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Identification of Cancer Stem Cell (CSC)-Associated Genes, Prognostic Value, and Candidate Drugs as Modulators of CSC-Associated Signaling in Carcinomas Through a Multiomics Data Analysis Approach

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Abstract: Background: Cancer stem cells (CSCs) are a small subpopulation of cancer cells that have the potential for self-renewal and a strong proliferative capacity, and sustain tumorigenesis capabilities. This ability of CSCs to escape immune responses makes the CSCs a primary source of functionally altered, immune-resistant, chemoresistant, aggressive tumor cells. These characteristics determine the potential advantage of targeting CSCs for the treatment of solid tumors. Method: First, we downloaded different gene expression datasets of CSCs from the NCBI-GEO (National Center for Biotechnology Information–Gene Expression Omnibus) database and identified common genes by using a suitable Venn tool. Subsequently, we explored the prognostic significance of the particular genes in particular cancers and analyzed the expression of these genes at the protein level in human solid tumors by using KM plotter (Kaplan-Meier plotter) and an HPA (The Human Protein Atlas) database, respectively. Finally, using a comparative toxicogenomic database, we selected several important drugs or chemicals. Result: From this study, we identified *APOC1* as a common upregulated gene in breast cancer and *SLC44A5* and *CAV2* as common up- and downregulated genes in lung cancer. In ovarian cancer, *PRRG4* is a commonly upregulated gene, and *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* are commonly downregulated genes. These genes also show prognostic significance in respective cancers. Several drugs that are capable of targeting the expression or signaling network of designated genes of CSC were also identified, which may contribute in CSC-targeted cancer therapy. Conclusion: Our study suggests a need for more in-depth experimental investigations to determine the actual functional activity and the mechanism of action of these CSC-associated genes.

Keywords: cancer stem cell; bioinformatics; breast cancer; lung adenocarcinoma; ovarian cancer

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1. Introduction

Cancer stem cells (CSCs) are a group of self-renewing cells with strong tumorigenic potential and an unlimited proliferation capacity. CSCs originate from either differentiated cells or adult tissue with resident stem cells at the stage of tumor onset [1]. A tumor is a heterogeneous mixture of cells with unsynchronized cycling cells that are in different phases of the cell cycle. Cells within the tumor mass also differ in terms of mutation, plasticity, level of dedifferentiation, and responses to stress. Compared to other cells inside a tumor, CSCs are more resistant to therapies and have greater adaptability based on their surrounding environment [2], and these cells are much more dedifferentiated. Because of their significant roles in resistance to tumor chemotherapy and radiation therapy, as well as tumor recurrence, CSCs have gained much attention [3]. Cancer stem cells maintain their self-renewal properties through different signaling pathways like *TGFB* signaling, *Wnt/B catenin* signaling, Notch signaling, Hedgehog signaling, etc. [4]. Chemotherapy

or radiotherapy may not always completely destroy CSCs, and after treatment, a small subset of residual CSCs may survive, which can cause cancer relapse and ultimately lead to invasiveness and therapy resistance [5]. CSCs induce relapse, metastasis, radiation resistance, and multidrug resistance due to their capacity to remain arrested in the G0 phase of the cell cycle and enter the cell cycle at an opportune moment. Ever since their discovery in leukemia in 1994, CSCs have been viewed as potential targets for cancer treatment. There are many intracellular and extracellular factors that control the activity of CSCs, so these factors can serve as drug targets [6]. Thus, in the past several years, CSC-targeted therapy has gained increasing interest from the research community, especially as potential targets in aggressive metastatic cancer.

Following skin cancer, the second most frequent type of cancer in women is breast cancer; in 2020, more than 2 million new cases were registered globally. Based on GLOBOCAN 2020 data, breast cancer ranks fifth globally in terms of cancer-related fatalities [7]. The majority of patients find out about their disease in routine screening, while the rest of such cases are discovered from a breast lump, a change in breast size, or discharge from the nipple. Genetic mutations and DNA damage, which further activate the secretion of estrogen, are the cause of breast cancer [8]. Based on GLOBOCON 2018 data, in the category of lung cancer, 2.09 million new cases and 1.76 million deaths were registered globally. In both genders, lung cancer is the most common cancer and also the leading cause of cancer-related deaths. Especially in women, this is the third most frequent cause of cancer and the second most lethal cancer [9]. A total of 90% of lung cancer cases are caused due to smoking. Men are affected more than women [10]. Ovarian cancer, another frequent type of cancer in women, ranks eighth in terms of cancer-related deaths, with a less than 45% five-year survival rate [11]. Ovarian cancer can strike anyone at any age; persons over 50 are more likely to have it [12]. Currently, the majority of clinical studies are concentrated on targeted methods, such as the latest attempts at immune therapies.

Here, we chose three types of cancer, including breast, lung, and ovarian cancer, for our study. These three types of cancer are highly aggressive, and there are various challenges linked with their treatment and early detection, including features that are closely linked with CSCs, such as the development of drug resistance and high metastatic potential [13]. Breast cancer and ovarian cancer are more frequent and deadly cancers among women, although ovarian cancer is less common than breast cancer. Lung cancer has a high mortality rate in both men and women. In summary, other types of solid tumors are also crucial, but breast, lung, and ovarian cancer are specifically mentioned here because of their high mortality rate, broad impact, and ongoing challenges related to enhancing early detection and treatment outcomes.

The functional activity of cancer stem cells in tumor progression was first described in acute myeloid leukemia (AML) [14]. After that study, several studies identified the involvement of CSCs in tumor growth, metastasis, and treatment resistance [15]. There are different types of identification markers for each type of cancer stem cells reviewed elsewhere [16]. *CD34* and *CD38* are the first identified cancer stem cell surface markers, and they are used to recognize the stem cells in AML [17]. Transcription factors like *OCT4*, *Sox2*, *Klf4*, *Nanog*, and *MYC* regulate the different activities of cancer stem cells [6]. In cases of breast cancer, *CD44*-high, *CD24*-low, and *ALDH*+ cells are characterized as markers of cancer stem cells [18]. CSCs of lung cancer are characterized by specific markers like *CD133*, *CD44*, *ABCG2*, and *ALDH1A1*. Cells with all these markers have the capability to form spheroid-like structures and also colonies [19]. Combinations of markers are used for the identification of ovarian cancer stem cells like *CD24*, $CD44^{+}/CD24^{-}$, $CD44^{+}/CD117^{+}$, *CD117*/*c-kit*, etc. [20]. Furthermore, the expressions of biomarkers of CSCs are variable and change depending on the cancer type and external conditions. Various signaling pathways that regulate the survival, proliferation, self-renewal, and differentiation of cancer stem cells include the Wnt signaling pathway, the Notch signaling pathway, the *JAK*/*STAT* signaling pathway, Hedgehog signaling, the *TGF* β/*SMAD* signaling pathway,

etc. All these signaling pathways stimulate the expression of various downstream genes like cytokines, apoptosis-related genes, growth factors, metastatic genes, etc., in CSC [6].

The use of bioinformatics for in silico studies in translational drug delivery is becoming more and more important in both the pharmaceutical industry and academics [21]. The roots of bioinformatics were discovered more than a half a century ago, although DNA sequencing techniques were not available at that time [22]. An increasing amount of data generated through the drug discovery process can now be computationally utilized to address major difficulties. In the present study, we focused on identifying CSC-linked genes, the expression of these genes, and their roles as prognostic biomarkers and potential targets for drug intervention in breast, lung, and ovarian cancer through in-depth in silico analysis.

2. Materials and Methods

2.1. Collection of Datasets

For this study, three datasets for breast, lung, and ovarian cancer gene expression profiles were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/ accessed on 2 January 2024). The GEO database is freely available for high-throughput gene expression and other computational datasets concerning genomes. GEO is a joint collaboration of National Centre for Biotechnology Information (NCBI) and National Library of Medicine (NLM). It collects the raw and processed data of experiments designed for the purpose of high-throughput gene expression and genomics studies [23]. Specific inclusion or exclusion criteria were not employed in our study, as available datasets in public databases were few, and data were also not selected on the basis of geographical location or demography. The datasets utilized are described in Table 1.

Table 1. Datasets used in this study.

Serial No.	Accession No.	Cancer Type	Sample Groups Used in This Study	Platform
1.	GSE7513	Breast Cancer	Cancer stem cell (n = 14) vs. noncancer stem cell (n = 15).	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
2.	GSE15192	Breast Cancer	Cancer stem cell (n = 4) vs. noncancer stem cell (n = 4).	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
3.	GSE136287	Breast Cancer	Cancer stem cell (n = 9) vs. noncancer stem cell (n = 9).	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].
4.	GSE35603	Lung Cancer	Cancer stem cell (n = 3) vs. parental tumor cell (n = 3).	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
5.	GSE50627	Lung Cancer	Normal stem cell (n = 6) vs. cancer stem cell (n = 9).	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].
6.	GSE160320	Lung Cancer	Cancer stem cell (n = 3) vs. noncancer stem cell (n = 3).	GPL26963 Agilent-085982 Arraystar human lncRNA V5 microarray.
7.	GSE28799	Ovarian Cancer	Cancer stem cell (n = 3) vs. noncancer stem cell (n = 3).	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
8.	GSE53759	Ovarian Cancer	Cancer stem cell (n = 3) vs. noncancer stem cell (n = 3).	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].
9.	GSE80373	Ovarian Cancer	Cancer stem cell (n = 4) vs. noncancer stem cell (n = 4).	GPL13667 [HG-U219] Affymetrix Human Genome U219 Array.

2.2. Identification of Differentially Expressed Genes (DEGs)

The DEGs were identified using the LIMMA [24] and DESeq2 [25] packages of R (Version 4.2.2) Programming. DESeq2 uses Wald statistics and LIMMA uses t statistics to generate p-values. Here, we use the log2 fold change with the cut-off of 0.5 and p-value kept under 0.05. Common genes were identified by using Bioinformatics and Evolutionary Genomics to generate Venn diagrams (https://bioinformatics.psb.ugent.be/webtools/Venn/ accessed on 22 April 2024). Generally, for the identification of differentially expressed genes, there should be a cut-off value for the log2 fold change. This cut-off varies according

to the requirements of the study and the type of analysis. There is no universal cut-off for the log2 fold change. Higher threshold values reduce the noise. Here, in our study, we used 0.5 as the threshold value for the log2 fold change so that we could identify the subtle changes in gene expression profiles. Various studies have also used 0.5 as the minimum threshold value for log2 fold change to identify suitable genes for analysis [26,27].

2.3. Validation of the Genes from the TCGA Database

The Cancer Genome Atlas (TCGA) database was utilized for validation of the genes. The Cancer Genome Atlas, or TCGA, was launched by The National Institute of Health (NIH) in 2005 for the advancement of comprehensive understanding of the genetics of cancer to discover innovative cancer therapies, methods, and preventions using genome analysis technologies [28]. We used Gene Expression Profiling Interactive Analysis (GEPIA), a freely available web-based tool for fast and customizable outcomes based on TCGA. Features including differential expression analysis, profile plotting, correlation analysis, survival analysis, and gene detection are offered by GEPIA [29]. The ShinyGO (Version 0.81) [30] web server was used further for the identification of related signaling pathways.

2.4. Survival Analysis

Kaplan–Meier Plotter (KM Plotter) (https://kmplot.com/analysis/ accessed on 20 May 2024) was used to perform survival analysis based on gene expression levels of three types of cancer. KM Plotter is an online global database that contains both survival data and gene expression data of ovarian, breast, lung, and gastric cancers, which are used to evaluate the relationship between the prognostic significance and genes of cancers. The Kaplan–Meier method was used to generate the survival plot and hazard ratio with 95% confidence intervals; in addition, the logrank *p*-values were calculated. Here, high vs. low expression is defined by using a cut-off point that divides the patients into two groups based on the median values of mRNA expression of a particular gene [31]. Overall survival defines the time duration from the date of cancer diagnosis or the start of treatment to the date of death. If the patient is still alive, the time duration should be from the diagnosis to the date of last contact [32]. Here, we chose 0.01 as the cut-off point that determines the more significant statistical threshold. It also minimizes the risk of obtaining higher false positive rates. One study also utilized 0.01 as the threshold value for survival analysis [33].

2.5. Identification of Pathological Stages

For the identification of pathological stages, the UALCAN (https://ualcan.path.uab.edu/ accessed on 12 July 2024) web server was used, which detects correlation of the genes with different stages of cancer [34]. UALCAN is an important web portal that analyzes TCGA gene expression data from different cancer. Different functions, like analysis of relative expression of particular genes across normal and tumor samples and also analysis of their expression in different tumors based on tumor grade, race, patient's age, nodal metastatic status, body weight, individual cancer stages, etc., were performed. Using the UALCAN web tool, we also analyzed the relation of the genes with nodal metastatic status in particular cancers. The Student's *t*-test was used to generate *p*-values.

2.6. Identification of the Protein Expression Levels of the Genes Using Human Protein Atlas (HPA)

The HPA database is used to identify the protein expression of particular genes in particular cancers. The immunohistochemical images were downloaded from HPA. HPA contains various immunohistochemical images of various cancers. Based on the staining intensity, the results were divided into low, moderate, and high. In our study, we used the HPA database for the identification of protein expression of prognostic genes in particular cancers [35]. We used tissue sections from the database for the normal tissue images (https://www.proteinatlas.org/humanproteome/tissue accessed on 16 July 2024) and the pathology section for cancerous tissue images (https://www.proteinatlas.org/humanproteome/pathology accessed on 16 July 2024).

2.7. Immune Infiltration Analysis

TIMER 2.0 was used for the analysis of the association of different immune cell infiltration levels with the expression of the particular genes in particular cancers. TIMER 2.0 mainly uses the TCGA data of different cancers and estimates the level of infiltration of different immune cells. Here, we used the CIBERSORT-ABS and TIMER algorithms to identify the M2 macrophage, dendritic cell, and neutrophil infiltration [36].

2.8. Drug Target Identification

To identify drugs targeting particular genes of each type of cancer, we searched in the Comparative Toxicogenomic Database (CTD) [37]. CTD (https://ctdbase.org/ accessed on 21 May 2024) is a free literature-based database resource that shows correlations among chemicals, genes/proteins, and diseases. This method combines cross-species reductionist data from the literature with high-throughput studies to improve understanding of the molecular actions of chemicals. We used the "Chemical-Gene interaction" part of the "Search" section, where we inputted our queries according to our requirements and obtained the chemicals or drugs. After the identification of targeting drugs or chemicals, the drugs were visualized via Cytoscape (Version 3.10.0) [38].

3. Results

3.1. Identification of DEGs from Cancer Stem Cell Gene Expression Datasets

Datasets of each cancer stem cells were examined individually to identify the differentially expressed genes (DEGs). Three types of cancer datasets were selected, including breast cancer, lung cancer, and ovarian cancer. The datasets of the breast cancers are GSE7513, GSE15192, and GSE136287. We identified 7578 DEGs (2555 upregulated and 3935 downregulated) in GSE7513, 11,509 DEGs (3950 upregulated and 4207 downregulated) in GSE15192, and 1312 DEGs (266 upregulated and 73 downregulated) in GSE136287. The datasets of lung cancers are GSE35603, GSE50627, and GSE160320, with 10,419 DEGs (5321 upregulated and 5098 downregulated) in GSE35603, 8100 DEGs (1809 upregulated and 1832 downregulated) in GSE50627, and 12,482 DEGs (1528 upregulated and 674 downregulated) in GSE160320. Similarly, the datasets of ovarian cancers are GSE28799, GSE53759, and GSE80373, with 9881 DEGs (3410 upregulated and 3422 downregulated) in GSE28799, 5884 DEGs (1123 upregulated and 813 downregulated) in GSE53759, and 13,308 DEGs (2310 upregulated and 2054 downregulated) in GSE80373. The numbers of up- and downregulated genes from each dataset are listed in Table 2.

Table 2. The numbers of up- and downregulated genes from each dataset.

Sl No.	Accession No.	DEG (p-Value < 0.05)	Up (logFC > 0.5)	Down (logFC < −0.05)
1	GSE7513	7578	2555	3935
2	GSE15192	11,509	3950	4207
3	GSE136287	1312	266	73
4	GSE35603	10,419	5321	5098
5	GSE50627	8100	1809	1832
6	GSE160320	12,482	1528	674
7	GSE28799	9881	3410	3422
8	GSE53759	5884	1123	813
9	GSE80373	13,308	2310	2054

Using Bioinformatics and Evolutionary Genomics, we generated Venn diagrams of common genes from 3 datasets of each type of cancer, where we obtained 5 upregulated common genes from the breast cancer datasets, 12 upregulated and 7 downregulated common genes from the lung cancer datasets, and 29 upregulated and 20 downregulated common genes from the ovarian datasets, as depicted in Figure 1. The common up-

and downregulated genes from each cancer are given in Supplementary Data S1, and the functional annotations of the common genes of cancer stem cells are described in Supplementary Table S1.

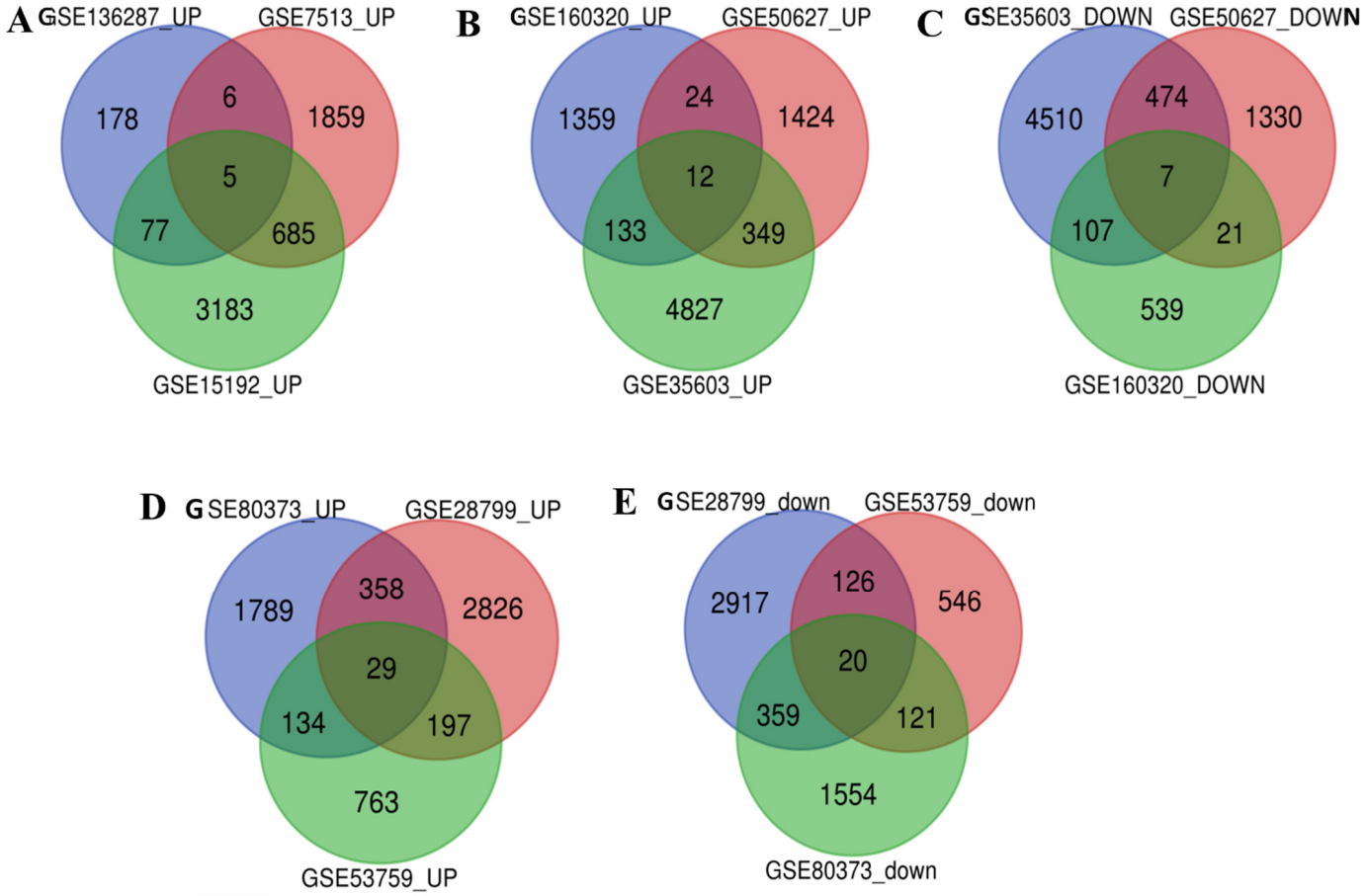

Figure 1. Common differentially expressed genes of different cancer stem cell gene expression datasets. (**A**) Common upregulated genes between three breast cancer stem cell gene expression datasets (there are no common downregulated genes found between the three BCSCs). (**B**) Common upregulated and (**C**) common downregulated genes among three lung cancer stem cell gene expression datasets. (**D**) Common upregulated and (**E**) common downregulated genes among three ovarian cancer stem cell gene expression datasets.

3.2. Validation of the CSC-Associated Genes from the TCGA Database

Genes that did not have sufficient clinical information for our analysis were excluded first. The final study included a total of 12 genes (up- and downregulated) from 9 datasets. We identified one upregulated gene from breast cancer, one upregulated and one downregulated gene from lung cancer, and four upregulated and five downregulated genes from ovarian cancer. The signaling pathways related to the up- and downregulated genes are listed in Table 3, where each gene can be involved in multiple signaling pathways, such as *ABCB7*, which is involved in the ABC transporter and the TGFB and HIF1 signaling pathways [39–41]. In the case of upregulated genes of breast cancer, the numbers of genes are too low for signaling pathways to be determined by the ShinyGO software. Validation of the genes from the TCGA database are shown in the box plots depicted in Figure 2.

Table 3. List of common up- and downregulated genes and related signaling pathways of three types of cancer. The mentioned genes are involved in multiple signaling pathways; for example, *ABCB7* is involved in various signaling pathways like the ABC transporter and the TGFB and HIF1 signaling pathways.

Cancer Type	Upregulated Common Genes	Signaling Pathways	Downregulated Common Genes	Signaling Pathways
Breast cancer	*VWA5A*, *LXN*, *CLIC4*, *APOC1*, *MCFD2*.	• NFkB signaling pathway [*VWA5A*, *LXN* [42,43]. • Apoptotic, angiogenic pathways [*CLIC4* [44]]. • MAP kinase pathway [*APOC1* [45]]. • Intracellular cargo transport pathway [*MCFD2* [46]].	-------	--------
Lung cancer	*ABCB7*, *SLC44A5*, *AIF1L*, *SYNE1*, *ID2*, *ID4*, *RPS6KA6*, *PPM1D*, *TP53BP2*, *ANGPT1*, *RHOBTB3*, *SLCO4C1*.	• TGFB signaling pathway. • Hippo signaling pathway. • Signaling pathway regulating pluripotency of cancer stem cell. • ABC transporter. • P53 signaling pathway. • HIF-1 signaling pathway.	*TRAM2*, *CAV2*, *CAP1*, *GLIPR1*, *TFPI2*, *PLAUR*, *MAN2A1*.	• Proteoglycans in cancer. • N-Glycan biosynthesis. • Focal adhesion. • Complement and coagulation cascades. • Endocytosis. • Prion disease.
Ovarian cancer	*ABCA3*, *DUSP4*, *EPHA4*, *ASAH1*, *FOXO1*, *MECOM*, *SLC6A12*, *PRRG4*, *ZDHHC14*, *AKR1C3*, *FRAS1*, *TSC22D1*, *MBD5*, *SLC5A3*, *PRDM1*, *ZFX*, *CTNS*, *AKR1B1*, *TGFBR3*, *AKR1C1*, *CAT*, *PKD2*, *C7ORF26*, *TMEM222*, *ARID5B*, *CCNG2*, *ABHD11*, *CDK19*, *PXK*.	• Folate biosynthesis. • FoxO signaling pathway. • Chemical carcinogenesis: reactive oxygen species. • Steroid hormone biosynthesis. • Longevity regulating pathways. • Metabolic pathways. • MAPK signaling pathways.	*ITGA3*, *ANXA3*, *NFE2L3*, *GJC1*, *CYR61*, *DARS2*, *UGCG*, *DEPDC1B*, *DUSP1(MKP-1)*, *BUB1*, *TBC1D1*, *ADCY7*, *TPX2*, *AURKA*, *AKAP12*, *TPM2*, *FLNC*, *PGM2*, *COTL1*, *HJURP*.	• Progesterone-mediated oocyte maturation. • Dilated cardiomyopathy. • Oocyte meiosis. • Focal adhesion. • Purine metabolism. • Adrenergic signaling in cardiomyocytes.

3.3. Survival Analysis of the CSC-Associated Genes

Survival analysis revealed 12 genes from different types of cancer, among which only 8 genes (*APOC1*, *SLC44A5*, *CAV2*, *PRRG4*, *ADCY7*, *AKAP12*, *TPM2*, and *FLNC*) had significant influence (p-value < 0.01) on the overall survival of patients of respective cancers, and the remaining 4 genes (*MECOM*, *SLC6A12*, *ABHD11*, and *MKP-1*) did not significantly influence overall survival. Therefore, in this study, eight prognostically significant genes were used for further analysis. As shown in Figure 3, the higher expression of *APOC1* is related to poor overall survival of breast cancer patients. In lung adenocarcinoma patients, the higher expression of *SLC44A5* is related to decreased overall survival and the higher expression of *CAV2* is associated with increased overall survival. However, in case of ovarian cancer, we found the opposite results: *PRRG4* expression is upregulated in both ovarian cancer and ovarian CSCs, but a higher expression of *PRRG4* is associated with increased overall survival. On the other hand, *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* are downregulated in ovarian cancer and also in ovarian CSCs but their higher expression is significantly associated with decreased overall survival. These observations suggest that more experimental investigation is necessary, particularly in the case of ovarian cancer stem cells.

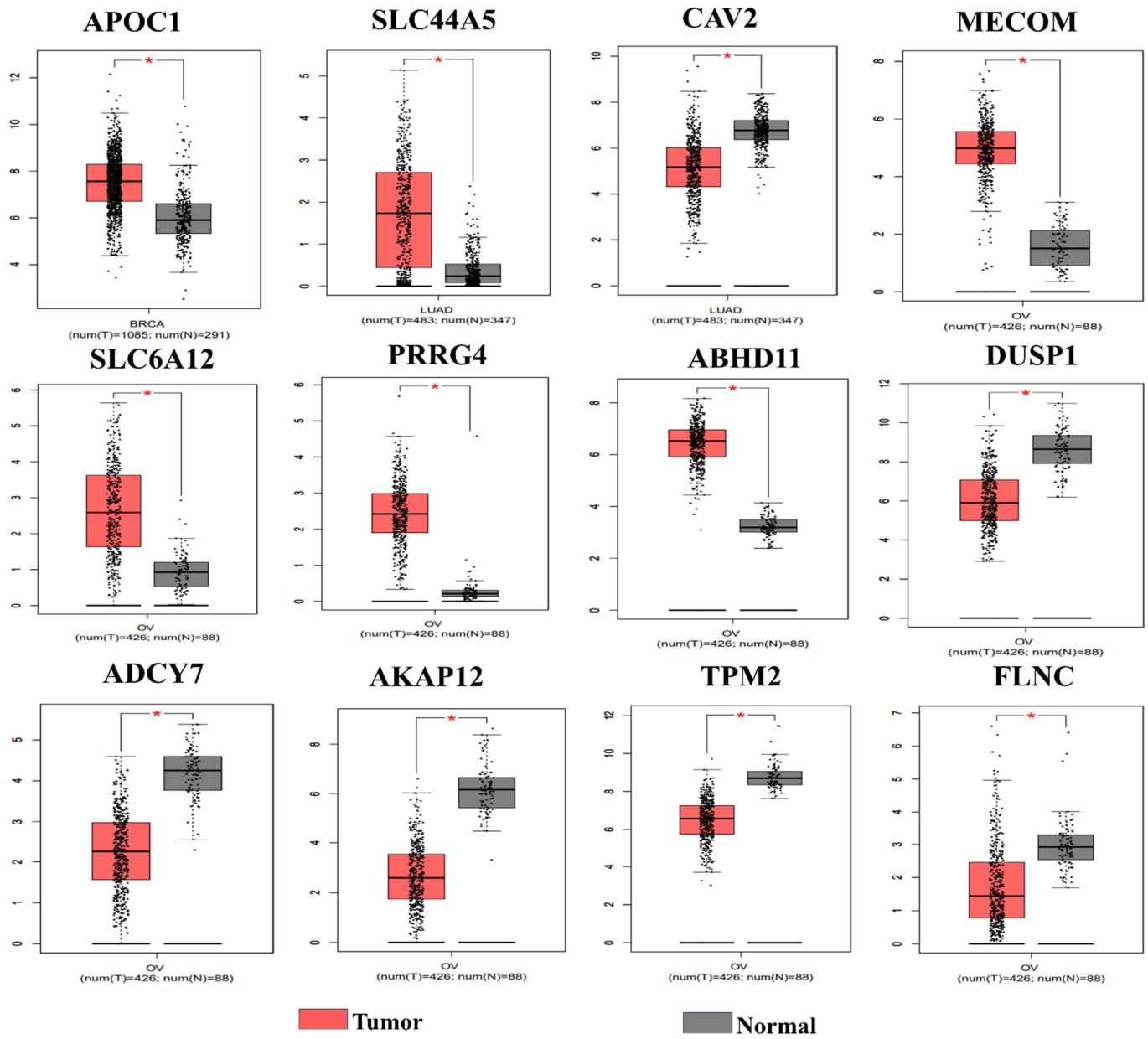

Figure 2. Expression of the selected genes validated from TCGA by using the GEPIA database for the respective cancers: *APOC1* in breast cancer; *SLC44A5* and *CAV2* in lung adenocarcinoma; MECOM, SLC6A12, PRRG4, ABHD11, DUSP1, ADCY7, AKAP12, TPM2, and FLNC in ovarian cancer. Here, the red color represents tumor sample, the black color represents normal samples, and red asterisks indicate that the expression of the gene is significant in the particular cancer. BRCA: Breast Cancer, LAUD: Lung Adenocarcinoma, OV: Ovarian Cancer.

3.4. Correlation Between the Expression of CSC-Associated Genes and Pathological Stages of Carcinomas

Using the UALCAN, we investigated the correlation between the expression of these eight genes and different pathological stages of particular cancers. The expression of *APOC1* was significantly increased (p-value < 0.01) in different stages of breast cancer from the normal persons but there were no significant changes between the stages (Figure 4A). A total of four stages are mentioned in this plot: stage 1, stage 2, stage 3, and stage 4. The *SLC44A5* expression was significantly upregulated and *CAV2* expression was significantly downregulated (p-value < 0.01) in different stages of lung adenocarcinoma patients compared to normal individuals (Figure 4B,C). However, there was no significant

variation between the stages of lung adenocarcinoma in the case of the expression of *SLC44A5* and *CAV2*. The other genes (*PRRG4*, *ADCY7*, *AKAP12*, *TPM2*, and *FLNC*) did not significantly differ between the stages of ovarian serous cystadenocarcinoma. In the case of ovarian serous cystadenocarcinoma, sufficient data were not available from normal individuals, indicating the need for more investigation into the detailed roles of identified genes in ovarian cancer (Figure 4D–H).

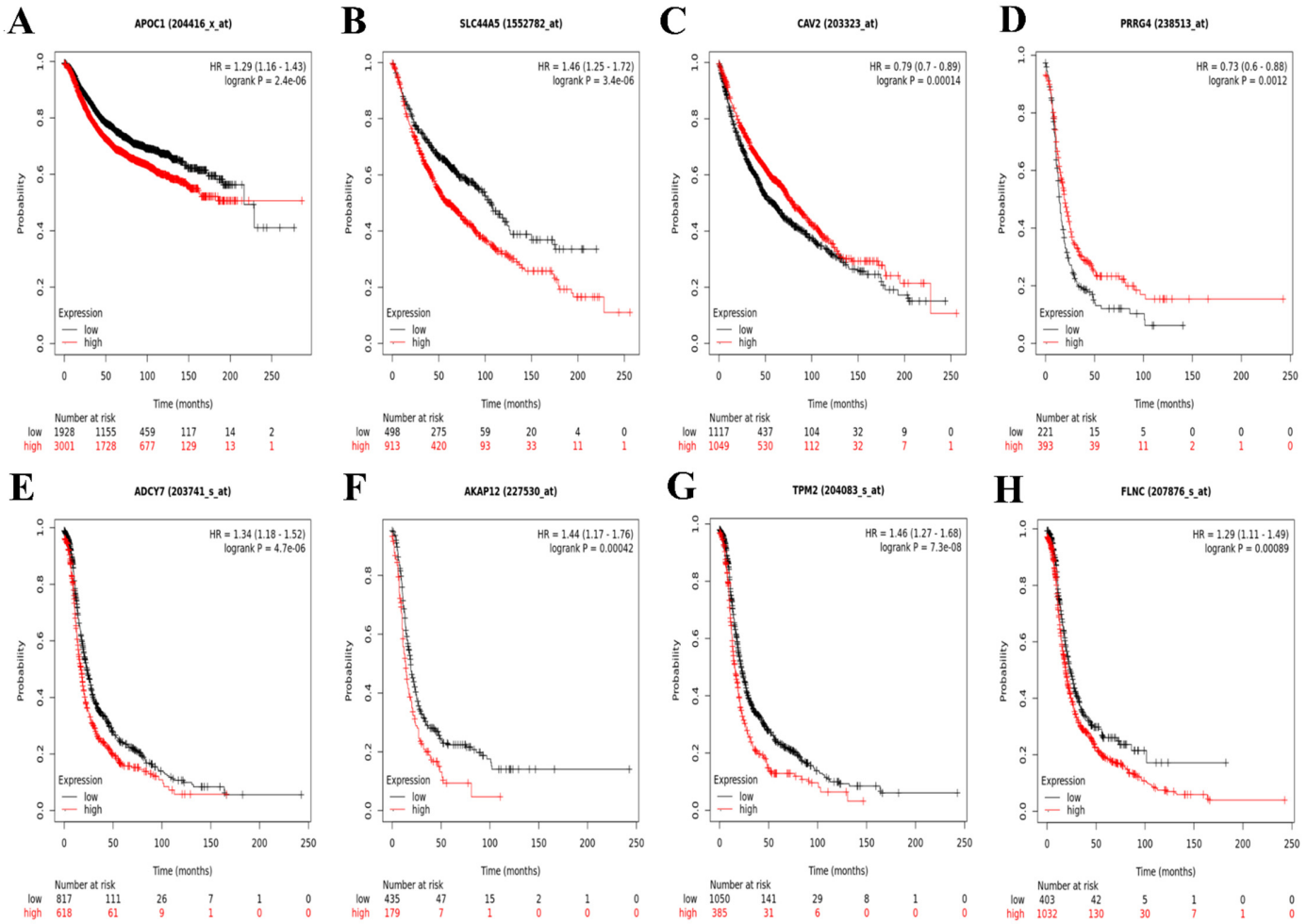

Figure 3. Prognostic value of mRNA expression of the genes in breast, lung adenocarcinoma, and ovarian cancer patients analyzed by KM plotter. (**A**) *APOC1* in breast cancer; (**B**) *SLC44A5* and (**C**) *CAV2* in lung adenocarcinoma; (**D**) *PRRG4*, (**E**) *ADCY7*, (**F**) *AKAP12*, (**G**) *TPM2*, and (**H**) *FLNC* in ovarian cancer. Here, logrank *p* value e- represents e^{-}. For example, logrank *p* value '4.76e-06' should be read as '4.76×10^{-6}'.

We also investigated the expression of these genes between tumor and normal samples on the basis of nodal metastatic status using UALCAN. Nodal metastatic status is indicated by the N0–N3 labels, where N0 represents no lymph node metastasis, N1 represents metastasis to 1–3 lymph nodes, N2 represents metastasis in 4–9 lymph nodes, and, lastly, N3 represents metastasis in 10 or more axillary lymph nodes. The results revealed that in patients with breast cancer, *APOC1* is significantly increased (p-value < 0.01) in N0, N1, N2, and N3 stages in comparison to normal healthy individuals (Figure 5A). Compared with the normal individuals, the expression of *SLC44A5* and *CAV2* in lung adenocarcinoma patients is significantly increased and decreased (p-value < 0.01), respectively, in all the nodal metastatic stages compared to normal persons (Figure 5B,C). In both breast cancer and lung adenocarcinoma, these three genes did not significantly change within the N0, N1, N2, and N3 stages. There are no data available for the genes associated with ovarian cancer.

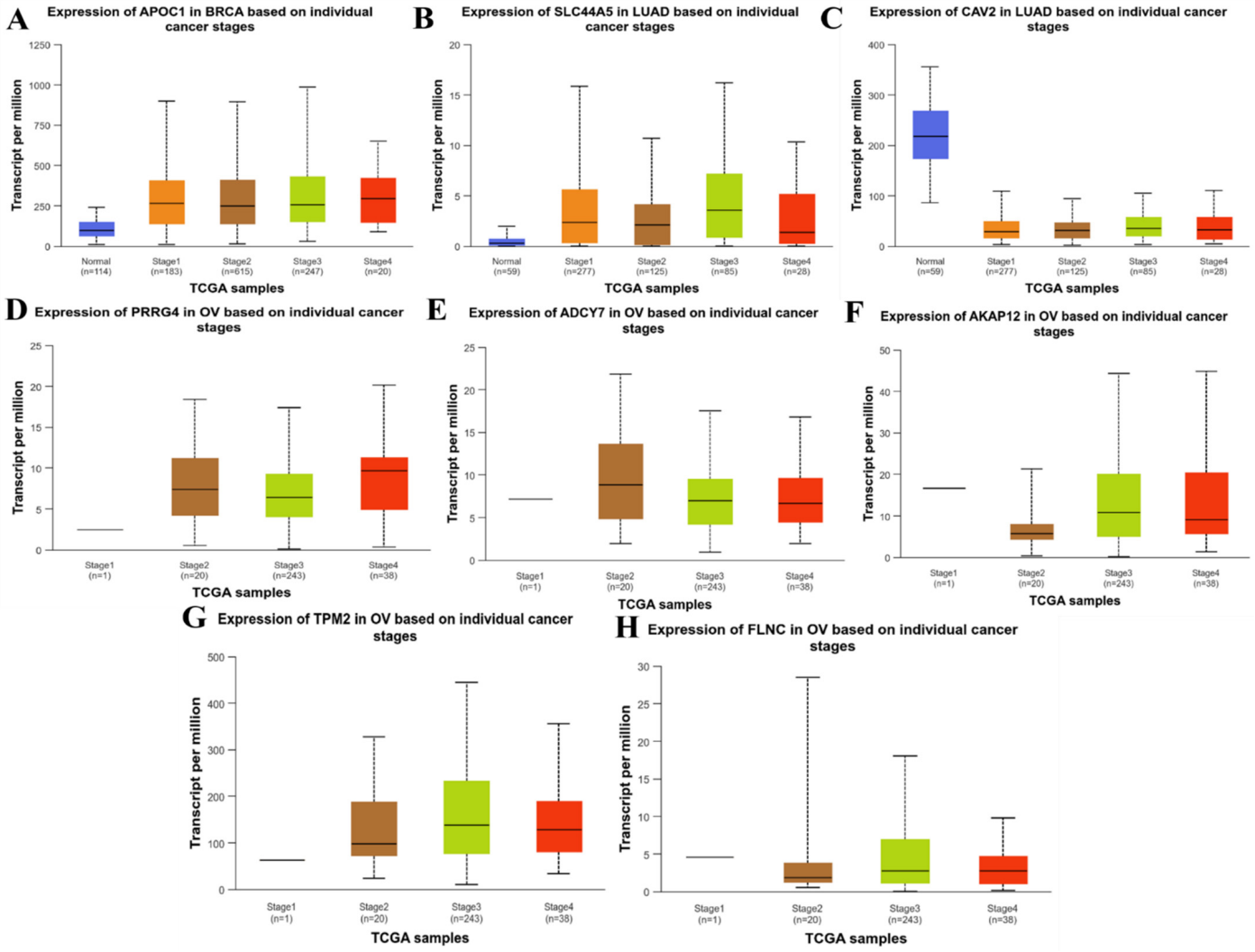

Figure 4. Expression of the genes associated with the different stages of particular cancers. (**A**) *APOC1* in breast cancer; (**B**) *SLC44A5* and (**C**) *CAV2* in lung adenocarcinoma; (**D**) *PRRG4*, (**E**) *ADCY7*, (**F**) *AKAP12*, (**G**) *TPM2*, and (**H**) *FLNC* in ovarian cancer.

3.5. Immunohistochemical Analysis

Using the HPA database, we explored the protein expression of the eight genes in respective cancers (depicted in Figure 6) and found that *APOC1* is not detected in normal breast tissue samples but shows medium expression in breast cancer tissue samples. The expression of *SLC44A5* shows lower expression in normal lung tissue but it shows medium expression in lung adenocarcinoma patients. However, *CAV2* expression is high in normal lung and shows medium expression in the case of patients with lung adenocarcinoma. The TCGA data revealed that the expression of *PRRG4* is upregulated in ovarian cancer, and the HPA data also support the TCGA report that in normal ovary, the expression of *PRRG4* is not detected but ovarian cancer patients show high intensity of *PRRG4*. However, other genes of ovarian cancer, like *ADCY7*, *AKAP12*, *TPM2*, and *FLNC*, show contrasting expression. Using the TCGA, these four genes were found to be downregulated in ovarian cancer. Using the HPA, we found that the expression of *ADCY7* and *FLNC* is not detected in normal ovary, but in the case of ovarian cancer patients, their expression is medium and low, respectively. *AKAP12* and *TPM2* expression is high in both normal and ovarian cancer samples.

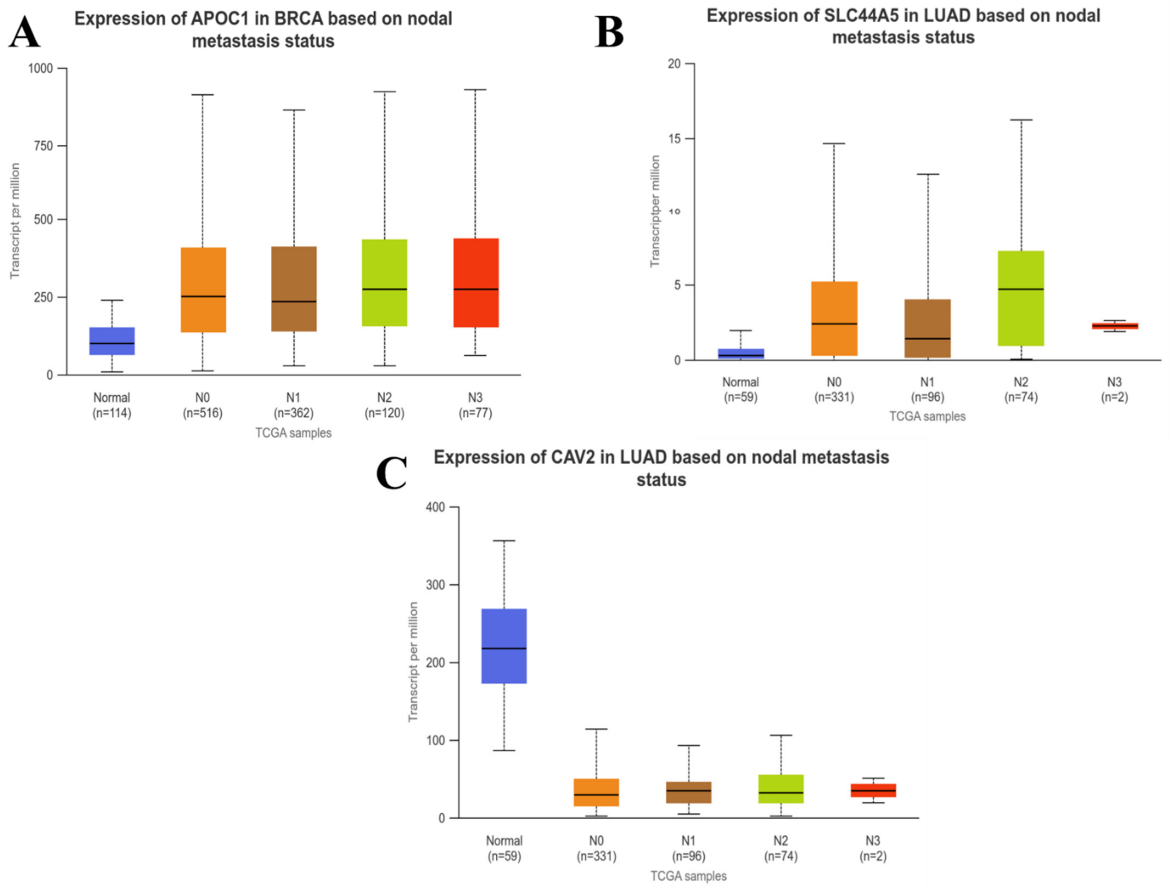

Figure 5. Expression of the genes based on the nodal metastatic status of particular cancer patients. (**A**) *APOC1* in case of breast cancer patients; (**B**) *SLC44A5* and (**C**) *CAV2* in case of lung adenocarcinoma patients. There are no data regarding the nodal metastatic status of ovarian cancer patients.

3.6. Correlation Between the Expression of Particular CSC-Associated Genes and Immune Infiltration Status in the Tumor Microenvironment

TIMER analysis revealed that expression of the identified genes was either positively or negatively correlated with the immune cell infiltration in particular cancers, as shown in Figure 7. Infiltration of M2 macrophages in the tumor microenvironment (TME) promotes tumor growth and metastasis by releasing the various proangiogenic factors and also suppressing the proliferation of T cells [47]. Neutrophils in the TME stimulate tumor angiogenesis by releasing MMP9 (Matrix metalloproteinases 9). They induce the recruitment of M2 macrophages and Treg cells and can also inhibit the function of NK cells [48]. Tumor-infiltrating dendritic cells have the capacity of immune suppression in the TME rather than stimulating the immune cells [49]. Our results revealed that *APOC1* in breast cancer (n = 1100) is positively correlated with the infiltration of M2 macrophage, neutrophil, and dendritic cells. In lung adenocarcinoma (n = 515), the expression of *CAV2* is positively correlated with the infiltration of M2 macrophage, neutrophil, and dendritic cells. However, the expression of *SLC44A5* is negatively correlated with the infiltration of these immune cells. On the other hand, M2 macrophage, neutrophil, and dendritic cell infiltration is positively related to the expression of *PRRG4* and *ADCY7* in ovarian cancer (n = 303). The expression of *AKAP12* is positively related to the infiltration of neutrophil cells but is negatively related to the infiltration of dendritic cells. *TPM2* expression is positively related to the infiltration of M2 macrophage and neutrophil cells, but their infiltration is not significantly related with the expression of *FLNC* in the case of ovarian cancer. However, the expression of *AKAP12* and *FLNC* is negatively correlated with the infiltration of dendritic cells in patients of ovarian cancer.

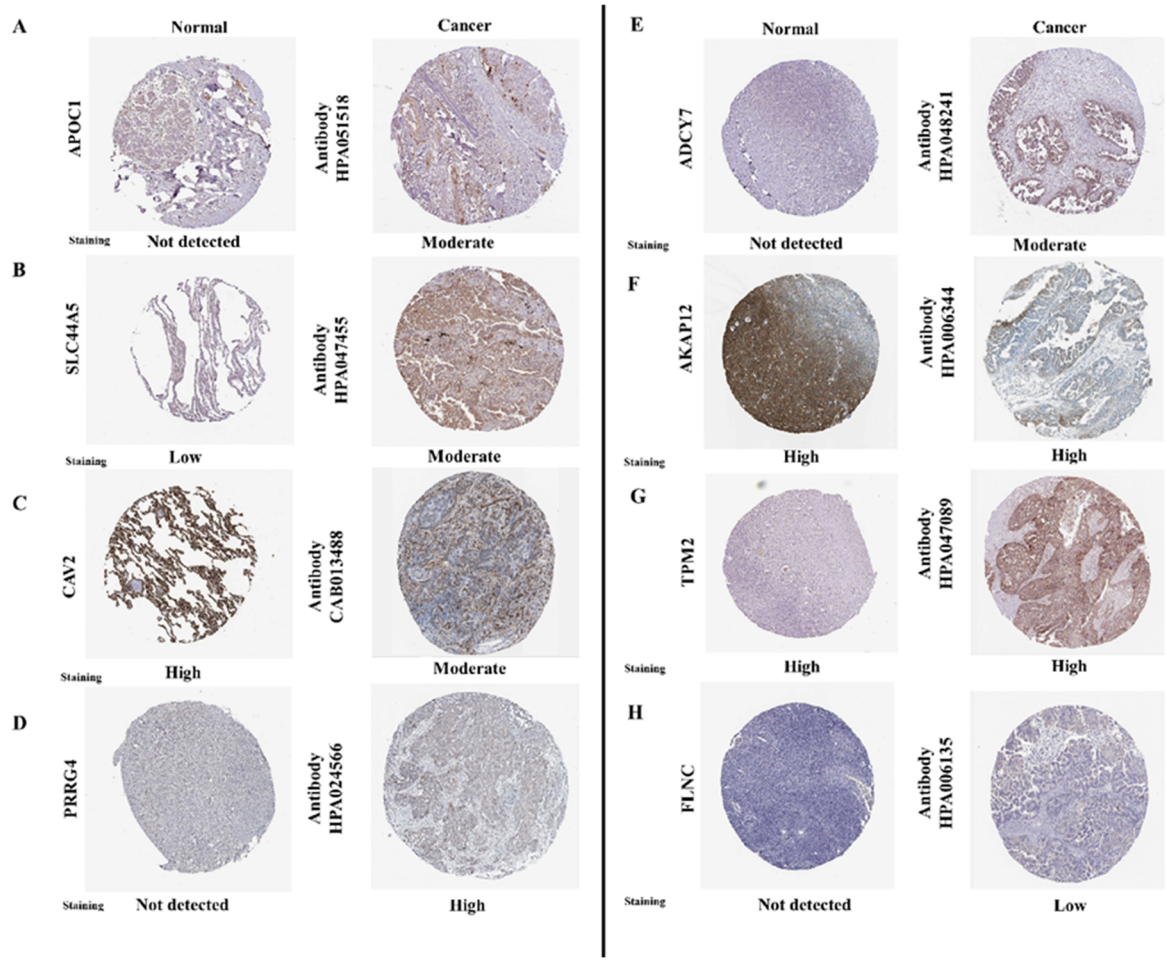

Figure 6. Protein expression of the genes acquired from the Human Protein Atlas database. (**A**) *APOC1* in breast cancer; (**B**) *SLC44A5* and (**C**) *CAV2* in lung adenocarcinoma; (**D**) *PRRG4,* (***E***) ADCY7, (**F**) *AKAP12*, (**G**) *TPM2*, and (**H**) *FLNC* in ovarian cancer. Here, staining is in four parts: not detected, low intensity, moderate intensity, and high intensity.

3.7. Identification of Drugs That Target the Particular CSC-Associated Genes

Using CTD, we identified several drugs that can influence the expression of particular genes. We selected the drugs on the basis of expression pattern of the target gene in CSCs and TCGA, i.e., inhibitors in the case of genes whose expression is upregulated, and for those genes whose expression is downregulated in CSC and cancer cells, we selected drugs that can induce the expression of these genes. From the analysis, we identified that *APOC1*, *SLC44A5*, and *PRRG4* are upregulated, so we searched for drugs that could decrease their expression (Figure 8A,B,D). Moreover, *CAV2*, *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* genes are downregulated, so, here, we searched for the drugs that could increase their expression (Figure 8C,E–H). We found that cyclosporine, valproic acid, ivermectin, and acetaminophen increase the expression of *ADCY7*. Doxorubicin, tretinoin, valproic acid, and cisplatin were found to induce the expression of *CAV2*. We found that drugs such as valproic acid, quercetin, and cyclosporin are able to decrease *APOC1*. Our results further revealed that cyclosporin, bisphenol S, sunitinib, and quercetin decrease expression of the *SLC44A5*. CTD results revealed various other drugs that can target the expression of the remaining genes. The drug datasheets derived from the CTD are given in Supplementary Data S2.

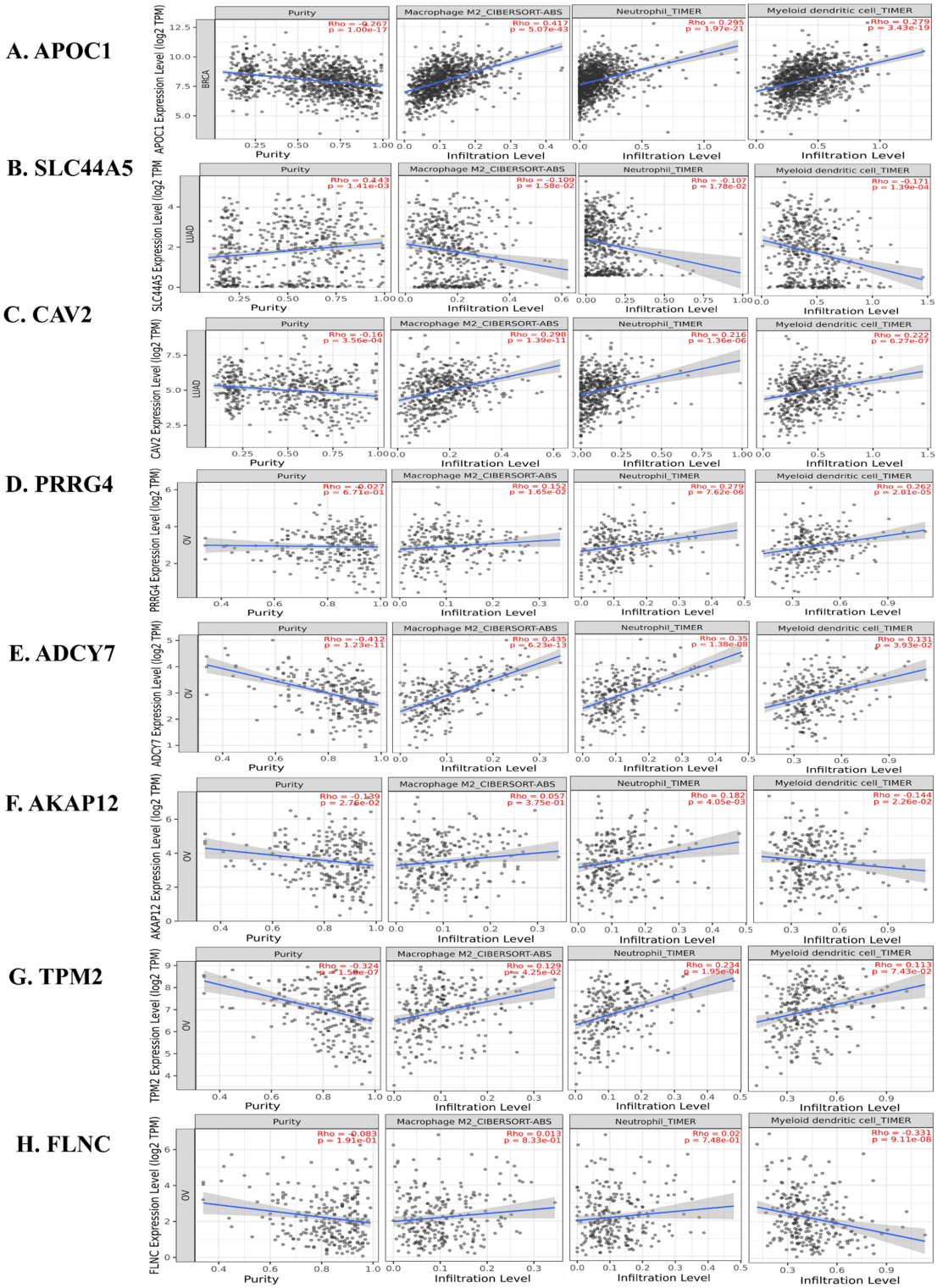

Figure 7. Prediction of immune cell infiltration with the expression of the genes in particular cancers. Correlation of immune cell infiltration with the expression of (**A**) *APOC1* in breast cancer; (**B**) *SLC44A5* and (**C**) *CAV2* in lung adenocarcinoma; (**D**) *PRRG4*, (**E**) *ADCY7*, (**F**) *AKAP12*, (**G**) *TPM2*, and (**H**) *FLNC* in ovarian cancer. Here, the blue line indicates the trend in this correlation analysis, and each dot in the scatter plot represents a single tumor sample. Here, a positive correlation is denoted by rho > 0, and a negative correlation is denoted by rho < 0. Here, *p* value e- represents e^{-}. For example, *p* value 9.11e-08 should be read as 9.11×10^{-8}.

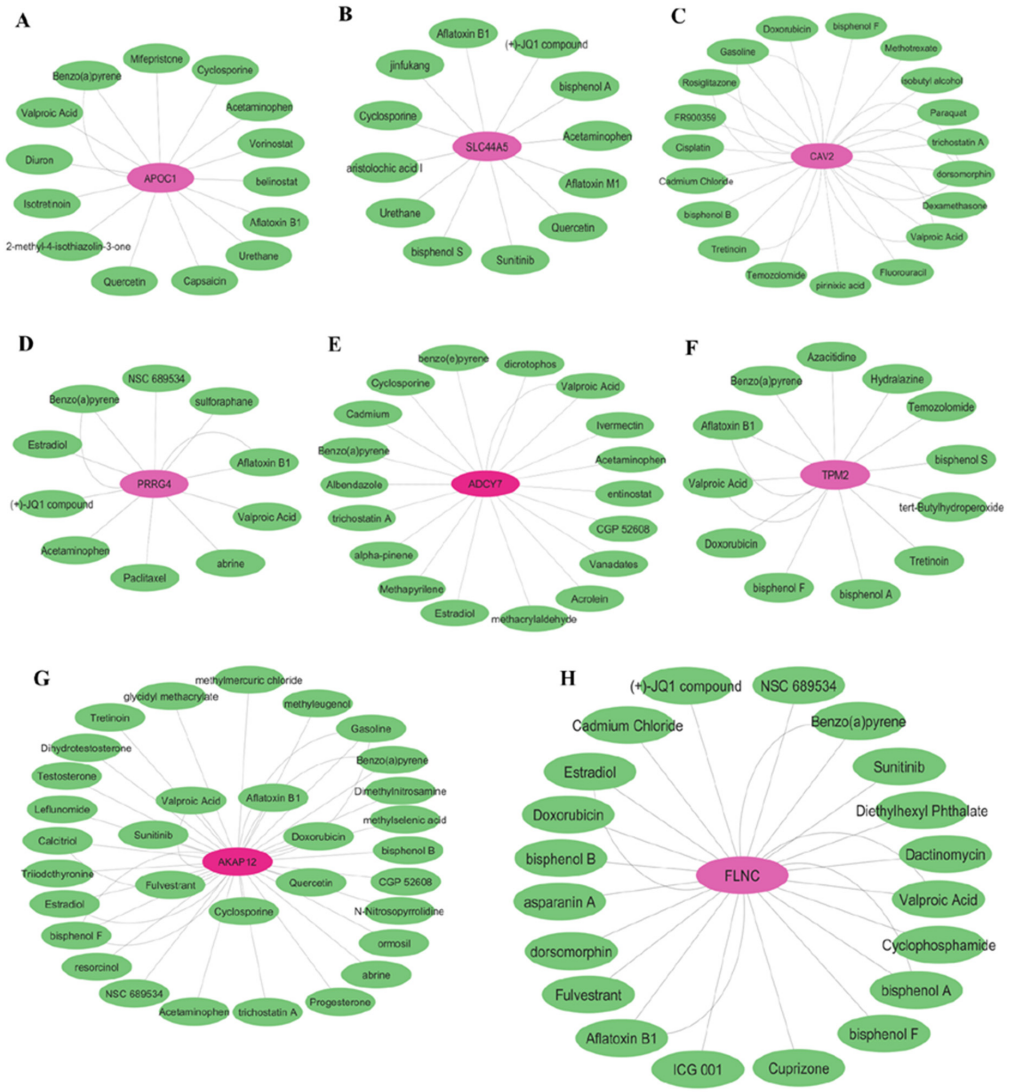

Figure 8. Chemical–gene interaction network established with the prognostically targeted genes. (**A**) *APOC1;* (**B**) *SLC44A5;* (**C**) *CAV2;* (**D**) *PRRG4;* (**E**) *ADCY7;* (**F**) *TPM2;* (**G**) *AKAP12;* (**H**) *FLNC*.

4. Discussion

CSCs are a small subpopulation of cancer cells that have the capability of self-renewal, which leads to their resistance to chemotherapy. Different signaling pathways like *JAK-STAT*, *Hedgehog*, *TGFB*, *Notch*, *Wnt-B* catenin, and *VEGF* regulate the development of CSCs. Different inhibitors, siRNAs, monoclonal antibodies, and enzyme inhibitors that interfere with those signaling pathways can target cancer stem cells. Recent studies involve ongoing clinical trials targeting CSCs [50]. Breast, lung, and ovarian cancer originate from epithelial cells and turn into the most aggressive types of cancer via the induction of epithelial–mesenchymal transition (EMT) [51–53]. Different strategies are available for targeting the stem cells of these cancers, although most of these strategies are yet to be clinically approved, and the plasticity and biology of CSCs need to be explored in more detail for the identification of novel targets. In the present study, we first downloaded the CSC gene expression datasets of these three different types of cancer and analyzed genes that are associated with CSCs from different datasets of the respective cancer types in order to identify genes that can be important intervention targets in these cancers. We aimed to identify important genes that are specifically expressed in cancer stem cells of these three types of cancer. There are few in silico analyses of different cancer-specific genes, and these studies identify several prognostic biomarkers and their significance in several cancers. However, notwithstanding our extensive search of the literature, we could not

find any such in silico studies that focus on the comparison of gene expression analyses of cancer stem cells. Cancer stem cell-specific genes in several cancers were identified through the comparison of gene expression datasets of cancer cells and normal cells. Few cancer stem cell-specific genes were identified using the stem-cell-related pathways or the mRNAsi (mRNA stemness index) or by using other criteria [54–57]. Recently, cancer stem cell (CSC)-targeted therapy has gained much attention since CSCs confer high drug resistance and show augmented metastatic capabilities. CSC-specific gene expression datasets are relatively scanty in public databases. Until now, no study has used CSC-specific gene expression datasets and identified CSC-specific genes and validated their prognostic significance in particular cancers. This study provides a comparative account of CSC-associated genes in breast, ovarian, and lung cancer and identifies several important CSC-specific gene targets in breast, lung, and ovarian cancer that can be further explored to determine their usefulness in CSC-targeted therapy. The datasets we analyzed did not reveal common differentially expressed genes between these three types of cancer stem cells. This observation necessitates further exploration of the possibility that some CSC-associated genes may be expressed in a cancer-tissue-specific manner. Next, we identified common important up- and downregulated genes in CSCs of particular types of cancer, i.e., breast, lung, and ovarian cancer, and investigated their relationships with different pathological stages of the respective types of cancer. We validated the identified genes in TCGA datasets of the respective cancers and carried out survival analysis to determine the prognostic significance of these genes. Finally, we identified several potential candidate drugs that can target these CSC-associated genes in particular cancers and are potentially able to reduce CSC-mediated cancer proliferation and metastasis.

From our analysis, we identified the *APOC1* (Apolipoprotein C1) gene in breast CSCs; *SLC44A5* and *CAV2* genes in lung CSCs; and *MECOM*, *SLC6A12*, *PRRG4*, *ABHD11*, *DUSP1*(*MKP-1*), *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* genes in ovarian CSCs as some of the most important genes. Out of these twelve genes, eight genes (*APOC1*, *SLC44A5*, *CAV2*, *PRRG4*, *ADCY7*, *AKAP12*, *TPM2*, and *FLNC*) showed significant overall prognostic value in respective cancers.

APOC1 has recently been identified in various types of cancers, including colorectal cancer [58], cervical cancer [59], and breast cancer [45]. An experimental study revealed that nanoparticle-mediated inhibition of *APOC1* reduces breast cancer growth and metastasis through the *MAPK*/*ERK* and *NFkB* pathways [60]. In esophageal cancer, reduced expression of *APOC1* has been found to be correlated with cancer inhibition [61]. Another experimental study revealed that GD2 is highly expressed in a small number of breast cancer cells, and these cells are also $CD44^{hi}CD24^{low}$. The Ganglioside GD2 helps in the identification of breast cancer stem cells, which ultimately induces tumorigenesis. The gene expression analysis of this study (GSE36643) revealed that *APOC1* expression is higher in the $CD44^{hi}CD24^{low}$ cell population compared to the $CD44^{low}CD24^{hi}$ population (Supplementary Table S2) [62]. In our analysis, we identified that *APOC1* is upregulated in human breast CSC as well as in breast tumor samples in the TCGA database (Figure 2). When we analyzed the effect of the expression status of this gene on overall survival, we found that higher expression of this gene is linked to worse overall survival (Figure 3A). We further analyzed the relationship between pathological stages of breast cancer with the expression of this gene, and found that its expression is significantly increased in different stages compared with normal condition, and nodal metastatic status is also increased in N0, N1, N2, and N3 stages of breast cancer. Protein expression data from HPA revealed that in normal breast tissue it is not detected, but in breast cancer tissue it is moderately expressed (Figure 6A). TIMER analysis revealed that in breast cancer patients, expression of *APOC1* is positively correlated with the infiltration of M2 macrophages, neutrophils, and dendritic cells, which contributes to the tumor-promoting environment in breast cancer (Figure 7A). We identified drugs that can decrease the expression of *APOC1*, including valproic acid, cyclosporin, quercetin, and isotretinoin.

Differential expression of *SLC44A5* (Solute Carrier Family 44 Member 5) and *CAV2* (Caveolin 2) is found in lung cancer stem cells, where *SLC44A5* shows upregulated expression and *CAV2* shows downregulated expression. *SLC44A5* is one type of choline transporter which plays an important role in increasing the viability and invasion of hepatocellular cancer cells and also inhibits their apoptosis. Knockdown of *SLC44A5* results in the suppression of cell viability and induces apoptosis [63]. There are no experimental data regarding the expression of *SLC44A5* in lung cancer. A gene expression study (GSE21656) involving a comparison between cisplatin-resistant H460 lung cancer cells and parental H460 cells revealed that the IGF1 signaling pathway is responsible for the resistance of H460 cancer cells. After analysis of gene expression data from cisplatin-resistant lung cancer cells (lung cancer stem cells), we found that the *SLC44A5* gene was unregulated in resistant cells compared with parental cells (Supplementary Table S2) [64]. The TCGA data show that its expression is significantly upregulated in lung adenocarcinoma samples compared with normal samples, and its higher expression is significantly associated with decreased overall survival of lung cancer patients. The expression of *SLC44A5* is significantly increased in the various pathological stages of lung adenocarcinoma patients but there are no significant changes within the stages of lung adenocarcinoma (Figure 4B). The expression of *SLC44A5* is also positively correlated with the nodal metastatic status of patients of lung adenocarcinoma. Several drugs like cyclosporine, sunitinib, bisphenol A, and quercetin can decrease the activity or expression of this gene. Caveolins are markers of caveolae which are small infoldings of the cell membrane involved in endocytosis, cellular trafficking, and signal transduction. *CAV2* is involved in the inhibition of the *TGFB* signaling pathway and it reduces the proliferation of mouse lung endothelial cells [65]. We were not able to find any experimental studies about the expression of *CAV2* in cancer stem cells; however, Liu et al. reported that *CAV2* knockout mice are unable to develop Lewis lung cancer, and B16F10 melanoma exhibits reduced tumor angiogenesis [66]. *CAV2* is also involved in the growth-promoting activities of renal cell carcinoma through the *PI3K*/*Akt* pathway [67]. The cisplatin-resistant lung cancer cells also show slightly upregulated expression of *CAV2*, although changes are slight (Supplementary Table S2). Our analysis and TCGA data revealed that its expression is downregulated in both lung cancer stem cells and lung adenocarcinoma patients and its higher expression is significantly related to increased overall survival, indicating that it could be an important prognostic marker for the patients of lung adenocarcinoma. Therefore, more experimental investigations are needed to determine the expression of this gene in lung cancer stem cells. Moreover, the expression of *CAV2* is significantly decreased across pathological stages and various nodal metastatic conditions from normal healthy individuals. The expression of the *CAV2* protein is found to be highly and moderately expressed in normal lung tissue and lung adenocarcinoma patients, respectively (Figure 6C). Immune infiltration analysis shows that the infiltration of M2 macrophage, neutrophil, and dendritic cells is positively correlated with the expression of *CAV2* but negatively correlated with the expression of *SLC44A5* in lung adenocarcinoma patients. As this gene is downregulated in lung cancer, we tried to identify drugs that could increase its expression, leading to perturbation in cancer proliferation. Drugs that can increase the expression of *CAV2* include doxorubicin, cisplatin, tretinoin, and valproic acid.

Next, *PRRG4* (Proline Rich and Gla Domain 4), *ADCY7* (Adenylate cyclase 7), *AKAP12* (A Kinase Anchor Protein 12), *TPM2* (Tropomyosin 2), and *FLNC* (Filamin C) genes were identified in ovarian cancer stem cells. *PRRG4* is upregulated and *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* are downregulated in ovarian cancer stem cells when compared with the nonstem cells. PRRG family proteins are involved in cell signaling and regulation, although the specific function of *PRRG4* has not been fully elucidated. Zhang et al. reported that upregulated expression of *PRRG4* induces breast cancer metastasis through downregulation of Robo1 via *NEDD4* [68]. As *PRRG4* is responsible for breast cancer metastasis, it may be involved in cancer stem cell development. Another study revealed that *PRRG4* regulates the mitochondrial function and migratory behavior of breast cancer cells via the Src–STAT3–

POLG axis [69]. In ovarian cancer, this gene has not been studied so far. In our study, this gene was found to be upregulated in ovarian cancer stem cells, but higher expression of this gene is significantly linked with increased overall survival, which highlights the paradoxical significance of this gene in ovarian cancer patients. However, few researchers have attempted to identify the characteristics of Aldehyde dehydrogenase 1 high ovarian cancer stem cells, which can be used for stem-cell-targeted therapy. They performed a microarray of $ALDH^{high}$ SKOV3 ovarian cancer cells and $ALDH^{low}$ SKOV3 ovarian cancer cells (GSE82304). Differential gene expression analysis revealed that PRRG4 is upregulated in $ALDH^{high}$ SKOV3 ovarian cancer cells compared with $ALDH^{low}$ SKOV3 ovarian cancer cells (Supplementary Table S2) [70]. The HPA data show that *PRRG4* is highly expressed in ovarian cancer, whereas it is not detected in normal ovary tissue (Figure 6D). *PRRG4* induces the M2 macrophage, neutrophil, and dendritic cell infiltration in the ovarian cancer microenvironment. Valproic acid, acetaminophen, and paclitaxel can reduce the activity of this gene in cancer.

ADCY7, *AKAP12*, *TPM2*, and *FLNC* genes showed reduced expression in ovarian cancer stem cells and also from the TCGA data. *ADCY7* is a membrane protein of the adenylate cyclase family that induces signal transduction. Reports indicate that *ADCY7* is associated with poor prognosis of myeloid leukemia patients [71]. An experimental study revealed that hypoxic conditions induce transient silencing of *ADCY6* and *ADCY7*, resulting in a reduction in *cAMP*/*PKA* signaling and reducing the migratory capacity of hypoxic cancer cells (HeLa and C33a cervical cancer, RKO colorectal cancer, MCF7 breast cancer cells). From the EMBL-EBL expression atlas and Oncomine database, it was also found that *ADCY7* mRNA expression is upregulated in ovarian cancer samples [72]. There are no experimental studies regarding the functional activity of *ADCY7* in ovarian cancer. However, in $ALDH^{high}$ SKOV3 ovarian cancer cells, ADCY7 expression is significantly downregulated (Supplementary Table S2). *AKAP12* acts as a tumor-suppressor gene in different types of cancer and can inhibit the proliferation, migration, invasion, and angiogenesis of cancer cells as well as block the cell cycle through the activation of protein kinase C [73]. Our study indicates that AKAP12 expression is downregulated in ovarian cancer stem cells. A similar result was observed, where AKAP12 was involved in the suppression of ovarian cancer cell proliferation via the Hippo pathway. It inhibited the proliferation, migration, and invasion of ovarian cancer cells [74]. Another gene expression study of $ALDH^{high}$ SKOV3 ovarian cancer cells reported that its expression was downregulated (Supplementary Table S2). Tropomyosins are actin-binding proteins that regulate the force generation and sensing stiffness. Downregulated expression of *TPM2* has been found to enhance the proliferation and migration of colorectal cancer cells [75] and also promote breast cancer metastasis and chemoresistance [76]. *TPM2* expression was noticed to be downregulated in our ovarian cancer stem cell gene expression study. Prostate cancer patient data reveal severe downregulation of *TPM2*. In vitro experiments concerning the overexpression of *TMP2* found it to be related with the inhibition of prostate cancer cell growth and proliferation [77]. The spread of glioblastoma in the brain was also found to be correlated with the loss of *TPM2* expression [78]. These data reveal that *TPM2* is downregulated in several cancers. A gene expression study of $ALDH^{high}$ SKOV3 ovarian cancer cells revealed that its expression is downregulated (Supplementary Table S2). Although detailed studies on *TPM2* expression in ovarian cancer, specifically on ovarian cancer stem cells, are not available, the abovementioned studies indicate that the upregulation of *TPM2* could provide favorable prognostic value in cancer. *FLNC* is an actin crosslinking cytoskeletal protein that maintains cellular morphology. In a study of gastric cancer, Filamin C suppressed metastasis and angiogenesis by reducing the expression of MMP2 (Matrix metalloproteinase 2). Immunohistochemical and qRT-PCR analysis revealed that *FLNC* expression was reduced in gastric cancer cells. Silencing of Filamin C affects the proliferation and colony-formation capabilities of gastric cancer cells [79]. $ALDH^{high}$ SKOV3 ovarian cancer cells also showed slightly reduced expression of FLNC (Supplementary Table S2). However, no detailed studies about the expression of FLNC in ovarian cancer are available, and our

study might help in the development of better experimental approaches for the study of ovarian cancer stem cells. Paradoxically, from the Kaplan–Meier plot, we found that high expression of the *ADCY7*, *AKAP12*, *TPM2*, and *FLNC* genes was significantly associated with decreased overall survival, indicating the need for detailed study of the functions of these genes in CSCs, tumor microenvironment, and survival of cancer cells. There is a lack of sufficient data concerning the relationships of these genes with different pathological stages and nodal metastatic status of ovarian cancer patients. However, when we investigated the immune infiltration status of these genes in ovarian cancer, we found that *ADCY7* expression was positively related to the infiltration of M2 macrophage, neutrophil, and dendritic cells but *AKAP12* expression was positively correlated with the infiltration of only neutrophils. *TPM2* expression was also positively related to M2 macrophage and neutrophil infiltration but not significantly related to dendritic cell infiltration, whereas the expression of *FLNC* was not significantly related to infiltration of M2 macrophages and neutrophils and was negatively related to dendritic cell infiltration in ovarian cancer. From immunohistochemical images, we found that *ADCY7* expression is not detected in normal ovary and is moderately expressed in cancerous tissue. *AKAP12* and *TPM2* are highly expressed in both normal ovaries and ovarian cancer. However, the expression of *FLNC* is not detected in normal conditions, and in ovarian cancer it shows lower intensity. Drugs like doxorubicin, quercetin, sunitinib, cyclosporine, and fulvestrant can target the *AKAP12*, and cyclosporine, valproic acid, and acetaminophen can increase the effect of *ADCY7*. Also, doxorubicin, valproic acid, and bisphenol A can effectively target the *TPM2* and *FLNC* genes of ovarian cancer stem cells. From our study, valproic acid, sunitinib, and quercetin emerged as drug candidates that can target most of the CSC-associated genes or signaling pathways in breast, ovarian, and lung cancer. It is important to note that quercetin has been actively explored as a potential candidate drug in cancer, but so far, clinical studies have failed to replicate the same level of impact shown in preclinical and in vitro studies [80], implying that the literature and high-throughput-study-based drug data bank may identify many well-researched candidate drugs that have failed to replicate the promising results revealed in in vitro/preclinical studies in clinical settings, and careful screening must be conducted to identify novel promising candidate drugs or small molecules.

5. Conclusions

Overall, our analysis suggests that there are no significant CSC-associated genes that have common expression patterns in the ovarian, lung, and breast cancer datasets, but we identified common genes amongst different datasets of ovarian, lung, and breast cancer, respectively. Our analysis of CSC gene expression revealed several important CSC-associated genes that are promising prognostic biomarkers for breast, lung, and ovarian cancers, and we also identified several candidate drugs that can modulate the function and expression of these genes or their protein products. Our study also indicates the need for thorough experimental analysis of the roles of these genes in different types of cancer stem cells, the molecular mechanisms of their actions, and their relationships with metastasis, survival, chemoresistance, and cancer relapse.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/informatics11040095/s1, Data S1, S2: Supplementary Data S1, Supplementary Data S2; Table S1: Supplementary Table S1; Table S2: Supplementary Table S2. Refs. [81–148].

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

Abbreviations

APOC1: Apolipoprotein C1, *SLC44A5*: Solute carrier family 44 member 5, *CAV2*: Caveolin 2, *PRRG4*: Proline rich Gla 4, *ADCY7*: Adenylate cyclase 7, *AKAP12*: A-kinase anchoring protein 12, *TPM2*: Tropomyosin 2, *FLNC*: Filamin C.

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