

HOXB13, a potential prognostic biomarker for prostate cancer

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1. ABSTRACT

HOXB13, a member of the homeobox proteins family, is a key regulator of the epithelial differentiation in the prostate gland. HOXB13 is overexpressed during malignant progression of the prostatic tissue and suspected to contribute in the pathogenesis of the prostate gland. In androgen deprived conditions, HOXB13 is thought to act through inhibition of the tumour suppressor protein p21. Since HOXB13 has a multifaceted role in ventral prostate development, its critical partners in the cascade need to be elucidated for a further understanding of its role in prostate malignancy. In this report, we review the functions attributed to HOXB13, by highlighting the most recent findings supporting the hypothesis that HOXB13 might serve as a novel biomarker for the prognosis of prostate cancer.

2. INTRODUCTION

The homeobox or Hox genes are a class of the regulatory genes encoding essentially for nuclear proteins, termed homeoproteins, and act mostly as transcription factors. They are characterized by the homeodomain, a protein domain encoded by the homeobox (HD) genes able to specifically recognize and bind DNA sequences.

The homeobox genes act as master switches for the development of various body structures during embryogenesis. They are highly conserved genes and have a key role in the regulation of anterior/posterior patterning (1). Homeodomain containing proteins encoded by Hox genes are the second largest class of

sequence specific transcription factors (2). The DNA binding homeodomain comprised of 60 aminoacids is encoded by a highly conserved sequence. Hox gene clusters control the polarity of the embryo, and body segmentation during embryogenesis in all species, including humans. Coordinated expression of these genes is observed in the limb and genital tubercle (3,4). In the human genome there are four groups of hox genes clustered in unlinked complexes, Hox (A, B, C, D) clusters located on chromosomes 7, 17, 12 and 2 successively. The hox genes of the four clusters are arranged into 13 paralog groups, and their (3'-5') position in the genome determines their expression along the anterior - posterior (AP) axis (from anterior to posterior) (2). Hox genes are expressed during and post-period of organogenesis in differentiated organs as well (1). As these genes play a pivotal role in the formation of body segments, any mutation can cause detrimental malformations. Recently, several studies have reported that altered Hox gene activities were associated with several forms of cancer as well (4-9).

HOXB13 gene, is the last identified paralog member found in the Hoxb cluster. Although it is separated from Hoxb-9 by almost 70kb, it is transcribed in the same orientation as the other Hoxb genes (10). Though it is physically separated from the cluster, HOXB13 exhibits both spatial and temporal co-linearity within the main body axis. It is expressed predominantly in the tail-bud and posterior domains of the spinal cord, digestive tract, and urogenital sinus (10,11). The HOXB13 gene

plays an essential role in prostate development and its secretory functions (12,13). Moreover, it has a polyvalent regulatory role on androgen receptors (AR), by either activating or repressing the transcription of distinct AR target genes (14).

There are controversial reports concerning the exact role of HOXB13 in prostate cancer (PCa). HOXB13 has a dual and contradictory role of inhibiting PCa cell proliferation in certain cases but also promotes malignancy in others. Our research group is interested in providing more evidence about HOXB13 role in PCa, and the credibility of its application in the prognosis and diagnosis of prostate malignancy.

3. STRUCTURE OF HOXB13

HOXB13 is a transcription factor encoded by the HOXB13 gene, which belongs to the Abdominal-B (Abd-B) homeobox family. Along with other hox genes, HOXB13 forms a cluster in the 17q21-22 region on chromosome 17. The transcript size, encoding for 284 amino acid length protein is estimated to 3467 bp. Hox proteins are characterized by sharing 60 amino acid residues (amino acids 216-275) termed the DNA binding domain or homeodomain (DBD), a highly conserved region between species. The ultrastructure analysis of DBD revealed the presence of three alpha helices and a highly conserved DNA binding domain (15) in addition to a distinct sequence located outside the homeodomain, to determine the tissue specific activity for different hox proteins (15).

4. PHYSIOLOGICAL FUNCTIONS OF HOXB13

In general all the hox proteins act as transcription factors, regulating the genes involved in body segmentation during embryogenesis (1). The anterior-posterior axial determination is controlled by hox genes. Loss of function of hox genes can cause serious malformations in the body. Hox genes also play various roles in the development of secondary sex characteristics such as the mammary glands (16,17), and male accessory sexual organs (3,18-20).

4.1. Role of HOXB13 in prostate gland- epithelial differentiation

The Hox13 paralogs are critically important in the development of the prostate. Mutant Hoxa13 and Hoxd13 have been found to reduce branching in prostate ducts of mice (18-21). Nkx3.1, a non-Hox homeodomain protein, is also required for the proper development of the prostate and displays an androgen-dependent expression profile (22,23). Expression of Hoxb 13 has been cited in the caudal extent of spinal cord, tail bud and urogenital sinus (10,11). The prostate gland derived from urogenital sinus shows high expression of Hoxb13 which has been found not to be regulated

by Androgen stimuli (11,13). Reports suggest that in adult mice, expression of HOXB13 is restricted to the prostate notably the ventral lobe, descending colon, and rectum, which are all derived from the posterior endoderm (11). The increased accumulation of HOXB13 mRNA in castrated mice, unlike other prostate specific genes, proves the androgenindependent expression of HOXB13 (11,24). Mice homozygous for mutant HOXB13 alleles showed 100% penetrance, complete absence of the secretory proteins p12 and p25 in the ventral prostate in addition to the unusual morphology of the luminal epithelium cells (12). HOXB13 plays a critical role in the regulation of maintaining a balance between cell growth and proliferation and in differentiation functions such as lipogenesis (14). The nuclear receptor family protein AR is co-regulated by HOXB13. In normal prostate gland, the cell proliferation and differentiation is regulated by the androgen stimuli via AR. The AR act together with many coactivators along with Hoxb 13 to initiate the expression of growth-specific genes, such as c-myc, fos and jun.

4.2. Role of HOXB13 in skin- response to wound healing

Along with its expression in posterior endoderm, studies have identified the cytoplasmic localization of the protein during fetal skin development. Experiments in SCID mice transplanted with human fetal and adult skin has shown that HOXB13 expression is down regulated in fetal skin that heals without scars, but remains unchanged in adult wounds (25). However, lower expression levels of nuclear HOXB13 tend to be localized in the hyper-proliferative epidermis associated with Kaposi sarcoma (26). Overexpression of HOXB13 in both human foreskin keratinocytes (26) and rat epidermal keratinocytes differentiation (27) demonstrated its role in adult skin development. The excessive cornified rat keratinocytes due to overexpresssion of HOXB13 underlines a putative role in skin pathogenesis as well (28). It is noticeable that the role of HOXB13 molecular partners and the related signaling cascade is nascent.

4.3. Role of HOXB13 in endothelial differentiation and angiogenesis

Recently study has reported significant changes in the expression patterns of four HOX genes, Hoxa7, Hoxb3, Hoxa3, and HOXB13, during angiogenesis in human bone marrow-derived mesenchymal stem cells (hMSCs). The expression levels of Hoxa7 and Hoxb3 were dramatically increased, whereas those of Hoxa3 and HOXB13 were decreased during endothelial cell differentiation. Evidence From these experiments suggest the hypothesi that these Hox genes, including HOXB13 are involved in angiogenesis of hMSCs (28).

4.4. Role of HOXB13 in prostate cancer

PCa is the fourth most common cancer worldwide, accounting for 15% of the cancers diagnosed

in men (29,30). In the US, PCa is the second most leading cause of death among American men (31). The incidence of PCa in the Middle East is approximately 6 per 100,000 with a mortality rate of 5 per 100,000 (32). Similarly, PCa is the second most common cancer in Oman, accounting for approximately 9.5.% of all cancers with a mortality rate of 8% (29,30). As compared to the West, where the rates of PCa are on the rise, in Oman statistics show low rates of prevalence; most likely due to the lack of awareness in Oman.

Upon binding to its receptor, the androgen ligand promotes prostate tumor cell survival and proliferation (33,34). The major therapy that reduces prostate malignancy is androgen ablation or refractor, using androgen antagonists. Androgen ablation induces cell cycle arrest of prostate cancer cells (35,36), but the major draw back in this treatment is the recurrence of androgen independent tumor. It has been clearly proven that the prostate malignancy still proceeds despite that the cells have been deprived of androgen (33,34,37). The reasons underlying the prostate tumor progression despite androgen ablation are not completely known due to the multifocal nature of the malignancy.

HOXB13 is a multifaceted regulator of AR leading to the transcription activation or repression of distinct AR target genes. The complex action of HOXB13 on AR transcriptional activity is essentially due to its dual function in positively and negatively regulating the interaction of AR with chromatin. Hence, the critical role of HOXB13 in the regulation of the cellular response to androgens (14). As reported earlier, HOXB13 suppresses prostate cell growth by inhibiting androgen mediated signaling. Moreover, HOXB13 regulates negatively the expression of T-Cell factor 4 (TCF-4), and in turn down-regulates or inhibits the TCF-4 responsive genes including c-myc and cyclin D1, thereby controlling prostate cancer cell proliferation (38). However, in a recent investigation, overexpression of HOXB13 in LNCaP prostate cancer cells and androgen independent growth progression of cancer cells, signaled by overexpressed HOXB13, was observed. HOXB13 seems to up-regulate E2F transcription factor through inhibition of the tumor suppressor gene p21; This leads to the release of cyclin-dependent kinase 2, and subsequently phosphorylating RB protein which allows the release of E2F1 and activation of cell proliferation factors such as c-myc, and p107 proteins. E2F induces positive growth signals to prostate cancer cells in conditions like androgen deprivation (9). These findings suggest that while HOXB13 suppresses prostate cancer cell proliferation in PC3 cell line by regulating androgen mediated signals, higher level of HOXB13 expression is associated with tumor growth progression in LNCaP cell line which is deprived of androgen. In a recent study, a recurrent germ line mutation G84E of HOXB13 was reported to be associated with an elevated risk of PCa

in men of European descent (39). The significance of this mutation in Pca development requires. Further investigation.

4.5. Role of HOXB13 in other cancers

HOXB13 is also involved in various other tumors including breast, ovarian, endometrial, renal, and malignant melanomas. In renal and malignant melanoma, HOXB13 appears to act as a tumor suppressor. In renal carcinoma cells, it has been shown that HOXB13 is inactivated by methylation and in case that exogenous HOXB13 is supplied, tumor invasion was reverted (7). Similarly, re-expression of HOXB13 in malignant melanoma cells inhibited tumor formation (40). On the other hand, in case of endocrine responsive tumors like ovarian, breast, and endometrium, HOXB13 functions as a growth promoter (5,6,8). Overexpression of HOXB13 is associated with the invasiveness of ovarian, breast and endometrial cancer cells. In breast cancer cells, the overexpression of hoxb 13 makes the cells unresponsive to tamoxifen therapy (41). Therefore, the relevance of HOXB13 in predicting breast cancer pathology status has already been established.

5. SUMMARY AND PERSPECTIVE

HOXB13 is required for the normal development of prostate gland. It is essential for the normal secretory function of prostate and also for the regular morphology of prostate epithelium and its expression in the gland is independent of androgen stimulation. The findings to date suggest that HOXB13 plays an important role in prostate malignancy. The overexpression of HOXB13 in androgen deprived prostate cancer cells needs further evaluation in order to provide clear evidence to consider Hoxb 13 as a biomarker for the prognosis of PCa.

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Key Words: HOXB13, prostate cancer, Androgen, Review

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