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Gene Expression Profile of Breast Cancer with Monoallelic Somatic *BRCA1* Inactivation

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Abstract: Background and Objectives: Most of the research of the *BRCA1* inactivation is focused on monoallelic germline alterations and loss of heterozygosity in tumour. The aim of this study was to identify characteristic transcriptomic pattern of monoallelic somatic *BRCA1* inactivation and estimate its impact to the event-free breast cancer survival prognosis. Materials and Methods: We conducted global transcriptome sequencing of breast cancer tissue samples to identify differentially expressed genes and signalling pathways associated with monoallelic somatic *BRCA1* inactivation. The study group involved 36 patient samples categorized based on *BRCA1* inactivation status. Subsequently, the differential gene expression and Kaplan-Meier analyses in the groups with and without monoallelic somatic *BRCA1* inactivation were performed. Results: Kaplan-Meier analysis showed tendency for longer event-free survival in patients with monoallelic somatic *BRCA1* inactivation, suggesting somatic *BRCA1* inactivation is favourable prognostic. Differential gene expression analysis followed by the STRING tool enrichment analysis showed significant enrichment of proteins in the extracellular region and extracellular space. Conclusion: In this study we identified transcriptomic profile of differentially expressed genes *TPSD1, FABP4, CARTPT* and *MMP9* as indicative for homologous recombination impaired tumours with tendency for better therapy results.

Keywords: breast cancer; transcriptome; *BRCA1* somatic inactivation; differential gene expression

1. Introduction

Breast cancer is the most common cancer among women worldwide and possesses a growing burden on global public health. It is a diverse disease, both biologically and molecularly, and is associated with environmental and genetic risk factors such as pathogenic variants in the *BRCA1* and *BRCA2* genes, which contribute to the development of malignancy [1].

The *BRCA1* and *BRCA2* genes have vital roles in preserving the integrity of the genome and suppressing tumour formation. Both genes facilitate DNA repair through homologous recombination and reactivating replication processes. They are essential for ensuring accurate and efficient restoration of damaged DNA, thereby preventing the accumulation of genetic changes that can lead to cancer development [2].

Germline alterations in the *BRCA1* and *BRCA2* genes are responsible for an increased susceptibility and higher likelihood of developing breast and ovarian cancers. These genetic variations, which result in the Hereditary Breast and Ovarian Cancer (HBOC) syndrome, account for around 16% from all breast cancer cases [3].

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Somatic inactivation of *BRCA1/2* genes plays a significant role in the development and progression of breast cancer. While germline *BRCA1/2* mutations are well-known risk factors, somatic mutations occurring specifically within the tumour cells which can also lead to the loss of *BRCA1/2* function [3]. Somatic *BRCA1/2* inactivation results in the disruption of crucial DNA repair mechanisms, including homologous recombination (HR), leading to genomic instability and an increased propensity for the accumulation of additional genetic alterations. These somatic mutations often manifest as large-scale genomic rearrangements, including deletions, insertions, and copy number alterations.

The inactivation of the *BRCA1* gene can occur in hereditary cases which is characterized by pathogenic variants in the gene sequence, and sporadic cases. The majority of breast cancer cases are considered sporadic, indicating they are not inherited or linked to genetic syndromes. While the sequence of the *BRCA1* gene remains mostly unchanged, there is a specific methylation pattern on the CpG Island region in the *BRCA1* promoter. Focal hypermethylation at the tip regions of this CpG island prevents the gene from being transcribed. Alterations in DNA methylation profiles can contribute to the development of sporadic breast cancer without changes in the underlying DNA sequence of the *BRCA1* gene [4,5].

The loss of BRCA1/2 function contributes to the impairment of DNA repair and renders cancer cells highly sensitive to DNA-damaging agents, such as platinum-based chemotherapy and poly(ADP-ribose) polymerase (PARP) inhibitors [6,7]. Beside the mutational epigenetic changes in the *BRCA1* gene, the breast cancer is also characterized by certain types of somatic mutational signatures, including "BRCAness" - an HR deficient tumour without pathogenic variants in *BRCA1* or *BRCA2*. Such tumours also are associated with better response to the platinum or PARP inhibitor therapy (Bodily et al., 2020; Polak et al., 2017; Nik-Zainal et al., 2016). The classification of cancer mutational signatures includes HR deficient cancers in base substitution signature 3 and 8, which are characterised with absence of *BRCA1* and *BRCA2* functions [8]. Combinations of base substitution, indel and rearrangement mutational signatures is proposed as predictive biomarkers of HR deficiency for responsiveness to cisplatin and PARP inhibitors treatment than *BRCA1/2* mutations or promoter methylation alone [8].

Drawing on the parallels with ovarian cancer, where *BRCA* (germline and somatic) mutations confer sensitivity to platinum-based chemotherapy and PARP inhibitors [9–11], incorporating *BRCA* status into clinical decision-making could improve outcomes and personalize therapy for breast cancer patients. Previous research by Maksimenko et al. [12] showed improved breast cancer-specific survival rates in triple-negative breast cancer patients with *BRCA1* founder mutations compared to non-carriers. Identifying somatic *BRCA1/2* inactivation in breast cancer patients can therefore have significant implications for treatment decisions and prognostic assessments. This enables the selection of targeted therapies that exploit the vulnerabilities of *BRCA1*/2-deficient tumours. Additionally, studying the patterns and consequences of somatic *BRCA1/2* inactivation can provide insights into the mechanisms driving tumour progression and aid in developing novel therapeutic strategies and biomarkers for breast cancer patients with these specific alterations.

2. Materials and Methods

Study Group

36 fresh frozen tissue samples isolated from surgery material were selected from the repository of Riga Stradins University, Institute of Oncology (RSU IO). All patient samples had confirmed breast cancer diagnosis and were not treated with systemic treatment before the surgery. Patients underwent surgery and subsequent systemic therapy at P. Stradins Clinical University Hospital from 2016 to 2018. Study group consisted of TNBC, luminal, HER2-positive samples. Patient's median age at diagnosis was 59 (ranging from 31 to 81). Only patients without germline *BRCA1*/*BRCA2* variants were included. Clinical information (Supplement 1) was collected from medical records, including information on cancer diagnosis, recurrence and survival status which was used for Kaplan-Mayer

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analysis and log-rank test to assess event-free survival, defined as the time from cancer diagnosis to recurrence with average follow-up 71 months (19-91).

This study was approved by the Central Medical Ethics Committee (Nr.1/18-09-19 (19.09.2018)). The informed consent was signed by all study participants.

MLPA

All tumour samples underwent MLPA testing for *BRCA1* gene. DNA was isolated using QIAamp DNA mini kit (Qiagen) according to manufacturer's protocol. Isolated DNA underwent MLPA analysis following manufacturer's protocols, ME001 Tumour suppressor probemix 1 (MRC Holland). Samples with *BRCA1* gene promoter deletion or methylation were defined as monoallelic somatic inactivation.

RNA-Sequencing

The total RNA was isolated using TRIzol (Life Technologies) reagent followed by Direct-zolTM RNA MiniPrep (Zymo Research) RNA purification according to the manufacturer's protocol. RNA concentration was measured with Qubit and NanoDrop according to manufacturer's protocols.

Library preparation: cDNA libraries were constructed using MGIEasy RNA directional library prep set (MGI) according to manufacturer's protocol followed by NGS sequencing with MGISEQ-200RS High-throughput Sequencing Set (PE100) (MGI).

Bioinformatics and Statistical Analysis

Obtained raw sequenced reads were analysed with CLC genomic workbench to filter raw reads, check sequencing error rate (Q20 and Q30) and CG content check. CLC Genomics Workbench (Qiagen) software was used for high-quality reads alignment to the reference genome; RPKMs (reads per kilobase of exon model per million mapped reads) were calculated for each annotated gene; differentially expressed genes (or transcripts) between two groups were determined.

The Negative binomial generalised Linear model embedded in CLC Genomic Workbench was used to capture differential gene expression (DEGs) between two study groups. Genes after Bonferroni correction ($p<0.05$) and with max group mean >10 of average expression were included in further analysis.

The STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis is a bioinformatics tool that evaluates and visualizes protein-protein interaction networks. It was used to predict and display interactions between proteins, analysing insights into cellular processes and functional relationships [13].

Statistical analysis was performed using R software, utilizing the 'survival' package for Kaplan-Meier analysis and employing the Peto & Peto method for log-rank testing.

3. Results

Based on MLPA results the group of 36 samples was divided in two study groups based on *BRCA1* status – with monoallelic somatic *BRCA1* inactivation and without inactivation. 16 samples had monoallelic promoter region deletion and one sample showed hypermethylation of *BRCA1* promoter region. 19 samples did not reveal deletion or methylation of the *BRCA1* promoter region. Based on these results two study groups were formed - "*BRCA1-*" with monoallelic somatic inactivation and "*BRCA1+*" with two active BRCA1 gene alleles. The analysis of clinical information (including grade, stage and molecular type of cancer, as well as Ki67 index) did not indicate statistically significant differences between the groups (Supplement 1).

The Kaplan-Mayer analysis indicated tendency for longer event-free survival ($p<0.09$; HR 5.17, 95% CI 0.60 and 44.3) in the group with the *BRCA1* inactivation (Figure 1).

The RNA sequencing data analysis revealed 39 DEGs between the study groups. Among these, 23 genes exhibited upregulation (Table 1) while 16 genes were downregulated (Table 2) in the *BRCA1-*

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group. All genes, except 4 are protein coding genes (the rest 4 are lncRNAs or rRNAs, not shown in the tables).

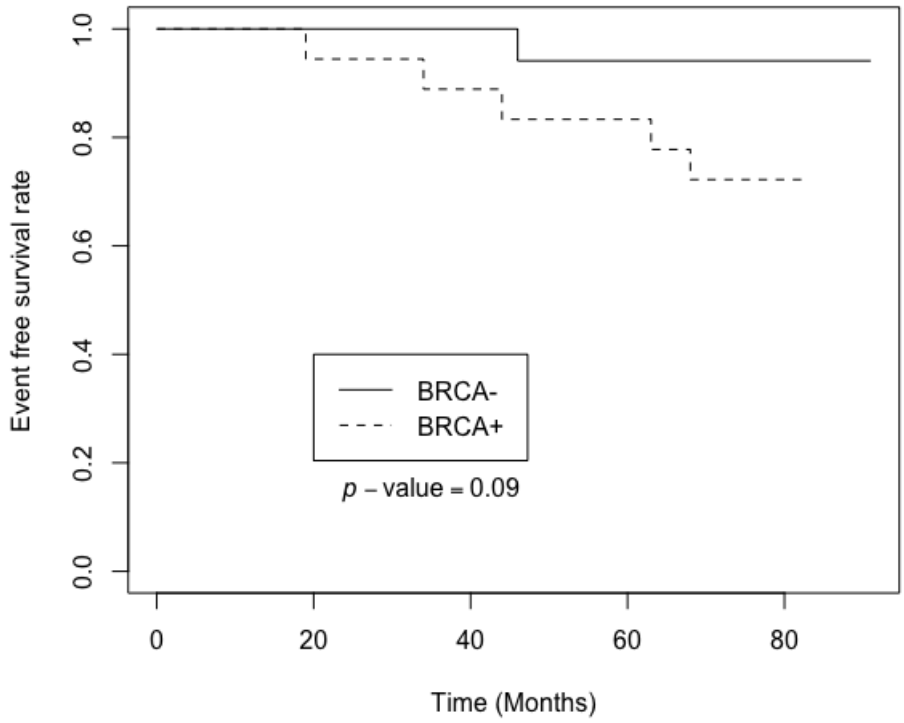

Figure 1. Kaplan-Meier plot of event-free survival based on *BRCA1* monoallelic inactivation status.

Table 1. The list of upregulated DEGs in *BRCA1*- group. Bonferroni adjustment p <0.05; Max group mean threshold of >10.

Gene	Log_2 fold change	Fold change	*p*-value	HGNC
NRIP3	2.37	5.17	1.81E-08	HGNC:1167
TUBGCP3	2.42	5.37	7.66E-08	HGNC:18598
GPX2	2.72	6.60	6.25E-08	HGNC:4554
PXDNL	2.84	7.15	3.21E-07	HGNC:26359
FSIP1	3.06	8.32	5.6E-09	HGNC:21674
IL20	3.35	10.22	1.31E-07	HGNC:6002
MMP9	3.75	13.46	6.45E-08	HGNC:7176
TPSD1	3.81	14.06	3.6E-07	HGNC:14118
TPSAB1	3.85	14.43	1.45E-08	HGNC:12019
TRH	4.36	20.56	2.56E-10	HGNC:12298
AKR1B10	4.37	20.75	5.45E-07	HGNC:382
ORM1	4.52	22.86	4.8E-07	HGNC:8498
CGA	4.75	26.90	1.18E-06	HGNC:1885
BEX1	4.87	29.27	5.66E-10	HGNC:1036
TBX10	5.72	52.82	9.21E-10	HGNC:11593
FGG	5.81	56.16	5.42E-08	HGNC:3694
CASP14	6.27	77.34	4.16E-10	HGNC:1502
CRISP3	7.06	133.61	1.41E-16	HGNC:16904
CSN3	7.10	137.58	1.92E-10	HGNC:2446

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HTN1	9.80	891.01	1.74E-12	HGNC:5283
ALPI	10.61	1566.05	4.67E-16	HGNC:437

Table 2. The list of downregulated DEGs in the *BRCA1-* group. Bonferroni adjustment p<0.05; Max group mean threshold of >10.

Gene	Log_2 fold change	Fold change	p-value	HGNC
CARTPT	-9.88	-944.90	4.29E-20	HGNC:24323
SBSN	-8.98	-504.29	2.71E-19	HGNC:24950
IRS4	-8.00	-256.83	1.06E-16	HGNC:6128
CHGB	-7.93	-243.21	2.68E-17	HGNC:1930
CYP2A7	-7.44	-173.43	4.19E-22	HGNC:2611
KRT6A	-5.37	-41.46	4.43E-10	HGNC:6443
DCD	-5.27	-38.53	4.59E-09	HGNC:14669
OBP2B	-4.04	-16.48	1.14E-10	HGNC:23381
KRT86	-3.62	-12.30	7.03E-09	HGNC:6463
DMKN	-3.30	-9.83	1.16E-08	HGNC:25063
FABP4	-3.09	-8.49	2.55E-07	HGNC:3559
IFITM10	-2.23	-4.68	3.22E-08	HGNC:40022
AOC3	-2.03	-4.08	3.7E-07	HGNC:550
ITGA7	-1.85	-3.61	1.25E-06	HGNC:6143

To explore functional association between proteins coded by the identified DEGs, the STRING analysis tool [13] was used. The acquired results provided insights into the molecular processes and pathways potentially associated with the monoallelic somatic *BRCA1* gene inactivation in breast cancer (Figure 2).

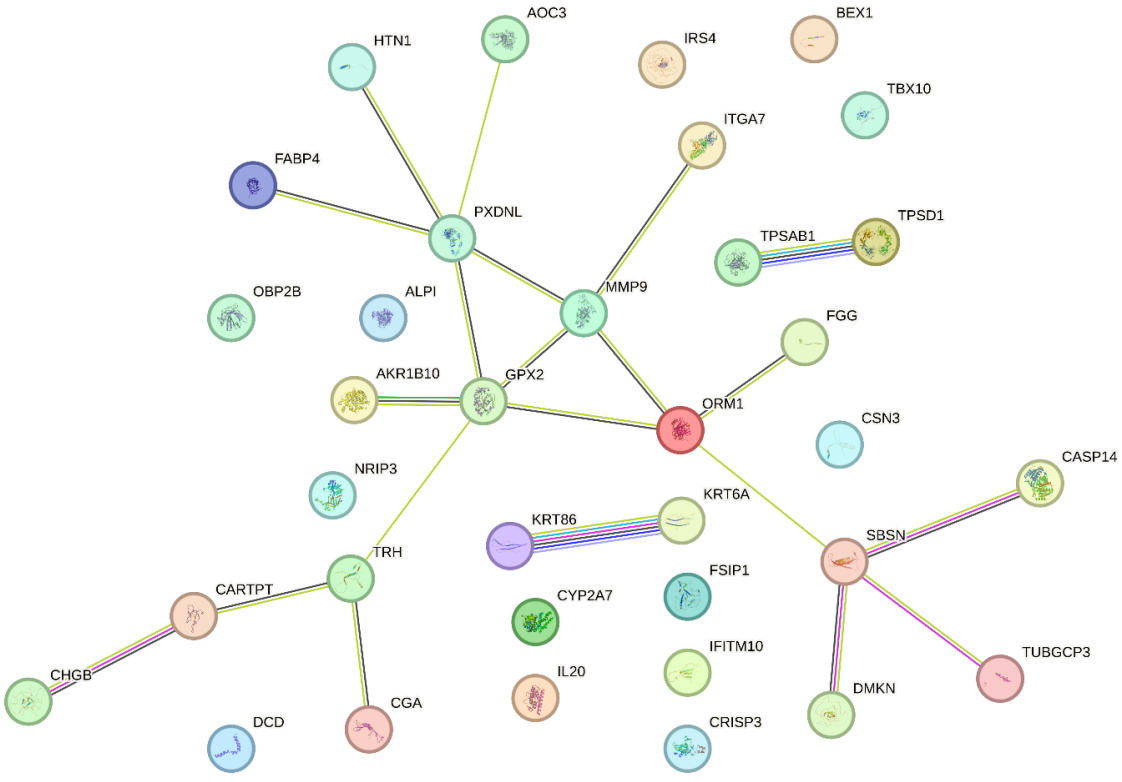

Figure 2. The DEGs coded protein interaction in *BRCA1-* group.

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The STRING tool enrichment analysis shows significant enrichment of proteins in the extracellular region (GO:0005576) and extracellular space (GO:0005615). Key genes implicated in these categories include *TPSD1, FABP4, ORM1, ALPI, CARTPT, TRH, CSN3, MMP9* among others.

The STRING tool enrichment analysis results also implies a potential connection or shared molecular pathways between breast cancer and thyroid dysfunction (not shown in the Table 3). This association is important as thyroid dysfunction has been implicated in various physiological and pathological conditions. Genes identified in both pathways are *TRH, IRS4, CHGB,* and *CGA.*

Table 3. Results of gene enrichment analysis.

#Category	Term ID	Term description	Genes from input	Strength	False discovery rate	Matching proteins in the network (labels)
GO Component	GO:0005576	Extracellular region	23/4175	0.49	3.74e-05	TPSD1, FABP4, ORM1, ALPI, CARTPT, TRH, CSN3, FGG, DMKN, TPSAB1, PXDNL, AKR1B10, IL20, MMP9, CHGB, KRT6A, CRISP3, DCD, HTN1, SBSN, KRT86, CGA, OBP2B
GO Component	GO:0005615	Extracellular space	20/3247	0.54	5.90e-05	TPSD1, FABP4, ORM1, CARTPT, CSN3, FGG, DMKN, TPSAB1, PXDNL, IL20, MMP9, CHGB, KRT6A, CRISP3, DCD, HTN1, SBSN, KRT86, CGA, OBP2B
COMPARTMENTS	GOCC:0005576	Extracellular region	17/2079	0.66	4.18e-05	FABP4, ORM1, ALPI, CARTPT, TRH, CSN3, FGG, TPSAB1, PXDNL, AKR1B10, IL20, MMP9, CHGB, CRISP3, DCD, HTN1, CGA

4. Discussion

Our study was based on global transcriptome sequencing of breast cancer tissue samples to identify differentially expressed genes and signalling pathways associated with monoallelic somatic *BRCA1* inactivation. The Kaplan-Meier analysis to assess the differences in event-free survival between two distinct groups was performed. Notably, the findings revealed tendency for positive effect on event-free survival in the group with monoallelic *BRCA1* inactivation. This observation, indicated by a $p<0.09$, suggests that breast cancer patients with *BRCA1* promoter inactivation may experience improved outcomes in terms of event-free survival. Further investigation into these finding could offer valuable insights into the underlying mechanisms driving the disease and would help to develop more targeted and effective therapeutic strategies for breast cancer patients.

The detailed analysis using the STRING database discovered functional associations of DEGs changes in the molecules pathways related to breast cancer with somatic monoallelic *BRCA1* inactivation. The enrichment analysis, focused on the extracellular region and extracellular space, has unveiled proteins with potential implications for the tumour microenvironment and intercellular

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communication in the context of cancer. Among the key genes found in these enriched categories, *TPSD1, FABP4, CARTPT, TRH, CSN3, MMP9* and others stand out, suggesting their critical roles in cancer progression.

The extracellular region and extracellular space are one of main components in the tumour microenvironment, contributing significantly to cancer progression and metastasis [14]. Proteins identified in these categories often participate in intricate signalling networks, modulating cell behaviour, angiogenesis, and immune responses within the tumour microenvironment. Furthermore, our previous study on the transcriptome of TNBC tumours revealed that differentially expressed genes (DEGs) were associated with processes such as extracellular matrix organization, collagen fibril organization, and the composition of collagen-containing extracellular matrix [15].

TPSD1 appears to be upregulated in study group with somatic monoallelic *BRCA1* inactivation. *TPSD1* gene codes for tryptase delta, which is secreted by mast cells. Mast cells (MCs) play a role in extracellular matrix degradation, angiogenesis, and immune responses through the release of various bioactive substances, including tryptases. Kankkunen et al observed a substantial increase in the presence of tryptase-containing MCs in malignant breast carcinomas compared to benign lesions [16]. The density of MCs, along with their release of tryptases, has been correlated with cancer growth, particularly in facilitating angiogenesis [16]. Mice deficient in mast cells, and subsequently, tryptase secretion, exhibit reduced susceptibility to carcinogenic agents [17,18]. Although TPSD1 shows increased expression in group with better event-free survival, its role in the context of BRCA1-deficient tumours requires further exploration.

Fatty Acid Binding Protein 4 (FABP4), also known as adipocyte protein 2 (aP2), is a member of the FABP family, playing a crucial role in lipid metabolism and cellular signalling. *FABP4* is primarily expressed in adipocytes and macrophages, where it facilitates the transportation of fatty acids and other lipophilic molecules within cells [19]. While its role in obesity-related metabolic disorders has been extensively studied, emerging evidence suggests its involvement in various cancers, including breast cancer. Recent studies demonstrate that adipose FABP4 promotes obesity-associated breast cancer development, thus suggesting FABP4 as a novel player linking obesity and breast cancer risk [20,21].

In breast cancer, *FABP4* expression and function is linked to the tumour microenvironment and cancer progression. Research indicates that *FABP4* is often upregulated in breast cancer tissues, promoting aggressive phenotypes. The higher expression of *FABP4* has been associated with increased cell proliferation, migration, and invasion, contributing to tumour growth and metastasis. Moreover, *FABP4* has been implicated in promoting angiogenesis, a critical process for the establishment and progression of solid tumours, including breast cancer [20,22]

Interestingly, our study group's observation of lower *FABP4* expression in breast cancer patients with monoallelic somatic *BRCA1* inactivation suggesting a potential regulatory role of *BRCA1* and *FABP4* expression. The crosstalk between *BRCA1* and *FABP4* may involve complex signalling pathways that influence lipid metabolism and tumour progression. This unique molecular profile, characterized by lower *FABP4* expression in the context of somatic *BRCA1* inactivation, could be associated with a less aggressive tumour phenotype and, consequently, better event-free survival outcomes.

Lower *FABP4* expression levels potentially influence response to therapy, including hormone-based treatments. While the exact mechanisms linking *FABP4* expression, *BRCA1* inactivation, and clinical outcomes require further exploration, these findings open avenues for personalized therapeutic strategies and highlight the importance of understanding the molecular complexity of breast cancer. Recent study by Kast et al, found association of higher BMI and weight gain in adult life were risk factors for postmenopausal breast cancer in *BRCA1* variant carriers [23]. The link between BMI, weight gain, and breast cancer risk in *BRCA1* carriers emphasizes the importance of lifestyle factors in modifying cancer susceptibility.

CARTPT, known for its role in cocaine- and amphetamine-regulated transcript signalling, has been implicated in breast cancer cell survival and tamoxifen resistance, underscoring its relevance in therapeutic response and tumour behaviour [24].

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Research into cocaine- and amphetamine-regulated transcript (CART) unveils its expression in both primary and metastatic breast cancer, appearing as an independent predictor of poor prognosis in oestrogen receptor-positive, lymph node-negative tumours [24]. CART plays a multifaceted role by amplifying the transcriptional activity of oestrogen receptor alpha (ERα) through the mitogen-activated protein kinase (MAPK) pathway in a ligand-independent manner. In various cancer cell lines, *CARTPT* acts as an oncogene, promoting cellular survival through the activation of the ERK pathway, stimulation of pro-survival molecules, inhibition of apoptosis, and an increase in cyclin D1 levels. Particularly in breast cancer, CART emerges as a safeguard, protecting tumour cells from tamoxifen-induced cell death and underscoring its pivotal role in cancer pathogenesis [24,25].

Study group with somatically inactivated *BRCA1* gene showed decreased *CARTPT* expression, it is conceivable that the compromised DNA repair mechanisms resulting from *BRCA1* inactivation may contribute to altered gene expression patterns, including downregulation of *CARTPT*.

This complexity may offer explanation into Kaplan-Meier plot (Figure 1), revealing that patient group with somatic monoallelic *BRCA1* inactivation experience prolonged event-free survival. Notably, half of these patients underwent hormone therapy, either tamoxifen or anastrozole. This observation suggests sensitivity to hormone therapy, potentially contributing to the extended event-free survival observed in this patient subgroup.

Matrix metalloproteinase 9 (MMP9) is a member of the matrix metalloproteinase family, a group of enzymes that play a crucial role in the degradation and remodelling of the extracellular matrix (ECM). The extracellular matrix is a complex network of proteins and carbohydrates that provides structural support to cells and regulates various cellular processes, including cell adhesion, migration, and signalling [26–28]. It's interesting that in our results increased matrix metalloproteinase 9 (*MMP9*) expression shows tendency for better event-free survival, although MMP9 is typically associated with promoting cancer progression, invasion, and metastasis. However, it is important to recognize that the role of MMP9 in cancer is complex. Several factors may contribute to this observation. MMP9 has both pro-tumorigenic and anti-tumorigenic functions.

While MMP9 is often linked with promoting invasion and metastasis, it can also have beneficial effects, such as influencing the immune response, modulating the tumour microenvironment, and facilitating tissue repair [27,29,30]. There are no studies investigating how somatic inactivation of *BRCA1* would be related to increased *MMP9* expression, however hypothetical explanation would be that genomic instability resulting from *BRCA1* inactivation may induce an inflammatory response within the tumour microenvironment. Inflammatory signals are known to influence *MMP9* expression, and this could contribute to increased *MMP9* levels.

In the context of BRCA1-related functions, these genes may contribute to the complex regulatory network associated with the BRCA1 pathway, influencing cellular responses, immune modulation, and therapeutic resistance in breast cancer.

The sample size and heterogeneity of molecular subtypes between the study groups may set some limitations to the current study. Further studies are needed for clinical validation of identified transcriptomic profile

5. Conclusions

Kaplan-Meier analysis indicates a favourable impact on event-free survival in patients with somatic monoallelic *BRCA1* inactivation, highlighting the potential clinical significance of this subtype for tailored patient care. STRING database analysis identifies alterations in key genes associated with cancer progression within the extracellular microenvironment, with genes like *TPSD1* and *FABP4* showing differential expression in patients with monoallelic somatic *BRCA1* inactivation, potentially contributing to improved outcomes. Additionally, the involvement of genes like *CARTPT* and *MMP9* hints at complex interactions between DNA repair mechanisms and endocrine therapies.

The transcriptomic profile of differentially expressed genes *TPSD1*, *FABP4*, *CARTPT* and *MMP9* identified in the group with monoallelic somatic inactivation can be indicative for homologous recombination impaired tumours with tendency for better therapy results.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and study was approved by the Central Medical Ethics Committee (Nr.1/18-09-19 (19.09.2018)).

Informed Consent Statement: The informed consent was signed by all study participants.

Data Availability Statement: The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

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

Appendix A

Clinical data of patients involved in the study

Characteristic	Study population (n=36)	*BRCA1* - group (n=17)	*BRCA1* + group (n=19)	*p*-value
Age, median (range)	59.5 (37-81)	58 (38-81)	68 (37-76)	
T stage, n (%)				0.16
T_{is}	1 (2.8)	0 (0)	1 (5)	
T1	10 (27.8)	4 (24)	6 (32)	
T2	21 (58.3)	13 76)	8 (42)	
T3	3 (8.3)	0 (0)	3 (16)	
T4	1 (2.8)	0 (0)	1 (5)	
N stage, n(%)				0.24
N0	26 (72.2)	13 (76.5)	13 (68.4)	
Nmic	2 (5.6)	2 (11.8)	0 (0)	
N1	7 (19.4)	2 (11.8)	5 (26.3)	
N3	1 (2.8)	0 (0)	1 (5.3)	
Clinical stage	n (%)			0.25
0	1 (2.8)	0 (0)	1 (5.2)	
IA	9 (25)	3 (17.6)	6 (31.8)	
IB	1 (2.8)	1 (5.9)	0 (0)	
IIA	14 (38.9)	10 (58.9)	4 (21.1)	
IIB	8 (22.1)	3 (17.6)	5 (26.3)	
IIIA	1 (2.8)	0 (0)	1 (5.2)	
IIIB	1 (2.8)	0 (0)	1 (5.2)	
IIIC	1 (2.8)	0 (0)	1 (5.2)	
Grade	n (%)			0.74
G1	5 (13.9)	3 (17.6)	2 (10.5)	
G2	22 (61.1)	10 (58.8)	12 (63.2)	
G3	6 (16.7)	2 (11.8)	4 (21.1)	
not known	3 (8.3)	2 (11.8)	1 (5.3)	
Ki67 index:	n (%)			0.34
<20%	15 (41.7)	5 (29.4)	10 (52.6)	
>20%	15 (41.7)	9 (52.9)	6 (31.6)	
Unknown	6 (16.7)	3 (17.6)	3 (15.8)	
Molecular type:	n (%)			0.28
Luminal A	10 (27.8)	3 (17.6)	7 (36.8)	

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Luminal B	13 (36.2)	6 (35.3)	7 (36.8)	
TNBC	7 (19.4)	4 (23.5)	3 (15.8)	
HER2 positive	3 (8.3)	3 (17.6)	0 (0)	
Unknown	3 (8.3)	1 (5.9)	2 (10.5)	
Adjuvant treatment:	n			
Chemotherapy	14	7	7	
Endocrine therapy (Tamoxifen or AI)	13	6	7	
Radiotherapy	26	11	15	
Trastuzumab	2	2	0	

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