

Research Article

Transporter Associated with Antigen Processing 1 (TAP1) as a Potential Biomarker for Breast, Lung, Liver and Ovarian Cancer using Health Informatics

Anika Tabassum¹, Md. Nazmus Samdani², Tarak Chandra Dhali³, Rahat Alam^{4,5}, Foysal Ahammad^{*5,6}, Abdus Samad^{*,4,5}, Tomasz M. Karpinski^{*,7}

- ¹ Biochemistry Department, School of Life Sciences, Independent University, Bangladesh; anikatabassumhiya@yahoo.com
- ² Department of Pharmacy, University of Dhaka, Dhaka, Bangladesh; nazmussamdani076@gmail.com
- ³ Department of Biotechnology and Genetic Engineering, Khulna University, Bangladesh; tarakbge16@gmail.com
- ⁴ Department of Genetic Engineering and Biotechnology, Faculty of biological science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh; rahat_160619@just.edu.bd (R.A.); kazisamad50@gmail.com (A.S.)
- ⁵ Biological Solution Centre (BioSol Centre), Farmgate, Dhaka-1215, Bangladesh. rahat_160619@just.edu.bd (R.A.), kazisamad50@gmail.com (A.S.); foysalgebt@gmail.com (F.A.)
- ⁶ Department of Biological Sciences, Faculty of Science, King Abdul-Aziz University (KAU), Jeddah- 21589, Saudi Arabia. foysalgebt@gmail.com
- ⁷ Chair and Department of Medical Microbiology, Poznań University of Medical Sciences, Wieniawskiego 3, 61-712 Poznan, Poland; tkarpin@ump.edu.pl
- * Correspondence Authors: foysalgebt@gmail.com (F.A.); kazisamad50@gmail.com (A.S.); tkarpin@ump.edu.pl (TMK)

Abstract: Transporter associated with antigen processing 1 (TAP1) gene codes for a transporter protein, which is responsible for tumor antigen presentation in the MHC I or HLA complex. A defect in the gene results in inadequate tumor tracking. TAP1 may also influence multidrug resistance, which is an extreme threat in providing treatment by chemotherapeutic drugs. The gene of TAP1 was analyzed bioinformatically. It gave us prognostic data as a confirmation of whether it should be used as a biomarker. The expression level and pattern analysis were conducted using ONCOMINE, GENT2, and GEPIA2 online platforms. Samples with different clinical outcomes were investigated for expression and promoter methylation analysis was done in cancer vs. normal tissues using UALCAN. The copy number alteration and mutation frequency and expression in different cancer studies were analyzed using cBioPortal. The Prognoscan and KM plotter survival analysis of significant data (p -value<0.05) was representing graphically. Pathway and Gene ontology analysis of genes correlated to the TAP1 gene was presented using bar charts. After arranging the data in a single panel and correlating expression to prognosis, understanding mutational and alterations and comparing pathways, TAP1 may be a potential novel target to evade a threat against chemotherapy and the study gives new aspects to consider for immunotherapy in human breast, lung, liver and ovarian cancer.

Keywords: TAP1; transcriptional expression; methylation analysis; survival analysis; co-expression; pathway analysis; health informatics

1. Introduction

Cancer has been a major contributor to mortality worldwide. In 2018, the global cancer death rate was estimated to be about 9.6 million [1] cancers rates were very high contributors [1]. In 2019, there were 1,762,450 new cancer cases, and in 2020, 606,520 cases of deaths were estimated in the United States of America alone [2]. At this rate, the significance of finding better treatments is

very high, even though we have options, such as chemotherapy, immunotherapy, or radiotherapy, but they, unfortunately, have their limitations [3]. We tend to dive into the cellular mechanisms and immunology to understand tumor progression better. A great part has been featured by ATP-Binding Cassette (ABC) transporters in the development of cancer and, also in the immune response towards cancer [4].

The ABC superfamily regulates the passage for ions and substrates, through cellular and organelle membranes [4]. Transporter associated with antigen processing 1 (TAP1) protein resides in the ABCB (ATP binding cassette subfamily B member) subfamily [4]. It forms a heterodimer with TAP2 and functions the transportation of cytosolic proteins into the endoplasmic reticulum. The proteins are provided to the major histocompatibility complex I (MHC-I), which presents antigenic peptides to get rid of the antigens by the help of cytotoxic T-cells. [5, 6] [7]. This immunological response can be used in detecting tumor cells by tumor antigen presentation. It has been reported that mutant TAP1 can hamper the MHC-I function of tumor surveillance [8]. As TAP1 influences the tumor detection and without TAP1 the function of transporter (TAP) protein family and antigen detection is insufficient, it can be utilized as a target to treat cancer patients via immunotherapy [9].

Most of the failure in chemotherapy during metastasis and invasion is occurring due to multidrug resistance [10]. Therefore, it is necessary to target novel molecules, causing drug resistance to decrease cancer deaths. The ABCB subfamily is known for the multidrug resistance (MDR) function [11], the ability in which the cells confer simultaneous resistance to drugs of different forms and structures. Thus, causes anti-cancer drug resistance [12]. ABC transporters limit anti-cancer drug uptake, increases the action of efflux pump, affects cell and organelle membranes [3], blocks cell death by anti-cancer drugs [13], detoxifies the drugs [14] and alters the cell cycle which nullifies the effect of the drugs on tumor cells [15]. It was discovered that TAP1 and P-glycoprotein domains, encoded by the ABCB1 gene, are homologically similar to binding sites and both might contribute to MDR [16]. The ABCB1 effluxes drugs/substrates from the cell membrane and is resistant to anti-cancer drugs like anthracyclines, taxanes, and many other compounds [17].

While TAP1 upregulation has been linked to better targeting of tumor cells, there is contradictory evidence that shows the overexpression of TAP1 in cancer cells [18]. There are associations of TAP1 in cancer involving various immune responses and analytical data of the TAP1 gene for specific cancers will help study the gene, as well as concluded if it should be used as a biomarker.

2. Results

2.1. The TAP1 Gene Expression in Different Kinds of Cancer

In order to analyses the mRNA expression for the TAP1 gene in different kinds of cancer, we used three databases. We used ONCOMINE to look into the comparison of TAP1 mRNA expression for different cancer and their healthy cells (Figure 1A). The mRNA levels were overexpressed in the bladder, brain and central nervous system (CNS), breast, cervical, head and neck, kidney, liver, lymphoma, ovarian and pancreatic cancers while under-expressed only in the lung cancer, unlike the healthy tissues. The GEPIA2 server was then used to study the profile of TAP1 expression levels in multiple cancer types (Figure 1B). The data was extracted from TCGA, where we inquired in 33 tumor types paired with their normal samples for the mRNA expression of TAP1. We saw that among other cancer types, BRCA (breast cancer), LIHC (liver hepatocellular carcinoma), LUAD

(lung adenocarcinoma), and OV (ovarian cancer) were significantly overexpressed. We also analyzed the TAP1 expression for different tumors and their respective counterparts using GENT2 (Figure 1C). The mRNA expression was high for breast, liver lung and ovary compared to the normal tissues.

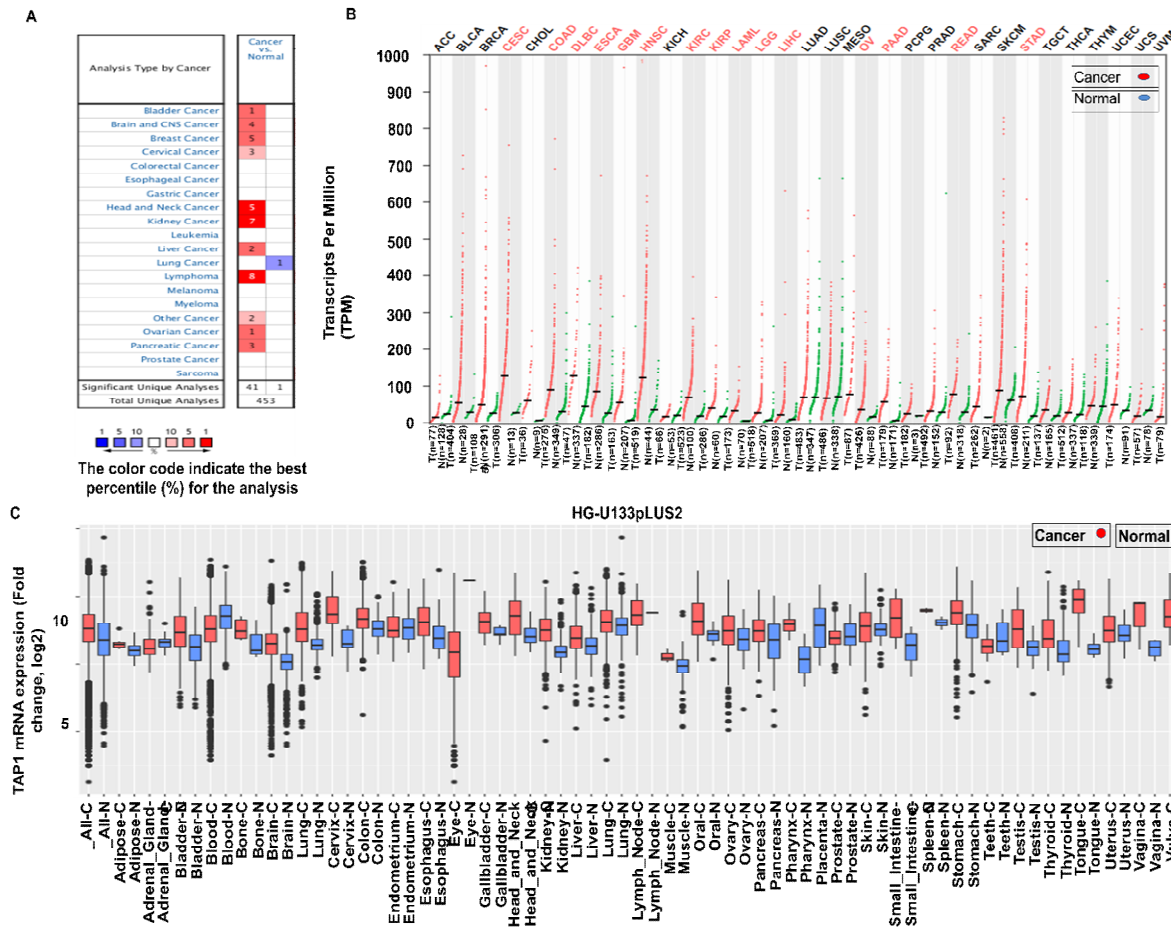


Figure 1. Expression of TAP1 in different cancers kinds: (A) cancer vs. normal upregulation (red) and downregulation (blue) in left and right columns, respectively, with default parameters of a p-value:1E-4, fold change: 2 and a % gene ranking: 10% for expression of mRNA in the ONCOMINE database (B) mRNA transcription profile for TAP1 in various cancer kinds were detected by TCGA database via GEPIA2 (Gene expression Profiling Interactive Analysis 2) website. In the dot plot, the red plot represents a tumor, and the green plot represents normal tissues. Each dot represented the expression of samples. The tumor specimens were compared to their counterparts to observe the expression criteria. (C) TAP1 expression profile in different cancers and its counterparts were detected through Gene Expression across normal and tumor tissue (GENT 2) with boxplot, where boxes with red color indicate cancer cells, boxes with blue color indicate normal cells, the middle line shows the median and the dots are the outliers.

2.2. The Pattern of TAP1 Expression in Breast, Lung, Liver and Ovarian Cancer

We further investigated the TAP1 gene expression pattern in various cancer kinds (Figure 2). We used the ONCOMINE database to observe the gene expression and fold changes. It was proceeded by considering four cancer types: breast, lung, liver, and ovarian cancers. The expression

was seen to be upregulated in breast, liver, ovary, and lung cancers (Figure 2A-2D). The GEPIA2 tool was used to look into the expression of the TAP1 gene (Figure 2E-2H), where the expression levels for LIHC and OV cancers were significantly higher than the normal tissues. The expression in the primary tumor and the normal was compared using the TCGA database in the UALCAN tool (Figure 2I-2K). A significant overexpression for the TAP1 expression was seen in the primary tumor in comparison with the normal in BRCA, LIHC, and LUAD.

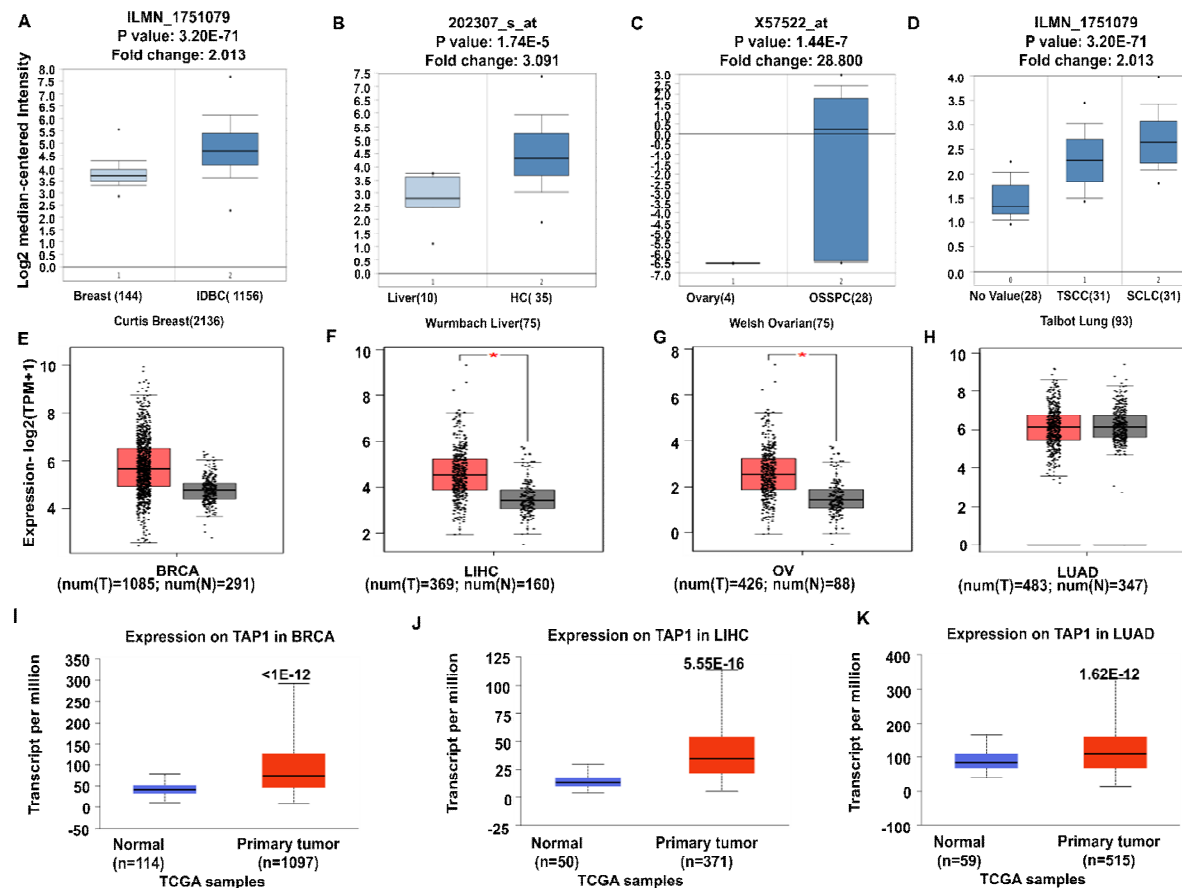


Figure 2. Cancer vs. normal comparison was observed in various cancers for the pattern of TAP1 expression. Box plot analysis for changes in the fold of TAP1 (log2 transformation of gene expression change) was conducted using four cancers namely: breast, liver, lung and ovarian cancer where the left plot represents normal and right plot represents tumor cells, and the highest and lowest levels are shown by an asterisk (A-D) using the ONCOMINE analytical tool. (A) Invasive Ductal Breast Carcinoma, (B) Hepatocellular Carcinoma, (C) Ovarian Serous Surface Papillary Carcinoma, (D) Tongue Squamous Cell Carcinoma, Squamous Cell Lung Carcinoma. (E-H) The analytical expression pattern investigation of TAP1 was conducted by GEPIA2 utilizing ANOVA differential method with threshold value: *p-value: 0.01. (I-K) UALCAN web and TCGA samples were used to analyses the expression of TAP1 in primary tumor vs. normal cells.

2.3. Expression Analysis of TAP1 Gene with Clinical Characteristics

We analyzed the expression of the TAP1 gene with different clinical characteristics using the UALCAN online database. The expression of the TAP1 gene in normal tissue was compared with tissues in patients with different clinical outcomes for breast, liver, lung, and ovarian cancers (Figure

3 and Supplementary Table 1). Overexpression of TAP1 gene in cancer patient compared to normal tissue was most in stage 2 for LUAD and LIHC cancer in four stages of cancer, stage 4 and stage 2 were among the highest for BRCA. Stage 3 had the least level of expression for TAP1 in BRCA, and stage 4 and stage 1 was among the lowest for LIHC and LUAD (Figure 3A, B, D). There was no significant TAP1 expression in ovarian cancer (Figure 3C). Overexpression of the TAP1 gene in cancer patients compared to normal tissue was most for Asians in BRCA, LUAD, and OV. African Americans had a high value for LIHC and lowest for OV and LUAD, whereas Caucasians had the lowest rate for BRCA and LIHC (Figure 3E-H). TAP1 gene was over-expressed in females having breast cancer than males (Figure 3I). TAP1 overexpression was more for females in comparison with males in all cancers (Figure 3J-K).

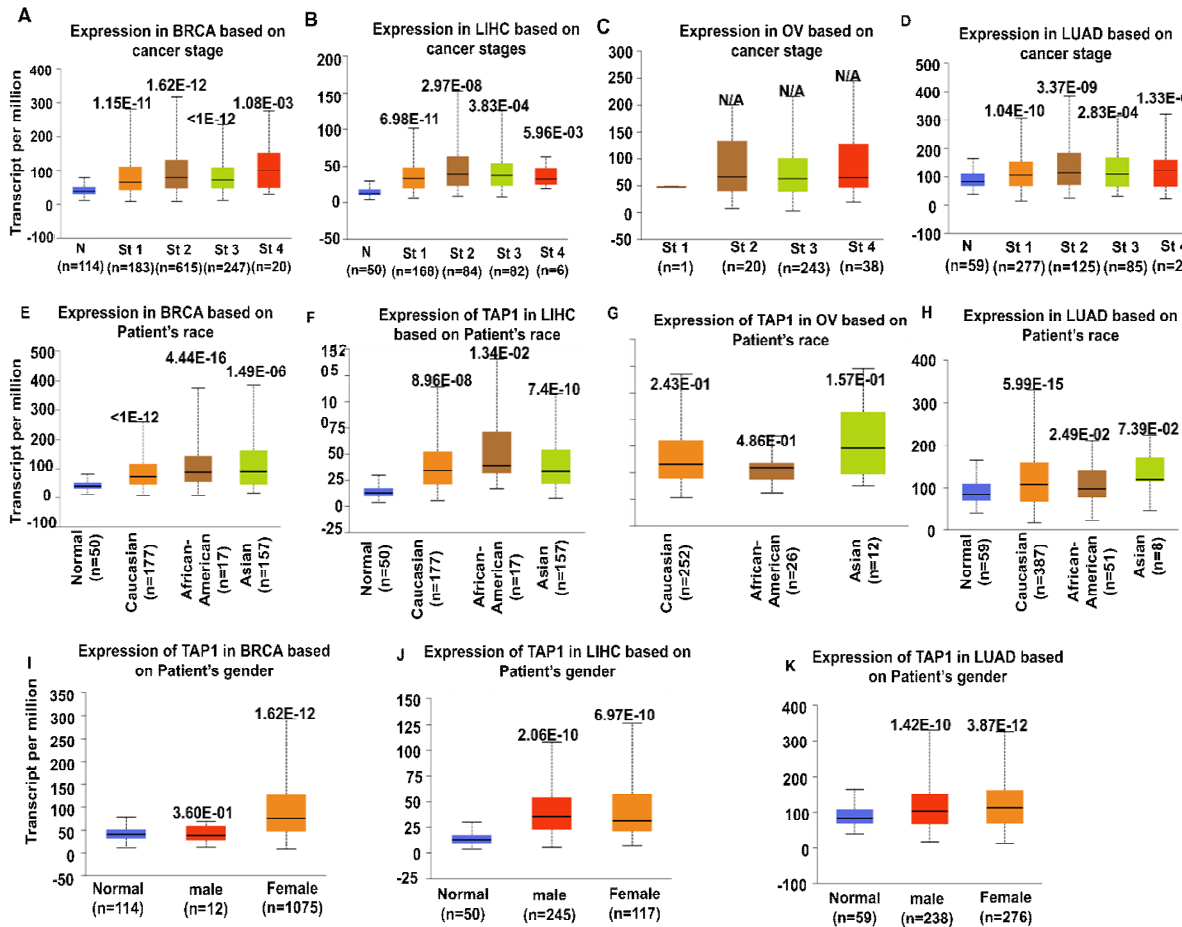


Figure 3. The relationship of the expression of TAP1 with different clinical characteristics in cancer affected people was shown in a box plot where the TAP1 mRNA expression level was detected through the UALCAN web. (A-D) Expression analysis for patient's attributes based on specific cancer stages for BRCA, LIHC, OV, and LUAD, respectively. (E-H) The expression for the patient's race for BRCA, LIHC, OV, and LUAD, and based on a patient's gender (I-k) for BRCA, LIHC, and LUAD, respectively.

2.4. Promoter Methylation of TAP1 Gene with Clinical Characteristics

We analyzed the level of TAP1 gene promoter methylation with different clinical characteristics using the UALCAN online database. The promoter methylation of the TAP1 gene in normal tissue was compared with tissues in patients with different clinical outcomes for breast, liver,

lung, and ovarian cancer (Figure 4 and Supplementary Table 2). Promoter methylation was increased slightly in tumor samples (Figure 4A-C). Compared to normal tissue, patients having stage1 and stage 3 breast cancer have increased promoter methylation and decreased promoter methylation for stage 4 (Figure 4D), patients having liver cancer had the highest promoter methylation in stage 3 and 1, and stage 4 had the lowest methylation in promoter among the four stages (Figure 4E), patients having stage 3 and stage 4 lung cancer had more increased promoter methylation than stage 1 and 2 in lung cancer (Figure 4F). Compared to normal tissue, Caucasian patients had increased promoter methylation in BRCA, LIHC, and OV, and African American patients had decreased methylation suffering from all cancers. Asians have the highest methylation in LUAD (Figure 4G-I). In breast, liver, and lung cancer, TAP1 promoter methylation has increased for males and is approximately unchanged for females compared to normal tissue (Figure 4J-L).

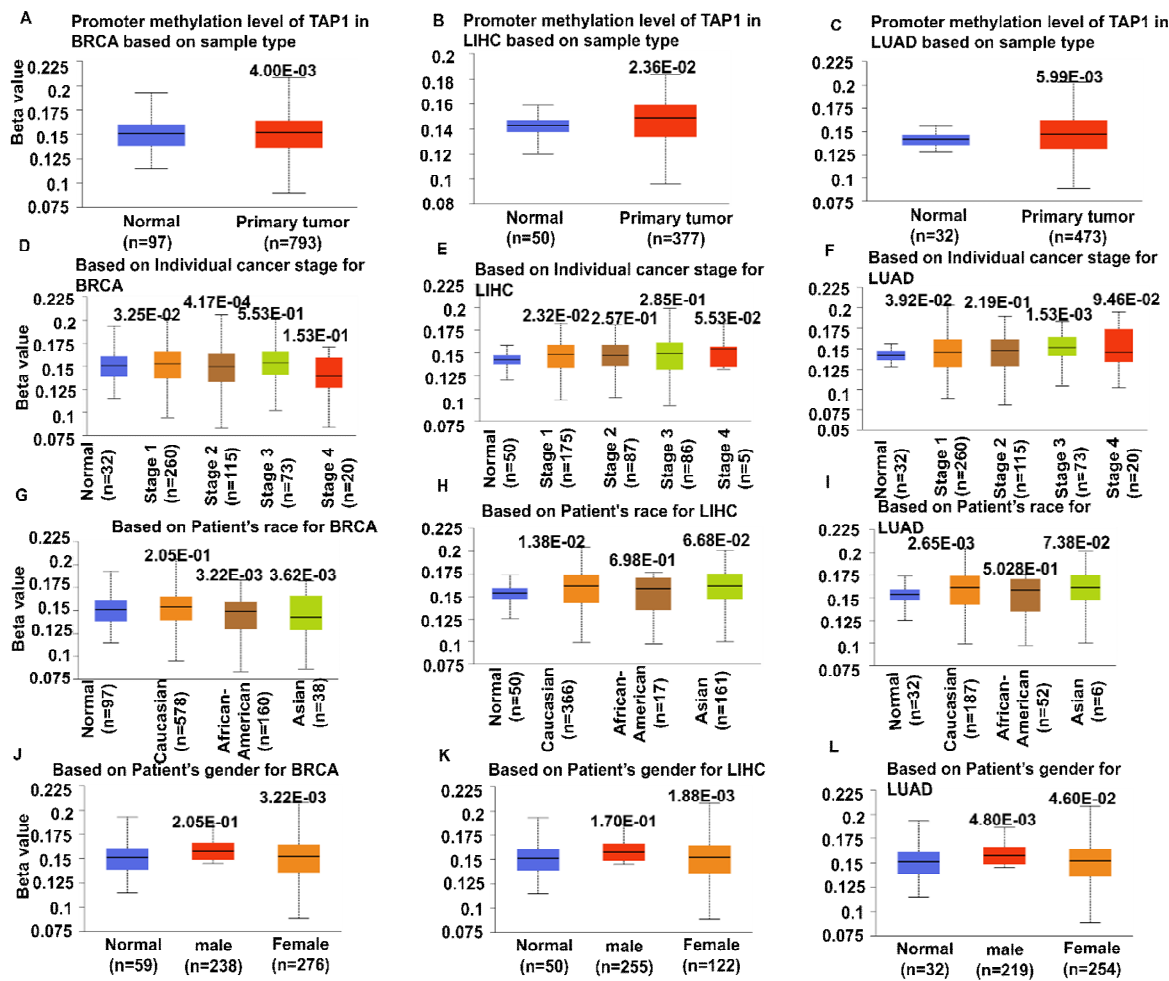


Figure 4. The relationship of the promoter methylation of TAP1 with different clinical characteristics in cancer affected people was shown in a box plot where the TAP1 mRNA promoter methylation level was detected through the UALCAN web. (A-C) The level of methylation of the primary tumor and in the counterparts in BRCA, LIHC, and LUAD, respectively. (D-F) Promoter methylation level for patient's characteristics based on specific cancer stages for BRCA, LIHC, and LUAD, respectively. (G-I) Promoter methylation level for the patient's race for BRCA, LIHC, and LUAD and based on a patient's gender (J-L) for BRCA, LIHC, and LUAD, respectively. The beta value ranges from 0.076-0.226 (unmethylated-full methylated), which indicates DNA methylation.

2.5. Mutant mRNA, Gene Mutations and Copy Number Alterations of TAP1

By utilizing the cBioPortal database, genetic alteration of TAP1 in different cancers were studied. Generated database queried to observe the genetic mutation of TAP1 in 7504 specimens from 28 cases of breast, lung, liver, and ovarian cancers. Of the total queried samples, there was a 2% alteration in the gene set or pathways with the somatic mutation frequency of 0.4%. Considering multiple sample studies, in total 21 mutations, including 12 duplications, were reported for the TAP1 gene area. (Figure 5A). We observed between 1 and 808 amino acids in the TAP1 pro-peptide and TAP1 domain for the query. Amidst those mutations, 19 were missense, and 2 truncating mutations were identified thoroughly. Breast adenocarcinoma and lung cancer had the highest level of mutations found in them, and the mutations laid among a hotspot in R547C/H. A complete formulation was identified in the R547C/H. The site contained mutations, such as missense mutations, that were discovered in 8 breast adenocarcinoma specimens, 2 lung cancer expressed nonsense mutations. For ovarian cancer datasets, alteration frequency was found highest (>6%) among four cancer types (Figure 5B). Consequently, it generated the expression of TAP1 mRNA (RNA Seq V2) among 12 cases of cancers by utilizing the cBioPortal (Figure 5C). Breast cancer, 6 cases, had the highest level of mutation in mRNA expression, and subsequently, the bladder cancer was next in mutation with 4 affected.

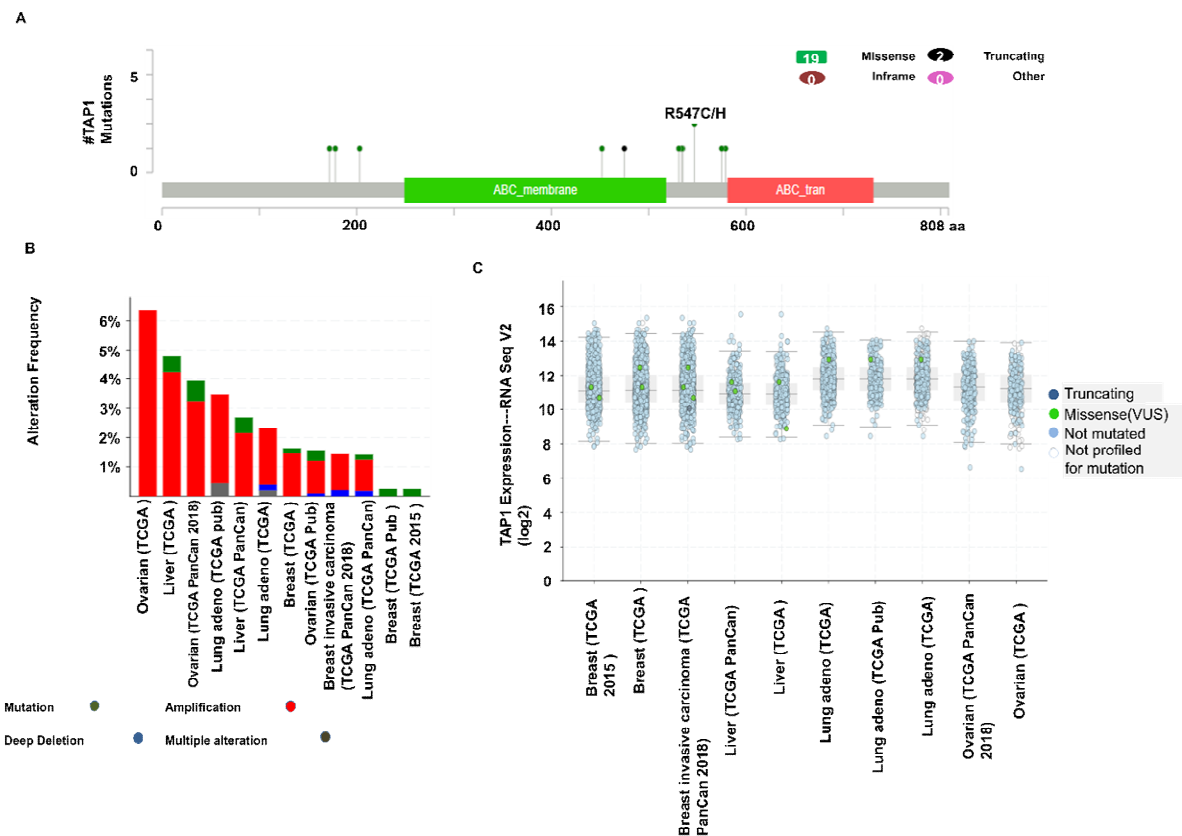


Figure 5. Analysis of expression of mutant mRNA, gene mutations, and copy number alterations of tap1 gene for different cancer studies using cBioPortal. (A) 21 Mutations were observed on samples between 1 and 808 amino acid residues in between pro-peptide and domain of TAP1 protein. (B) The frequency of alteration showing: mutations (green), amplification (red), deep deletion (blue), and multiple alterations (gray) was presented graphically. Datasets having a minimum of 100 cases were plotted. (C) Truncating mutation (deep

blue), a missense mutation (green), no mutation (light blue), and mutation not profiled (white) in TAP1 expression by RNA-Seq V2 method for a sample of 12 studies.

2.6. Prognostic Investigation for the TAP1 Expression among Cancer Patients

Different categories of cancer were considered for the prognosis of TPA1 mRNA expression and we summarized the data using the prognostic databases with Cox p-value of a significance of ($p<0.05$). To analyze the interaction as for the expression of TAP1 with the ratio of survival for breast, lung, liver, and ovarian cancer patients, the PrognoScan database was used. In case of breast cancer, GSE9195 and GSE1456-GPL96 sets provided data that patients with decreased expression of TAP1 gene ($n = 57$ and $n = 139$ respectively) had significant higher relapse-free survival in comparison with the ones with a greater expression of TAP1 ($n = 20$, for both) (Figure 6A-6B). The results found for the lung cancer dataset had the same consequences as the breast cancer results, for OS and RFS (Figure 6C-6D). Here the GSE31210 and GSE31210 datasets of lung cancer exhibited that the low expression ($n = 180$, for both) of TAP1 mRNA exhibited a significantly higher OS in comparison with the higher TAP1 mRNA expressed ($n= 24$, for both cases) group. On the other hand, the analysis of dataset GSE26712 and GSE26712 of PrognoScan showed significantly lower OS and DFS of ovarian cancer, for the lower TAP1 mRNA expression ($n = 62$ and $n = 149$, respectively) in contrast with the higher levels of counterparts ($n= 49$ and $n = 36$, respectively) (Figure 6 E-6F). For in lower TAP1 expression group ($n= 203$ and $n= 228$ respectively) on KM plotter for liver cancer high survival ratio was reported in case of OS and RFS, respectively, compared to the higher expression of the counterparts ($n =161$ and $n = 88$, respectively) (Figure 6G-6H). Primarily, the data-focused that indifferent to the single ovarian cancer difference in the expression, high TAP1 expression is in a positive correlation with the low recovery rate in breast, lung, and liver cancers.

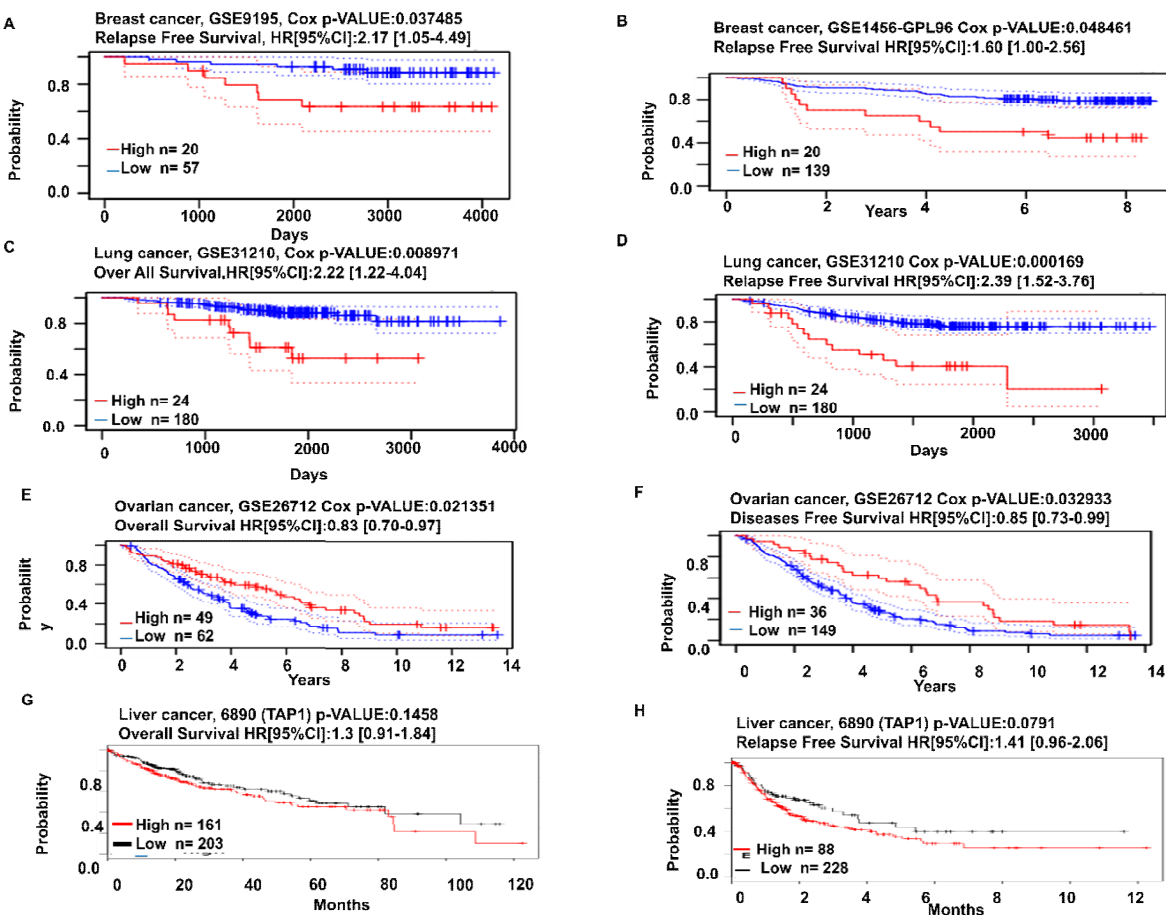


Figure 6. Analysis of patient survival on TAP1 expression in different cancers. The probability of affected people surviving with high (red) and low (blue) TAP1 expression. The plots were analyzed for cancers: (A–B) relapse-free survival in breast, (C–D) overall survival and relapse-free survival in lung, (E–F) overall survival and disease-free survival in ovarian and (G–H) overall survival and relapse-free survival in the liver, were retrieved from the Prognoscan Database, with cox p-value of 0.05. The probability of survival with high (red) and low (black) TAP1 expression curve concerning time in liver cancer (G–H) were retrieved from the KM plotter.

2.7. Analyses of Pathway and Gene Ontologies of co-expressed genes of TAP1

Lastly, we figured out the genes that positively correlated with the TAP1 gene in BRCA, LIHC, LUAD, and OV cancer by using the R2 genomics analysis and visualization platform (Supplementary Table 3). The correlated genes were used in Venn Draw to draw a Venn diagram giving us the common correlated genes in BRCA (3101 genes), LIHC (5580 genes), LUAD (7235 genes), and OV cancer (2572 genes) (Figure 7 and Supplementary Table 4).

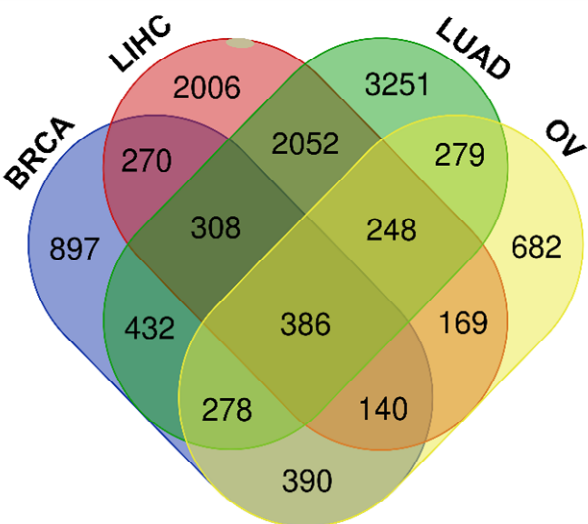
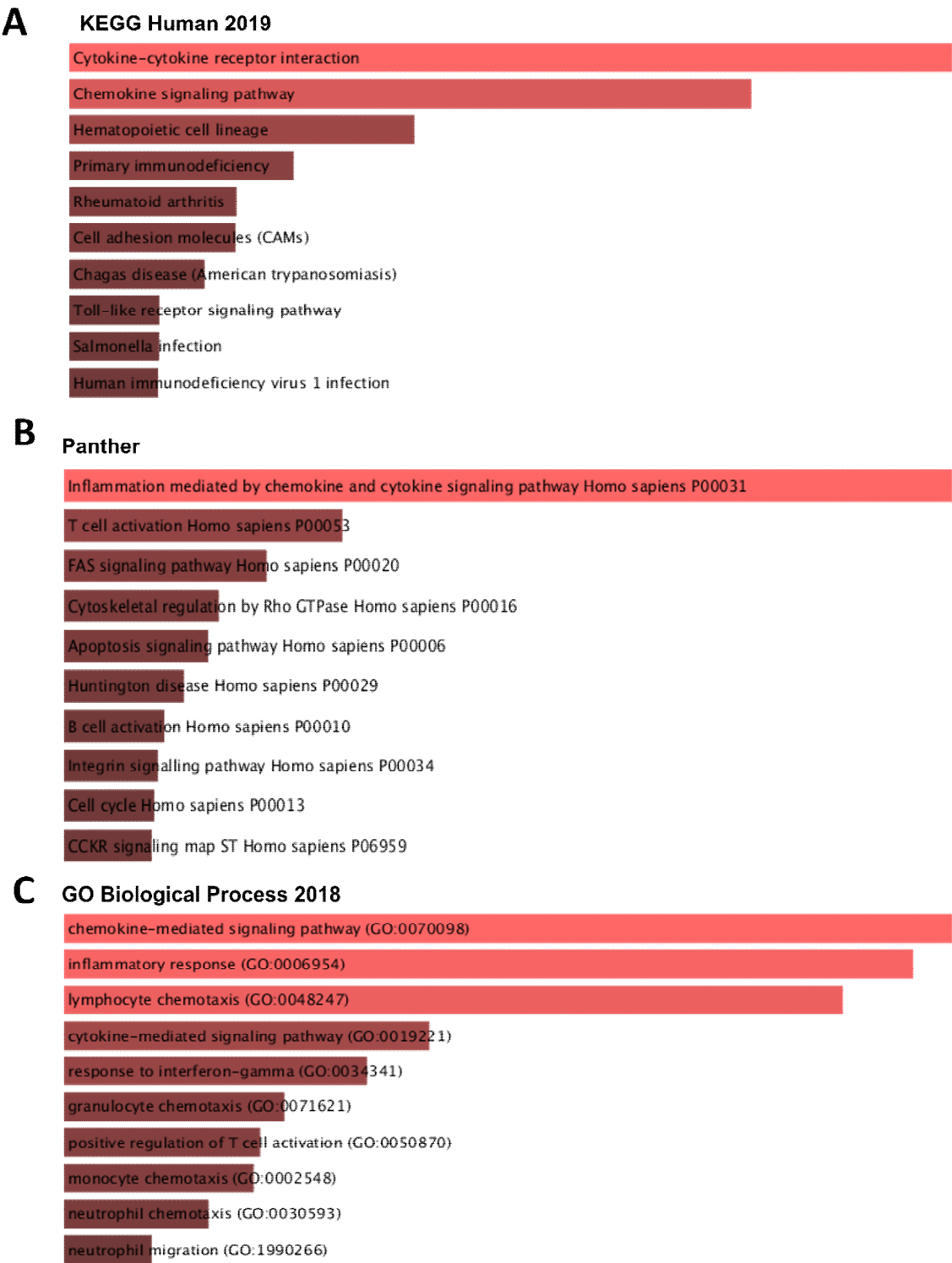
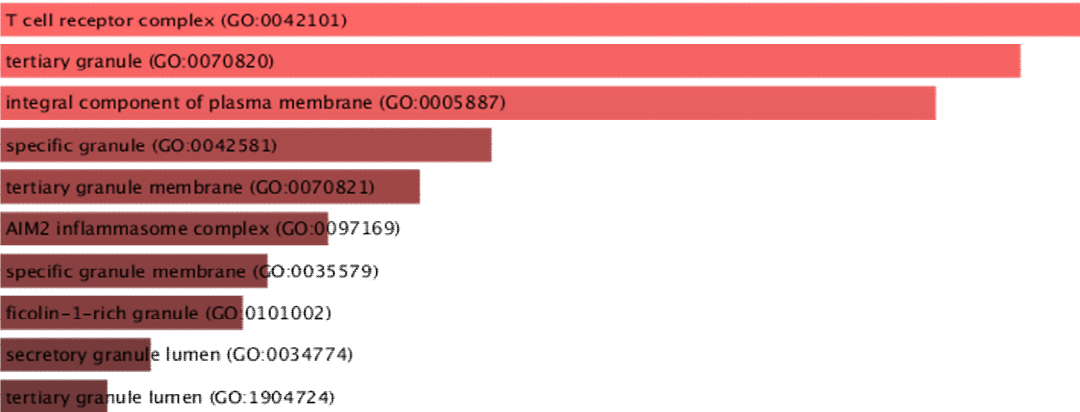


Figure 7. Venn diagram of positively correlated genes to the TAP1 gene for BRCA, LUAD, LIHC, and OV, collected from the Draw Venn web tool and R2 genomics web-based tool.

We extracted the positively correlated common genes to conduct an ontology investigation. We used the Enrichr software in order to understand which signaling pathways were influenced by the positively co-expressed genes and the TAP1 gene in BRCA, LIHC, LUAD, and OV. In the pathway analysis of the KEGG database (Figure 8A), We saw that the most correlated 10 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of TAP1 and the gene which are in positive correlation with TAP1, were primarily associated with cytokine-cytokine receptor pathway, chemokine signaling pathway, hematopoietic cell lineage, primary immunodeficiency, rheumatoid arthritis, cell adhesion molecules, Chagas disease, toll-like receptor signaling pathway, *Salmonella* infection, and human immunodeficiency virus-1 infection. Cytokine-cytokine receptor pathway and chemokine signaling pathway being the most significant pathways to be influenced (Figure 8A). Panther database showed more significant interaction with inflammation mediated by chemokine and cytokine signaling pathways. It also showed pathways like T cell activation, FAS signaling pathway, cytoskeletal regulation by Rho GTPase, apoptosis signaling pathway, Huntington disease, B cell activation, integrin signaling pathway, cell cycle and SSKR signaling map ST (Figure 8B). The Enrichr tool also performed a gene ontology analysis to explore the different processes based on the co-expressed genes. The co-expression in biological processes was most significant for chemokine mediated signaling pathway (GO 0070098), inflammatory response, and lymphocyte chemotaxis (Figure 8C). In molecular functions, the T cell receptor complex, tertiary granule, and integral component of the plasma membrane (Figure 8D), and in GO cellular component, the chemokine receptor binding and chemokine activity were mostly influenced (Figure 8E).



D Go Molecular Function 2018



E Go Cellular Component 2018

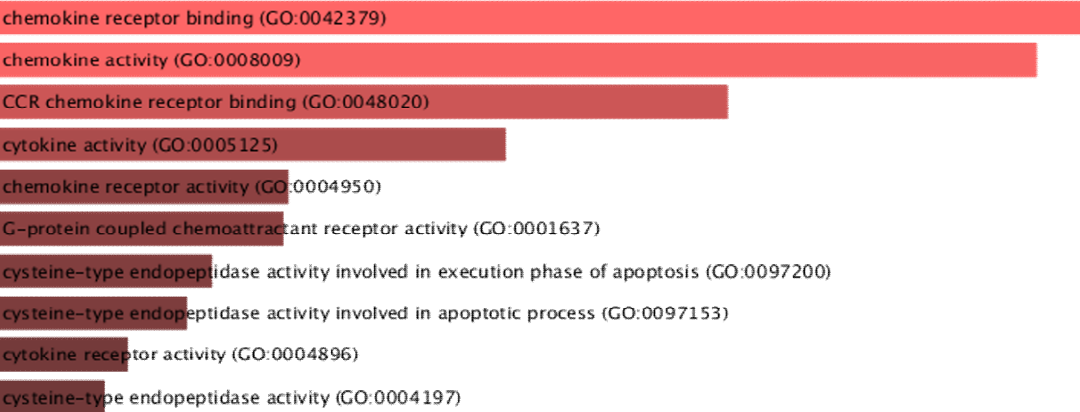


Figure 8. Signaling analysis of pathways and ontologies of genes with positive correlation with the TAP1 gene in BRCA, LUAD, LIHC, and OV was presented graphically using Enricher Web. (A) Kyoto Encyclopedia of Gene and Genomes (KEGG) Human pathways 2019, (B) Panther 2016, (C) Enrichment of GO Biological Process (2018) terms in proteomic analysis, (D) Enrichment of GO Molecular Function (2018) terms in proteomic analysis, (E) Enrichment of GO Cellular Component (2018) terms in proteomic analysis. Generated bars, representing the ratio: % composition of terms in proteomic data: % composition of genomic annotation, are ranked by p-value.

3. Materials and Methods

Differential expression of TCGA (The Cancer Genome Atlas) samples for different cancer was observed in comparison with normal complements through the ONCOMINE database (<https://www.ONCOMINE.org/resource/login.html>) [19]. The analysis of expression level was conducted considering a threshold parameter of; *p*-value: 1E-4, fold change: 2, gene ranking: 10%. The expression profile for genes throughout different cancer and their complementary normal specimens was detected through the GEPIA2 website (<http://gepia2.cancer-pku.cn/>) [20] and GENT2 online platform (<http://gent2.appex.kr/gent2/>) [21] with default parameters. The GTEx (Genotype-Tissue Expression) data and TCGA data were matched by the ANOVA differential method and were used to detect the expression with default threshold settings through the GEPIA2 website.

TCGA sample gene expression and promoter methylation analysis was conducted with the UALCAN website (<http://ualcan.path.uab.edu/index.html>) by comparing the TPM (transcript per

million) and beta value count of a healthy patient with patients having cancer, cancers patients with different cancer stage, cancer patients of different race and gender [22].

The number and location of the mutations in the peptide sequence were detected using cBioPortal (<http://www.cbioportal.org/>) [23, 24]. Frequency of alteration (Mutation, Amplification, Deep Deletion and Multiple Alterations) was investigated using the cBioPortal web. It helped to analyze the RNA seq V2 profile sorted by cancer study for TAP1 expression [23, 24].

Transcriptional expression of TAP1 protein and its relation to patient survival in cancer patients was examined via PrognScan and Kaplan–Meier plotter was utilized in examining the effect on overall survival (OS), relapse-free survival (RFS) and Diseases Free Survival (DFS) by multivariate and univariate investigation of TAP1 expression with p -value: 0.05 as a parameter [25].

The genomic investigation and visualization platform R2: Genomics Analysis and Visualization Platform (<https://hgserver1.amc.nl/>) was used to find the positively co-expressed genes of TAP1 OF TCGA sample for breast, liver, lung, and ovarian cancer. Venn diagrams (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to determine the common positively co-expressed genes of all the cancers. Enricher (<http://amp.pharm.mssm.edu/Enrichr/enrich#>) was used to present the pathways and ontologies sorted by p -value ranking as a bar diagram for the common positively co-expressed genes with default parameters [26].

4. Discussion

TAP protein portrays a crucial role in the MHC I pathway as it is responsible for delivering proteins to endoplasmic reticulum from cytosol, utilizing the energy harvested by ATP hydrolysis. The peptides are then displayed by MHC I in the infected cells so that it will be identified by the T cells [7]. Because of its role in tumor antigen presentation, it can be used in cancer immunotherapy [27]. The TAP protein constitutes of TAP1 and TAP2 heterodimers that are highly significant for the antigen presentation and a defect in one of the genes can conclude to an unstable TAP protein [28]. An adequate TAP protein cannot perform properly and lead to improper tumor antigen presentation and evasion of cancer immunity.

The tumor cells present tumor antigens, which are detected and killed by cytotoxic T cells. TAP1 gene codes, a transporter protein which being a component of MDR/TAP of ABC transporter family, has a vital role in chemotherapeutic drug resistance [29]. The TAP1 encoded protein is involved in the processing of antigens it contributes to anti-cancer drug resistance and hence, has an effect on the survival rates of patients and can play a part in cancer progression due to its MDR activity.

As tumor deaths depend on the transportation of proteins with the help of TAP1, it becomes a prospective biomarker for cancer. Though low TAP1 expression is related with tumor development and alteration of TAP1 may lead to evasion of cytotoxic T-cell killing of some cancer cells [30], but studies are downregulating TAP1 as a way to promote neoantigens as tumor immunity [31]. The consideration of microenvironment for tumor seems essential regarding the expression of a gene as TAP1 as its regulation may be determined by proteins like STAT1 and IRF1. Therefore, resulting in varied expressions of the gene in different cancers [32]. We need analytical data to show the standpoint of TAP1 in various cancers. It is vital to have a systematic report for TAP1 in order to evaluate approaches towards future treatment. We, here, gather computational integrative omics data analysis for TAP1 in multiple cancers.

In previous studies, we have seen defective TAP protein resulting in an inadequate tumor antigen presentation, leading to tumor progression [33]. A decreased level of TAP1 was related to various cancers, for example, cancer of the colon [34], lung [35], and cervix [36]. A differential TAP1 expression has been linked to the liver [37] and renal cancer [32]. Studies also reported that a higher expression of TAP1 mRNA was found in stage II breast tumors and an increased level of expression was identified in high-grade breast cancers [38]. To understand the role of TAP1 expression in different cancers, we used ONCOMINE, GEPIA2, and GENT2 databases, where we got an increase in expression for breast, liver, lung, and ovarian cancers, and a contrasting underexpression for lung cancer in the ONCOMINE analysis. We could sum up from our analysis that overall, TAP1 has a high expression in these cancers.

We focused on the expression of TAP1 in four cancers kinds, breast, liver, lung, and ovarian cancers, next. The analytical expression pattern in these cancers gave us a clearer idea about the regulation of the gene in each cancer and the fold change in the expression in cancer in comparison with the normal one in the ONCOMINE database. It provided us with data regarding the quantitative difference between normal and cancer cells, where all the cancers showed a noticeable fold change in the TAP1 expression. A significant rise in the expression of Liver Hepatocellular Carcinoma and ovarian cancer was seen in the ULCAN web. The TCGA database showed primary tumors to have a significant increase in expression for BRCA, LIHC, and LUAD. For both the analysis, we found that LIHC had a higher expression. As reported earlier, TAP1 expression is increased multi-fold times in patients affected with the hepatitis C virus, which is a significant factor of liver cancer [39] [40].

Furthermore, we wanted to see if a patient with cancer had any clinical characteristics related to the TAP1 gene and how it varies in different cancers. We found that for BRCA, LIHC, and LUAD, all had a high expression of TAP1 gene for stage 2 carcinomas, providing us with evidence that TAP1 is still present with an increased amount in stage 2 tumor in various cancers. The expression pattern fluctuates throughout the stages. In BRCA, it was low for the first stage and increased in the second, decreased substantially in stage 3, and increased again in stage 4. LIHC and LUAD, both had a similar pattern in terms of expression. We reported an increase in expression for stage 2 and stage 3, and a decrease for stage 1 and stage 4. An explanation for such fluctuating results could be that the immune infiltrates are present in the cells, which induce interferon-gamma, and that induces TAP1 [38]. The IFN- γ induction results when tumor cells escape targeting and INF- γ induces TAP1 to trigger tumor detection as a part of the immune response. The study highlighted the relationship of ethnicity and gender with the pattern of TAP1 expression, with significant results.

Epigenetically changed DNA sequences contribute to gene expression and promoter methylation can downregulate the gene expression in cancers. It is expected that proteins in HLA/MHC complex will be downregulated in tumors [41]. The promoters of the TAP1 gene were seen to be methylated in primary tumors, but the Stage 2 tumors tend to have a lower methylation rate, which acknowledges our result with expression patterns. Furthermore, lung cancer patients, stage 3 and stage 4 lung cancer have more increased methylation, and for breast cancer, the lowest promoter methylation was for stage 4, and LIHC had the highest methylation for stage 3. Methylation in the promoter may conclude in inhibition of the gene transcription [42] and thus, contribute to the expression pattern according to this study. The ULCAN analysis for different races TAP1 expression we found Caucasians to have high methylation for BRCA, LIHC, and OV, and Asians were found to

have the highest methylation for LUAD. Males had more methylated promoters than females, congruent with the high expression patterns in females for TAP1.

Exploration of the copy numbers, types of mutations, and alteration of the genome will help understand the function of the TAP gene in cancer progression. A change such as these can result in altered gene expression [43]. Previously, we saw that lung cancer caused by impaired human leukocyte antigen (HLA)-1 complex had altered the TAP1 gene. The TAP1 gene influences the HLA-1 complex maturation. An altered TAP1 gene results in an ineffective HLA-1 complex and helps escape tumor detection by the immune cells [44, 45]. So, we looked into the mutations in the TAP1 domain using the cBioPortal database. Mutation sites were analyzed, and 21 mutations were found between 1 and 808 amino acids of TAP1 propeptide and TAP1 domain, in which 19 were missense and 2 were truncating. The mutation hotspot, R547C/H, was in between 500 and 600 amino acid residues. In query for the frequency of alteration, the highest frequency occurred in ovarian cancer with (>6%), and the highest mutant mRNA in RNA Seq V2 was found in breast cancer.

It was essential to see if TAP1 affected the survival rate of patients with various cancers. We investigated breast, liver, lung, and ovarian cancers patients' prognosis related to the TAP1 expression. The database PrognScan, a microarray database, and km plotter were used to achieve the data for survival rate. Prognostic analysis on the liver, lung, and breast cancers showed a negative correlation for TAP1 expression and overall, relapse-free and/or disease-free patient survival. However, for ovarian cancer, the survival curve was higher for higher TAP1 expression. Giving us an expression that with an increased level of TAP1, there is a weaker probability of survival for the mentioned cancers. We analyzed the positively coexpressed genes with TAP1 and produced a Venn diagram using R2 genomics and Venn Draw to get the common correlated genes in BRCA, LIHC, LUAD, and OV. The identification of different pathways influenced by TAP1 and the common correlated genes can give us an idea about the mechanisms possibly being affected. An ontology analysis was performed using Enrichr. In KEGG pathway analysis, cytokine-cytokine receptor and chemokines seem to be most influenced. Cytokine receptors are involved in the initiation of JAK and signal transducer and activation of the transcriptions (STAT) pathway. JAK-STAT pathway stops the progression of tumor cells via tumor surveillance, but an excessive activation of this pathway was found in malignant tumors [46]. An inflammatory response caused by cancer results in a rise in chemokines [47]. Panther pathway showed a positive correlation with inflammation mediated by chemokine and cytokine signaling pathways. The GO pathways looked into the biological, cellular, and molecular pathways. Other than chemokines and inflammatory response, a significant interaction was with lymphocytes chemotaxis, which is responsible for migrating T cells into the tumor microenvironment [48]. Moreover, T cell receptor complex and tertiary granule and integral component of the plasma membrane were influenced, too, which are an indication of the immune response.

5. Conclusion

In our study, we did a computational analysis of the TAP1 gene in multiple cancers. We found a correlation between the TAP1 gene and tumor progression. The analytical mining sites were used, such as ONCOMINE, GEPIA2, GENT2, KM plotter, UALCAN, etc., to get relevant data. Accompanied by the gene expression pattern in different cancer stages, ethnicity, and gender, we looked into the promoter methylation too. We figured out the downregulation of promoter

methylation can play a part in TAP1 regulation, analyzed a negative correlation for gene expression and the survival rate, reported the mutation in TAP1, frequency alteration, and the pathways of correlated genes. We used different prognostic tools to get an idea about the gene in different cancers and our data had some interesting insights. Further investigations are needed in order to understand the TAP1 role in cancer progression, tumor immune evasion and possible biomarker.

Supplementary Materials: Table S1: Correlation of TAP1 expression for normal vs. different cancer patients and the clinicopathological parameters (TCGA data), Table S2: Promoter methylation of TAP1 in various clinicopathological parameters of different cancers (TCGA data), Table S3: R2 genomics analysis and visualization platform, Table S4: Common positively co-expressed genes.

Authors Contributions: AS, FA and TMK designed the project; TCD performed the analysis of data; AT and MSS evaluated and interpreted the results; RA, TCD, AT and MSS prepared the draft manuscript; AS, TMK and FA critically reviewed and finalized the manuscript. Finally, all authors approved the final version for journal submission.

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Abbreviations:

| | |
|------------------|--|
| ABC | ATP-binding cassette |
| ABCB | ATP binding cassette subfamily B member |
| BRCA | Breast cancer |
| CNS | Central nervous system |
| DFS | Diseases free survival |
| GTE _x | Genotype -tissue expression |
| HLA | Human leukocyte antigen |
| IRF | Interferon regulatory factor |
| LIHC | Liver hepatocellular carcinoma |
| LUAD | Lung adenocarcinoma |
| MHC-I | Major histocompatibility complex |
| MDR | Multidrug resistance |
| OV | Ovarian cancer |
| OS | Overall survival |
| RFS | Relapse free survival |
| STAT | Signal transducer and activator of transcription |
| TCGA | The cancer genome atlas |
| TPM | Transcript per million |
| TAP | Transporter |
| TAP1 | Transporter associated with Antigen Processing 1 |

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