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Gene of the month: NKX3.1

Jon Griffin, Yuqing Chen, James Catto, Sherif El-Khamisy

Abstract

NKX3.1 is a multi-faceted protein with roles in prostate development and protection from oxidative stress. Acting as a pioneer factor, NKX3.1 interacts with chromatin at enhancers to help integrate androgen regulated signalling. In prostate cancer, NKX3.1 activity is frequently reduced through a combination of mutational and post translational events. Owing to its specificity for prostate tissue, NKX3.1 has found use as an immunohistochemical marker in routine histopathology practice.

Structure and function of NKX3.1

NKX3.1 is an androgen regulated homeobox gene whose expression is almost uniquely restricted to the prostate. Early work identified a novel coding sequence with homology to the Drosophila NK3 gene and further characterisation localised NKX3.1 to chromosome 8p21.2 [1]. The NKX3.1 gene comprises two exons encoding a 234 amino acid protein. As well as N- and C- terminal domains, NKX3.1 has a homeodomain with the characteristic arrangement of three alpha helices (figure 1A). These facilitate binding of NKX3.1 to the consensus sequence TAAGTA[2] (figure 1B). DNA binding of NKX3.1 is essential for specifying prostatic differentiation during embryological development. Physiologically, NKX3.1 marks nascent prostatic epithelium before the appearance of glandular structures and, in the absence of NKX3.1, defective prostate development is seen[3]. In tissue recombination experiments in mice, NKX3.1 over expression was sufficient to induce seminal vesicle to differentiate into prostate-like tissue[4]. In this model, NKX3.1 interacted with G9a a methyltransferase that regulates the expression of genes involved in prostate development and differentiation.

In the adult prostate NKX3.1 acts as an androgen receptor (AR) cofactor, co-occupying enhancers with other pioneer factors such as FOXA1, GATA2 and HOXB13 which mark chromatin prior to AR translocation [5]. This association is supported ChIP-seq data showing that more than 90% of NKX3.1 co-localise with AR after androgen stimulation of prostate cancer cells.[6] NKX3.1 appears to play a specific role at enhancers, stimulating repair of transcription induced DNA damage through an interaction with topoisomerase 1, an enzyme that resolves DNA topological stress generated by transcription [7] (figure 1C). This interaction is mediated by the homeodomain of NKX3.1 and the Top1-NKX3.1 association is required for *in-vitro* DNA relaxation[8].

Inflammation, oxidative stress and post translational modification of NKX3.1

Multiple lines of evidence point towards a reduction of functional NKX3.1 secondary to an inflammatory micro-environment seen in early prostate cancer. This reduction occurs at the protein level and may cooperate with pre-existing haploinsufficiency of NKX3.1 to drive

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prostate cancer. Two studies reported a reduction in NKX3.1 expression in mouse prostate after inoculation with uropathogenic *E. Coli.* [9,10]. In these models, increased T and B cell infiltration was associated with loss of NKX3.1 expression by immunohistochemistry in mouse and human tissue. Furthermore, NKX3.1 loss and inflammation cooperated to induce a more aggressive basal phenotype. Mechanistically, exposure to inflammatory cytokines such as IL1-b and TNF-a rapidly reduces NKX3-1 protein levels by phosphorylation of the C-terminal domain and subsequent labelling for proteasomal degradation by ubiquitination[11]. Interestingly, TOPORS, a ubiquitin ligase associated with Topoisomerase 1, has been implicated as an enzyme responsible for NKX3.1 ubiquitination [12] providing a possible link between inflammation and enhancer occupancy of NKX3.1.

Recently NKX3.1 was implicated in the response to oxidative stress. Free radicals resulting from hydrogen peroxide or paraquat treatment resulted in translocation of NKX3.1 to the mitochondria to upregulate genes in the electron transport chain. This challenged the idea that NKX3.1 is exclusively nuclear in location and highlighted a role separate from androgen signalling [13]. A separate study suggested that NKX3.1 may act as a paracrine transcription factor to regulate gene expression programs of neighbouring cells. Interestingly the common NKX3.1 germline mutant T164A could not be exported into extracellular fluid similar to the loss of mitochondrial import seen in this mutant.

Regulation of NKX3.1 gene expression

Transcriptional regulation of NKX3.1 is not as well described as post translational modifications. A recent study of the transition from castration responsive to castration resistant prostate cancer identified a putative enhancer for NKX3.1 65 kb downstream of the transcription start site [14]. A separate regulatory region was identified 6 kb downstream of the NKX3.1 gene which exhibited androgen receptor binding and drove NKX3.1 expression in a mouse model[15]. Inhibitory elements have also been identified in the NKX3.1 promoter which could themselves be inhibited by an oligonucleotide decoy resulting in increased NKX3.1 expression and a reduction in proliferation.

Hypermethylation of CpG islands is implicated in the downregulation of tumour suppressor genes in many cancers. No CpG islands have been detected in the NKX3.1 promoter, however methylation of CpG dinucleotides was seen in a tumour specific manner [16]. Moreover, the cooperation of CpG dinucleotide methylation and mono-allelic deletion of NKX3.1 was associated with a more pronounced decrease in NKX3.1 expression than either mechanism alone.

Loss of NKX3.1 in prostate cancer

The chromosome region 8p21 undergoes frequent loss of heterozygosity in prostate cancer. The resulting haploinsufficiency of NKX3.1 is sufficient for loss of tumour suppressor function and this appears to be an early event in prostate carcinogenesis [17]. Futhermore, mouse prostate cancer models with conditional NKX3.1 loss exhibit specific gene expression profiles, corroborating the role of NKX3.1 in gene expression regulation. Additional

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reduction in NKX3.1 protein levels is mediated by dysregulation of various post translational modifications (PTM; figure 1D).

Mono- and bi-allelic loss of PTEN is a common genomic event in prostate cancer, particularly in cases with the TMPRSS2-ERG fusion [18,19]. PTEN normally translocates to the nucleus following TNF alpha administration and antagonises the phosphorylation of the C-terminal domain serine 185 of NKX3.1. In prostate cancer cells with reduced or absent PTEN levels, phosphorylation at this site by DYRK1B proceeds without regulation leading to degradation of NKX3.1[20]. In a CRISPR mouse model, mutation of the analogous serine increased NKX3.1 stability and led to reduced DNA damage and an abrogation of PIN[21]. In contrast, Pim-1, a serine/threonine protein kinase, stabilises NKX3.1 through phosphorylation of N and C terminal lysine and serine residues. Specifically, phosphorylation of serine 186, immediately adjacent to the serine responsible for PTEN mediated NKX degradation is important for NKX3.1 stability. These context specific modifications suggest a balance between specific PTMs to maintain NKX3.1 homeostasis[22].

The oncoprotein MYC is commonly over expressed in prostate cancer[19]. Using mouse models and human prostate cancer cells Anderson et al showed that NKX3.1 and MYC interact and regulated a shared subset of target genes. NKX3.1 loss resulted in upregulation of these genes suggesting NKX3.1 might exert its anti-proliferative effects through suppression of MYC signalling. In clinical prostate cancer samples low expression of the shared MYC/NKX3.1 target genes was associated with relapse [23]. Overall, the reduction of NKX3.1 expression and activity are associated with prostate cancer initiation and progression, and levels of NKX3.1 measured by immunohistochemistry have a negative correlation with tumour stage and grade.

Whilst reduced NKX3.1 expression is associated with prostate cancer progression, NKX3.1 is also required for prostate regeneration after periods of androgen deprivation. Wang et al identified a subset of luminal stem cells that expressed NKX3.1 in the androgen deprived state [24]. Importantly these cells could undergo self-renewal and could form prostate tissue in single-cell engraftment experiments. Overall, NKX3.1 seems to act as a tumour suppressor in the normal prostate but in advanced prostate cancer can act as a signal for cancer regrowth in stem cells that remain following androgen deprivation therapy.

NKX3.1 in diagnostic histopathology

In an immunohistochemistry study of over 4000 samples NKX3.1 was more restricted to prostate cancer tissues than PSA, however expression was demonstrated rarely in other tissues and tumours including lobular breast cancer, pulmonary mucus glands and testis[25]. Immunohistochemistry for NKX3.1 is useful in three common diagnostic situations: 1) Distinguishing high grade bladder cancer from high grade prostate cancer, 2) identifying prostate metastases (figure 2) and 3) differentiating seminal vesicle from prostate cancer. In a series of 249 cases of bladder and prostate cancer, NKX3.1 had a sensitivity of 88.3% but stained none of the bladder cancer cases[26]. In a cohort of metastatic tumours NKX3.1 had a sensitivity and specificity of 98.6% and 99.7% respectively[27]. In both studies, NKX3.1 was

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more specific than PSA for identifying prostate cancer. Lastly, NKX3.1 expression was absent in a series of 63 cases containing seminal vesicles and benign prostate or concurrent malignancy [28]. Together these studies highlight the use of NKX3.1 as part of a panel for common challenging scenarios in uropathology.

Take home messages

NKX3.1 is a prostate-specific transcription/ pioneer factor which functions to specify prostate development and as a tumour suppressor

Loss of NKX3.1 is an early event in prostate cancer and reduction in expression correlates with more aggressive disease

Inflammation can down regulate NKX3.1 at the protein level, providing a potential explanation for the cooperation of inflammatory stimuli and proliferation in the development of prostate intraepithelial neoplasia.

NKX3.1 is a sensitive and specific marker of prostate cancer making it useful in diagnostic dilemmas such as differentiating high-grade bladder and prostate cancer, and in diagnosing prostate cancer metastases.

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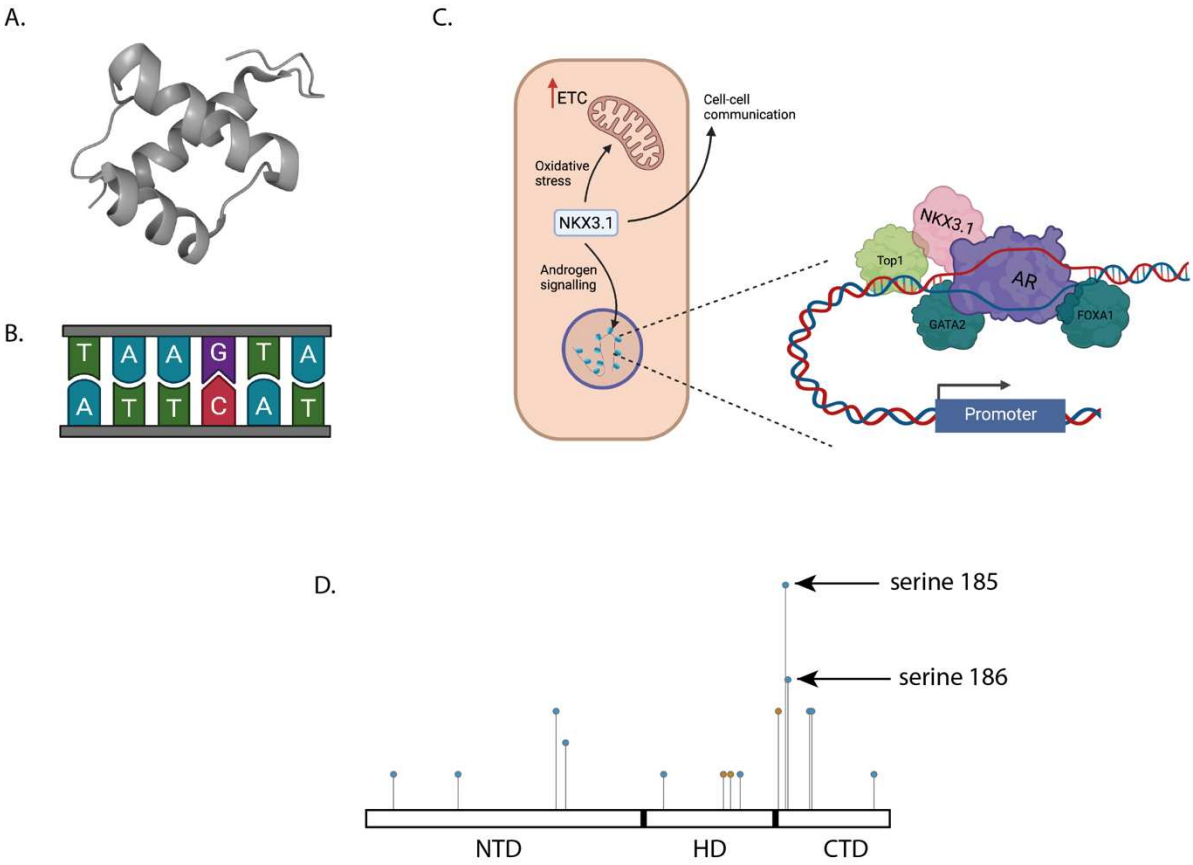

Figure 1. Structure and functions of NKX3.1. A: Protein ribbon diagram demonstrating triple alpha helix structure of NKX3.1; B: The consensus DNA sequence for NKX3.1; C: The diverse roles of NKX3.1 including interaction with the chromatin looping model of androgen signalling. NKX3.1 occupies enhancers and interacts with topoisomerase 1 (Top1); D: NKX3.1 gene structure and post translational modifications (adapted from PhosphoSite Plus). Blue circles are phosphorylation sites; yellow circles indicate ubiquitylation. Arrows indicate the adjacent serine 185 and 186 PTM with opposing effects on protein stability. ETC: Electron transport chain. NTD: N-terminal domain, CTD: C-terminal domain, HD: Homeobox domain.

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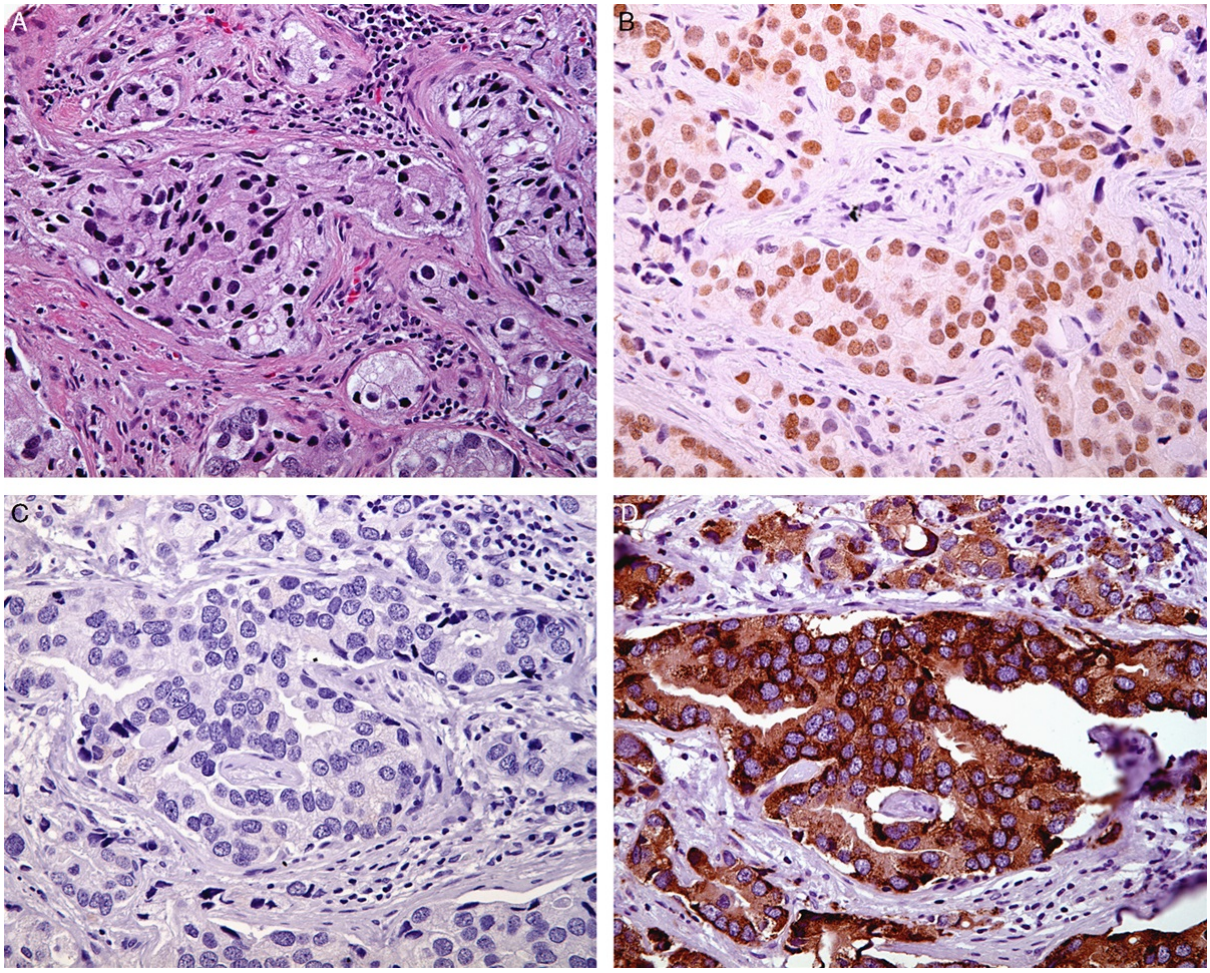

Figure 2. Immunohistochemistry of NKX3.1, PSA and PSAP in a soft tissue metastasis. A: H&E; B: NKX3.1; C: PSA; D: PSAP. Reproduced from Gurel et al. NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumours. The American Journal of Surgical Pathology34(8):1097-1105, August 2010. Used with permission.

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