

IN THE SPOTLIGHT

Unlikely Suspects Identified in Neuroblastoma Conspiracy

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Summary: *KIF1B* is a candidate tumor-suppressor gene in neuroblastoma whose function is to mediate apoptosis when nerve growth factor becomes limiting in the developing nervous system. Chen and colleagues now provide mechanistic insight into how one of the protein products of this locus, *KIF1B β* , induces apoptosis. *Cancer Discov*; 4(4); 392–3. ©2014 AACR.

See related article by Chen et al., p. 434 (5).

Neuroblastoma and pheochromocytomas are tumors of the sympathetic nervous system that are thought to result from a failure of neural progenitors to die when nerve growth factor (NGF) becomes limiting for their survival during development. Analysis of the genes responsible for familial predisposition to these cancers has identified a number of components of a signaling pathway that induces apoptosis when NGF is limiting. Thus, genes such as *VHL*, *RET*, *NF1*, *SDHB*, *SDHC*, and *SDHD* have been identified as components of a signaling network that links the NGF receptor (TRKA) to the prolyl hydroxylase EGLN3, which is required for the execution of apoptosis upon NGF withdrawal (1, 2). These data have led to a model in which mutations in any of these genes causes a defect in culling of neuronal progenitors during development, setting the stage for malignant transformation of the surplus of these progenitors (Fig. 1).

In 2008, the Kaelin laboratory published an elegant loss-of-function genetic screen in which they searched for genes that are required to execute apoptosis downstream of EGLN3. They identified an unlikely suspect, the β splice variant of a member of the kinesin family, named *KIF1B β* , as both necessary and sufficient to induce apoptosis downstream of EGLN3 (3). Although a protein involved in the transport of synaptic vesicles may not sound like a likely suspect for playing a major part in an oncogenic pathway, the genomic address of *KIF1B*, 1p36.22, certainly is a hot zip code when it comes to neuronal cancers. This locus was identified as early as 1977 as a putative tumor-suppressor locus in neuroblastoma (4). Indeed, when Schlisio and colleagues sequenced *KIF1B* genes of neuroblastomas and pheochromocytomas, they found missense mutations. Importantly, these variant *KIF1B β* proteins were impaired in their ability to induce apoptosis when introduced into primary rat sympathetic neurons, supporting a role for these variants in oncogenesis (3).

Having established a role for *KIF1B β* in neuronal apoptosis, the Schlisio laboratory went on to study how EGLN3

regulates *KIF1B β* and how *KIF1B β* promotes cell death, the results of which are reported in this issue (5). Through the analysis of a series of *KIF1B β* deletion mutants, Chen and colleagues (5) found that the motor function of *KIF1B β* is not required for the induction of apoptosis. They also found that *EGLN3* expression induced *KIF1B β* , establishing a further link between these two proteins. To study how *KIF1B β* induces apoptosis, they searched for proteins that bind *KIF1B β* through immunoprecipitation followed by mass spectrometry. This revealed another unlikely suspect in causing cancer, the DNA and RNA helicase DHX9. In a series of elegant experiments, the authors show that suppression of *DHX9* inhibits *KIF1B β* -induced apoptosis, establishing DHX9 as a relevant component of *KIF1B β* apoptosis signaling. Consistent with this notion, they found that *KIF1B β* and DHX9 synergize to induce neuronal apoptosis.

So how does DHX9 conspire with *KIF1B β* to induce apoptosis? DHX9 has two functional domains, a nuclear translocation domain and a transcriptional activation domain, in agreement with its established role in transcription activation. Mutational analysis revealed that both domains are required for apoptosis induction in combination with *KIF1B β* . Important in this context is that the authors found that DHX9 was predominantly nuclear in neuroblastoma cells with a non-deleted 1p36 locus (thus having expression of *KIF1B β*), whereas DHX9 was mostly cytoplasmic in 1p36-mutant neuroblastoma cell lines. Moreover, the cancer-associated mutants of *KIF1B β* discussed above failed to cause nuclear translocation of DHX9. These data indicate a key role for nuclear translocation of DHX9 through *KIF1B β* . To understand this further, the authors went back to their list of *KIF1B β* -associated proteins identified through mass spectrometry. This list also contained Exportin-2 (XPO2), a protein involved in nuclear import and export of cellular proteins. Indeed, knockdown of XPO2 reduced nuclear accumulation of DHX9 and also blocked apoptosis by *KIF1B β* . These data indicate that XPO2 and *KIF1B β* are required for nuclear localization of DHX9, and that this nuclear DHX9 somehow induces apoptosis. The absence of *KIF1B β* in 1p36-deleted neuroblastomas predicts that such tumors will have predominantly cytoplasmic DHX9. Indeed, when 13 primary neuroblastoma tumors were analyzed for *KIF1B β* expression and DHX9 localization, this relationship could be mostly confirmed.

The finding that mutant versions of DHX9 defective in transactivation are unable to induce apoptosis suggested that a downstream transcriptional target of DHX9 is required for

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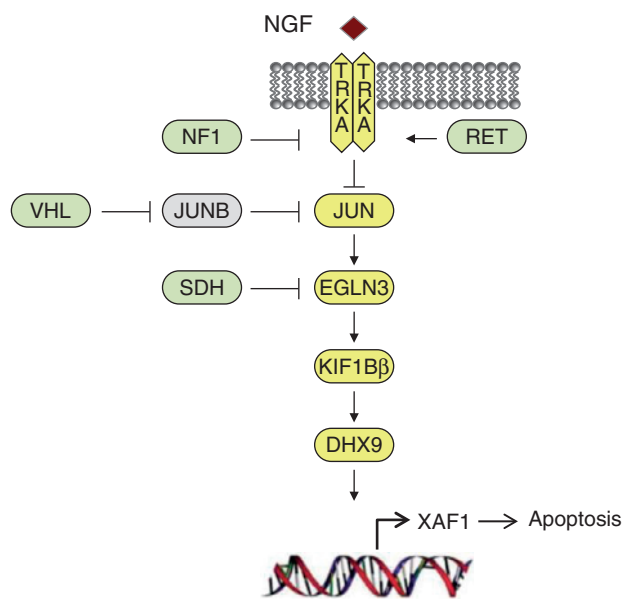


Figure 1. Model linking NGF signaling to apoptosis induction in tumors of the sympathetic nervous system. When NGF levels become limiting in the developing sympathetic nervous system, a signaling cascade is activated that involves activation of JUN, which in turn activates EGLN3. This prolyl hydroxylase activates KIF1B β , which mediates nuclear translocation of the RNA helicase DHX9, leading to transcriptional activation of the proapoptotic factor XAF1. The modulators of this pathway that are mutated in familial sympathetic nervous system tumors are highlighted in green.

apoptosis. Transcriptomic analysis of *KIF1B*^{-/-} NB1 neuroblastoma cells in which KIF1B β expression was reestablished in the presence and absence of DHX9 revealed a set of 18 genes that depended on the presence of both KIF1B β and DHX9 for activation. Among this short list was XIAP-associated factor 1 (*XAF1*), a negative regulator of the inhibitor of apoptosis (IAP) proteins. That XAF1 is involved in apoptosis in neuronal cells following NGF withdrawal is suggested by experiments in PC12 pheochromocytoma cells. In these cells, NGF withdrawal caused induction of KIF1B β protein, nuclear DHX9, and *XAF1* mRNA and protein expression. It is well established that the removal of XIAP inhibition is required for caspase activation in sympathetic neurons (6), which supports a role for XAF1 induction in the execution of apoptosis.

Together, these data establish a line of command in apoptosis regulation from NGF via TRKA–JUN–EGLN3–KIF1B β /XPO2/DHX9 to XAF1 (Fig. 1). Indeed, the authors show that in the developing mouse sympathetic nervous system, *KIF1B β* is most abundantly expressed in the sympathetic ganglia when apoptosis peaks, and this coincides with nuclear

accumulation of DHX9 in these cells. Mutations in any of the components of this signaling pathway could in principle contribute to a failure to cull neuronal progenitors during embryonic development and thereby contribute to the early stages of tumor formation. However, once full-blown neuroblastomas have formed, the absence of KIF1B β could still aggravate the oncogenic phenotype by suppressing apoptosis. Indeed, the authors show that low *KIF1B β* expression is a marker of poor outcome in neuroblastoma, which further supports its role as a tumor-suppressor gene in this cancer.

Given that so many components of the pathway from NGF to XAF1 are mutated in neuronal tumors, the authors searched public databases for cancer-associated *DHX9* mutations. They found mutations in vital domains of *DHX9* in 44 of 144 unique tumor samples, raising the possibility that this gene is another tumor suppressor in cancer.

Cancer genome analyses have identified a number of likely suspects that contribute to cancers of the peripheral nervous system. The article by Chen and colleagues (5) highlights that rigorous biochemical analyses can uncover another layer of less likely suspects that, nevertheless, play key parts in the malignant transformation of neuronal precursors. It is gratifying to see that old-fashioned biochemistry still has a place in today's world of high-throughput biology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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