RESEARCH ARTICLE

Comprehensive Expression Analysis Suggests Functional Overlapping of Human FOX Transcription Factors in Cancer

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Abstract

Forkhead-box (FOX) transcription factors comprise a large gene family that contains more than 50 members in man. Extensive studies have revealed that they not only have functions in control of growth and development, but also play important roles in different diseases, especially in cancer. However, biological functions for most of the members in the *FOX* family remain unknown. In the present study, the expression of 39 *FOX* genes in 48 kinds of cancer was mined from the Gene Expression Atlas database of European Bioinformatics Institute. The analysis results showed that some *FOX* genes demonstrate overlapping expression in various cancers, which suggests particular biological functions. The pleiotropic features of the *FOX* genes make them excellent candidates in efforts aimed to give medical treatment for cancers at the genetic level. The results also indicated that different *FOX* genes may have the synergy or antagonistics effects in the same cancers. The study provides clues for further functional analysis of *FOX* genes, especially for the pleiotropic biological functions and crosstalk of *FOX* genes in human cancers.

Keywords: *FOX* transcription factors - overlapping expression - cancer diseases - gene expression atlas

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Introduction

The *FOX* transcription factors (Forkhead box, *FOX*), as one of the largest gene families, are characterized by the highly conserved 110 amino acids DNA-binding domain (Wijchers et al., 2006). The *FOX* domain is also known as winged-helix domain with two wing-like loops and three α-helices structure (Kaestner et al., 2000). Genome-based bioinformatics analyses have showed that *FOX* proteins comprise a large family members identified in species ranging from yeasts to humans, and have been classified into different subfamilies (Kaestner et al., 2000; Mazet et al., 2003; Wotton et al., 2006; Tu et al., 2006; Benayoun et al., 2011). Animals appeared to have more *FOX* genes than fungi, for example, with 4 genes identified in Saccharomyces and Schizosaccharomyces, and over 50 members in the human (Wotton et al., 2006; Benayoun et al., 2011). Up to date, the members of the *FOX* gene family have been demonstrated to play important roles in diverse biological processes, including cell differentiation, embryonic development, morphogenesis, metabolism, and effectors of signal transduction (Kaestner et al., 2000; Carlsson et al., 2002; Pohl et al., 2005).

The *FOX* transcription factors also play key roles in the health and disease (Hannenhalli et al., 2009; Benayoun et al., 2011; Katoh et al., 2013). Mutations in eight different *FOX* genes including *FOXC1, FOXC2, FOXE1, FOXE3, FOXL2, FOXN1, FOXP2,* and *FOXP*3 have been associated with human congenital disorders or hereditary diseases (Carlsson et al., 2002). For example, *FOXC1* has been identified in patients with defects in development of the anterior chamber of the eye (Lehmann et al., 2000). Mutations in *FOXC2* lead to distichiasis, or double rows of eyelashes, together with lymphedema (Bell et al., 2001). Mutations in *FOXL2* cause variable eyelid defects and also associated with ovarian failure (Crisponi et al., 2001; De et al., 2001). *FOX* family genes are also involved in carcinogenesis (Katoh et al., 2013). Several *FOX* genes such as *FOXA1, FOXM1, FOXO1, FOXO2 (FOXO6), FOXO3, FOXO4, FOXP1, FOXR1* have been reported to play important roles in carcinogenesis as oncogenes and/or tumor suppressor genes (Katoh et al., 2013).

Although several *FOX* genes of human have been characterized functionally in the cancer diseases, the biological functions for the most members in this family remain unknown. To extend our understanding the biological function of the human *FOX* genes, we analyzed expression of human *FOX* genes in different cancer diseases from the European Bioinformatics Institute (EBI) (http://www-test.ebi.ac.uk/gxa/). The results suggested that most of human *FOX* family genes are different expression in cancer diseases. The results also provide

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clues for further functional analysis of *FOX* genes, especially for the pleiotropic biological functions and the crosstalk of *FOX* genes in cancer diseases of human.

Materials and Methods

The sequence analysis of human FOX transcription factors

Using "forkhead human" to search the human *FOX* family genes in the gene mode of National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/gene), the *FOX* genes were obtained. The protein sequences of human *FOX* transcription factors were obtained from National Center of Biotechnology Information. In addition, the chromosome, sites, location, and protein length of human *FOX* genes were obtained. Protein domain architectures of the *FOX* transcription factors were analyzed in SMART database (http://smart.embl-heidelberg.de/). The physics and chemistry features of *FOX* protein including molecular weight and isoelectric point, instability index, aliphatic index, and grand average hydropathicity were obtained from protParam (http://web.expasy.org/protparam/). The subcellular localization was predicted by Protcomp 9.0 (http://linux1.softberry.com/berry.phtml?).

Multiple sequence alignment of full-length protein sequences of human *FOX* transcription factors was performed using ClustalW2 program with default parameters. Phylogenetic tree was plotted using MEGA5.05 software by the Neighbor-joining method with 1000 bootstrap replicates. The conserved motifs in full length human *FOX* proteins were identified using Multiple Expectation Maximization for Motif Elicitation (MEME) program version 4.6.1 (http://meme.nbcr.net/meme/) with default parameters.

Expression profiling analysis in cancer diseases

The differential expression of human *FOX* genes in different cancer diseases was mined from Gene Expression Atlas database of European Bioinformatics Institute (http://www-test.ebi.ac.uk/gxa/). The Gene Expression Atlas is an added-value database providing information about gene expression in different cell types, organism parts, developmental stages, disease states, sample treatments and other biological/experimental conditions. The content of this database derives from re-annotation and statistical analysis of selected data from the arrayExpress archive of functional genomics data. The query results are ranked using various statistical measures and many independent studies in the database to show the particular gene-condition association. An interface allows us to query for differential gene expression *(i)* by *FOX* gene names; *(ii)* by cancer diseases. By the condition of P-value <0.05 of the automated screening, we have obtained the differential expression of 39 *FOX* genes in different cancer diseases. Moreover, P-value of up-regulated *FOX* genes was set as positive number, while the down-regulated genes as negative number. In order to illustrate the expression of *FOX* genes in the same cancer diseases, P-value was taken to the base 10 of a negative logarithm. Then invert P-value of up-regulated genes was set as positive number and down-regulated genes as negative number. The histograms were plotted with invert P-value and its corresponding *FOX* genes.

Results and Discussion

Identification of human FOX genes

Using "forkhead human" to search the human *FOX* family genes in the gene mode of National Center of Biotechnology Information, the 50 *FOX* genes of human were obtained (Table 1). The *FOX* transcription factors of human have been named by a captial letter, and a number is used to distinguish members in the same subclass. The method of definition is similar with previous report (Kaestner et al., 2000). The 50 protein sequences of human *FOX* genes were obtained from National Center of Biotechnology Information. Protein domain architectures of the *FOX* transcription factors were analyzed in SMART database. The physics and chemistry features of *FOX* protein are analyzed by protparam (Table 1). The amino acid number of *FOX* protein is from 292 to 748 with average 473. The weight of protein is from 33 to 82.7 kDa with average 50.5 kDa. There is positive correlation between protein molecular weight and the number of amino acid. The isoelectric point of protein is from 4.92 to 9.82 with average 7.95. The instability index of protein is from 49.44 to 86.35. The aliphatic index of protein is from 50 to 75.37 with average 64.67. The grand average hydropathicity of protein is from -0.887 to -0.163 with average -0.496. The subcellular localization was predicted by Protcomp 9.0. The results showed that almost of predicted nuclear location scores are above 9.9, which indicated that most of *FOX* transcription factors can locate in nuclear.

Chromosomal location and Phylogenetic analysis of human FOX genes

50 *FOX* genes distribute on 19 chromosomes of human (Table 1). Among them, 8 *FOX* genes locate on the Chromosome 9. Chromosome 1 and 6 contains 5 *FOX* genes, Chromosome 2, 12, 14, 16, 17 and X contains 3 *FOX* genes, Chromosome 3, 5, 7, and 20 contains 2 *FOX* genes, Chromosome 8, 10, 11, 13, 15, 19 contains 1 *FOX* genes, respectively. The phylogenic analysis suggested that human *FOX* transcription factors can be divided into A to S 19 subgroups (Figure 1). According to the phylogenetic tree and gene location analysis, it was found that there are fragments replication and string copying phenomenon of *FOX* transcription factors. For example, *FOXA1*/*FOXA2*, *FOXC1*/*FOXC2*, *FOXD1*/*FOXD2*, *FOXK1*/*FOXK2*, *FOXP1*/*FOXP2*, *FOXN1*/*FOXN4*, and *FOXN2*/*FOXN3* are fragments replication. Several genes *FOXD4L2*, *FOXD4L3*, *FOXD4L4*, *FOXD4L5*, and *FOXD4L6* are string copying. In order to analyze the diversity of human *FOX* proteins, the conserved motifs were identified. There were 2 or 3 conservative *FOX* motifs in the structure of human *FOX* domain. Among them, FOXH1, FOXJ2, FOXP1, FOXP2, FOXP3, FOXP4, FOXR1, and FOXR2 only have 2 motifs (motif 1 and motif 2). For the motif 1, the possible match is "QGWKNSIRHNLSLNDCFVKVPRE". For the motif 2, the possible match is

"PYSYIALITMAIQQSPEKRLTLNEIYQWI". For the motif 3, the possible match is "PGKGNYWTLDPDCEDMFENGSFLRRRKRF". Most of *FOX* motifs in the N end of the sequence, suggests that the presence of these elements for *FOX* execution of protein function is required.

Expression profiling analysis of FOX genes in cancer diseases

To study the possible involvement of *FOX* genes in different cancer diseases, we mined expression of human *FOX* genes in different cancer diseases from the European Bioinformatics Institute (EBI) (http://www-test.ebi.ac.uk/gxa/). The results showed that the differential expression of 39 *FOX* genes in various diseases, including 48 kind of cancer diseases.

Overlapping expression patterns of the *FOX* genes in response to different cancer diseases were analyzed. The results showed that *FOX* genes exhibited overlapping expression patterns in response to different cancer diseases. It is noteworthy that *FOX* genes exhibited overlapping expression patterns in response to brain tumor, adenoma, metastatic prostate cancer, renal cell carcinoma, acute myeloid leukemia, myeloma, B-cell lymphoma, papillary thyroid carcinoma, breast cancer, leiomyoma, oral squamous cell carcinoma, invasive ductal carcinoma (Table 1). For example, *FOXE1* was overlapping expression in eight cancer diseases. *FOXA2, FOXC1, FOXD3, FOXG1, FOXJ1 and FOXM1,* were overlapping expression in seven cancer diseases.

Table 1. The Distribution of FOX Transcription Factor Family on the Scaffolds of Genome and Physico-chemical analysis in Human

Gene	Chromo-some	Site	Location	Length/aa	Molecular Weight/Kda	Isoelectric point	Instability index	Aliphatic index	Grand average hydropathicity	Nuclear location score
FOXA1	14	NC_000014.9	37589552..37595120	472	49.1	8.93	55.56	50.61	-0.483	10
FOXA2	20	NC_000020.11	22581004..22585463	463	48.9	8.82	65.88	49.96	-0.532	10
FOXA3	19	NC_000019.10	45795710..45902604	350	37.1	7.01	55.93	60.57	-0.447	10
FOXB1	15	NC_000015.10	59871343..60397986	325	35	9.66	65.89	71.32	-0.279	9.9
FOXB2	9	NC_000019.12	77019655..770200953	432	45.6	9.55	61.54	65	-0.372	9.9
FOXC1	6	NC_000006.12	1528364..2245634	553	56.8	8.7	65.39	54.72	-0.534	9.9
FOXC2	16	NC_000016.10	86530176..86737841	501	53.6	8.68	53.6	60.3	-0.588	9.9
FOXD1	5	NC_000005.10	73168253..73509715	465	46.1	5.03	70.37	68.19	-0.215	10
FOXD2	1	NC_000001.11	47416072..47550750	495	48.7	6.76	55.24	63.84	-0.163	9.9
FOXD3	1	NC_000001.11	63159083..63361181	478	47.6	6.01	53.49	71.55	-0.181	9.9
FOXD4	9	NC_000009.12	45408..465259	439	47.3	9.38	74.03	68.18	-0.575	9.9
FOXD4L1	2	NC_000002.12	113406388..113572564	408	43.6	9.36	71.37	68.09	-0.554	10
FOXD4L2	9	NC_000009.11	42717234..42720342	416	45.9	9.74	70.8	67.69	-0.614	9.9
FOXD4L3	9	NC_000009.12	68302867..68305084	417	45.8	9.82	72.74	68.25	-0.579	10
FOXD4L4	9	NC_000009.12	65736314..65739422	416	45.9	9.74	70.8	67.69	-0.614	9.9
FOXD4L5	9	NC_000009.12	65282101..65285209	416	45.8	9.58	70.35	67.69	-0.59	9.9
FOXD4L6	9	NC_000009.12	41126251..41128975	417	45.8	9.76	72.39	68.01	-0.576	9.9
FOXE1	9	NC_000009.12	97698872..97922570	373	38.1	9.62	59.99	57.94	-0.279	10
FOXE3	1	NC_000001.11	47333797..47440691	319	33.2	9.72	72.06	71	-0.25	10
FOXF1	16	NC_000016.10	86434844..86555235	379	40.1	9.24	62.23	54.43	-0.468	9.9
FOXF2	6	NC_000006.12	961003..1515582	444	46	9.2	80.9	52.27	-0.395	9.9
FOXG1	14	NC_000014.9	28593773..28832060	489	52.4	8.99	51.19	53.95	-0.749	9.9
FOXH1	8	NC_000008.11	144428780..144507172	365	39.3	9.6	65.23	71.67	-0.416	10
FOXI1	5	NC_000005.10	169637247..170199141	378	41	5.89	64.71	55.21	-0.625	9.9
FOXI2	10	NC_000010.11	127737274..127741186	318	33	8.93	62.21	66.23	-0.291	9.9
FOXI3	2	NC_000002.12	88448207..88452535	420	43.3	8.51	71.92	55.74	-0.387	9.9
FOXJ1	17	NC_000017.11	76080993..76240367	421	45.2	5.04	56.8	71.54	-0.439	9.9
FOXJ2	12	NC_000012.12	7994770..8097777	574	62.4	6.2	72.69	50	-0.887	9.9
FOXJ3	1	NC_000001.11	42153421..42456267	622	69	6.59	60.88	60.05	-0.784	10
FOXK1	7	NC_000007.14	4682299..4771443	733	75.5	9.41	67.98	74.15	-0.17	10
FOXK2	17	NC_000017.11	82458184..82698722	660	69.1	9.56	57.53	74.55	-0.361	9.9
FOXL1	16	NC_000016.10	86555320..87063991	345	36.5	9.64	53.77	66.96	-0.481	10
FOXL2	3	NC_000003.12	138652698..139006268	376	38.8	9.26	66.04	55.35	-0.445	9.9
FOXM1	12	NC_000012.12	2812621..3040676	748	82.7	8.57	76.13	75.37	-0.609	9.9
FOXN1	17	NC_000017.11	28467778..28571869	648	68.9	5.93	62.97	64.35	-0.462	9.9
FOXN2	2	NC_000002.12	47989625..48776517	431	47.2	5.98	49.44	73.16	-0.565	10
FOXN3	14	NC_000014.9	88979098..89954777	468	51.5	6.71	65.6	56.5	-0.859	9.9
FOXN4	12	NC_000012.12	109277978..109309220	517	55.2	5.93	65.03	73.83	-0.36	9.9
FOXO1	13	NC_000013.11	40194509..40771211	655	69.7	6.28	61.9	57.13	-0.571	2.5
FOXO3	6	NC_000006.12	108559823..108684769	673	71.3	4.98	66.77	62.11	-0.594	6
FOXO4	X	NC_000023.11	70987306..71111631	505	53.7	5.13	66.38	72.89	-0.45	4.8
FOXO6	1	NC_000001.11	41361656..41383591	492	50.6	5.25	58.65	69.72	-0.362	6.1
FOXP1	3	NC_000003.12	70751067..71785206	677	75.3	6.19	60.95	75.23	-0.647	8.7
FOXP2	7	NC_000007.14	113116718..115125475	740	82.6	6.03	78.39	74.73	-0.743	8.9
FOXP3	X	NC_000023.11	49205063..49301464	431	47.2	9.52	55.68	73.41	-0.353	3.4
FOXP4	6	NC_000006.12	41381392..41667655	680	73.5	5.97	65.4	70.93	-0.574	7.2
FOXQ1	6	NC_000006.12	909119..1502550	403	41.5	9.52	67.45	69.58	-0.318	9.9
FOXR1	11	NC_000011.10	118896140..119018347	292	33.3	9.3	86.35	63.53	-0.757	5.3
FOXR2	X	NC_000023.11	55484616..55759333	311	35.9	4.92	77.51	64.28	-0.785	4.4
FOXS1	20	NC_000020.11	31739101..31968710	330	35.4	9.28	63.82	53.33	-0.492	9.9

FOXH1, *FOXJ3*, *FOXN2*, *FOXP1*, and *FOXP3* were overlapping expression in six cancer diseases. *FOXF2*, *FOXJ2*, *FOXK2*, *FOXO1*, and *FOXP2* were overlapping expression in five cancer diseases. *FOXB1*, *FOXC2*, *FOXD2*, *FOXI1*, *FOXL2* and *FOXO4* were overlapping expression in four cancer diseases. *FOXA1*, *FOXD4*, *FOXE3*, *FOXF1*, *FOXL1*, *FOXN1*, and *FOXQ1* were overlapping expression in three cancer diseases. Several *FOX* genes in this study showed the similar expression pattern with previous reported and these genes have been proved to play important roles in cancer diseases (Katoh et al., 2013). Among them, *FOXA1* is upregulated in estrogen receptor (ER)-positive breast cancer (Schneider et al., 2006) and *FOXA1* point mutations also occur in prostate cancer (Grasso et al., 2012). Overexpression of *FOXA1* is associated with estrogen receptor (ER)-positive breast cancer (Hu et al., 2009), lung cancer, esophageal

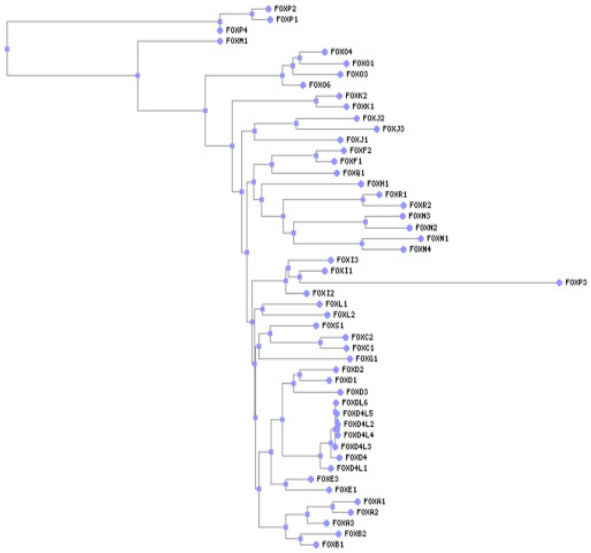

Figure 1. Phylogenetic Tree Analysis of Human FOX Genes. Multiple sequence alignment of full-length protein sequences of human FOX genes was performed using ClustalW2 program with default parameters. Phylogenetic tree was plotted using MEGA5.05 software by the Neighbor-joining method with 1000 bootstrap replicates

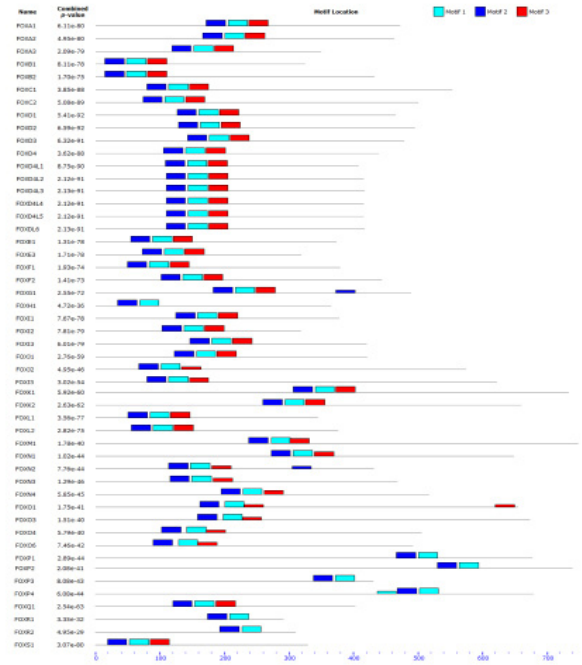

Figure 2. The Motif Analysis of Human FOX genes. The conserved motifs in full length human FOX proteins. The motifs were identified using Multiple Expectation Maximization for Motif Elicitation (MEME) program version 4.6.1 with default parameters and the maximum number of motifs to find was set to 12

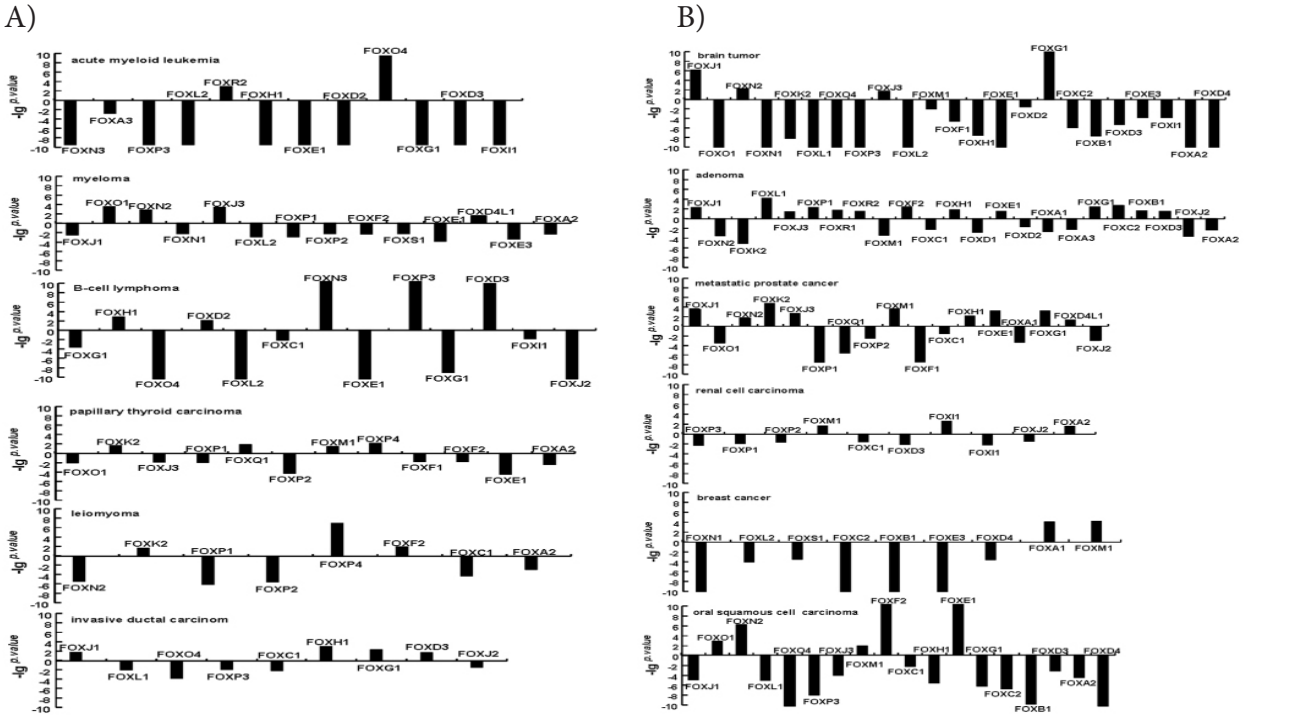

Figure 3. Differential Expressions of FOX Genes in Same Cancer Diseases. P-value was taken to the base 10 of a negative logarithm and copied out the number of P-value after changing. We set the up-regulated genes as positive number, while the down-regulated genes as negative number. Finally, we use each value and its corresponding FOX gene made a histogram

Table 2. Differential Expression of FOX Genes in Response to Cancer Diseases

Gene	brain tumor	adenoma	metastatic prostate cancer	renal cell carcinoma	breast cancer	oral squamous cell carcinoma	acute myeloid leukemia	myeloma	B-cell lymphoma	papillary thyroid carcinoma	leiomyoma	invasive ductal carcinoma
FOXA1	-	-0.004	-0.00034	-	6.00E-05	-	-	-	-	-	-	-
FOXA2	-1.00E-10	-0.009	-	0.026	-	-0.0000413	-	-0.007	-	-0.004	-0.002	-
FOXA3	-	-0.01	-	-	-	-	0.002	-	-	-	-	-
FOXB1	-2.15E-08	0.028	-	-	-1.00E-10	-2.11E-08	-	-	-	-	-	-
FOXC1	-	-0.011	-0.028	-0.033	-	-0.007	-	-	-0.011	-	-0.00008	-0.01
FOXC2	-0.000001	0.003	-	-	-1.00E-10	-0.00000023	-	-	-	-	-	-
FOXD1	-	-0.003	-	-		-	-	-	-	-	-	-
FOXD2	-0.029	-0.035	-	-	-	-	1.00E-10	-	0.011	-	-	-
FOXD3	-6.00E-06	0.037	-	-0.011	-	-0.000875	-1.00E-10	-	2.40E-10	-	-	0.017
FOXD4	-1.00E-10	-	-	-	-3.00E-04	-1.00E-10	-	-	-	-	-	-
FOXD4L1	-	-	0.048	-	-	-	-	0.026	-	-	-	-
FOXE1	-1.00E-10	0.037	0.000516	-	-	1.00E-10	1.00E-10	-0.0002	-1.00E-10	-3.00E-05	-	-
FOXE3	-1.00E-04	-	-	-	-1.00E-10	-	-	-0.0007	-	-	-	-
FOXF1	-3.10E-04	-	-2.26E-08	-	-	-	-	-	-	-0.017	-	-
FOXF2	-	0.005	-	-	-	1.00E-10	-	-0.006	-	-0.02	0.012	-
FOXG1	1.00E-10	0.005	0.000501	-	-	-0.00000082	1.00E-10	-	-1.76E-09	-	-	0.004
FOXH1	-	0.019	0.006	-	-	-0.00000374	1.00E-10	-	0.002	-	-	0.001
FOXI1	-1.00E-04	-	-	0.003	-	-	-1.00E-10	-	-0.018	-	-	-
FOXJ1	1.00E-06	0.007	0.00025	-	-	-0.000016	-	-0.004	-0.0004	-	-	0.014
FOXJ2	-	0.00058	-0.001	-0.039	-	-	-	-	-1.00E-10	-	-	-0.049
FOXJ3	0.019	0.04	0.002	-	-	-0.000135	-	0.00056	-	-0.015	-	-
FOXK2	-6.21E+09	-0.00002	0.0000124	-	-	-	-	-	-	0.025	0.024	-
FOXL1	-1.00E-10	0.00013	-	-	-	-0.0000117	-	-	-	-	-	-
FOXL2	-1.00E-10	-	-	-	-	-	-1.00E-10	-0.002	-1.00E-10	-	-	-
FOXM1	-0.011	-0.00076	0.000179	0.024	1.00E-05	0.015	-	-	-	0.038	-	-
FOXN1	-1.00E-10	-	-	-	-1.00E-10	-	-	-0.008	-	-	-	-
FOXN2	0.005	-0.00065	0.02	-	-	0.000001	-	0.002	-	-	-0.00001	-
FOXN3	-	-	-	-	-	-	1.00E-10	-	1.00E-10	-	-	-
FOXO1	1.00E-10	-	-0.00028	-	-	0.002	-	0.0004	-	-0.01	-	-
FOXO4	-1.00E-10	-	-	-	-	-1.00E-10	1.00E-10	-	-1.00E-10	-	-	-
FOXP1	-	0.006	-1.69E-08	-0.016	-	-	-	-0.001	-	-0.012	-1.00E-06	-
FOXP2	-	0	-0.003	-0.028	-	-	-	0.009	-	-0.0001	-4.00E-06	-
FOXP3	-1.00E-10	-	-	-0.006	-	-1.65E-08	1.00E-10	-	1.00E-10	-	-	0.018
FOXP4	-	-	-	-	-	-	-	-	-	0.007	1.00E-07	-
FOXQ1	-	-	-0.000002	-	-0.025	-	-	-	-	0.013	-	-
FOXR1	-	0.023	-	-	-	-	-	-	-	-	-	-
FOXR2	-	0.032	-	-	-	-	0.000714	-	-	-	-	-
FOXS1	-	-	-	-	-3.00E-04	-	-	-0.009	-	-	-	-

*The P-value of differential expressions of FOX genes in response to cancer diseases. The up-regulated genes as positive number and the down-regulated genes as negative number

cancer (Lin et al., 2002), and anaplastic thyroid cancer (Nucera et al., 2009). *FOXM1* is overexpressed in basal-type breast cancer (Curtis et al., 2012), non-Hodgkins lymphoma (Green et al., 2011), and malignant peripheral nerve sheath tumors (Yu et al., 2011). Down-regulation of *FOXM1* in laryngeal squamous carcinoma cells resulted in an inhibition of cell proliferation, migration, and invasion, which indicated that inhibition of *FOXM1* represents an attractive target for cancer therapy (Chen et al., 2011). Xu et al. (2012) indicated that *FOXM1* expression in tumor tissue had clinical significance for predicting recurrence in patients with non-small cell lung cancer after tumor surgery. *FOXO1* gene is located within the commonly deleted region in prostate cancer and the expression *FOXO1* is frequently down regulated in prostate cancer (Dong et al., 2006). *FOXO4* is fused to the MLL gene as a result of chromosomal translocation in acute lymphoblastic leukemia (ALL) (Dansen et al., 2008). *FOXC2* acts as regulators of Lymphangiogenesis and Angiogenesis in Oral Squamous Cell Carcinoma (Sasahira et al., 2014). The expression of *FOXC2* gene also increases with malignancy of Cervical Cancer, especially with blood vessel hyperplasia and invasion degree (Zheng et al., 2014). *FOXL1* plays an inhibitory role in renal tumor progression and over-expression of *FOXL1* can inhibit tumor cell growth, migration and invasion in renal cancer cells (Yang et al., 2013). The responsiveness of these cancer-responsive *FOX* genes with overlapping expression patterns might imply that they have pleiotropic biological functions in cancer diseases, therefore, the detailed functional analysis using mutants and/or overexpression transgenic lines is required. Taken together, the pleiotropic feature of the biological functions of the *FOX* genes make them become excellent candidates in efforts aimed to give medical treatment for cancer diseases at genetic.

The expression characteristics of *FOX* genes in the same kind of cancer disease were also analyzed (Figure 2, and Figure 3). Twenty-three *FOX* genes (4 up-regulated and 19 down-regulated) were differentially expressed in brain tumor. It is noted that most of them were down-regulated expression in the brain tumor indicated that the *FOX* family genes may play important roles in brain tumor. The study suggested that *FOXD2* and *FOXE3* maybe play suppressor roles in the meningioma tumor, which is a kind of brain tumor (Sulman et al., 2004). Twenty-three *FOX* genes (13 up-regulated and 10 down-regulated) were differentially expressed in adenoma. Seventeen *FOX* genes (9 up-regulated and 8 down-regulated) were found

to be differentially expressed in metastatic prostate cancer. Among them, *FOXA1* (Grasso et al., 2012), *FOXM1* (Lokody., 2014), and *FOXO1* (Dong et al., 2006) have been reported to play key roles in prostate cancer. Ten *FOX* genes (3 up-regulated and 7 down-regulated) were found to be differentially expressed in renal cell carcinoma. Nine *FOX* genes (2 up-regulated and 7 down-regulated) were differentially expressed in breast cancer. For example, *FOXA1* (Schneider et al., 2006; Hu et al., 2009), and *FOXM1* (Curtis et al., 2012) were upregulated expression in breast cancer, which play an important roles in breast cancer. In addition, eighteen *FOX* genes (5 up-regulated and 13 down-regulated) were found to be differentially expressed in oral squamous cell carcinoma. Twelve *FOX* genes (2 up-regulated and 10 down-regulated) were found to be differentially expressed in acute myeloid leukemia. Fourteen *FOX* genes (4 up-regulated and 10 down-regulated) were found to be differentially expressed in myeloma. Thirteen *FOX* genes (5 up-regulated and 8 down-regulated) were found to be differentially expressed in B-cell lymphoma. Twelve *FOX* genes (4 up-regulated and 8 down-regulated) were found to be differentially expressed in papillary thyroid carcinoma. Eight *FOX* genes (3 up-regulated and 5 down-regulated) were found to be differentially expressed in leiomyoma. Nine *FOX* genes (4 up-regulated and 5 down-regulated) were found to be differentially expressed in invasive ductal carcinoma. All of these results showed that multiple genes may have the synergy or antagonism effects in the same kind of cancer disease, which suggested that the *FOX* genes involved redundant function in the cancer disease.

In conclusions, the present study, we analyzed the expression of *FOX* genes in different cancer diseases. The cancer-responsive *FOX* genes with overlapping expression patterns might imply that they have pleiotropic biological functions in cancer diseases. The pleiotropic feature of the biological functions of the *FOX* genes make them become excellent candidates in efforts aimed to give medical treatment for cancer diseases at genetic. The cancer-responsive *FOX* genes are also different expression in the same disease, which indicated that they may have the synergy or antagonism effects in the same kind of cancer disease. In all, the results of this study provide clues for further functional analysis of *FOX* genes, especially for the pleiotropic biological functions and the crosstalk of *FOX* genes in cancer diseases of human

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