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Potential Functions of the Human Homeobox *TGIFLX/Y* Genes in Normal and Abnormal Development

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ABSTRACT

Homeobox genes encode transcription factors that play important roles in the developmental and normal cellular processes in all metazoans. *TGIFLX/Y* (*TGIFLX* and *TGIFLY*) are members of the homeobox superfamily of genes. Their expression is specifically detected in human adult testis but their functions remain to be investigated. Identification of relevant target genes should make a key contribution to a complete understanding of the mechanisms by which *TGIFLX/Y* functions in both normal and abnormal developmental processes. In this review, we provide an overview of recent studies on different aspects of *TGIFLX/Y* with a focus on the current state of research about their roles in tumorigenesis and azoospermia.

Keywords: homeobox genes, TALE class, TGIF family, *TGIFLX*, *TGIFLY*, azoospermia, prostate cancer

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INTRODUCTION

In humans there are about 230 Hox genes, a group of evolutionarily conserved genes encoding a large family of transcription factors that play important roles in various developmental processes.[1,2] They are characterized by the presence of a specific sequence motif known as the homeobox translated to the 60-amino acid homeodomain, a helix-turn-helix DNA binding domain.[1,3,4] The three-amino acid loop extension (TALE) group of homeodomain proteins have a 3-amino acid insertion between helices 1 and 2 of the homeodomain resulting in a 63-amino acid homeodomain instead of the typical 60-amino acid one.[5] Cooperative function among TALE family members is critical for transcription regulation[6] and it is well established that they can act as transcriptional activators and repressors.[7]

The TGIF (TG-interacting factor) family belongs to the TALE class of homeobox-containing genes known to play critical roles in developmental process such as cell proliferation, differentiation, and cell fate.[8,9] The human TGIF family includes *TGIF* (also known as *TGIF1*), *TGIF2*, and *TGIF2LX/Y* (TGIF2-like on X or Y chromosome), also known as *TGIFLX/Y*.[10] Studies in humans have indicated that loss-of-function mutations in *TGIF* cause holoprosencephaly (HPE), a severe genetic disease affecting the forebrain and facial structures.[11,12] TGIF2 protein is overexpressed in many ovarian cancer cell lines.[13] It seems that TGIF and TGIF2 act as transcriptional repressors of TGF-ß signaling pathway.[14–16]

The *TGIFLX/Y* genes were originally identified in human adult testis and suggest implication in testicular development and spermatogenesis.[17] However, the mechanisms by which TGIFLX/Y genes act in normal and abnormal development remain to be elucidated. In this review, we take a detailed look at the evolution, expression, and function of the *TGIFLX/Y* genes that are implicated in both normal and abnormal development.

TGIFLX/Y GENE EXPRESSION AND EVOLUTION

The *TGIFLX* and *TGIFLY* loci were mapped to chromosomal regions Xq21.3 and Yp11.2, respectively.[17] The *TGIFLY* locus is located at the distal part of a male-specific region (MSY) on ''p'' arm of the Y chromosome (**Figure 1**). The MSY, which was previously known as nonrecombining region (NRY), accounts for 95% of the length of the Y chromosome. The MSY consists of four different fragments including a heterochromatic block and three classes of euchromatic sequences: X-transposed, X-degenerate, and ampliconic.[18] The X-transposed region (XTR) comprises a sequence of 3.4 Mb in distal Yp and exhibits 99% homology to XTR in Xq21, a chromosomal band in the ''p'' arm of the human X chromosome with a? middle position.[18,19] The X-transposed sequences are so named because their presence in the human MSY is the result of a massive X-to-Y transposition that occurred about three to four million years ago after the divergence of the human and chimpanzee lineages.[20–23] Three protein-coding genes, *PABPC5*,[24] *PCDHX*,[25] and *TGIFLX*[17] have been identified in the XTR of Xq21. Different studies have revealed that the XTR of the Y chromosome harbors two coding sequences, *PCDHXY*

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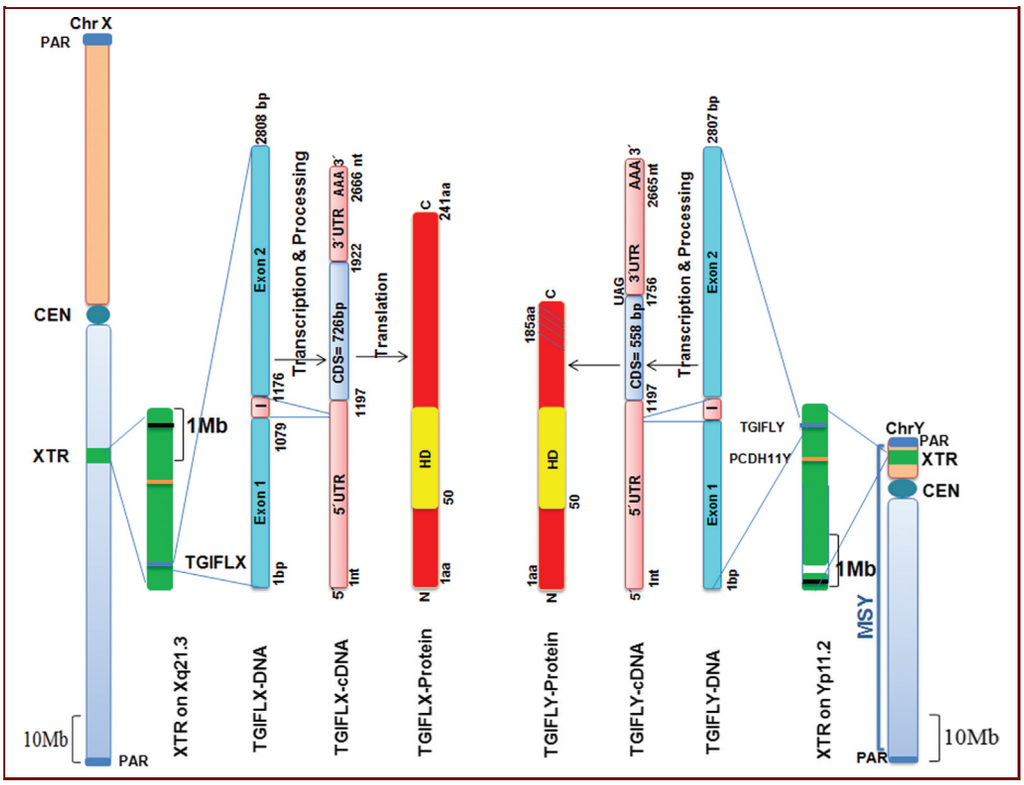

Figure 1. Chromosomal locations and mRNA transcript structures of *TGIFLX* and *TGIFLY*. (Left) The X-transposed region (XTR) comprises a 3.4 Mb sequence at chromosomal region Xq21.3. One of the three protein-coding genes in this region, *TGIFLX* encodes a 2666 bp mature transcript. The coding sequence (CDS), contained within exon 2, produces a 241-amino acid polypeptide with two conserved domains including a 63-amino acid homeodomain and a 21-amino acid segment known as CCD (C-terminus conserved domain). (Right) XTR of Yp11.2 close to pseudoautosomal region (PAR) of "p" arm of Y chromosome contains two genes, *PCDH11Y* and *TGIFLY*. The *TGIFLY* gene has undergone a single nucleotide deletion in codon 149, producing a frameshift and leading to the production of a 185-amino acid truncated polypeptide. The C-terminal 37-amino acids of the *TGIFLY* protein after the frameshift are different from its counterpart *TGIFLX* protein. Abbreviations: CEN = centromere, ChrX = X chromosome, ChrY = Y chromosome, I = intron, 5′ UTR = 5′ untranslated region, 3′ UTR = 3′ untranslated region, AAA = poly (A) tail.

and *TGIFLY*.[17,25] The Y chromosomal version of PABPC5 has been lost due to XTR inversion and cleavage during human evolution.[24] Comparative DNA analysis has shown that *TGIFLX* originated from retrotransposition of *TGIF2*, located on the long arm of chromosome 20 (q11.2-12), onto its current position on the X chromosome in a common ancestor of rodents and primates.[17,26]

TGIFLX is 2808 base pairs (bp) in length and comprises two exons and one intron, with the coding region being located within exon 2. The mRNA transcript encoded by the *TGIFLX* gene has a length of 2666 bp. However, the majority of the transcript sequence has been found to be 5′-untranslated region (5′-UTR) and 3′ -UTR. Therefore, the *TGIFLX* coding region is just 726 bp, which expresses a 26.675 kDa protein of 241 amino acids (**Figure 1**). Amino acid sequence alignment of this polypeptide showed two conserved domains including a 63-amino acid homeodomain near the N-terminus and a 21-amino acid segment known as the CCD (C-terminus conserved domain) at the C-terminus.[17] It is believed that the *TGIFLX* protein, like other homeoproteins, regulates its target genes via direct interaction of the homeodomain with specific DNA sequences, whereas the CCD is thought to be involved in protein–protein interactions.[17] To study its target sequences, production of *TGIFLX* recombinant protein is useful. A GST-TGIFLX fusion protein has been expressed in a prokaryotic system.[27] The recombinant protein is a valuable tool that enables investigation of target genes, as well as the identification of cofactors or partner proteins involved in normal or abnormal *TGIFLX* function.

Similar to *TGIFLX*, the *TGIFLY* sequence is 2807 bp in length containing two exons and one intron with a 558 bp open reading frame that encodes a 23 kDa protein (**Figure 1**). *TGIFLY* shows a remarkable similarity to *TGIFLX* within the first 148 amino acids except for a single amino acid substitution at the third helix of the homeodomain. A single nucleotide deletion in codon 149 of *TGIFLY* produces a frameshift that leads to a truncated protein, without the CCD, that is 56 amino acids shorter than the *TGIFLX* protein.[17] Sarkargar et al.[28] have produced GST-TGIFLY fusion protein in a bacterial system.

To date, few studies have been carried out to examine associations of the *TGIFLX/Y* gene with human developmental or malignant disorders. Most recent reports have demonstrated a significant association of *TGIFLX* with prostate cancer development as well as with azoospermia in infertile men (see below).[29,30] In the context of *Drosophila* development, knock-in expression of human versions of *TGIFLX* and *TGIF2*, but not *TGIFLY* and *TGIF*, genes led to reduced size of various tissues such as epithelial and neuronal.[31] This forced expression in the fly decreased both cell size and number.

Significantly, overexpression of *Drosophila* versions of *Myc* and *Cyclin* E, but not insulin receptor (INSR) and *PI3-K* genes, reverted the phenotypic effects of forced expression of *TGIFLX* and *TGIF2* in fly tissues.[31]

Homeobox genes are evolutionarily conserved in their sequences due to their critical role in developmental processes. However, the *TGIFLX* gene as a testis-specific and X-linked homeobox locus appears to have evolved rapidly at an extraordinarily high rate under positive Darwinian selection. The nature of this selective pressure on the *TGIFLX* gene and the benefit(s) of this selection are unknown, but it has been proposed that it contributes to developmental isolation and accelerates biological speciation.[26] In general, X-linked genes evolve more rapidly than autosomal genes.[32] This evolutionary change is higher when the product of the gene (like *TGIFLX*) is expressed exclusively in male reproductive system. It has been demonstrated that positive selection acts often on sex and reproduction-related genes and that hemizygous expression facilitates positive selection.[26] Thus, X-linked genes expressed in sperm cells should reveal larger consequences of positive selection than those on autosomes. Wang et al. suggested that nucleotide changes involving two codons in the homeobox as well as others outside of the homeobox have been strongly positively selected in the orthologous *TGIFLX* gene found in the anthropoid primates including humans.[26] As a further demonstration that the rate of evolution in *TGIFLX* has been exceptionally high, of 1880 orthologous human and rodent genes analyzed, only six genes were found to have had substitution rates greater than that of *TGIFLX*.[33]

TGIFLX, SPERMATOGENESIS, AND FERTILITY

TGIFLX/Y and *Tex1* are TGIF family members that were originally reported to be specifically expressed in human and mouse testis, respectively.[17,34] Moreover, analysis of knockouts of two TGIF like genes, *vis* and *achi*, in *Drosophila* revealed that these genes have no essential role in embryonic development but affected males were sterile.[35] The mechanisms by which these genes act are unknown, but flies defective for both genes showed blocked spermatogenesis before the first meiotic division.[35] Consistent with this evidence, we recently reported an association between *TGIFLX* mRNA expression with nonobstructive azoospermia (lack of live spermatozoa in the semen of males with no sign of vasa deferentia blockage) in infertile men.[29] In our study, mRNA expression of *TGIFLX* and *TGIFLY* were measured on testicular samples from 110 patients. It was found that 51 patients had detectable levels of *TGIFLY* expression, while none of the patients showed any evidence of the *TGIFLX* gene expression found in normal testes. These findings suggest that although there was no significant association between azoospermia and *TGIFLY*, *TGIFLX* can be considered a regulator of spermatogenesis and a potential biomarker for the evaluation of infertility in males. Despite genetic redundancy occurring among some homeobox genes, the results of this study may suggest a lack of compensatory function between *TGIFLX* and *TGIFLY*.[29]

TGIFLX/Y GENES AND CANCER

As master regulators of developmental programs, homeobox genes have crucial roles in many aspects of differentiation (acquisition of tissue-specific functions) and morphogenesis (the physical creation of normal architecture).[36] Although association between loss and gain of homeobox gene expression and tumorigenesis has frequently been reported, relatively few studies have addressed their causal roles in carcinogenesis. There is an emerging concept in which those homeobox genes that are associated with undifferentiated and proliferating cells are upregulated in cancer. This is in contrast to those related to the differentiated status of cells, which are generally downregulated in the process of oncogenesis.[37]

Our recent work has suggested a possible involvement of *TGIFLX* and *TGIFLY* in malignant transformation by examining mRNA expression in 60 cases of prostate cancer and 15 cases of benign prostate hyperplasia (BPH). Whereas expression of *TGIFLX/Y* mRNA in BPH samples could not be detected, we found a significant correlation between more advanced prostate cancer (defined as a Gleason score of equal or higher than six, which correlates with increased likelihood of metastasis and mortality) and ectopic expression of the *TGIFLX* and *TGIFLY* genes.[30] Interestingly, *TGIFLX/Y* gene expression did not show any significant correlation with PSA level, patient age, or prostate size. These results suggest a correlation of *TGIFLX/Y* expressions with a less differentiated status of prostate cancer.

Besides *TGIFLX* and *TGIFLY*, expression of other homeobox genes such as *HOXC6* and *HOXC8*, have been correlated with higher Gleason scores and a less differentiated status in human prostate cancer.[38–41] This reflects the now well-established view that homeobox genes can play a critical role in the process of carcinogenesis by acting in different pathways.[42,43] Conversely, human *NKX3.1*, which is expressed in fully differentiated prostate epithelium and behaves like a tumor suppressor gene, is downregulated in tumors with high Gleason scores.[44] Due to a lack of *TGIGLX/Y* mRNA expression in normal prostate, ectopic expression of these genes can be considered as a ''gain'' of expression in prostate cancer.[28]

CONCLUSIONS AND RECOMMENDATIONS

There are many observed parallels between normal development and neoplasia, one of which is the functional expression of homeobox proteins. Recent significant correlations found between *TGIFLX/Y* homeobox gene expression levels and human diseases implicate *TGIFLX/Y* not only in the process of normal spermatogenesis but also in the progression of human prostate cancer. *TGIFLX/Y* factors either directly or indirectly play critical roles in normal and abnormal biological processes by regulating the transcription of specific target genes. However, these remain to be elucidated. As one expedient approach, we recommend the utilization of GST-TGIFLX/Y recombinant proteins and specific antibodies for their precise quantification and localization, as well as for

measurement of their interactions with other proteins. This will be a practical first step in addressing the involvement of these genes in both their physiological and pathological contexts.

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