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 KIF1Bβ, 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 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 KIF1Bβ. Importantly, these variant KIF1Bβ 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 KIF1Bβ in neuronal apoptosis, the Schlisio laboratory went on to study how EGLN3 regulates KIF1Bβ and how KIF1Bβ promotes cell death, the results of which are reported in this issue (5). Through the analysis of a series of KIF1Bβ deletion mutants, Chen and colleagues (5) found that the motor function of KIF1Bβ is not required for the induction of apoptosis. They also found that EGLN3 expression induced KIF1Bβ, establishing a further link between these two proteins. To study how KIF1Bβ induces apoptosis, they searched for proteins that bind KIF1Bβ 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 KIF1Bβ-induced apoptosis, establishing DHX9 as a relevant component of KIF1Bβ apoptosis signaling. Consistent with this notion, they found that KIF1Bβ and DHX9 synergize to induce neuronal apoptosis.

So how does DHX9 conspire with KIF1Bβ 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. 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...
apoptosis. Transcriptomic analysis of KIF1Bβ−/− NB1 neuroblastoma cells in which KIF1Bβ expression was reestablished in the presence and absence of DHX9 revealed a set of 18 genes that depended on the presence of both KIF1Bβ 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 KIF1Bβ 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–KIF1Bβ–XPO2/DHX9 to XAF1 (Fig. 1). Indeed, the authors show that in the developing mouse sympathetic nervous system, KIF1Bβ 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 KIF1Bβ could still aggravate the oncogenic phenotype by suppressing apoptosis. Indeed, the authors show that low KIF1Bβ 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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Unlikely Suspects Identified in Neuroblastoma Conspiracy

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