.* **2006**, *72*, 1622–1631.

245. Petsa, A.; Pergialiotis, V.; Konstantopoulos, P.; Katsichti, A.; Petrou, K.; Giannopoulos, A.; Botsis, D.; Perrea, D. Serum Calprotectin and Prealbumin Levels among Ovarian Cancer Patients Aged 30 - 45 Years. *Open J. Obstet. Gynecol.* **2017**, *07*, 303–311.

246. Ni Bhriain, H.; Trovik, J.; Wik, E.; Stefansson, I. M.; Akslen, L. A.; Salvesen, H. B.; Staff, A. C. Plasma calprotectin concentrations in women with endometrial carcinoma. *Gynecol. Oncol.* **2009**, *114*, 491–495.

247. Markowitz, J.; Carson, W. E. 3rd Review of S100A9 biology and its role in cancer. *Biochim. Biophys. Acta* **2013**, *1835*, 100–109.

248. Begum, F. D.; Høgdall, C. K.; Kjaer, S. K.; Christensen, L.; Blaakaer, J.; Bock, J. E.; Glud, E.; Høyer-Hansen, G.; Ring-Larsen, H.; Høgdall, E. V. S. The prognostic value of plasma soluble urokinase plasminogen activator receptor (suPAR) levels in stage III ovarian cancer patients. *Anticancer Res.* **2004**, *24*, 1981–1985.

249. Arellano-Garcia, M. E.; Li, R.; Liu, X.; Xie, Y.; Yan, X.; Loo, J. A.; Hu, S. Identification of tetranectin as a potential biomarker for metastatic oral cancer. *Int. J. Mol. Sci.* **2010**, *11*, 3106–3121.

250. Terui, S.; Moriya, Y.; Yamamoto, H.; Koyama, Y. Thyroxine-binding globulin as a marker of liver tumors. *Cancer Detect. Prev.* **1987**, *10*, 371–378.

251. Sieg, A.; Schönpflug, A.; Seitz, H. K.; Kommerell, B. [Thyroxine-binding globulin--not a tumor marker of hepatocellular cancer]. *Z. Gastroenterol.* **1991**, *29*, 101–104.

252. Cui, S.-X.; Yu, X.-F.; Qu, X.-J. Roles and Signaling Pathways of Des-γ-Carboxyprothrombin in the Progression of Hepatocellular Carcinoma. *Cancer Invest.* **2016**, *34*, 459–464.

253. Yang, Y.; Li, G.; Lu, Z.; Liu, Y.; Kong, J.; Liu, J. Progression of Prothrombin Induced by Vitamin K Absence-II in Hepatocellular Carcinoma. *Front. Oncol.* **2021**, *11*.

254. Jain S, Gautam V, N. S. Acute-phase proteins: As diagnostic tool. *J Pharm Bioallied Sci.* **2011**, *3*, 118–127.

255. Lee, C.-C.; Ding, X.; Zhao, T.; Wu, L.; Perkins, S.; Du, H.; Yan, C. Transthyretin Stimulates Tumor Growth through Regulation of Tumor, Immune, and Endothelial Cells. *J. Immunol.* **2019**, *202*, 991–1002.

256. Shimura, T.; Shibata, M.; Gonda, K.; Okayama, H.; Saito, M.; Momma, T.; Ohki, S.; Kono, K. Serum transthyretin level is associated with prognosis of patients with gastric cancer. *J. Surg. Res.* **2018**, *227*, 145–150.

257. Gericke, B.; Raila, J.; Sehouli, J.; Haebel, S.; Könsgen, D.; Mustea, A.; Schweigert, F. J. Microheterogeneity of transthyretin in serum and ascitic fluid of ovarian cancer patients. *BMC Cancer* **2005**, *5*, 133.

258. Malik, S.; Fu, L.; Juras, D. J.; Karmali, M.; Wong, B. Y. L.; Gozdzik, A.; Cole, D. E. C. Common variants of the vitamin D binding protein gene and adverse health outcomes. *Crit. Rev. Clin. Lab. Sci.* **2013**, *50*, 1–22.

259. Speeckaert, M.; Huang, G.; Delanghe, J. R.; Taes, Y. E. C. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin. Chim. Acta* **2006**, *372*, 33–42.

260. Bera, A.; Subramanian, M.; Karaian, J.; Eklund, M.; Radhakrishnan, S.; Gana, N.; Rothwell, S.;

Pollard, H.; Hu, H.; Shriver, C. D.; Srivastava, M. Functional role of vitronectin in breast cancer. *PLoS One* **2020**, *15*, e0242141.

261. Hassan, M. I.; Waheed, A.; Yadav, S.; Singh, T. P.; Ahmad, F. Zinc alpha 2-glycoprotein: a multidisciplinary protein. *Mol. Cancer Res.* **2008**, *6*, 892–906.

262. Hale, L. P.; Price, D. T.; Sanchez, L. M.; Demark-Wahnefried, W.; Madden, J. F. Zinc alpha-2-glycoprotein is expressed by malignant prostatic epithelium and may serve as a potential serum marker for prostate cancer. *Clin. cancer Res. an Off. J. Am. Assoc. Cancer Res.* **2001**, *7*, 846–853.

263. Bundred, N. J.; Scott, W. N.; Davies, S. J.; Miller, W. R.; Mansel, R. E. Zinc alpha-2 glycoprotein levels in serum and breast fluids: a potential marker of apocrine activity. *Eur. J. Cancer* **1991**, *27*, 549–552.

264. Xue, Y.; Yu, F.; Yan, D.; Cui, F.; Tang, H.; Wang, X.; Chen, J.; Lu, H.; Zhao, S.; Peng, Z. Zinc-α-2-glycoprotein: a candidate biomarker for colon cancer diagnosis in Chinese population. *Int. J. Mol. Sci.* **2014**, *16*, 691–703.

265. Sanders, C. L.; Ponte, A.; Kueppers, F. The Effects of Inflammation on Alpha 1 Antitrypsin Levels in a National Screening Cohort. *COPD J. Chronic Obstr. Pulm. Dis.* **2018**, *15*, 10–16.

266. Torres-Durán, M.; Lopez-Campos, J. L.; Barrecheguren, M.; Miravitlles, M.; Martinez-Delgado, B.; Castillo, S.; Escribano, A.; Baloira, A.; Navarro-Garcia, M. M.; Pellicer, D.; Bañuls, L.; Magallón, M.; Casas, F.; Dasí, F. Alpha-1 antitrypsin deficiency: outstanding questions and future directions. *Orphanet J. Rare Dis.* **2018**, *13*, 114.

267. Tanash, H. A.; Piitulainen, E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. *J. Gastroenterol.* **2019**, *54*, 541–548.

268. Hughes, G. J.; Frutiger, S.; Paquet, N.; Ravier, F.; Pasquali, C.; Sanchez, J. C.; James, R.; Tissot, J. D.; Bjellqvist, B.; Hochstrasser, D. F. Plasma protein map: an update by microsequencing. *Electrophoresis* **1992**, *13*, 707–714.

269. York, W. S.; Mazumder, R.; Ranzinger, R.; Edwards, N.; Kahsay, R.; Aoki-Kinoshita, K. F.; Campbell, M. P.; Cummings, R. D.; Feizi, T.; Martin, M.; others GlyGen: computational and informatics resources for glycoscience. *Glycobiology* **2020**, *30*, 72–73.

270. Singh, S.; Saleem, S.; Reed, G. L. Alpha2-Antiplasmin: The Devil You Don't Know in Cerebrovascular and Cardiovascular Disease. *Front. Cardiovasc. Med.* **2020**, *7*.

271. Harwood, S. L.; Lyngsø, J.; Zarantonello, A.; Kjøge, K.; Nielsen, P. K.; Andersen, G. R.; Pedersen, J. S.; Enghild, J. J. Structural Investigations of Human A2M Identify a Hollow Native Conformation That Underlies Its Distinctive Protease-Trapping Mechanism. *Mol. Cell. Proteomics* **2021**, *20*, 100090.

272. Kalsheker, N. A. Alpha 1-antichymotrypsin. *Int. J. Biochem. Cell Biol.* **1996**, *28*, 961–964.

273. Lichenstein, H. S.; Lyons, D. E.; Wurfel, M. M.; Johnson, D. A.; McGinley, M. D.; Leidli, J. C.; Trollinger, D. B.; Mayer, J. P.; Wright, S. D.; Zukowski, M. M. Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding protein gene family. *J. Biol. Chem.* **1994**, *269*, 18149–18154.

274. Merlot, A. M.; Kalinowski, D. S.; Richardson, D. R. Unraveling the mysteries of serum albumin—more than just a serum protein . *Front. Physiol.* 2014, *5*.

275. Stan, S.; Delvin, E.; Lambert, M.; Seidman, E.; Levy, E. Apo A-IV: an update on regulation and physiologic functions. *Biochim. Biophys. Acta* **2003**, *1631*, 177–187.

276. Jacobson, T. A. Opening a new lipid "apo-thecary": incorporating apolipoproteins as potential risk factors and treatment targets to reduce cardiovascular risk. *Mayo Clin. Proc.* **2011**, *86*, 762–780.

277. Kei, A. A.; Filippatos, T. D.; Tsimihodimos, V.; Elisaf, M. S. A review of the role of apolipoprotein C-II in lipoprotein metabolism and cardiovascular disease. *Metabolism.* **2012**, *61*, 906–921.

278. Netti, G. S.; Franzin, R.; Stasi, A.; Spadaccino, F.; Dello Strologo, A.; Infante, B.; Gesualdo, L.; Castellano, G.; Ranieri, E.; Stallone, G. Role of Complement in Regulating Inflammation Processes in Renal and Prostate Cancers. *Cells* **2021**, *10*.

279. Zhang, R.; Liu, Q.; Li, T.; Liao, Q.; Zhao, Y. Role of the complement system in the tumor microenvironment. *Cancer Cell Int.* **2019**, *19*, 300.

280. Revel, M.; Daugan, M. V; Sautés-Fridman, C.; Fridman, W. H.; Roumenina, L. T. Complement System: Promoter or Suppressor of Cancer Progression? *Antibodies (Basel, Switzerland)* **2020**, *9*.

281. Thurman, J. M.; Laskowski, J.; Nemenoff, R. A. Complement and Cancer-A Dysfunctional Relationship? *Antibodies (Basel, Switzerland)* **2020**, *9*.

282. Afshar-Kharghan, V. The role of the complement system in cancer. *J. Clin. Invest.* **2017**, *127*, 780–789.

283. Roumenina, L. T.; Daugan, M. V; Petitprez, F.; Sautès-Fridman, C.; Fridman, W. H. Context-dependent roles of complement in cancer. *Nat. Rev. Cancer* **2019**, *19*, 698–715.

284. Sjöholm, A. G.; Jönsson, G.; Braconier, J. H.; Sturfelt, G.; Truedsson, L. Complement deficiency and disease: an update. *Mol. Immunol.* **2006**, *43*, 78–85.

285. Hellman, N. E.; Gitlin, J. D. CERULOPLASMIN METABOLISM AND FUNCTION. *Annu. Rev. Nutr.* **2002**, *22*, 439–458.

286. Ran, H.; Ma, J.; Cai, L.; Zhou, H.; Yuan, Z.; Chen, Y.; Chang, W.; Huang, Y.; Xiao, Y. Serum cholinesterase may independently predict prognosis in non-small-cell lung cancer. *BMC Cancer* **2022**, *22*, 93.

287. Jones, S. E.; Jomary, C. Clusterin. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 427–431.

288. Lenney, J. F.; George, R. P.; Weiss, A. M.; Kucera, C. M.; Chan, P. W.; Rinzler, G. S. Human serum carnosinase: characterization, distinction from cellular carnosinase, and activation by cadmium. *Clin. Chim. Acta.* **1982**, *123*, 221–231.

289. Alpert E, Doysdale JW, I. K. Human alphafetoprotein: isolation, characterisation and demonstration of microheterogeneity. *J Biol Chem* **1972**, *247*, 3792–8.

290. Yamashita K, Hitoi A, T. Y. Sugar chain of alphafetoprotein producing human yolk sac tumor. *Cancer Res* **1983**, *43*, 4691–5.

291. Zhao YJ, Ju Q, L. G. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol.* **2013**, *1*, 593–598.

292. Singhal A, Jayaraman M, D. D. and K. V Molecular and serum markers in hepatocellular carcinoma: predictive tools for prognosis and recurrence. *Crit Rev Oncol Hematol* **2012**, *82*, 116–140.

293. Kim, H.; Kim, K.; Jin, J.; Park, J.; Yu, S. J.; Yoon, J.-H.; Kim, Y. Measurement of glycosylated alpha-fetoprotein improves diagnostic power over the native form in hepatocellular carcinoma. *PLoS One* **2014**, *9*, e110366.

294. Wang, M.; Zhu, J.; Lubman, D. M.; Gao, C. Aberrant glycosylation and cancer biomarker discovery: a promising and thorny journey. *Clin. Chem. Lab. Med.* **2019**, *57*, 407–416.

295. Pucci P., Siciliano R., Malorni A., Marino G., Tecce M.F., Ceccarini C., T. B. Human alpha-fetoprotein primary structure: a mass spectrometric study. *Biochemistry* **1991**, *30*, 5061–5066.

296. Brown, W. M.; Saunders, N. R.; Møllgård, K.; Dziegielewska, K. M. Fetuin--an old friend revisited. *Bioessays* **1992**, *14*, 749–755.

297. Lee, W. M.; Galbraith, R. M. The extracellular actin-scavenger system and actin toxicity. *N. Engl. J. Med.* **1992**, *326*, 1335–1341.

298. Tripathi, A.; Lammers, K. M.; Goldblum, S.; Shea-Donohue, T.; Netzel-Arnett, S.; Buzza, M. S.;

Antalis, T. M.; Vogel, S. N.; Zhao, A.; Yang, S.; Arrietta, M. C.; Meddings, J. B.; Fasano, A. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. *Proc. Natl. Acad. Sci. U. S. A.* **2009**.

299. Fasano, A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity, and cancer. *Physiol. Rev.* **2011**, *91*, 151–175.

300. Wakabayashi, S. Chapter Nine - New Insights into the Functions of Histidine-Rich Glycoprotein. In; Jeon, K. W., Ed.; International Review of Cell and Molecular Biology; Academic Press, 2013; Vol. 304, pp. 467–493.

301. Johnson, L. D. S.; Goubran, H. A.; Kotb, R. R. Histidine rich glycoprotein and cancer: a multi-faceted relationship. *Anticancer Res.* **2014**, *34*, 593–603.

302. Law, R. H. P.; Zhang, Q.; McGowan, S.; Buckle, A. M.; Silverman, G. A.; Wong, W.; Rosado, C. J.; Langendorf, C. G.; Pike, R. N.; Bird, P. I.; Whisstock, J. C. An overview of the serpin superfamily. *Genome Biol.* **2006**, *7*, 216.

303. Cicardi, M.; Zingale, L.; Zanichelli, A.; Pappalardo, E.; Cicardi, B. C1 inhibitor: molecular and clinical aspects. *Springer Semin. Immunopathol.* **2005**, *27*, 286–298.

304. Zhuo, L.; Hascall, V. C.; Kimata, K. Inter-alpha-trypsin inhibitor, a covalent protein-glycosaminoglycan-protein complex. *J. Biol. Chem.* **2004**, *279*, 38079–38082.

305. Belkacemi, L.; Zhang, S. X. Anti-tumor effects of pigment epithelium-derived factor (PEDF): implication for cancer therapy. A mini-review. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 4.

306. Li, H.-L.; Liu, D.-P.; Liang, C.-C. Paraoxonase gene polymorphisms, oxidative stress, and diseases. *J. Mol. Med. (Berl).* **2003**, *81*, 766–779.

307. Sack, G. H. J. Serum Amyloid A (SAA) Proteins. *Subcell. Biochem.* **2020**, *94*, 421–436.

308. Lee, S. E.; Chung, J. S.; Han, B. K.; Park, C. S.; Moon, K. H.; Byun, S.-S.; Choe, G.; Hong, S. K. Preoperative serum sex hormone-binding globulin as a predictive marker for extraprostatic extension of tumor in patients with clinically localized prostate cancer. *Eur. Urol.* **2008**, *54*, 1324–1332.

309. Bikle, D. D.; Schwartz, J. Vitamin D Binding Protein, Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. *Front. Endocrinol. (Lausanne).* **2019**, *10*.

310. Garbett, N. C.; Mekmaysy, C. S.; Helm, C. W.; Jenson, A. B.; Chaires, J. B. Differential scanning calorimetry of blood plasma for clinical diagnosis and monitoring. *Exp. Mol. Pathol.* **2009**, *86*, 186–191.

311. Chagovetz, A. A.; Quinn, C.; Damarse, N.; Hansen, L. D.; Chagovetz, A. M.; Jensen, R. L. Differential scanning calorimetry of gliomas: a new tool in brain cancer diagnostics? *Neurosurgery* **2013**, *73*, 289–95; discussion 295.

312. McMillan, D. C. An inflammation-based prognostic score and its role in the nutrition-based management of patients with cancer. *Proc. Nutr. Soc.* **2008**, *67*, 257–262.

313. Zhang, H.; Ren, D.; Jin, X.; Wu, H. The prognostic value of modified Glasgow Prognostic Score in pancreatic cancer: a meta-analysis. *Cancer Cell Int.* **2020**, *20*, 462.

314. Naryzhny, S.; Volnitskiy, A.; Kopylov, A.; Zorina, E.; Kamyshinsky, R.; Bairamukov, V.; Garaeva, L.; Shlikht, A.; Shtam, T. Proteome of Glioblastoma-Derived Exosomes as a Source of Biomarkers. *Biomedicines* **2020**, *8*, 216.

315. Naryzhny, S.; Zgoda, V.; Kopylov, A.; Petrenko, E.; Kleist, O.; Archakov, A. Variety and Dynamics of Proteoforms in the Human Proteome: Aspects of Markers for Hepatocellular Carcinoma. *Proteomes* **2017**, *5*, 33.

316. Naryzhny, S. N.; Zgoda, V. G.; Maynskova, M. A.; Novikova, S. E.; Ronzhina, N. L.; Vakhrushev, I. V.; Khryapova, E. V.; Lisitsa, A. V.; Tikhonova, O. V.; Ponomarenko, E. A.; Archakov, A. I.

Combination of virtual and experimental 2DE together with ESI LC-MS/MS gives a clearer view about proteomes of human cells and plasma. *Electrophoresis* **2016**, *37*, 302–309.

317. Naryzhny, S. N.; Maynskova, M. A.; Zgoda, V. G.; Ronzhina, N. L.; Kleyst, O. A.; Vakhrushev, I. V.; Archakov, A. I. Virtual-Experimental 2DE Approach in Chromosome-Centric Human Proteome Project. *J. Proteome Res.* **2016**, *15*, 525–530.

318. Vaudel, M.; Barsnes, H.; Berven, F. S.; Sickmann, A.; Martens, L. SearchGUI: An open-source graphical user interface for simultaneous OMSSA and X!Tandem searches. *Proteomics* **2011**, *11*, 996–999.

319. Ishihama, Y.; Oda, Y.; Tabata, T.; Sato, T.; Nagasu, T.; Rappsilber, J.; Mann, M. Exponentially Modified Protein Abundance Index (emPAI) for Estimation of Absolute Protein Amount in Proteomics by the Number of Sequenced Peptides per Protein. *Mol. Cell. Proteomics* **2005**, *4*, 1265–1272.

320. Ignjatovic, V.; Geyer, P. E.; Palaniappan, K. K.; Chaaban, J. E.; Omenn, G. S.; Baker, M. S.; Deutsch, E. W.; Schwenk, J. M. Mass Spectrometry-Based Plasma Proteomics: Considerations from Sample Collection to Achieving Translational Data. *J. Proteome Res.* **2019**, *18*, 4085–4097.

321. Geyer, P. E.; Arend, F. M.; Doll, S.; Louiset, M.-L.; Virreira Winter, S.; Müller-Reif, J. B.; Torun, F. M.; Weigand, M.; Eichhorn, P.; Bruegel, M.; Strauss, M. T.; Holdt, L. M.; Mann, M.; Teupser, D. High-resolution serum proteome trajectories in COVID-19 reveal patient-specific seroconversion. *EMBO Mol. Med.* **2021**, *13*, e14167.