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Association of *TGIFLX/Y* mRNA expression with prostate cancer

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Abstract

Prostate cancer is the most common type of solid tumor and a leading cause of cancer-related death of men living in the developed world. In recent years, the molecular mechanisms involved in prostate cancer development and/or progression have been intensely studied and several genes have been identified. *TGIFLX/Y* (*TGIFLX* and *TGIFLY*) are members of the homeobox superfamily of genes whose function(s) is unknown. To investigate *TGIFLX/Y*mRNA expression in prostate cancer, we studied two different types of clinical samples, namely 60 prostate tumors and 15 cases of benign prostate hyperplasia (BPH), by RT-PCR. Our results revealed that most prostate tumors (73.5%) express at least one of these genes, although different patterns of *TGIFLX/Y* mRNA expression were observed. In some tumor samples the expression of both genes was detected, while in others no expression of either gene was observed. Notably, there was a significant correlation between expression of both *TGIFLX* and *TGIFLY* and a Gleason score of ≥6 ($P = 0.038$). By contrast, expression of *TGIFLX/Y* mRNA in BPH samples could not be detected. These results suggest an association of *TGIFLX/Y* expression with the progression of prostate cancer.

Keywords: Prostate cancer; TGIFLX/Y; Homeobox gene; RT-PCR

Introduction

Prostate cancer (PCa) is the most frequently diagnosed non-cutaneous malignancy and the second leading cause of cancer death of men living in the developed world [1]. Despite the identification of several genes that are important in PCa development, the molecular mechanisms that contribute to the onset or progression of PCa are not fully understood. One group of genes implicated in PCa are the

homeobox genes, which encode transcription factors involved in the regulation of gene expression [2, 3, 4], that function in normal and abnormal developmental processes [5, 6, 7]. They are categorized into six distinctive groups, one of which is the TALE (three amino acid loop extension) class. Like other homeobox classes, TALE proteins possess a helix-turn-helix DNA binding motif known as the homeodomain and act to regulate various cellular processes by specifically binding to the transcriptional control regions of target genes [8, 9, 10].

TGIF (TG-interacting factor) is a TALE-class homeodomain protein that serves as a multifunctional repressor of TGF beta-induced transcription. The related TGIF2 shows distinct homology with TGIF and is ubiquitously expressed in human tissues, with the highest levels being found in heart, kidney, and testis as well as in ovarian cancer cells [11, 12]. TGIF-like homeodomain proteins are those that have homology with TGIF. *TGIFLX/Y* is a homeobox locus related to the TALE superclass gene family and consists of two genes: *TGIFLX* (X-linked) and *TGIFLY* (Y-linked). *TGIFLX*, which is thought to have originated from the retrotransposition of TGIF2 [reference], is 2808 bp in length and comprises two exons, with the coding region being located within exon 2. In spite of the fact that *TGIFLY* shows a remarkable similarity to *TGIFLX*, a single nucleotide deletion in codon 149 of *TGIFLY*produces a frameshift that leads to a truncated protein (56 amino acids shorter than TGIFLX). The biological roles of *TGIFLX/Y* remain poorly understood, although both these genes are specifically expressed in human adult testis [13]. Consistent with this, we recently reported an association of *TGIFLX/Y* mRNA expression with azoospermia in infertile men [14]. An accumulating body of data also suggests a possible involvement of this class of homeobox gene in malignant transformation. In the present study, therefore, we investigated whether the expression of *TGIFLX* and *TGIFLY* may be associated with human PCa. We utilized the RT-PCR technique to examine *TGIFLX* and *TGIFLY* mRNA expression in both PCa and benign prostatic hyperplasia (BPH).

Materials and methods

Subjects

The study group consisted of 60 previously untreated PCa patients with known histological information and 15 age-matched patients with untreated BPH. Indication for PCa and BPH was elevated serum levels of prostate-specific antigen (PSA), and further diagnosis was made by transrectal ultrasound (TRUS)-guided biopsies. Pathological examination confirmed the presence of cancerous cells or benign hyperplasias. For all patients, relevant clinical and pathological data were collected.

Tissue collection and RNA extraction

Tissue samples from patients were collected at Imam Khomeini Hospital and Bistoon Ultrasound Clinic, immediately placed in liquid nitrogen and carried to the laboratory for either total RNA isolation or storage at −70°C. Informed consent was obtained from all the participants in this study. During serial slide preparation samples were homogenized according to the microscopic characteristics of cancerous cells with more than 80% of cells that underwent RNA extraction. Tripure isolation reagent (Roche, Mannheim, Germany) was used for extraction of total RNA according to the manufacturer's instructions with minor modifications. Briefly, the tumor specimen was homogenized in Tripure reagent, mixed with chloroform, then shaken vigorously for 15 s and centrifuged at 12,000*g* for 15 min at 4°C. The RNA present in the upper colorless aqueous phase was precipitated by addition of isopropanol, washed with 75% ethanol, air-dried, and then dissolved in RNase-free water. Following incubation for 10 min at 55°C to allow for complete dissolution, the RNA was stored at −70°C.

cDNA synthesis and RT-PCR

For the synthesis of cDNA 1 μg of total RNA was added to a master mix that included 4 μl of 5× reverse transcriptase (RT) buffer (Fermentas, Burlington, Canada) 10 mM of each dNTP, 20 pmol/μl random primer, 20 U RNase inhibitor (Roche, Mannheim, Germany), 200 U Moloney Murine Leukemia Virus (M-MuLV) RT (RevertAid^{TM}, Fermentas, Burlington, Canada), and 4 μl of DEPC-treated water. The RT reaction was performed at 42°C for 60 min, followed by heat inactivation at 70°C for 10 min. The integrity of cDNA was checked using the housekeeping gene phosphoglucomutase 1 (PGM1) primers, which amplify the region 1718–2104 (from exon 10 to exon 11). The sequences of the used forward and reverse primers are: PGMF: 5′-GCCCGCAGGTCCTCTTTCCCTCACA-3′ and PGMR 5′-TCCGACTGAGCGGCACTGGGAGTGC-3′. Samples with satisfactory cDNA quality as judged by the *PGM1* PCR were stored at −20°C for subsequent investigation. Differential expression of *TGIFLX* and *TGIFLY* in PCa tissues was carried out by amplification refractory mutation system RT-PCR. Briefly, a specific common forward primer corresponding to the 5′ untranslated region of both the *TGIFLX* and *TGIFLY* transcribed sequences (5′-TCGAAACAACAGTAACGATAAGCCT-3′) was used with gene-specific reverse primers 5′-CATTGATAAACCAGTTAGAAATCT-3′ for *TGIFLX* and to 5′-CATTGATAAACCAGTTAGAAATCC-3′ for *TGIFLY*. Thermocycling was performed using a Touch-down amplification program on an ABI thermocycler. The PCR conditions included an initial denaturation step for 3 min at 95°C, 30 s at 95°C, 45 s at 64°C with a 1°C decrease every second cycle down to 55°C, then 55°C for 14 cycles, 1 min at 72°C for extension, and finally 10 min at 72°C. PCR products were separated on 2% agarose gels and visualized with ethidium bromide. Positive controls for all RT-PCR assays were carried out using *PGM1* and the specificity of *TGIFLX/Y* primers was tested using genomic DNA (gDNA).

Statistical analysis

Microsoft Excel spreadsheet and the Statistical Package for Social Sciences (SPSS) version 11.2 were used for data entry and analysis. Correlations between *TGIFLX*/Y mRNA expression and Gleason score were examined using the Fisher test and an alpha level of 0.05 was used to indicate statistical significance.

Results

TGIFLX/Y mRNA expression in prostate cancer and BPH biopsy

Sixty PCa patient's, relevant clinical and pathological data were collected and showed that 13.4%, 58.3%, and 28.3% of prostate cancer patients represented Gleason scores with (4–5), (6–7), and (8–10), respectively. To examine the expression of *TGIFLX* and *TGIFLY* in human PCa, total RNA was obtained from 60 PCa tumors as well as 15 BPH specimens. The principal characteristics of these study populations are presented in Table 1. In the first instance, we confirmed RNA quality by RT-PCR for *PGM1* gene expression and representative results of four PCa tumors are shown in Fig. 1. A survey of *TGIFLX* and *TGIFLY* expression revealed that, whereas most PCa specimens (73.5%) contained transcripts from at least one of these genes (Table 2, Fig. 2), neither gene was expressed in cases of BPH (data not shown).

The expression of *TGIFLX* and *TGIFLY* showed different patterns among the various PCa patients (Table 2). *TGIFLY* was expressed in 36 out of 60 (60%) and *TGIFLX* expression in 31 out of 60 (51.6%) cases. When the expression of both genes was analyzed, 38.3% patients were positive for both genes, 21.6% had only *TGIFLY* expression and were negative for *TGIFLX*gene, 13.3% showed *TGIFLX* expression only, and 26.6% had no expression of either gene.

Expression of *TGIFLX/Y* is associated with higher-grade prostate cancer

We analyzed the correlation between *TGIFLX*/*Y* gene expression and Gleason score in the PCa specimens by SPSS and Fisher's exact test for each of the genes (Table 3). In samples in which one, or both, of these genes were expressed ($TGIFLX^+/Y^+$, $TGIFLX^+/Y^-$, $TGIFLX^-/Y^+$), a significant association between their expression and a Gleason score of ≥6 was observed ($P = 0.038$). By contrast, *TGIFLX/Y* gene expression did not show any significant correlation with PSA level, patient age, or prostate size.

Discussion

Several studies have demonstrated that heterogeneity increases markedly with progression from benign prostate hyperplasia through prostatic intraepithelial neoplasia (PIN) to localized PCa and metastases [15]. By using a genome-wide scan, several candidate genes have been identified for malignant transformation in the prostate [16, 17, 18].

In a previous gene expression study, we identified an association between human homeobox *TGIFLX/Y* mRNA expression with azoospermia in men [14]. The *TGIFLX/Y* gene is a member of TALE group of homeobox genes, which is specifically expressed in human adult testis [13]. In male infertility, *TGIFLX/Y* has been implicated in the azoospermia phenotype [14]. The results of the present work show a significant correlation between *TGIFLX/Y* gene expression and Gleason score in prostate cancer. The *TGIFLX/Y* gene is not the first homeobox gene to be implicated in the development of prostate cancer. Aberrant HOXC8 expression was reported in human prostate tumors where it was associated with loss of differentiation [19]. Subsequently, Miller et al. [20] found overexpression of four *HOXC* cluster genes (*HOXC4*, *HOXC5*, *HOXC6*, and *HOXC8*) in malignant prostate cell lines and lymph node metastases using RT-PCR. By RT-PCR, we detected the expression of both *TGIFLX* and *TGIFLY* in 23 out of 60 (38.3%) prostate tumors, but not in BPH samples. This dysregulation points to a possible involvement of *TGIFLX/Y* expression with malignancy in prostate cells.

The expression patterns of *HOX* genes, which are normally expressed in proliferating and/or differentiated tissues, are often different in comparison to cancerous cells [17, 21]. This reflects the now well established view that *HOX* genes can play a role in the process of carcinogenesis by acting in different pathways [22, 23]. In several cancers, *HOX* genes function by either gain or loss of expression, which is associated with their oncogene-like or tumor suppressor-like activities, respectively. The loss-of-function of the *NKX3.1* homeobox gene is an important example of the latter in respect to prostate cancer. Since *TGIFLX/Y* gene expression is normally lacking in the prostate, its presence can be categorized as a "gain" of expression in prostate cancer. Although different patterns of *TGIFLX/Y* mRNA expression in PCa biopsies could be due to genetic heterogeneity, our results suggest that *TGIFLX/Y* may have a role in cancer development, especially since mRNA expression of *TGIFLX* and *TGIFLY*were not detected among BPH samples.

Significantly, expression of *TGIFLX/Y* genes was associated with a Gleason score of ≥6. Other genes whose aberrant expression have been reported to be associated with a higher Gleason score in prostate cancer include *HOXC8* [19], E-cadherin [17, 24], and *DNp73* [25], a p53 homologue that induces apoptosis and inhibits cell proliferation in benign and malignant tumors of the prostate. Our results suggest interactive effects between the *TGIFLX* and *TGIFLY* genes in prostate cancer progression. However, it is also possible that *TGIFLX* and *TGIFLY* act as redundant regulatory proteins, at least in the context of their abnormal function in tumor development. Several studies have reported redundancy between some homeobox genes [26, 27, 28]. The best examples to date are two nearly identical homeobox genes of the *TGIF* subclass, *vismay* and *achinta (vis/achi)*, in *Drosophila melanogaster* that are normally expressed in the testes. Wang and Mann [28] indicated that Vis and

Achi are redundant transcriptional regulators that function at the same step in normal sperm development. Thus, it remains a possibility that expression of either the *TGIFLX* or *TGIFLY*genes could have a role malignancy.

In conclusion, our results indicate a significant correlation between prostate cancer and the abnormal expression of the *TGIFLX* and *TGIFLY* genes. Nevertheless, the precise mechanisms by which these genes are involved in tumorigenesis remain to be elucidated. The use of specific antibodies for precise quantification and localization, together with measurement of interaction with other proteins, will be a first step in addressing the direct or indirect involvement of the *TGIFLX/Y* genes in normal and abnormal biological functions.

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References

1. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1998. CA Cancer J Clin. 1998;48:6–29. doi:10.3322/canjclin. 48.1.6.

2. Berthelsen J, Zappavigna V, Ferretti E, Mavilio F, Blasi F. The novel homeoprotein Prep1 modulates Pbx-Hox protein cooperativity. EMBO J. 1998;17:1434–45. doi:10.1093/emboj/17.5.1434

3. Jacobs Y, Schnabel CA, Cleary ML. Trimeric association of Hox and TALE homeodomain proteins mediates Hoxb2 hindbrain enhancer activity. Mol Cell Biol. 1999;19:5134–42.

4. Kamps MP, Murre C, Sun XH, Baltimore D. A new homeobox gene contributes the DNA binding domain of the t(1;19) translocation protein in pre-B ALL. Cell. 1990;60:547–55. doi:10.1016/0092-8674(90)90658-2.

5. Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. Oncogene. 2007;26:6766–76. doi:10.1038/sj.onc.1210760.

6. Yu YY, Pan YS, Zhu ZG. Homeobox genes and their functions on development and neoplasm in gastrointestinal tract. Eur J Surg Oncol. 2007;33:129–32. doi:10.1016/j.ejso.2006.09.010.

7. Wigle JT, Eisenstat DD. Homeobox genes in vertebrate forebrain development and disease. Clin Genet. 2008;73:212–26.

8. Banerjee-Basu S, Baxevanis AD. Molecular evolution of the homeodomain family of transcription factors. Nucleic Acids Res. 2001;29:3258–69. doi:10.1093/nar/29.15.3258.

9. Bertolino E, Reimund B, Wildt-Perinic D, Clerc RG. A novel homeobox protein which recognizes a TGT core and functionally interferes with a retinoid-responsive motif. J Biol Chem. 1995;270:31178–88. doi:10.1074/jbc.270.52.31178.

10. Burglin TR. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucleic Acids Res. 1997;25:4173–80. doi:10.1093/nar/25.21.4173.

11. Imoto I, Pimkhaokham A, Watanabe T, Saito-Ohara F, Soeda E, Inazawa J. Amplification and overexpression of TGIF2, a novel homeobox gene of the TALE superclass, in ovarian cancer cell lines. Biochem Biophys Res Commun. 2000;276:264–70. doi:10.1006/bbrc.2000.3449.

12. Jin L, Zhou Y, Kuang C, Lin L, Chen Y. Expression pattern of TG-interacting factor 2 during mouse development. Gene Expr Patterns. 2005;5:457–62.

13. Blanco-Arias P, Sargent CA, Affara NA. The human-specific Yp11.2/Xq21.3 homology block encodes a potentially functional testis-specific TGIF-like retroposon. Mamm Genome. 2002;13:463–8. doi:10.1007/s00335-002-3010-9.

14. Aarabi M, Ousati-Ashtiani Z, Nazarian A, Modarressi MH, Heidari M. Association of TGIFLX/Y mRNA expression with azoospermia in infertile men. Mol Reprod Dev 2008.

15. Magi-Galluzzi C, Xu X, Hlatky L, Hahnfeldt P, Kaplan I, Hsiao P, et al. Heterogeneity of androgen receptor content in advanced prostate cancer. Mod Pathol. 1997;10:839–45.

16. Suarez BK, Gerhard DS, Lin J, Haberer B, Nguyen L, Kesterson NK, et al. Polymorphisms in the prostate cancer susceptibility gene HPC2/ELAC2 in multiplex families and healthy controls. Cancer Res. 2001;61:4982–4.

17. Hsu Y-S, Wang J-S, Wu TT. E-cadherin expression in prostate adenocarcinomas in Chinese and its pathological correlates. Urol Int. 2004;73:36–40. doi:10.1159/000078802.

18. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. CA Cancer J Clin. 2008;58:71–96.

19. Waltregny D, Alami Y, Clausse N, de Leval J, Castronovo V. Overexpression of the homeobox gene HOXC8 in human prostate cancer correlates with loss of tumor differentiation. Prostate. 2002;50:162–9. doi:10.1002/pros.10045.

20. Miller GJ, Miller HL, van Bokhoven A, Lambert JR, Werahera PN, Schirripa O, et al. Aberrant HOXC expression accompanies the malignant phenotype in human prostate. Cancer Res. 2003;63:5879–88.

21. Ouyang X, DeWeese TL, Nelson WG, Abate-Shen C. Loss-of-function of Nkx3.1 promotes increased oxidative damage in prostate carcinogenesis. Cancer Res. 2005;65:6773–9. doi:10.1158/0008-5472.CAN-05-1948.

22. Crijns APG, de Graeff P, Geerts D, Ten Hoor KA, Hollema H, van der Sluis T, et al. MEIS and PBX homeobox proteins in ovarian cancer. Eur J Cancer. 2007;43:2495–505. doi:10.1016/j.ejca.2007.08.025.

23. Wong P, Iwasaki M, Somervaille TCP, So CWE, So CWE, Cleary ML. Meis1 is an essential and rate-limiting regulator of MLL leukemia stem cell potential. Genes Dev. 2007;21:2762–74. doi:10.1101/gad.1602107.

24. Richmond PJ, Karayiannakis AJ, Nagafuchi A, Kaisary AV, Pignatelli M. Aberrant E-cadherin and alpha-catenin expression in prostate cancer: correlation with patient survival. Cancer Res. 1997;57:3189–93.

25. Guan M, Chen Y. Aberrant expression of DeltaNp73 in benign and malignant tumours of the prostate: correlation with Gleason score. J Clin Pathol. 2005;58:1175–9. doi:10.1136/jcp. 2005.026955.

26. Lappin TRJ, Grier DG, Thompson A, Halliday HL. HOX genes: seductive science, mysterious mechanisms. Ulster Med J. 2006;75:23–31.

27. Grier DG, Thompson A, Kwasniewska A, McGonigle GJ, Halliday HL, Lappin TR. The pathophysiology of HOX genes and their role in cancer. J Pathol. 2005;205:154–71. doi:10.1002/path.1710.

28. Wang Z, Mann RS. Requirement for two nearly identical TGIF-related homeobox genes in Drosophila spermatogenesis. Development. 2003;130:2853–65. doi:10.1242/dev.00510.

Table 1 Principle characteristics of the subjects

Characteristics		**Cancer patients (*n*)**	**BPH patients (*n*)**
Age (years)	Mean (95% CI)	69.2(67–71)	64.5(62.9–66.1)
	Minimum	48	46
	Maximum	85	82
PSA (ng/ml)	Mean (95% CI)	20.7(13.5–27.8)	12.7(9.32–16.18)
	Minimum	1.2	0.8
	Maximum	130	95
Prostate weight (g)	Mean (95% CI)	50.6(43.3–57.8)	64.2(57.97–70.60)
	Minimum	17	26
	Maximum	153	156
Gleason score	Mean (95% CI)	6.9(6.5–7.3)	–
	Minimum	4	–
	Maximum	10	–

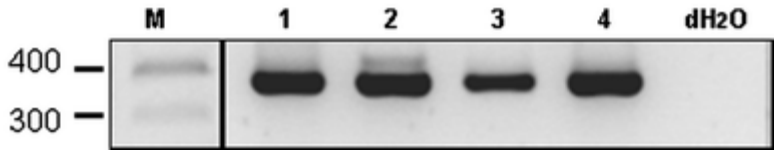
Table 2 Expression of *TGIFLX* and *TGIFLY* genes in prostate cancers

Expression pattern of *TGIFLX* (X) and *TGIFLY* (Y)	**Number**	**Percentage (%)**
$X^+ Y^+$	23	39
$X^+ Y^-$	8	13
$X^- Y^+$	13	21.5
$X^- Y^-$	16	26.5
Total	60	100

Table 3 Correlation between *TGIFLX* and *TGIFLY* gene expression and Gleason score in 60 prostate cancers

	Gleason score		
	4–5 (%)	**6–7 (%)**	**8–10 (%)**
$X^+ Y^+$	0	25.92	11.11
$X^+ Y^-$	0	11.11	0
$X^- Y^+$	3.70	7.40	11.11
$X^- Y^-$	9.25	14.81	5.55

Fig. 1 Confirmation of RNA quality by RT-PCR of the *PGM1* housekeeping control gene. Shown are representative RT-PCR products resolved on a 2% agarose gel stained with ethidium bromide. Lanes 1–4: prostate cancer patients; lane 5: negative control (ddH_2O); M, molecular weight marker (100 bp)



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