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Research Article

In Silico Analysis of the FOXP3 Transcription Factor Associated with T-Cell Oncogenesis

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ABSTRACT

Background: The X chromosome encoded FOXP3 gene is a unique regulator of the T-cell differentiation and immunosuppressive function. The nuclear transcription factor FOXP3 gene regulates lineage-specific differentiation in the Treg crucially maintenance of the immune homeostasis. The regulatory T-cell (Treg or CD4+ cells) play a role in the immune response for self-antigens, allergens, and tumours. However, FOXP3 gene function is inconsistent in tumorigenesis such as tumour-suppressive and tumour-promoting. A recent report suggested the FOXP3 gene repress tumorigenesis per effects on proliferation and apoptosis.

Objective: My objective was to investigate the FOXP3 gene from the FOX family in between *Homo sapiens* and *Mus musculus*.The study of the FOXP3 gene is currently mandatory to explore the molecular mechanisms of the Treg differentiation and immunosuppressive function in a particular organism.

Methods: I perform bioinformatics and computational tools and technique to the current knowledge of the FOX family in the mammalian genome. My procedure may be useful for future functional analysis of specific gene family in particular organisms.

Results: In this study, I conducted a compressive genome-wide survey of the FOX family in mammals. My findings documented the FOX family play an essential role during development. The functional regulation of the FOXP3 gene exhibits tumour suppressor activity. The specific structure, domain, motifs, phylogeny, gene expression, and chromosome locationanalysis suggested that the FOXP3 gene is a T-cell dependent gene.

Conclusion: My analysis data concluded the FOX family plays a crucial role during development. In contrast, the restricted expression of the FOXP3 gene in the T-cell is an immune-privileged. The ultimate function of the FOXP3 gene in tumour cells may represent a novel mechanism in the immune system.

Keywords: FOXP3; FOX family; Treg or CD+ cells; T-Cell Oncogenesis

INTRODUCTION

The genetic lesions of several autosomal tumour-suppressor genes intimates in the molecular pathogenesis of cancer. The cancer pathogenesis involves both inactivations of tumour-suppressor genes and activation of oncogenes. One of the most compelling aspects of cancer biology is the influence between caner-suppressor genes and oncogenes. The encoded protein of tumour-suppressor genes can inactivate oncogenes. Conversely, oncogenes may defeat the tumour-suppressor proteins. The epidemiology study suggested a role of X-linked genes controls the susceptibility of the cancers. The breakthrough of X chromosome encoded gene FOXP3 function in human leading autoimmune disorders. The FOXP3 gene function assigns critical novel insight into the biology of Treg and cellular mechanism of immune homeostasis. The immunosuppressive activity and regulatory T-cells (Treg) or CD4+ cells contribute to the progression of cancer and prevent the induction of specific immune response [1-3]. The regulatory T-cell has distinct

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lymphocyte lineage ability with an inhibitory property that affects the activation of the immune system. The FOXP3 gene characterizes by the specific forkhead box domain involved in the development of mammals [4,5]. The FOXP3 gene is a forkhead box DNA binding domain is conserved in evolution and consists of precise amino acid residues. The FOX (Forkhead-box) family contains 43 genes in the human genome [6]. A recent study suggested the FOXP3 gene expressed in normal breast, prostate, ovarian epithelium and also down-regulated expression in corresponding to the tumours [7-9]. The overexpression of FOXP3 gene reported in pancreatic adenocarcinoma [10], melanoma [11-13], hepatocellular carcinoma [14], leukaemia [15], bladder cancer [16], thyroid carcinoma [17] and cervical cancer [18] demonstrated critical question in the human cancer genome sequencing [19]. In contrast, the FOXP3 gene function control tumorigenesis by enabling tumour cells to lose tumour immunity. Thus previous reports supported the FOXP3 expression suppresses Treg proliferation in pancreatic carcinoma and melanoma cells [10, 13]. The FOXP3 gene expression in tumours suggested worse overall survival of the breast, bladder, and colorectal cancers [16, 20,21]. This study suggested either a pro-tumorigenic or anti-tumorigenic activity depend on clinical criteria. Numerous study in the animal model suggested that the inhibiting of Treg dramatically improves tumours and survival [22,23]. Treg plays a unique role in the prevention and development of effective autoimmunity. Therefore, the notable objective of this study is to evaluate the encoded FOXP3 gene that is immunogenic in nature and potential used as biomarker and target for immunotherapy. In this study, I reviewed the FOX family develops a novel therapeutics and preventives strategy in mammals. In contrast, FOXP3 gene functions play important roles in the biology of Treg and cellular mechanism of the immune homeostasis. One of the exciting developments in the cancer research findings prompted exploration of the fundamental genetic, immunogenic and molecular mechanism of the differentiation and function of Treg in oncogenesis.

MATERIALS AND METHODS

Sequence and database

Primary sequence retrieves from the different specific database (UniProt, KEGG, GenBank, EMBL, DDBJ and NCBI), and performed web base application SMART for identification of the particular domain in the query sequence. Pfam searched for retrieving protein family information. SWISS-MODEL performs for the structure prediction. The SWISS-MODEL is structural bioinformatics web-server for comparative modelling of 3D structure. The most accurate method for generating rational 3D structure and routinely used in many practical applications. This method makes the experimental protein structure to a build model of evolutionarily related proteins. The SWISS-MODEL is a continuously updated database of homology or comparative model of the organism proteome for biomedical research. Genome: The genome sequences download from genomic data in different specialized databases (NCBI and Ensemble).

Standalone tools and GO annotation

HMMER executes using multiple sequence alignments of the specific domain as a profile search. HMMER is a statistical algorithm, making multiple sequence alignment (MSA) of the particular domain as a profile search and implemented methods using probabilistic models called the profile hidden Markov model. The standalone BLAST performs for homologs gene in selected organisms. The BLAST2GO performs for the gene ontology annotation, is a bioinformatics and computational tool for high-throughput gene ontology annotation of the particular sequence. The practical information of the genes retrieves via Gene Ontology (GO) annotation, a controlled vocabulary of the functional attribute.

Domain, motif, and phylogeny

Multiple sequence alignment (MSA) method for calculate the best match of the homologs sequences and line them up so identities, similarities, and differences can observe. MSA of highest hits sequence analysis carried out by web-based tool MultAlin for identification of conserved domain. The identification of the molecular evolutionary relationship of the specific gene in the *Homo sapiens* and *Mus musculus,* MEGA7 performed for constructing a phylogenetic tree using Neighbor-Joining Methods. The MEME suite retrieves for the sequence motifs is a computational web-based tool for discovery and analysis of specific motifs.

Gene expression and chromosome location

The expression analysis carried out using the GENEVESTIGATOR tool is a high-performance search engine of gene expression in different biological contexts. GENEVESTIGATOR use to identify and validate novel targets. Chromosome location retrieves using gene card is a database of the human genes provide genomic information of all known and predicted human genes. This database is currently available for biomedical research such as gene, encoded protein and associated disease.

RESULTS

Structural Analysis

The primary sequence demonstrated the specific composition of nucleotides and peptides. The sequence composed of 1296 nucleotides and 431 peptides with 83 peptides binding to the DNA sequence is well-known as a forkhead domain (Table 1a and 1b). The peptide structure illustrated the forkhead domain involved in DNA binding. The forkhead domain also is known as "winged helix" domain. The 3D structure demonstrated alpha and beta proteins binds with B-DNA as a monomer interacts with the DNA backbone. The alpha helices assume a

compact structure that presents in the third helix to the major grove. The protein comprises a twisted antiparallel beta structure and random coil that interact with the minor groove (Figure 1).

Table 1 (a): Primary Structure –Nucleotide.

>FOXP3

ATGCCCAACCCCAGGCCTGGCAAGCCCTCGGCCCCTTCCTTGGCCCTTGGCCCATCCCCAGGAGCCTCGCCCAGCTGGAGGGCT
GCACCCAAAGCCTCAGACCTGCTGGGGGCCCGGGGCCCAGGGGGAACCTTCCAGGGCCGAGATCTTCGAGGCGGGGCCCATGC
CTCCTCTTCTTCCTTGAACCCCATGCCACCATCGCAGCTGCAGCTCTCAACGGTGGATGCCCACGCCCGGACCCCTGTGCTGCAG
GTGCACCCCCTGGAGAGCCCAGCCATGATCAGCCTCACACCACCCACCACCGCCACTGGGGTCTTCTCCCTCAAGGCCCGGCCT
GGCCTCCCACCTGGGATCAACGTGGCCAGCCTGGAATGGGTGTCCAGGGAGCCGGCACTGCTCTGCACCTTCCCAAATCCCAGT
GCACCCAGGAAGGACAGCACCCTTTCGGCTGTGCCCCAGAGCTCCTACCCACTGCTGGCAAATGGTGTCTGCAAGTGGCCCGGAT
GTGAGAAGGTCTTCGAAGAGCCAGAGGACTTCCTCAAGCACTGCCAGGCGGACCATCTTCTGGATGAGAAGGGCAGGGCACAATG
TCTCCTCCAGAGAGAGATGGTACAGTCTCTGGAGCAGCAGCTGGTGCTGGAGAAGGAGAAGCTGAGTGCCATGCAGGCCCACCTG
GCTGGGAAAATGGCACTGACCAAGGCTTCATCTGTGGCATCATCCGACAAGGGCTCCTGCTGCATCGTAGCTGCTGGCAGCCAAG
GCCCTGTCGTCCCAGCCTGGTCTGGCCCCCGGGAGGCCCCTGACAGCCTGTTTGCTGTCCGGAGGCACCTGTGGGGTAGCCATG
GAAACAGCACATTCCCAGAGTTCCTCCACAACATGGACTACTTCAAGTTCCACAACATGCGACCCCCTTTCACCTACGCCACGCTCA
TCCGCTGGGCCATCCTGGAGGCTCCAGAGAAGCAGCGGACACTCAATGAGATCTACCACTGGTTCACACGCATGTTTGCCTTCTTC
AGAAACCATCCTGCCACCTGGAAGAACGCCATCCGCCACAACCTGAGTCTGCACAAGTGCTTTGTGCGGGTGGAGAGCGAGAAGG
GGGCTGTGTGGACCGTGGATGAGCTGGAGTTCCGCAAGAAACGGAGCCAGAGGCCCAGCAGGTGTTCCAACCCTACACCTGGCC
CCTGA

Table 1 (b): Primary Structure -Peptide.

>FOXP3

MPNPRPGKPSAPSLALGPSPGASPSWRAAPKASDLLGARGPGGTFQGRDLRGGAHASSSSLNPMPPSQLQLPTLPLVMVAPSGARLGPLPHLQ
ALLQDRPHFMHQLSTVDAHARTPVLQVHPLESPAMISLTPPTTATGVFSLKARPGLPPGINVASLEWVSREPALLCTFPNPSAPRKDSTLSAVPQ
SSYPLLANGVCKWPGCEKVFEEPEDFLKHCQADHLLDEKGRAQCLLQREMVQSLEQQLVLEKEKLSAMQAHLAGKMALTKASSVASSDKG
SCCIVAAGSQGPVVPAWSGPREAPDSLFAVRRHLWGSHGNSTFPEFLHNMDYFKFHNMRPPFTYATLIRWAILEAPEKQRTLNEIYHWFTRMF
AFFRNHPATWKNAIRHNLSLHKCFVRVESEKGAVWTVDELEFRKKRSQRPSRCSNPTPGP

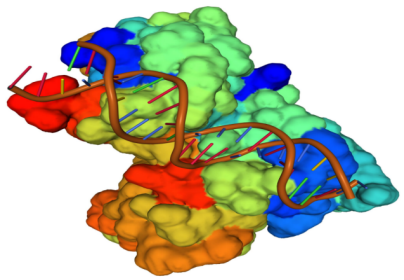

Figure 1: Tertiary structure (FOXP3).

Genome-wide Analysis

The genome-wide analysis by HMMER algorithm results shown that the multiple hits of 114 and 88 of the particular forkhead domains in *Homo sapiens* and *Musmusculus* respectively (Table 2). The standalone BLAST results represent 116 and 88 of homologs in *Homo sapiens* and *Musmusculus*, respectively (Table 2). Multiple hits selected from both organisms for gene ontology (GO) annotation (Table 3). The gene ontology annotation confirms that a total of 6 and 4 of the FOXP3 gene in *Homo sapiens* and *Musmusculus* respectively (Table 2).

Table 2: Summary of the (a) Forkhead domain and (b) FOX family

Organisms	**HMMER Hits**	**BLAST Hits**
Homo sapiens	114	116
Musmusculus	88	88
Total	202	204
Summary of the Fork head domain		
Gene	*Homo sapiens*	*Musmusculus*
FOXP3	6	4
FOXP1	11	13
FOXP4	5	4
FOXP2	11	4
FOXJ3	7	3
FOXJ2	2	5

FOXC2	1	1
FOXJ1	1	1
FOXC1	1	1
FOXN3	10	5
FOXL1	3	2
FOXG1	1	2
FOXE3	1	1
FOXK2	4	1
FOXE1	1	1
FOXI3	1	2
FOXI1	2	1
FOXN2	3	2
FOXA1	1	1
FOXA2	2	3
FOXA3	1	1
FOXS1	1	1
FOXI2	1	1
FOXF1	1	1
FOXD1	1	2
FOXD2	1	0
FOXF2	1	1
FOXL2	1	1
FOXD3	1	1
FOXM1	5	4
FOXO6	2	1
FOXD4	6	1
FOXO4	2	2
FOXK1	1	1
FOXO3	2	4
FOXO1	1	1
FOXN1	2	1
FOXQ1	1	1
FOXB1	1	1
FOXB2	1	1
FOXN4	3	1
FOXH1	1	1
FOXR1	2	0
FOXR2	1	2
FOXD5	0	0
FOXD6	0	0
FOXE2	0	0
Total	114	87

Table 3: Summary of the Gene Ontology annotation (a) *Homo sapiens* and (b) *Mus musculus*.

Gene Id	Gene	Protein
ENSP00000365372.2	FOXP 3	Forkhead box P3
ENSP00000428952.1	FOXP 3	Forkhead box P3
ENSP00000365380.4	FOXP 3	forkhead box protein P3 isoform X3
ENSP00000396415.3	FOXP 3	forkhead box protein P3 isoform X1
ENSP00000365369.1	FOXP 3	forkhead box protein P3 isoform X1
ENSP00000451208.1	FOXP 3	forkhead box protein P3 isoform X1
ENSP00000418225.1	FOXP 1	forkhead box protein P1 isoform X1
ENSP00000482847.1	FOXP 1	forkhead box protein P1 isoform X1
ENSP00000333560.4	FOXP 1	forkhead box protein P1 isoform X4
ENSP00000417857.1	FOXP 1	forkhead box protein P1 isoform X2
ENSP00000420736.1	FOXP 1	forkhead box protein P1 isoform X4
ENSP00000418524.1	FOXP 1	forkhead box protein P1 isoform X3

ENSP00000318902.4	FOXP1	forkhead box protein P1 isoform X4
ENSP00000418102.1	FOXP1	forkhead box protein P1 isoform X4
ENSP00000419393.1	FOXP1	forkhead box protein P1 isoform X4
ENSP00000362154.3	FOXP4	forkhead box protein P4 isoform X5
ENSP00000386958.1	FOXP4	forkhead box protein P4 isoform X4
ENSP00000362148.3	FOXP4	forkhead box protein P4 isoform X2
ENSP00000309823.4	FOXP4	forkhead box protein P4 isoform X1
ENSP00000362151.1	FOXP4	forkhead box protein P4 isoform X1
ENSP00000488944.1	FOXP2	forkhead box protein P2 isoform X3
ENSP00000377129.3	FOXP2	forkhead box protein P2
ENSP00000385069.4	FOXP2	forkhead box protein P2
ENSP00000377135.2	FOXP2	forkhead box protein P2
ENSP00000489135.1	FOXP2	forkhead box protein P2
ENSP00000386200.3	FOXP2	forkhead box protein P2
ENSP00000265436.7	FOXP2	forkhead box protein P2
ENSP00000377132.2	FOXP2	forkhead box protein P2
ENSP00000489073.1	FOXP2	forkhead box protein P2
ENSP00000489229.1	FOXP2	forkhead box protein P2
ENSP00000484803.1	FOXP1	forkhead box protein P1 isoform X1
ENSP00000377130.3	FOXP2	forkhead box protein P2
ENSP00000418883.1	FOXP1	forkhead box protein P1 isoform X4
ENSP00000403060.1	FOXJ3	forkhead box protein J3-like
ENSP00000393408.1	FOXJ3	forkhead box protein J3 isoform X3
ENSP00000354449.1	FOXJ3	forkhead box protein J3 isoform X3
ENSP00000354620.1	FOXJ3	forkhead box protein J3 isoform X1
ENSP00000361653.1	FOXJ3	forkhead box protein J3 isoform X1
ENSP00000361654.1	FOXJ3	forkhead box protein J3 isoform X1
ENSP00000439044.1	FOXJ3	forkhead box protein J3 isoform X1
ENSP00000403411.2	FOXJ2	forkhead box protein J2
ENSP00000162391.3	FOXJ2	forkhead box protein J2 isoform X1
ENSP00000326371.4	FOXC2	forkhead box protein C2
ENSP00000323880.4	FOXJ1	forkhead box protein J1
ENSP00000370256.2	FOXC1	forkhead box protein C1
ENSP00000451135.1	FOXN3	forkhead box protein N3 isoform X3
ENSP00000326272.3	FOXL1	forkhead box protein L1
ENSP00000339004.3	FOXG1	forkhead box protein G1
ENSP00000334472.2	FOXE3	forkhead box protein E3
ENSP00000452005.1	FOXN3	forkhead box protein N3 isoform X1
ENSP00000452227.1	FOXN3	forkhead box protein N3 isoform X1
ENSP00000479114.1	FOXN3	forkhead box protein N3 isoform X1
ENSP00000261302.5	FOXN3	forkhead box protein N3 isoform X1
ENSP00000343288.4	FOXN3	forkhead box protein N3 isoform X1
ENSP00000472376.1	FOXL1	forkhead box protein L1
ENSP00000433167.1	FOXK2	forkhead box protein K2
ENSP00000364265.3	FOXE1	forkhead box protein E1
ENSP00000478384.2	FOXI3	forkhead box protein I3

ENSP00000304286.5	FOXI1	forkhead box I1
ENSP00000432663.2	FOXK2	forkhead box protein K2
ENSP00000388486.1	FOXN2	forkhead box protein N2
ENSP00000250448.2	FOXA1	hepatocyte nuclear factor 3-alpha
ENSP00000484534.1	FOXN2	forkhead box protein N2
ENSP00000343633.3	FOXN2	forkhead box protein N2
ENSP00000304004.1	FOXA3	hepatocyte nuclear factor 3-gamma
ENSP00000436108.2	FOXK2	forkhead box protein K2
ENSP00000366319.4	FOXA2	hepatocyte nuclear factor 3-beta isoform X2
ENSP00000335677.5	FOXK2	forkhead box protein K2
ENSP00000400341.3	FOXA2	hepatocyte nuclear factor 3-beta isoform X1
ENSP00000365145.3	FOXS1	forkhead box protein S1
ENSP00000373572.4	FOXI2	forkhead box protein I2
ENSP00000262426.4	FOXF1	forkhead box protein F1
ENSP00000481581.1	FOXD1	forkhead box protein D1
ENSP00000335493.5	FOXD2	forkhead box protein D2
ENSP00000259806.1	FOXF2	forkhead box protein F2
ENSP00000333188.3	FOXL2	forkhead box protein L2
ENSP00000415483.2	FOXI1	forkhead box protein I1 isoform X2
ENSP00000360157.2	FOXD3	forkhead box protein D3
ENSP00000354492.3	FOXM1	Forkhead box M1
ENSP00000486536.1	FOXM1	Forkhead box M1
ENSP00000352901.3	FOXM1	forkhead box protein M1 isoform X1
ENSP00000342307.2	FOXM1	forkhead box protein M1 isoform X1
ENSP00000492766.1	FOXL1	forkhead box L1-like
ENSP00000493184.1	FOXO6	forkhead box protein O6
ENSP00000486069.5	FOXO6	forkhead box protein O6
ENSP00000302756.5	FOXD4	forkhead box protein D4-like 1
ENSP00000363377.3	FOXO4	forkhead box protein O4
ENSP00000328720.4	FOXK1	forkhead box protein K1
ENSP00000484875.1	FOXD4	Forkhead box D4-like 6
ENSP00000341961.2	FOXD4	Forkhead box D4-like 6
ENSP00000366637.1	FOXD4	Forkhead box D4-like 6
ENSP00000366630.1	FOXD4	Forkhead box D4-like 6
ENSP00000339527.6	FOXO3	forkhead box protein O3
ENSP00000385824.1	FOXO3	forkhead box protein O3
ENSP00000371940.2	FOXD4	Forkhead box D4
ENSP00000368880.4	FOXO1	forkhead box protein O1
ENSP00000226247.2	FOXN1	forkhead box protein N1
ENSP00000464645.1	FOXN1	forkhead box protein N1
ENSP00000296839.2	FOXQ1	forkhead box protein Q1
ENSP00000379369.4	FOXB1	forkhead box protein B1
ENSP00000342209.3	FOXO4	forkhead box protein O4 isoform X2

ENSP00000365898.1	FOXB2	forkhead box protein B2
ENSP00000299162.5	FOXN4	forkhead box protein N4
ENSP00000366534.4	FOXH1	forkhead box protein H1
ENSP00000450684.1	FOXN3	forkhead box protein N3 isoform X1
ENSP00000347354.1	FOXN4	forkhead box protein N4
ENSP00000474754.1	FOXN4	forkhead box protein N4
ENSP00000451024.1	FOXN3	forkhead box protein N3 isoform X3
ENSP00000314806.3	FOXR1	forkhead box protein R1
ENSP00000487495.3	FOXR1	forkhead box protein R1
ENSP00000450833.1	FOXN3	forkhead box protein N3 isoform X1
ENSP00000451437.1	FOXN3	forkhead box protein N3 isoform X3
ENSP00000442309.1	FOXM1	forkhead box protein M1 isoform X1
ENSP00000427329.2	FOXR2	forkhead box protein R2

Homo sapiens

Gene Id	Gene	Protein
ENSMUSP00000041953.6	FOXP3	forkhead box protein P3
ENSMUSP00000111403.1	FOXP3	forkhead box protein P3
ENSMUSP00000111404.1	FOXP3	forkhead box protein P3
ENSMUSP00000111405.1	FOXP3	forkhead box protein P3
ENSMUSP00000135809.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000134817.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000135098.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000135635.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000135041.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000108952.2	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000135181.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000135764.1	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000108954.2	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000073953.5	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000108948.2	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000108950.2	FOXP1	forkhead box protein P1 isoform X1
ENSMUSP00000108890.1	FOXP4	forkhead box protein P4 isoform X4
ENSMUSP00000108887.1	FOXP4	forkhead box protein P4 isoform X1
ENSMUSP00000108888.1	FOXP4	forkhead box protein P4 isoform X1
ENSMUSP00000094916.2	FOXP4	forkhead box protein P4 isoform X1
ENSMUSP00000111134.1	FOXP2	forkhead box protein P2 isoform X4
ENSMUSP00000111132.1	FOXP2	forkhead box protein P2 isoform X4
ENSMUSP00000031545.7	FOXP2	forkhead box protein P2 isoform X4
ENSMUSP00000111137.1	FOXP2	forkhead box protein P2 isoform X4
ENSMUSP00000108955.3	FOXP1	forkhead box protein P1 isoform X5
ENSMUSP00000145438.1	FOXJ2	forkhead box protein J2
ENSMUSP00000123815.1	FOXJ3	forkhead box protein J3 isoform X3
ENSMUSP00000124806.1	FOXJ3	forkhead box protein J3 isoform X3

ENSMUSP00000101917.2	FOXJ3	forkhead box protein J3 isoform X2
ENSMUSP00000035746.8	FOXJ3	forkhead box protein J3 isoform X1
ENSMUSP00000003238.7	FOXJ2	forkhead box protein J2
ENSMUSP00000137645.1	FOXJ2	forkhead box protein J2
ENSMUSP00000055290.6	FOXC2	forkhead box protein C2
ENSMUSP00000038351.6	FOXJ1	forkhead box protein J1
ENSMUSP00000052196.2	FOXC1	forkhead box protein C1
ENSMUSP00000137732.1	FOXL1	forkhead box protein L1
ENSMUSP00000050445.2	FOXE3	forkhead box protein E3
ENSMUSP00000021333.3	FOXG1	forkhead box protein G1
ENSMUSP00000136372.1	FOXG1	forkhead box protein G1
ENSMUSP00000036035.4	FOXN3	forkhead box protein N3 isoform X3
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ENSMUSP00000065664.5	FOXI3	forkhead box protein I3
ENSMUSP00000125380.1	FOXI3	forkhead box protein I3
ENSMUSP00000041118.6	FOXA1	hepatocyte nuclear factor 3-alpha
ENSMUSP00000145473.1	FOXM1	forkhead box protein M1 isoform X1
ENSMUSP00000043173.5	FOXA3	hepatocyte nuclear factor 3-gamma
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ENSMUSP00000100725.3	FOXD1	forkhead box protein D1
ENSMUSP00000058651.2	FOXI1	forkhead box protein I1
ENSMUSP00000101719.1	FOXK2	forkhead box protein K2
ENSMUSP00000045918.3	FOXA2	hepatocyte nuclear factor 3-beta isoform X2
ENSMUSP00000105590.1	FOXA2	hepatocyte nuclear factor 3-beta isoform X1
ENSMUSP00000096806.2	FOXS1	forkhead box protein S1
ENSMUSP00000137662.1	FOXF1	forkhead box protein F1
ENSMUSP00000066868.3	FOXD1	forkhead box protein D1
ENSMUSP00000053641.5	FOXI2	forkhead box protein I2
ENSMUSP00000046789.2	FOXF2	forkhead box protein F2
ENSMUSP00000053297.2	FOXL2	forkhead box protein L2
ENSMUSP00000118378.1	FOXN2	forkhead box protein N2
ENSMUSP00000145305.1	FOXM1	forkhead box protein M1 isoform X1
ENSMUSP00000084541.3	FOXD3	forkhead box protein D3
ENSMUSP00000107776.1	FOXM1	Forkhead box M1
ENSMUSP00000073041.6	FOXM1	forkhead box protein M1
ENSMUSP00000137099.1	FOXL1	forkhead box L1-like
ENSMUSP00000099716.3	FOXO6	forkhead box protein O6
ENSMUSP00000058575.2	FOXD4	forkhead box protein D4

ENSMUSP00000059420.4	FOXO4	forkhead box protein O4
ENSMUSP00000050683.3	FOXO3	forkhead box protein O3
ENSMUSP00000101141.1	FOXO3	forkhead box protein O3
ENSMUSP00000135380.1	FOXO3	forkhead box protein O3
ENSMUSP00000072616.5	FOXK1	forkhead box protein K1
ENSMUSP00000055308.5	FOXO1	forkhead box protein O1
ENSMUSP00000103929.1	FOXN1	forkhead box protein N1 isoform X1
ENSMUSP00000036952.8	FOXQ1	forkhead box protein Q1
ENSMUSP00000096197.2	FOXB1	forkhead box protein B1
ENSMUSP00000116165.1	FOXO4	forkhead box protein O4
ENSMUSP00000072687.2	FOXB2	forkhead box protein B2
ENSMUSP00000047951.5	FOXN4	forkhead box protein N4
ENSMUSP00000036591.4	FOXH1	forkhead box protein H1
ENSMUSP00000093984.3	FOXR2	forkhead box protein R2
ENSMUSP00000128452.1	FOXR2	forkhead box protein R2
ENSMUSP00000101140.1	FOXO3	forkhead box protein O3
ENSMUSP00000123923.1	FOXJ3	forkhead box protein J3 isoform X4
ENSMUSP00000134081.1	FOXA2	hepatocyte nuclear factor 3-beta isoform X2

Musmusculus

Analysis of the domain, motifs, and phylogeny

The highest hits of the FOXP3 gene listed from both organisms for sequence alignment, a multiple sequence alignment (MSA) determine the conserved domain (Figure 2). The high consensus (90%) sequence indicates the extended forkhead domain and their specific motifs (Figure 3a, 3b, and 3c). The phylogenetic tree demonstrated the molecular evolutionary relationship of the FOXP3 gene in between *Homo sapiens* and *Musmusculus*. Particular clades represent the multifunctional forkhead domain involved genes in both organisms (Figure 4).

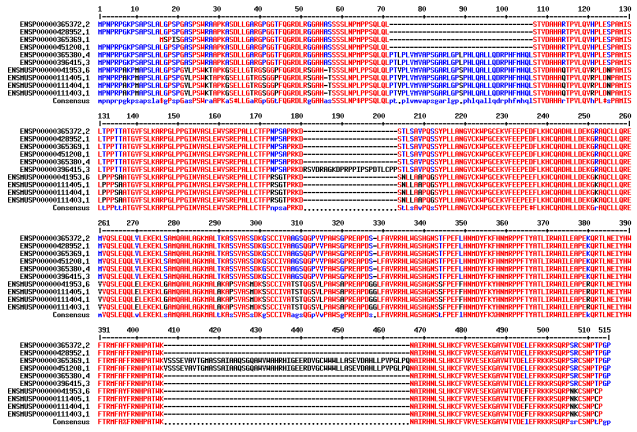

Figure 2: Multiple sequence alignment (Forkhead domain).

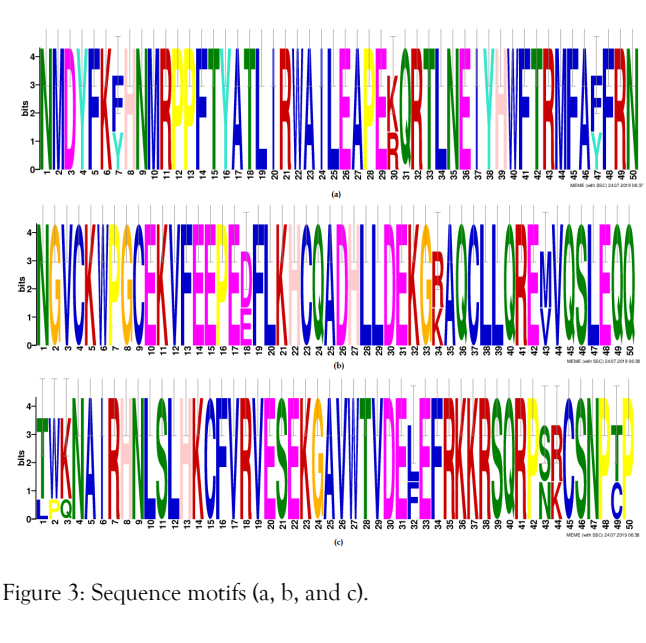

Figure 3: Sequence motifs (a, b, and c).

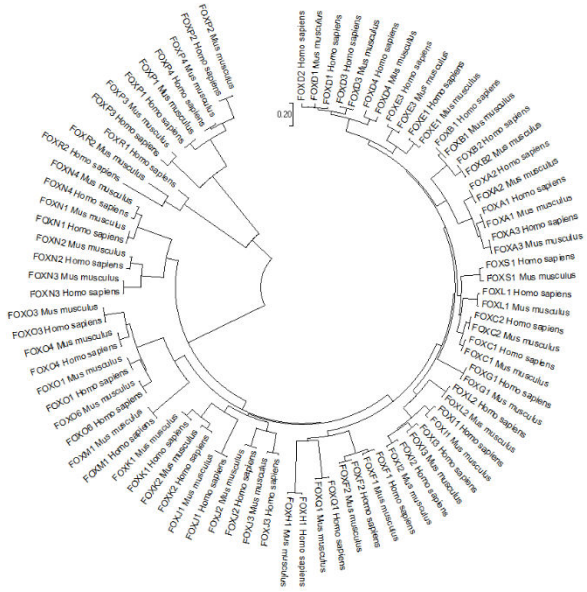

Figure 4: Phylogenetic tree (FOX family).

Analysis of the gene expression and chromosome location

The gene expression analysis of four anatomical cancer categories shown that the low level of FOXP3 expression and express in neoplasm of secondary/ill-defined, malignant neoplasm, malignant melanoma (unstable behavior) is shown in Figure 5. The chromosome location study demonstrated that the FOXP3 located band Xp11.23, start 49,250,436 bp and end 49,270,477 bp is shown in Figure 6.

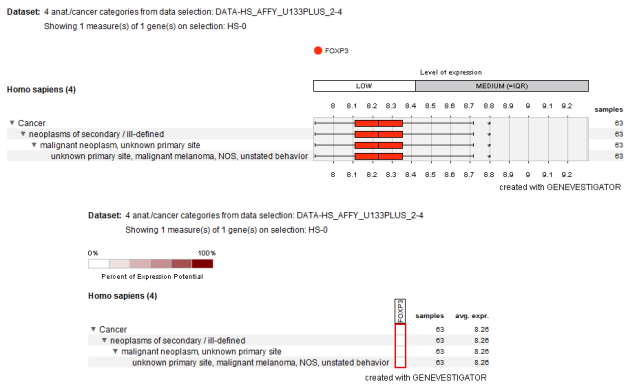

Figure 5: Gene expression(FOXP3).

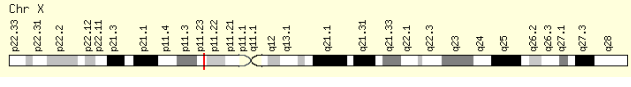

Figure 6: Chromosome location(FOXP3).

DISCUSSION

The immune system is a subject of the self and no self-discrimination both is directly fighting against the pathogens and maintains tolerance to self-antigens in mammals. Immune tolerance of the T-cells is achieving through central and peripheral strategies. The identification and characterization of regulatory T-cells (Treg or CD4+ cells) play unique roles in immune tolerance to self and transplant antigens [24-28].

Since the T-cell deficiency is a subject of lack of Treg in individual, it remains unknown the Treg function in the adult develop the immune system is not critical in the newborn. According to the genome, sequencing study suggested the elimination of Treg or CD4+ cells in the adult's restively mild immune-mediated lesions and compression associated with congenital T-cell deficiency. In this study, my findings suggested the fundamental roles of FOXP3 gene dependent for differentiation and Treg function in the immune system and raise a question, how numerous inherent mechanisms prevent differentiation of Treg in the thymus and restrain their activity in the periphery to ensure self-tolerance.

FOXP3 gene considers as a master regulatory function in typically derived naturally occurring Treg (CD4+ cells). The FOXP3 express in Treg cells found in the major histocompatibility complex (MHC) class 2 restricted CD4+ cells and express high levels of CD25 (IL-2). An addition of FOXP3 also express CD4+ cells and CD25+ cells appears in minor histocompatibility complex (MHC) class 1 restricted CD8+ cells expression in Treg [29,30].

Therefore, the expression of CD8+ cells can kill tumour cells by the release of perforin and granzymes. The Treg also included Type 1 regulatory cells (Th1 or CD4+ cells), T helper 3 cells (Th3), CD8, CD28, and HLA-E restricted T-cells. The Treg function is associated with CD28 and CTLA-4 receptor molecules both potentially bind with two natural ligands such as CD80 and CD86 are capable of Treg-suppressive capacity [31].

The CD86 (B7-2) strongly enhances suppression by CD4+ CD25+ and Treg cells, the blocking of CD80 (B7-1) enhance proliferative responses by reducing Treg suppression. Comparative expression of CD80 and CD86 on dendritic cells regulates during the progression of abnormal to a normal state with the ability of Treg-suppressive responses. The CD80 and CD86 have rival functions through CD28 and CD152 (CTLA-4) on Treg that has remarkable effect for control of immune responses. The initiation of Treg cells and expression of immune checkpoint molecules includes CTLA-4, PD-1 (CD279), TIM-3, LAG-3, and TIGIT [32,33].

The prominent rule of PD-1 receptor is mainly express on T cells and interacts with their two ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) play roles in the initiation and maintenance of peripheral tolerance mechanism [34]. The PD-L1 and PD-L2 express on antigen-presenting cells (APCs) and tumours release a negative signal to T cells, which is called T-cell exhaustion. Antibodies binding CTLA-4, PD-1 and PD-L1 have striking ability to enhance anti-cancer immunotherapy [35,36].

Therefore, Treg activity can evoke anti-tumour immunity and acknowledge inhibition of the T-cells function. The peptide inhibiter protein probably binds to the forkhead/winged-helix transcription factor 3 (FOXP3) which necessary for the development and function of Treg (CD4+ cells). The CD4+ cells produce transforming growth factor-beta and IL10 for suppressive function in the immune system and allow cell survival. The peptide P60 enter into the cells and inhibits FOXP3 nuclear translocation and reduces its ability to suppress the transcription factors NF-kB and NFAT both regulate transcription of DNA, cytokine production and cell survival. The functional evidence of the FOXP3 gene represses the expression of CD340, SKP2, SATB1 and MYC oncogenes and induces expression of tumour suppressor genes P21 and LATS2. The FOXP3 gene also pioneers of the anti-tumourenzymes such as CD39 and CD8 [37].

The functional inhibition of Treg by the FOXP3-inhibitory peptide initiates a strategy to enhance antitumor immunotherapies [3]. This study describes the significant advances of the FOXP3 gene as an X-linked tumour suppressor gene in between *Homo sapiens* and *Musmusculus*. This work represents a compelling exception and widely accepted theory of the inactivation of tumour suppressor genes. The oncogenes are a heterogeneous group that is generally expressed in the cells and trophoblast and also aberrantly activated in various cancers [38].

A subset of oncogenes encodes antigens that are immunogenic and elicit humoral and cellular immune responses in cancers [39]. Limited evidence supported the FOXP3 gene is a tumour suppressor gene. In this report, my finding suggested the expression of FOXP3 gene in cancer cells provide evidence that this could be important in tumour escape mechanisms. In this study, I discuss the multiple in-silico analysis and comprehensive genome-wide survey of the FOX family is involved in the development process in mammals. In contrast, the restricted expression of FOXP3 gene in the genome is an immune-privileged and therapeutic strategy in cancers. The conserved domain, motifs, phylogeny, gene expression, and chromosome location analysis suggested the mechanism of the FOXP3 gene is conserved in evolution. The phylogeny analysis has apparent the large gene family consist of specific genes is closely related to each other.

Thus study suggested the FOXP3 gene acts as transcriptional activator and repressor and also targets makeup as a T-cell dependent gene. The fundamental contribution of the FOXP3 gene in tumour cells may represent a novel mechanism in the immune system. This study is consistent with the results of an in-silico analysis of FOXP3 gene target in T-cells. Therefore, my finding supported the genetic, immunologic and molecular mechanisms of the X chromosome encoded FOXP3 gene associated with T-cell oncogenesis.

CONCLUSION

My analysis data concluded that the FOX family of transcription factors developed therapeutics and preventives strategy in mammals. In contrast, the restricted expression of FOXP3 in the cell is an immune-privilege and therapeutic strategy. The ultimate transcriptional regulation of FOXP3 in tumour cells may represent a novel mechanism in the immune system.

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