Review Article
The structure and function of NKAIN2-a candidate tumor suppressor

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Abstract: The deletion of chromosomal region 6q was commonly found in several types of human cancers, although the tumor suppressor genes (TSGs) located within this genomic region are not well established. Our recent work detected recurrent chromosomal truncation at the Na⁺/K⁺ transporting ATPase interacting 2 (NKAIN2) gene in prostate cancer, which was also found to be truncated in leukemia and lymphoma, suggesting that NKAIN2 is potentially one of the TSGs located in the 6q commonly deleted region in human cancers. NKAIN2 gene consists of eight coding exons that span approximately 1 Mb of genomic DNA on chromosome 6q and there are four main splice variants. The function of this gene is not well investigated and the limited knowledge of this gene pointed to nervous system development. The chromosomal translocations in nervous development disorders usually lead to inactivation of this gene. In human tumors, both chromosomal deletion and translocation may also inactivate this gene and consequently contribute to tumorigenesis. Further genetic and cellular functional studies are required to establish its tumor suppressor role.

Keywords: Chromosomal deletion, genomic rearrangement, tumor suppressor gene, NKAIN2 (TCBA1)

Introduction
Deletions of 6q are frequently found in human cancers, including prostate cancer [1-3], breast cancer [4], pancreatic cancer [5], renal cell carcinoma [6], lung adenocarcinoma [7], malignant melanoma [8], esophageal squamous cell cancer [9], lymphoblastic leukemia [10], and non-Hodgkin’s B-cell lymphomas [11]. Several studies on the loss of heterozygosity (LOH) have also suggested that chromosome 6q is involved in the pathogenesis of various human malignancies, including prostate cancer [12], acute lymphoblastic leukemia [13], non-Hodgkin’s B-cell lymphoma [13], ovarian carcinoma [14], breast carcinoma [15], malignant melanoma [16], renal cell carcinoma [17], hepatocellular carcinoma [18], salivary gland adenocarcinoma [19], pancreatic cancer [20], and parathyroid adenoma [21]. Re-introducing a normal chromosome 6 into melanoma cells suppresses tumorigenesis [3] and a potential tumor metastasis suppressor locus has been functionally linked to 6q16.3-q23 [3]. These studies suggest that chromosome 6 may harbor one or more genes that suppress tumorigenesis and metastasis. However, while several candidate TSGs have been proposed, such as MAP3K7 [22], PLAGL1 [23], and LATS1 [24], no TSGs have been well established. In our recent study of prostate cancer genomic alterations, we found that in prostate cancer the majority of genomic truncations occurred at TSGs rather than oncogenes, indicating that genomic truncation is also a major mechanism causing TSG inactivation in prostate cancer [25]. Na⁺/K⁺ transporting ATPase interacting 2 (NKAIN2) is one of the genes recurrently truncated by chromosomal rearrangement [25] and our unpublished data showed that NKAIN2 was generally under-expressed in prostate cancer cell compare to adjacent non-malignant prostate tissues, suggesting that NKAIN2 is potentially one of the TSGs located on 6q. In the present article, we review the biological function of NKAIN2 and existing evidence to support its role in tumorigenesis. We speculate that NKAIN2 could be a novel tumor suppressor on the 6q
commonly deleted chromosomal region in human cancer and propose further researches required to investigate its potential tumor suppressor role. With the deepening of its involvement in human cancer and its cellular functions, the role of NKAIN2 in tumorigenesis will be uncovered, which may impact the treatment of human malignancy.

**Basic structure and cellular function of NKAIN2**

NKAIN2 was originally named T-cell lymphoma breakpoint associated target 1 (TCBA1), because it was involved in chromosome 6q aberrations in T-cell lymphoma and leukemia cell lines [26]. Potential functions of NKAIN2 can be predicted based on its genetic structure. Searching the Sanger Center Pfam (Protein Families) database (http://www.sanger.ac.uk/resources/databases/pfam.html) for the product of NKAIN2 identifies a family of proteins (Pfam05640/DUF798) with several members that are phylogenetically well conserved. The common feature shared by these proteins is the presence of the DUF798 domain, the function of which is not known. Most of the members of this family are classified as hypothetical proteins and no information is available about their function. In human, NKAIN2 belongs to a superfamily of transmembrane proteins that interact with the b1 subunits of Na⁺/K⁺-ATPase and that are encoded by four genes, NKAIN1, NKAIN2, NKAIN3 and NKAIN4 [27] (Table 1).

NKAIN genes do not show any similarities to any other known genes, but they do share striking evolutionary conservation among species, there is a striking amino acid conservation in the first two transmembrane domains from Drosophila to human, which is considered a good indicator of their functional significance [27]. Preliminary data concerning the putative C. elegans homolog gene (T13H5.6) suggest that its expression pattern is also conserved among phylogenetically diverse species [28]. The NKAIN2 gene consists of eight coding exons that span approximately 1 Mb of genomic DNA on chromosome 6q [28]. Four main splice variants have been identified, including two short isoforms (B and C) that contain four exons and two long isoforms (D and E) containing seven exons.

**Figure 1.** (A) Schematic representation of the NKAIN2 (TCBA1) gene. The gene consists of eight coding exons spanning about 1 Mb of genomic DNA on chromosome 6q (proportions are not respected in this picture). (B-E) Four main splice variants of NKAIN2, including two short isoforms (B and C) that contain four exons and two long isoforms (D and E) containing seven exons.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Expression</th>
<th>Interaction</th>
<th>Involvement in diseases</th>
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<td>1p35.2</td>
<td>Neuron-specific</td>
<td>Interact with the b1 subunit of the Na, K-ATPase</td>
<td>Alcohol dependence, alcohol dependence, genital herpes, alcohol dependence, lymphoma, neuroblastoma, prostate cancer, type 2 diabetes mellitus.</td>
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<tr>
<td>NKAIN2</td>
<td>6q22.31</td>
<td>Neuron-specific</td>
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<td>NKAIN3</td>
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<td>NKAIN4</td>
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Involvement of NKAIN2 in human malignance

Chromosomal rearrangements, including translocations, inversions, deletions, and duplications, are the hallmarks of human cancer [33, 34]. Those chromosomal rearrangements can either activate oncogenes by deregulating them and even generate novel tumorigenic fusion product or inactivate TSGs by deleting the whole or part of the genes. As mentioned above, NKAIN2 is located within the 6q commonly deleted region in human cancers, thus reducing expression by loss of genomic copies and potentially causing haploid insufficiency. In addition, chromosome translocations affecting NKAIN2 genomic region can also lead to loss of function of this gene, both in developmental diseases and human malignancies [26, 28, 35].

It is well established that translocation and genomic fusion can deregulate oncogenes or generate gain of function tumorigenic genes. The generation of fusion proteins is a well-known mechanism capable of promoting oncogene activation, because the fusion proteins often have novel functional features such as the unscheduled expression or constitutive activation of an intrinsic function [36, 37]. Nowell and Hungerford’s discovery of the Philadelphia chromosome in chronic myeloid leukemia (CML) [38], the first consistent chromosome change seen in human malignancy, clearly supports the notion that chromosomal abnormalities, especially translocations and their corresponding gene fusions, play an important role in the initiation of carcinogenesis. Fusion genes and the accompanying deregulation of oncogenes associated with chromosomal rearrangements have been extensively studied in hematological malignancies and soft tissue sarcomas [33], and can frequently be used to define tumor subtypes and predict prognosis. In last 10 years, the commonness and importance of fusion genes in carcinomas have also been revealed [39]. TMPRSS2:ERG, for instance, is the most frequently found fusion gene in human malignancies and occurs in about 50% of prostate cancer cases [2, 33]. However, in the case of chromosomal translocation and genomic fusion involving NKAIN2, this gene is inactivated rather than gain of function both in malignant and non-malignant diseases.

Translocation involving NKAIN2 has been identified in both T-cell lymphoma and leukemia cell lines [26]. In a T-cell lymphoblastic lymphoma cell line, HT-1, NKAIN2 was found to be fused to SUSP1 (SUMO-1-specific protease), creating a SUSP1-NKAIN2 chimeric gene. However, this fusion was not in frame, which led to the production of a abnormal SUSP1 protein and loss of NKAIN2 protein from this chromosome [26]. In an adult T-cell leukemia cell line, ATN-1, aberrant NKAIN2 transcripts were produced and no chimeric gene was detected. Therefore, in both cases, the chromosomal rearrangements affecting this genomic region led to loss of function of NKAIN2 [26]. Translocations of t(1;6)(q32.3;q22.3) and t(2;6)(q24.3;q22.31) with breakpoint at NKAIN2 have been reported in developmental delay [35] and neurological disorders [28] respectively, and in both cases, the consequence is constitutional inactivation of the NKAIN2 gene.

In our SNP array analysis of 71 samples from patients with prostate cancer, NKAIN2 was truncated in 5 cases and deleted in 10 cases [25]. Importantly, in two of the five cases where NKAIN2 was truncated, a small genomic deletion event occurred that covered nearly the entire gene but very little of the adjacent genomic regions [25]. This finding suggests that NKAIN2 is also inactivated by not only chromosome deletion but also genomic truncation in prostate cancer. Further supporting this proposition, Kanishka Sircar et al. [40] have reported that NKAIN2 is downregulated and deleted in castration-resistant prostate cancer (CRPC), which appears at the early stage of the development of castration resistance. These data support the hypothesis that NKAIN2 is an important prostate cancer TSG in the frequently deleted 6q region.

In addition to chromosome loss, mutation and promoter methylation can also result in inactivation of TSG. The expression of NKAIN2 is downregulated in human brain and CNS cancer (Data from Oncomine database http://www.oncomine.org/resource/login). In addition, 80 mutations affecting the NKAIN2 coding region have been characterized and NKAIN2 gene mutation recurrence has been found in breast carcinoma (4/1233 samples), endometrioid carcinoma (4/494 samples), clear cell renal cell carcinoma (2/878 samples), lung adenocarcinoma (7/639 samples), lung squamous cell...
carcinoma (4/531 samples), oesophagus adenocarcinoma (2/151 samples), skin malignant melanoma (13/526 samples), stomach adenocarcinoma (6/338 samples), bladder carcinoma (2/327 samples) (Data from COSMIC database http://cancer.sanger.ac.uk/cosmic and International Genome Cancer Consortium database http://dcc.icgc.org/). All these data reveals that NKAIN2 maybe a TSG inactivated in many types of human cancers.

However, based on the tissue type involved, NKAIN2 may not only act as a TSG, but also promotes tumorigenesis in tissue types where it is required for cell proliferation, such as the neurons. In fact, Romania et al. revealed that NKAIN2 expressed at high mRNA levels in affected siblings in peripheral blood and tumor samples, in a study involving an Italian family with three neuroblastoma patients [41]. Elevated NKAIN2 was also detected in MYCN-amplified neuroblastoma cell lines, in the most aggressive neuroblastoma lesions and on the cell membranes of neuroblasts in the peripheral blood of a large cohort of neuroblastoma patients. These data indicate that NKAIN2 contribute to neuroblastoma development and/or progression, which is consistent with its role in neuron cell growth.

Biological function of NKAIN2 and its involvement in human diseases

The biological function of NKAIN2 remains unclear. NKAIN2 is transcribed in different splice variants and abundantly expressed in the brain tissues [27]. Several studies have suggested that NKAIN2 is highly specific to the central nervous system and is necessary for nervous system health and development [28, 35, 42-44]. Bocciardi et al. described the characterization of a de novo balanced translocation, t(2;6)(q24.3;q22.31), in a patient with a severe neurologic phenotype that included epileptic encephalopathy with spastic tetraparesis, severe psychomotor retardation associated with cerebral atrophy and involvement of the periventricular white matter [28]. Calboli et al. analyzed 430,000 autosomal SNPs together with an additional 1.2 million SNPs with high estimated quality from the International Hap Map Project’s CEU samples and demonstrated that neuroticism is one of the primary effects associated with SNPs in NKAIN2 [42]. Yue et al. found that NKAIN2 is disrupted in intron 4 by a de novo balanced translocation, t(1;6)(q32.2;q22.3), in a child with developmental delay and recurrent infections [35]. In an Australian twin-family based association analysis of alcohol dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) study of two cohorts of Australian twins and their spouses, Wang et al. revealed that rs637547 in NKAIN2 at 6q21 showed a strong association with alcohol dependence [43]. Lind et al. performed a meta-analysis of Australian and Dutch Data and found that rs594664 in the NKAIN2 intron was the thirty most significant association with nicotine dependence, although it failed to reach genome-wide significance ($P = 2.63 \times 10^{-5}$) [44].

In addition to the strong expression in brain tissues [26, 35], NKAIN2 was also expression in thymus [26], skeletal muscle [28], spinal cord, and ovary (Data from GeneCards http://www.genecards.org). However, the role of NKAIN2 in those tissues is unknown. NKAIN2 expression was detected in hematological malignancy cell lines but not those T cell derived malignant cell lines with NKAIN2 truncation, suggesting its potential involvement in hematological cell differentiation, which is also supported by the recurrent infection in the case with constitutional t(1;6)(q32.2;q22.3) [35].

NKAIN2 in prostate cancer and the difference between Chinese and western populations

Despite numerous investigations into the molecular mechanisms underlying the pathogenesis of the disease, the genetic changes driving prostate cancer development and progression are incompletely characterized [45]. The chromosomal regions that are most commonly lost in prostate cancer are 1p, 6q, 8p21.2, 10p15, 10q21, 10q23.31, 12p12-13, 13q21, 16q22, 21q22.2 [2, 46]. Prostate cancer has been shown commonly to involve very complex chromosome rearrangements, which lead to many chromosome breakpoints and gene fusions, including the most common fusion gene in human malignancies, TMPRSS2:ERG [47, 48]. The fusions frequently involve the ETS family of transcription factors, which are placed under the control of genes highly active in prostate epithelial cells, predominately as TMPRSS2, but also including SLC45A3, HERV-K_22q11.23, C15orf21, and HNRPA2B1 [49-52]. However, in our recent work, we found that...
chromosome breakpoints in prostate cancer may more frequently lead to TSG inactivation than oncogene activation [25].

By analyzing 71 clinical prostate cancer cases and 6 prostate cancer cell lines using Affymetrix array 6.0 and 500K SNP microarrays, we identified many recurrent breakpoints (n ≥ 2) and 41 located at known TSGs, oncogenes and genes previously identified as partner genes in gene fusion events. While the ERG and TMPRSS2 genes were affected by the frequent breakpoints (identified in 18/77 and 15/77 cases, respectively), there was a preferential involvement of TSGs (n = 27) compared to oncogenes (n = 6) at the breakpoints [25]. NKAIN2 was also recurrently truncated by chromosomal rearrangement [25]. Together with our unpublished data that NKAIN2 was generally underexpressed in prostate cancer cell compare to adjacent non-malignant prostate tissues, it suggests that NKAIN2 is potentially a TSG in prostate cancer.

Interestingly, NKAIN2 was truncated in four of the 39 Chinese prostate cancer cases but not in any of the 32 cases from UK. Remarkable disparities of prostate cancer incidence and mortality exist among different racial groups and the prevalence of prostate cancer in Asian countries is much lower than that observed in Western countries [53-56]. In our previous study, we have revealed that deletions of chromosomes 21 (causing TMPRSS2:ERG and consequently ERG overexpression) and 10q (inactivating PTEN) in Chinese patients are far less common than those reported in Western populations [53, 57]. Magi-Galluzzi et al. confirmed the difference in ERG rearrangement frequency between Western and Asian patients [58]. We have also found that different ERG proteins are expressed in prostate cancer from UK or Chinese patients and that this difference was detected in prostate cancer precursor lesion, high-grade prostatic intraepithelial neoplasia [59]. PTEN is a well-characterized TSG, recurrently deleted as a result of chromosome rearrangements [60]. AKT pathway activation resulting from PTEN inactivity is a commonly detected phenomenon in prostate cancers from Western countries, particularly during the progression of cancer from localized tumors to metastatic cancer lesions [53]. However, the activation of the AKT pathway through the inactivation of PTEN may have limited contribution to Chinese prostate cancer [53]. While TMPRSS2:ERG gene fusion and PTEN loss were detected much less frequently in Chinese than Western prostate cancer, recently we also found a higher frequency of BRAF and RAF1 alterations in Chinese patients with prostate cancer than was observed in their Western counterparts [61]. All those observations suggest that UK and Chinese prostate cancer tumorigenesis may result from different genetic mechanisms and loss of function of NKAIN2 may contribute more significantly to prostate carcinogenesis in Chinese than the Western population. In fact, deletion of 6q was also more frequent in prostate cancers in Chinese than Western men, although 6q deletion occurred at a high frequency in the prostate cancer in the Western population.

Future directions

Clearly, there are limited studies on NKAIN2. It has been found to be involved in the nervous system developmental and genetic alterations affect its function lead to developmental diseases and mental disorders [28, 35, 42-44]. There are several lines of evidence suggest that NKAIN2 is potentially a TSG [25, 26, 40], but much more extensive investigations are required to confirm its tumor suppressor role. It is necessary to continue to study the chromosomal alterations of NKAIN2 gene and the functional consequence of those genomic alterations in prostate cancer and other human cancers. NKAIN2 is currently known as a transmembrane protein, which interacts with Na+/K+-ATPase. Apart from this, little is known of its cellular functions; therefore it is unclear how it may play the role to suppress tumor growth. Extensive biological study of the cellular function of NAKIN2 is urgently required, in particular its involvement in tumorigenesis pathways. Until the cellular and biological functions of NKAIN2 revealed, we will not be able to use it for the diagnosis and treatment of related diseases.

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Disclosure of conflict of interest

None.

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