An Integrated Study on the Differential Expression of the FOX Gene Family in Cancer and Their Response to Chemotherapy Drugs

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Abstract: The Forkhead-box (FOX) transcription factors, as one of the largest gene families in humans, play key roles in cancer. Although studies have suggested that several FOX transcription factors have a significant impact on cancer, the functions of most of the FOX genes in cancer remain elusive. In the study, the expression of 43 FOX genes in 63 kinds of cancer diseases (including many subtypes of same cancer) and in response to 60 chemical substances was obtained from the Gene Expression Atlas database of the European Bioinformatics Institute. Based on the high degree of overlap in FOXO family members differentially expressed in various cancers and their particular responses to chemotherapeutic drugs, our data disclosed the FOX genes that played an important role in the development and progression of cancer. More importantly, we predicted the role of one or several combinatorial FOX genes in the diagnosis and prognostic assessment of a specific cancer and evaluated the potential of a certain anticancer drug therapy for this type of cancer by integrating patterns of FOX genes expression with anticancer drugs sensitivity.

Keywords: Forkhead-box family genes; overlapping expression; cancer diseases; anticancer drugs

1. Introduction

The Forkhead-box (FOX) transcription factors contain a highly conserved winged DNA-binding domain, which is composed of about 100 similar amino acids [1]. Genome-based bioinformatics analyses have shown that FOX genes were classified into 23 subfamilies [2–4]. In humans, there are more than 50 members from FOXA to FOXS subfamilies in the FOX superfamily [2–4]. FOX transcription factors have been demonstrated to regulate diverse biological processes related to cell differentiation, cell cycle control, embryonic development, metabolism, longevity, etc. [3]. Mutations of many FOX genes have been associated with human genetic diseases in different tissues, such as the brain, heart, liver, kidney, lung, breast, prostate, pancreas, vascular tissue, and immune cells [3–5]. Most importantly, FOX family genes, such as FOXA1, FOXC2, FOXE1, FOXF1, FOXM1, FOXO1, FOXO3, FOXO4, FOXO6, FOXP1, FOXP3, FOXQ1, and FOXR1, have been documented to play key roles in cancer by acting as tumor suppressors and/or oncogenes genes [3,4,6,7]. Although the function of several FOX transcription factors in cancers has been characterized, the functions of most FOX genes in cancer, especially in the subtypes of same cancer and in the response to anticancer drugs, are still unknown. In the study, we analyzed that the expression of 43 FOX genes in 63 kinds of cancer diseases (including many subtypes of the same cancer) and in response to 60 chemical substances. By integrating patterns of FOX genes expression with anticancer drugs sensitivity, we speculated that six FOX genes, including FOXO3, FOXO1, FOXN3, FOXX2, FOXXN2, and FOXX3, might be important for the development and treatment of a wide range of cancers. Furthermore, we revealed the value of the combination of several FOX genes in the diagnosis of the corresponding cancer and assessed the novel therapeutic potential of anticancer drugs by targeting these genes.
Our study aimed to put up new clues for the further functional study of FOX genes in the occurrence and therapy of cancers.

2. Materials and Methods

2.1. Gene Correlation Analysis in Gene Expression Atlas

The differential expression of human FOX genes in different diseases and response to different chemical substances were obtained from the Gene Expression Atlas database of the European Bioinformatics Institute (http://www.ebi.ac.uk/gxa, accessed on 20 May 2021) [8]. The Gene Expression Atlas is a comprehensive p-value database, based on many statistical methods and various independent studies, that provides information on the expression of genes or proteins in different species and conditions, including the cell types, different tissues, diseases, and chemical substances. An interface enables us to search for the expression of genes (i) by FOX genes (gene/gene properties); (ii) by Homo sapiens (organism); and (iii) by cancer diseases or chemical treatment response (biological conditions). Using the condition of a p-value < 0.05 for querying, among all the FOX genes in humans, the differential expression of 43 FOX genes in different cancer diseases and response to different chemical substances were obtained, except FOXD4L2-FOXD4L6, because of their lack of expression. To reflect the significant difference level, a p-value < 0.0001 of downregulated genes was set as the number 1 and is shown in deep green; 0.0001 < p-value < 0.05 of downregulated genes was set as the number 2 and is shown in light green; the p-value > 0.05 indicates a lack of differential expression, was set as the number 3, and is shown in black; 0.0001 < p-value < 0.05 of upregulated genes was set as the number 4 and is shown in light red; and a p-value < 0.0001 of upregulated genes was set as the number 5 and is shown in deep red. Moreover, the inverted p-values and their corresponding FOX genes are shown in the figures.

2.2. Other Gene Correlation Analysis Methods

The Gene Expression Profiling Interactive Analysis (GEPIA2) (http://gepia2.cancer-pku.cn/#index, accessed on 22 August 2022) online database was used to testify the notably related genes between clinically visible tumors and normal tissues [9]. GEPIA2 is an online commonly tool to calculate survival analysis automatically by analyzing the RNA-seq results. In addition, the University of Alabama at Birmingham Cancer data analysis portal (UALCAN) (http://ualcan.path.uab.edu/index.html, accessed on 2 May 2022) was also applied to the prognosis survival analysis [10]. The representative immunohistochemistry plots of the certain molecular was detected from The Human Protein Atlas (HPA) (https://www.proteinatlas.org/, accessed on 2 May 2022) website [11]. To depict the genetic characteristics of cancer cells, several data were obtained from a comprehensive interactive web resource—Cancer Cell Line Encyclopedia (CCLE) (https://portals.broadinstitute.org/ccle/data, accessed on 10 October 2021) [12]. STRING (https://string-db.org/, accessed on 10 October 2021) is a database of known and predicted functional protein-protein interaction (PPI) networks [13]. The TCGA database (https://portal.gdc.cancer.gov/, accessed on 4 September 2022) was used to obtain clinical information on breast cancer patients and the relating RNA-Seq and gene mutation data [14]. The co-occurrence and co-exclusion analysis was performed by maftools package in R software [15].

3. Results

3.1. Identification of FOX Genes That Play Important Roles in Various Types of Cancer

As shown in Figure S1, the differential expression of 43 FOX genes in 63 kinds of cancers (including many subtypes of same cancer), such as leukemia, lymphoma, brain tumors, prostate cancer, breast carcinoma, kidney carcinoma, lung carcinoma, and pancreatic cancer, and might imply that these FOX genes have pleiotropic effect in cancers. The expressions of FOXF1, FOXJ2, FOXJ3, FOXX2, FOXM1, FOXN2, FOXN3, FOXX1, FOXX3, and FOXO4, and
were differentially expressed in at least six subtypes of leukemia (Figure 1A). In parallel, we explored the correlation of these two genes in the GEPIA2 database and found that they were positively correlated in AML (Figure 1C). AML patients with high FOXP3 expression tend to have a poor prognosis as well (Figure 1D).

3.1.1. Expression Profile of FOX Genes in Leukemia and Identification of Several FOX Genes with High Expression in AML

Leukemia, a common tumor disease of the blood or bone marrow, characterized by the presence of immature leukocytes, is a major cause of death from cancer [16,17]. Leukemia can be divided into different subtypes, including chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL) [18–20]. The expression levels of FOXA2, FOXC1, FOXD1, FOXE1, FOXF1, FOXF2, and FOXG1 were downregulated in all types of leukemia, while those of FOXJ2, FOX3, FOXM1, FOXN2, FOXN3, FOXO3, FOXO4, and FOXP3 were upregulated in at least four subtypes of leukemia (Figure 1A). In addition, FOXK2, FOXL1, FOXO1, and FOXP3 were differentially expressed in at least six subtypes of leukemia (Figure 1A). In parallel, we compared the mRNA expression of FOXJ2, FOXN2, FOXN3, FOXO3, and FOXP1 between AML and normal samples by using the GEPIA2 database. Results indicated that these five genes were all upregulated in AML samples (Figure 1B). Given that FOXO4 and FOXP3 were highly expressed in combination in precursor T lymphoblastic leukemia, acute promyelocytic leukemia, leukemia, and acute myeloid leukemia, we explored the correlation of these two genes in the GEPIA2 database and found that they were positively correlated in AML (Figure 1C). AML patients with high FOXP3 expression tend to have a poor prognosis as well (Figure 1D).

Figure 1. Differential expression of FOX genes in leukemia (A). (B) The expression of FOXJ2, FOXN2, FOXN3, FOXO3, and FOXP1 in AML and normal samples. (C) The Pearson Correlation analysis of FOXO4 and FOXP3 expression in AML. (D) Survival analysis curves of FOXP3 in AML.

3.1.2. Importance of FOXO1 and FOXO4 from the Expression Profile of FOX Genes in Lymphoma

Lymphoma, as a type of neoplastic disease, starts in lymphocytes or white blood cells and can spread quickly from the lymph nodes to other body parts through the lymphatic system [21,22]. Hodgkin lymphoma is believed to involve lymphocytes, eosinophilic granulocytes, plasma cells, and specific Reed–Sternberg cells in tumor tissues. Non-Hodgkin
lymphoma, the most common type, comprises many subtypes, such as T-cell, B-cell, and NK lymphoma [21,22]. Ten subtypes of lymphoma were investigated in our study (Figure 2A). FOXF1, FOXK2, FOXO1, FOXO3, and FOXP1 were differentially expressed in at least five subtypes of lymphoma. Twenty-five FOX genes were differentially expressed in B-cell lymphoma (Figure 2A). Interestingly, both belong to the FOXO family, and compared with normal samples, FOXO1 was highly expressed in lymphoid neoplasm diffuse large B-cell lymphoma (DLBC); however, FOXO4 was down expression in DLBC (Figure 2B). Particularly, FOXO4 is downregulated only in DLBC, but not in other types of lymphomas (Figure 2A). In addition, high expression of FOXO1 was observed in lymphoma cell lines (Figure 2C).

**Figure 2.** Differential expression of FOX genes in lymphoma (A). (B) The expression level of FOXO1 and FOXO4 between DLBC and normal tissues. (C) Characterization of FOXO1 in cancer cell lines. *p* values less than 0.05 were considered to be statistically significant, *p* < 0.05.

3.1.3. Upregulation of FOXD1 Based on TP53 Mutation Is a Poor Prognosis Factor for LGG Patients

Central nervous system tumors are types of high mortality in nervous system diseases [23]. There were 11 kinds of nervous system tumors in our study (Figure 3A). In total, 25, 27, 24, and 25 FOX genes were differentially expressed in brain tumors (8 upregulated and 17 downregulated), glioblastoma (10 upregulated and 17 downregulated), glioma (14 upregulated and 10 downregulated), and neuroblastoma (4 upregulated and 21 downregulated), respectively. FOXD2, FOXD3, FOXH1, FOXJ3, FOXK2, FOXL1, FOXM1, FOXN2, FOXN3, FOXO1, FOXO3, and FOXP3 were differentially expressed in at least five types of nervous system tumors. The same was true for the upregulation of FOXM1 and downregulation of FOXO4 in glioblastoma multiforme (GBM) (Figure 3B). Besides, the FOXD1 expression in the brain lower-grade glioma (LGG) group was higher than that in the control group (Figure 3C). It is well known that TP53 is a tumor suppresser, and according to the ALCAN-LGG dataset, the expression level of FOXD1 was high in the TP53 mutant group (Figure 3D). Furthermore, the high expression of FOXD1 conferred a poor prognosis of survival in LGG patients (Figure 3E).

![Figure 2](image-url)
prognostic marker in breast cancer [33]. FOXA1 overexpression is involved in estrogen prostate cancer cells and prostate adenocarcinoma (PRAD). To explore novel molecules that prostate carcinoma progress [27]. As shown in Figure 4B,C, FOXA1 is highly expressed in FOXD1 and FOXM1 and the control group (Figure 3C). It is well known that TP53 is a tumor suppresser, and ac-
downregulation of types of nervous system tumors. The same was true for the upregulation of according to the ALCAN-LGG dataset, the expression level of prognosis of survival in LGG patients (Figure 3E). mutant group (Figure 3D). Furthermore, the high expression of 0.05, *** downregulated), respectively. FOXD2, FOXD3, FOXH1, FOXJ3, FOXK2, FOXL1, ... in PRAD (Figure 4E). Recent studies have revealed that the GATA3/miR −503/ZNF217 axis influenced the epithelial-mesenchymal transition process in PRAD [28], and GATA3-dependent mitotic spindle orientation was crucial for prostate lineage specification [29]. The roles of PGR-related genes in prostate cancer tissues have also been summarized [30]. These results indicated that the downregulation of GATA3 and PGR is a critical determinant of the progression in PRAD, possibly modulated by FOXA1. The underlying mechanism is under further investigation.

3.1.4. The Importance of FOXA1 in Prostate as Well as Its Interaction with GATA3 and PGR

An investigative report in 2012 showed that 1.1 million men, accounting for more than 15% of the cancers diagnosed in men, were diagnosed with prostate cancer [24]. The FOX transcription factors have been regarded as direct or indirect factors for predicting, monitoring, and curing prostate cancer [25]. As shown in Figure 4A, almost all of the FOX genes were differentially expressed in prostate cancer, prostate carcinoma, and metastatic prostate cancer, except FOXA3, FOXB2, FOXC2, FOXI2, FOXI3, FOXP4, and FOXR1. The FOXA1 was identified as a recurrent mutation in prostate carcinoma by exome sequencing [26]. The finding of Jin’s group illustrated that recurrent mutation of FOXA1 lead to prostate carcinoma progress [27]. As shown in Figure 4B,C, FOXA1 is highly expressed in prostate cancer cells and prostate adenocarcinoma (PRAD). To explore novel molecules that interacted with FOXA1 in PRAD, we utilized the existing interactive gene network from the STRING software (Figure 4D). Surprisingly, although GATA3 and PGR both closely interacted with FOXA1, they were downregulated in PRAD (Figure 4E). Recent studies have revealed that the GATA3/miR−503/ZNF217 axis influenced the epithelial-mesenchymal transition process in PRAD [28], and GATA3-dependent mitotic spindle orientation was crucial for prostate lineage specification [29]. The roles of PGR-related genes in prostate cancer tissues have also been summarized [30]. These results indicated that the downregulation of GATA3 and PGR is a critical determinant of the progression in PRAD, possibly modulated by FOXA1. The underlying mechanism is under further investigation.

3.1.5. Multiple Roles of FOX Gene in Breast Cancer and Identification of Several FOX Genes with High Expression in TNBC

Breast cancer, as a high morbidity and mortality kind of cancer in women, is expected to increase significantly in the future [31,32]. The expression of FOXO3a is a favorable prognostic marker in breast cancer [33]. FOXA1 overexpression is involved in estrogen
receptor-positive breast cancer [34]. Compared with its expression in normal breast tissue, FOXM1 has shown a different expression pattern in breast neoplasms [35]. As shown in Figure 5A, among the 6 kinds of breast carcinoma/cancers, 32 (14 upregulated and 18 downregulated), 31 (4 upregulated and 27 downregulated), and 28 (17 upregulated and 11 downregulated) FOX genes were differentially expressed in breast carcinoma, breast cancer, and invasive ductal carcinoma, respectively. Data-mining results implied that FOXA1 expressed at a high level in breast carcinoma (Figure 5B). According to the HPA dataset, the positive rate of FOXA1 expression was high in tumor tissues, whereas all the normal breast tissues were not stained (Figure 5C). It is documented that promoter hypermethylation is associated with gene silencing. Based on the predicted results from the UALCAN database, promoter methylation levels of FOXA1 were lower in breast carcinoma compared to normal tissues (Figure 5D). These results highlight the crucial role that FOXA1 upregulation play in breast carcinoma development and progression. In addition, FOXO3 exerted a higher expression level in breast cancer (Figure 5A) and was significantly correlated with a poor prognosis (Figure 5E). Based on the “person neoplasm cancer status” in the TCGA database, we regarded the “with tumor” group as a recurrence group and the “tumor free” group as a non-recurrence group. The prognosis for breast cancer patients with relapsed tumors is poor (Figure 5F). In addition, FOXN2 expression was elevated slightly in the recurrence group, compared to the non-recurrence group (Figure 5G). Since the prevalence of breast cancer increases drastically in individuals with BRCA1/2 mutation [36], we performed a collinear analysis of the FOX family and BRCA1/2 gene mutations in breast cancer patients based on data from TCGA. The results suggested that mutations in FOXO1, FOXO3, FOXJ2, and FOXO4 are associated with mutations in BRCA1/2 (Figure 5H). Taken together, FOX genes are widely involved in breast cancer including occurrence, prognosis, and recurrence of breast cancer.

3.1.6. The Importance of FOXO1 and FOXO4 in KIRC from Expression, Prognosis, and Correlation Analysis

About 431,288 persons were diagnosed with kidney carcinoma in 2020, accounting for 2.2% of the total number of all cancers diagnosed globally [38]. Most of the FOX genes were differentially expressed in renal cell carcinoma (23 FOX genes) and clear cell renal carcinoma (26 FOX genes), while only 4 FOX genes were differentially expressed in chromophobe renal cell carcinoma (Figure 6A). The disordered expression of FOXD4L2, FOXK2, and FOXL1 could be prognostic markers for kidney renal clear cell carcinoma (KIRC) patients [39]. As can be seen from Figure 6A, FOXO1 and FOXO4 exerted a lower expression level in KIRC. By querying the GEPIA2 database, high expression of FOXO1 and FOXO4 were associated with a good prognosis of KIRC (Figure 6B,C). Simultaneously, positive correlation between FOXO1 and FOXO4 expression was observed in Figure 6D.

Lung cancer is one of the main cancers to cause people death in the world [40]. A total of 36 FOX genes were differentially expressed in lung carcinoma, lung cancer, non-small cell lung cancer, and lung adenocarcinoma. Among the FOX genes, FOXA2, FOXD3, FOXE1, FOXF1, FOXF2, FOXJ3, FOXK2, FOXL1, FOXM1, FOXN1, FOXN2, FOXN3, FOXO1, FOXO3, FOXO4, and FOXP3 were differentially expressed in at least three kinds of lung cancer (Figure 6A). Compared to normal tissues, either in lung adenocarcinoma (LUAD)
or lung squamous cell carcinoma (LUSC), the expression of FOXM1 is at a higher level (Figure 6E).

Figure 4. Differential expression of FOX genes in prostate cancer (A). (B) The expression of FOXA1 in different cell lines. (C) The Differential expression analysis of FOXA1 was carried out between PRAD and normal tissues. (D) The respective PPI network of FOXA1. (E) Difference of GATA3 and PGR expression between PRAD and normal tissues. *p values less than 0.05 were considered to be statistically significant, * p < 0.05.
Figure 5. Expression profile of FOX genes in breast cancer (A). (B) The representative expression of FOXA1 in BRCA.
FOXAI in the GEPIA2 dataset. (C) Differential expression of FOXAI in normal breast tissue and breast cancer tissue was retrieved from the HPA database. (D) Box plot of FOXAI promoter methylation expression level in UALCAN-BRCA dataset. (E) The survival curve of BRCA patients with different FOXO3 expression levels. (F) A prognostic analysis based on the TCGA database of breast carcinoma patients with and without relapsed tumor groups. (G) The differential expression of FOXN2 in breast carcinoma patients between “with tumor” and “tumor free” groups. (H) The co-occurrence and co-exclusion analysis of the FOX family and BRCA1/2 mutated genes. (I) The relative expression of FOXA1, FOXC1, FOXK2, and FOXM1 in three subtypes of breast cancer (TNBC; Triple-Negative Breast Cancer; HER2+: human epidermal growth factor 2 positive; HR+: estrogen and progesterone receptors positive). p values less than 0.05 were considered to be statistically significant, * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 6. Differential expression of FOX genes in renal cell carcinoma and lung cancer (A). (B,C) Effects of the respective expression level of FOXO1 and FOXO4 on the survival rate of KIRC patients. (D) Pearson correlation coefficient analysis of FOXO1 and FOXO4 in KIRC tumor and normal tissues. (E) The differential expression of FOXM1 in LUAD, LUSC, and normal tissues. p values less than 0.05 were considered to be statistically significant, * p < 0.05.

3.1.7. The Importance of FOXM1 and FOXP1 in PAAD Is Underlined by Their Expression and Prognosis

FOX genes were differentially expressed in other cancers, such as thyroid adenocarcinoma, papillary thyroid carcinoma, pancreatic cancer, synovial sarcoma, embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, muscle-invasive carcinoma, cholangiocarcinoma, hepatocellular carcinoma, and gastric cancer (Figure 7A). Through GEPIA, FOXM1 exerted a higher expression in pancreatic adenocarcinoma (PAAD), and corresponded with a poor prognosis (Figure 7B,C). Notably, FOXP1 was highly expressed in PAAD (Figure 7D), with different expression levels in different stages (Figure 7E).
Figure 7. Differential expression of FOX genes in other cancers (A). (B) GEPIA2 data of FOXM1 expression in PAAD and normal samples. (C) The survival curve of PAAD patients with different FOXM1 expression level. (D) Difference of FOXP1 expression between PAAD and normal tissues. (E) Violin plots of the expression of FOXP1 with tumor stage in PAAD. * p values less than 0.05 were considered to be statistically significant, * p < 0.05.

3.2. Integrative Analysis of FOX Genes Differential Expression and Response to Chemicals

3.2.1. Screening for FOX Genes Which Are Important for the Progression and Treatment of a Wide Range of Cancers

The differential expression of human FOX genes in response to different chemical substances was also analyzed. A total of 16 kinds of anticancer drugs, including interferon γ, fulvestrant, camptothecin, tamoxifen, lipopolysaccharide, and colchicine, were analyzed, as shown in Figure 8A. The results showed that FOX genes were also differentially expressed in response to 60 different chemical substances (Figure S2), including some anticancer drugs.

We found that almost the majority of FOX genes differentially expressed in multiple cancers (>30) showed sensitivity to multiple chemotherapeutic agents (>30), suggesting that these FOX genes, including FOXJ3, FOXK2, FOXN2, FOXN3, FOXO1, and FOXO3, play an important role in the initiation and progression of cancers and could serve as important therapeutic targets for cancers (Figure 8B,C).

3.2.2. Evaluating the Potential of Anticancer Drug Therapy for a Specific Cancer

Based on the above research results, we wondered whether we could actively explore new anticancer effects of known anticancer drugs through the differential expression of FOX genes in tumors and the different responses to drugs. In order to find novel matching anticancer drugs for the above-mentioned malignant tumors, we analyzed the FOX genes in Figure S1 and Figure 8A. As shown in Figure 8D, the expression of FOXJ2, FOXN2, FOXN3, and FOXO3, which were originally highly expressed in AML, was suppressed after the stimulation of MTX. The treatment of Tamoxifen could cause the decrease of FOXO1 and the increase of FOXO4, which were differently expressed in DLBC. In similar, MTX, Etoposide, Fulvestrant, and Resveratrol could suppress the expression of FOXD1, FOXA1, FOXM1, and FOXP1, respectively, which exhibited oncogenic potential in LGG, PRCA/breast cancer, LUAD/LUSC, and PAAD. More research is needed to explore and identify the new applications of known anticancer drugs.
Figure 8. Differential expression of FOX genes response to anticancer drugs (A). (B) Statistics of FOX genes differentially expressed in various tumors (>30) and sensitive to multiple chemotherapeutic agents (>30). (C) The corresponding numbers of tumors (in the red circle) and chemical substances (in the blue circle) of six FOX genes. (D) The potential therapeutic roles of anticancer drugs to certain tumors due to opposing regulatory effects on the same FOX genes.

4. Discussion

The FOX transcription factors are a family of evolutionarily conserved transcriptional factors characterized by a winged helix/forkhead DNA-binding domain, which has a consensus sequence GTAAATAAA [41]. Apart from its common role in physiological processes, dysregulation of FOX transcription factors has been reported to be closely associated with tumor initiation and development, raising the possibility that FOX transcriptional factors combination could serve as effective diagnostic markers and novel therapeutic targets for cancer. Moreover, chemical substances targeting specific FOX genes could be considered as effective curative options.

In this study, we conducted a differential expression analysis of 43 FOX genes in 16 leukemia subtypes. Among the 43 FOX genes, we found that FOXA2, FOXC1, FOXD1, FOXE1, FOXF1, FOXF2, FOXG1, FOXJ2, FOXJ3, FOXM1, FOXN2, FOXN3, FOXO3, FOXO4, and FOXP3 were differentially expressed in a large number of leukemias with significant changes, suggesting that these genes play an important role in the pathogenesis of leukemia (Figure 1A). Over the past decade, AML has been the most common form of acute leukemia among adults, with little advancement in cure rates. The highly expressed FOXJ2, FOXN2, FOXN3, FOXO3, and FOXP1 were observed in AML (Figure 1B). Among all the regulated FOX genes in leukemia, the high expression of FOXO4 and FOXP3 in multiple leukemia types and their correlation, as well as the prognostic value of FOXP3 in AML, suggest the importance of FOXO4 and FOXP3 in leukemia, especially AML, and the potential of their combination as biomarkers for AML diagnosis and drug sensitivity.
Consistent with our finding, several lines of evidence have recently highlighted the roles of FOXN3 [42], FOXO3 [43], FOXO4 [16], and FOXP3 [44] in leukemia. In this light, based on the fully elucidation of pathogenic mechanisms of these genes, the identification of new medicine targeting these genes could be considered as a novel clue provided for effective systemic therapy. As illustrated in Figure 8A, the expression of FOXJ2, FOXN2, FOXN3, FOXO3, and FOXO4 are decreased after Methotrexate (MTX) stimulation. Zhao et al. demonstrated the potential clinical value of MTX by an aptamer-drug conjugation, which resulted in the apoptosis of AML cells [45]. It is reasonable to speculate that the combination therapy with MTX may be an attractive way to give a possibility in improving the therapeutic efficacy of AML.

It is reported that FOXO1 confers long-term maintenance of the dark zone proliferation program and can acted as a pharmacologically target in adult Burkitt Lymphoma [46]. This report is in line with our results that FOXO1 was not only differentially expressed in six types of lymphomas, but FOXO1 expression was significantly higher in lymphomas than in other cancer cell lines. These results indicates that FOXO1 may play an important role in the pathogenesis of lymphoma and be an attractive target for lymphoma therapy. DLBC is the most common type of lymphoma worldwide, and patients with treatment failure after the chemoradiotherapy regimen of R-CHOP often have dismal outcomes with limited therapeutic options [47]. The importance of FOXO1 in DLBC is underlined by the evidence that its mutation leads to competitive expansion of B cells during germinal center responses [48]. In the study, we found that the FOXO1 gene was remarkably upregulated, while the FOXO4 gene was decreased in DLBC, which belongs to B-cell lymphoma (Figure 2B). Of note, we found FOXO4 expression was reduced only in B-cell lymphoma and not in other types of lymphoma (Figure 2A). Considering the importance of FOXO1 and specificity of FOXO4 in DLBC, we hypothesized that the combination of high expression of FOXO1 with low expression of FOXO4 could be diagnostic indicators for DLBC. Tamoxifen, an antiestrogen widely used in advanced ovarian and breast cancer has a therapeutic effect in DLBC by targeting estrogen receptor β [49]. In particular, we found the stimulation of Tamoxifen can reduce the expression of FOXO1 and increase the expression of FOXO4. Accordingly, the clinical significance of tamoxifen in DLBC and its interaction with FOXO1 and FOXO4 deserved to be investigated.

In general, GBM is the most common and aggressive malignant primary brain tumor; thus, research on pathogenic mechanisms and new therapeutic modalities merits further attention. Evidences revealed that dysregulation of FOX genes contribute to aggressive tumor biology and therapy resistance in GBM patients [50]. Compared to normal tissues, FOXM1 expressed at a higher level in GBM (Figure 3B). A circBFAR/miR−548b/FOXM1 axis [51] and a FOXM1/ADAM17 feedback loop [52] have been identified as involved in the development of GBM. In this light, FOXM1 might serve as a potential target for GBM. As can be seen in Figure 3A, FOXO4 is decreased in several types of nervous system tumors, including GBM. It is reported that FOXO4 possesses an anticancer glioma activity [53]. Therefore, molecular mechanism studies are warranted to clarify and assess whether elevation of FOXM1 and simultaneously decrease of FOXO4 could act as a diagnostic marker for GBM. Typically, LGG occurs in young adults. It has a high risk of turning malignant, which leads to neurological problems and ultimately death. Figure 3C,E clearly show that the expression of FOXD1 is increased in LGG and its high expression predicts a poor prognosis of LGG patients, suggesting that FOXD1 plays an important role in the pathogenesis of LGG. Then, we found a possible link between the high expression of FOXD1 in LGG and the mutation of the tumor suppressor TP53 (Figure 3D). Since FOXD1-KD could potentiate the anticancer effects of radiotherapy by fostering the expression of TXNIP, an activator of TP53 in oral cancer cells, we speculate that FOXD1 might have a similar role in LGG. Since MTX is the only anticancer drug that can inhibit the expression of FOXD1 in Figure 8A, and there is evidence that MTX decreases the cells viability of glioma cells [54], further validation is required to clarify the role of MTX in this tumor.
In recent years, the incidence of hormone-dependent malignant tumors, led by breast cancer and prostate cancer, has been increasing year by year worldwide. FOXA1, an androgen receptor (AR) chromatin-binding precursor in the prostate epithelium, was reprogrammed to neuroendocrine-specific regulatory elements [55]. In addition, a previous study revealed that FOXA1, which established estrogen-responsive transcriptomes, was a pioneer of nuclear receptor action in breast cancer [56]. The interaction of FOXA1 with cofactors such as ESR1 and E2F1 enhanced the susceptibility of breast cancer [57]. Clinically, combining gene therapy of FOXA1 knockdown with ultrasound targeted microbubble destruction technology has been proved effective in the non-invasive therapy of breast cancer [58]. Consistently, the importance of FOXA1 in breast and prostate cancer is underlined by our results that FOXA1 is highly expressed in various prostate and breast cancers, and that FOXA1 expression at mRNA in prostate cancer is significantly higher than that in other cancer lines (Figure 4A, B and Figure 5A). As an aggressive and highly lethal disease, TNBC is characterized by the lack of hormone receptors (HR). Of note, the expression of FOXA1 is low in TNBC but high in HR + breast cancer patients (Figure 5I). Therefore, it is clear that FOXA1 is a therapeutic target for HR + breast cancer. More importantly, the chemotherapeutic drug Etoposide, which has been shown to inhibit the growth of prostate and breast cancer [59, 60], reduced FOXA1 expression in our study (Figure 8A), concluding that FOXA1 may be useful as potential prognostic marker and therapeutic target. All in all, our and others’ studies provide clear evidence for the role of FOXA1 in breast cancer and prostate cancer progression and treatment response, suggesting that the targeting of FOXA1 may be useful, in combination with standard therapy, in treatment.

It is noteworthy that TNBC and basal-like breast cancer (BLBC) are different molecular classes of breast cancer with a high degree of overlap, and the overlap ratio of gene expression profile between TNBC and BLBC can be as high as 80% [61]. Thus, we compared and analyzed the data from Figure 5A, I; the expression trend of FOXC1, FOXK2, and FOXM1 in TNBC was indeed consistent with that in BLBC. Simultaneous elevation of these genes may serve as oncogenic targets of TNBC. Combining the results of Figure 5I and Figure S2, the use of MTX led to the inhibition of FOXC1, FOXK2, and FOXM1 expression, which was highly expressed in TNBC. Consistent with our results, MTX has a spectrum of antitumor activity and can be used in the treatment of breast cancer [62]. Beware of the inhibitory effect of Trichostatin A (TSA) on FOXC1 and FOXK2 expression; despite the present study confirming its tumor suppressor effect on TNBC cells [63], more research is needed to prove its actual roles in TNBC patients.

As the most prevalent type of renal cell carcinoma, KIRC is characterized by genetic mutations in factors governing the hypoxia signaling pathway [64]. Our study also found that the expression of FOXO1 and FOXO4 was significantly downregulated in KIRC, which was strongly correlated with worse survival outcomes (Figure 6B, C). Meanwhile, there is a positive relationship between the two genes (Figure 6D). Therefore, the combined effect of FOXO1 and FOXO4 could be developed as a promising therapeutic strategy of KIRC. Lung cancer is a lethal tumor, which could be split into two basic types—small cell lung cancer and non-small-cell lung cancer (NSCLC). Lung cancer is largely caused by NSCLCs, with LUAD and LUSC being the most common forms. As displayed in Figure 6E, compared with other FOX family genes, FOXM1 is significantly higher in these two types of lung cancer. Similar to our results, it is proved that FOXM1 could be served as a predictive factor for patients with NSCLC [14]. Moreover, FOXM1 could also promote the metastasis of lung cancer cells through the activation of the AKT/p70S6K signal pathway [65]. These new insights may contribute to further investigations about the role of FOXM1 in therapeutic response of lung cancer. Given that Fulvestrant can reduce FOXM1 expression (Figure 8A) and that the combination of Fulvestrant and TLR4-specific inhibitor CLI-095 prevents the metastasis of NSCLC effectively [66], it is of potential significance to investigate the role of FOXM1 in Fulvestrant against NSCLC.

PAAD is the most devastating type of cancer, with a five-year survival rate of 10% [67]. Patients’ advanced disease at diagnosis remains a major challenge in treatment. Reliable
predictors for diagnosis and individual risk of progression of PAAD are not available. In this report, *FOXM1* were expressed at relatively higher levels in PAAD tissues (Figure 7A,B) and predicted poor prognosis in PAAD (Figure 7C). In line with our report, *FOXM1* upregulation is critical in the initiation, advancement, and diversion of pancreatic cancer [68]. In Figure 7D,E, we paid attention to the role of potential oncogenic factor *FOXP1* in PAAD and assessed the possibility of *FOXP1* expression with cancer grade and disease progression. Zhao’s article demonstrated a novel TTN-AS1/microRNA−589−5p/*FOXP1* feedback loop in PAAD malignant phenotype [69], providing evidence for our results. In summary, these results implied a significant direction for further investigation of *FOXM1* and *FOXP1* during PAAD progression. As can be seen in Figure 8A, the treatment of Resveratrol lead to a decrease expression both in *FOXM1* and *FOXP1*. Moreover, in pancreatic cancer stem cells, Resveratrol can reverse epithelial-mesenchymal transition directly [70]. This discovery may result in novel targeted therapies after detailed mechanistic studies.

5. Conclusions

In this study, we revealed the differential expression of 43 *FOX* genes in 63 types of cancer diseases, and the responses of the *FOX* family to 60 chemical substances. On the one hand, based on the intersection of *FOX* genes that are differentially expressed in more than 30 cancers and respond to more than 30 chemotherapeutics simultaneously, the current study has identified six *FOX* genes, such as *FOXJ3*, *FOXK2*, *FOXN2*, *FOXN3*, *FOXO1*, and *FOXO3*, which play an important role in the development of a wide range of cancers and may share similar pathogenic mechanisms. On the other hand, some *FOX* genes were specifically expressed in certain cancer types; for instance, *FOXJ3* is highly expressed only in GBM. The specific expression makes them excellent targets to cure cancers. More importantly, based on the differential expression of some *FOX* genes combination in a specific cancer and the response to related chemotherapeutic drugs, this study speculated the possibility of the combination of several *FOX* genes in the diagnosis and treatment of the certain tumors and the new application of old chemical substances. In addition, a detailed functional analysis of more *FOX* genes using mutants or overexpressing transgenic lines is required. Overall, our study provides clues for further functional analysis of *FOX* genes in the occurrence and therapy of cancers, which will help to open up new avenues for cancer prevention, diagnosis, and treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13101754/s1, Figure S1: Differential expression of *FOX* genes in different cancer diseases. Figure S2: *FOX* genes were expressed in response to 60 different chemical sub-stances.

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