Live or Let Die: CCM2 Provides the Link

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TrkA receptors are well known for promoting neuronal cell survival. However, in some neuroblastic tumors, TrkA activation can instead induce apoptosis. In this issue of *Neuron*, Harel et al. identify CCM2 as a mediator of TrkA-dependent cell death, suggesting that CCM2 is a distinctive type of tumor suppressor that modulates tyrosine kinase signaling.

The Trk family of neurotrophin receptors plays a major role in promoting neuronal survival during development and maintenance of both the central and peripheral nervous systems. When neurotrophins bind their cognate Trks, the activated receptors initiate a number of signaling pathways critical for suppressing apoptosis and promoting neuronal survival, including PI3Kinase/Akt and Ras/Raf/MAPK. These signaling molecules participate in the activation of a complex group of transcription factors to promote survival and also induce acute changes within the cell to block apoptotic pathways (Reichardt, 2006).

In recent years, a growing body of literature has also documented an additional, paradoxical role for Trk signaling: the ability to induce cell death in the two most common malignant solid tumors in childhood: medulloblastoma and neuroblastoma (Brodeur et al., 2009; Pomeroy et al., 1997). Both tumors arise from cells of neural origin—neuroblastoma from the developing sympathetic nervous system and medulloblastoma from the neuronal precursors in the developing cerebellum (De Bont et al., 2008; Brodeur, 2003).

While both neuroblastomas and medulloblastomas are malignant tumors, the biological and clinical behavior of each type of tumor is remarkably heterogeneous. In neuroblastoma, some tumors can undergo spontaneous regression, while others instead progress to extensive metastatic disease. The Trk family of neurotrophin receptors plays critical roles in determining this behavior. Neuroblastomas expressing TrkA are biologically favorable and prone to spontaneous regression or differentiation, whereas TrkB-expressing tumors are very aggressive

and often fatal (Brodeur et al., 2009). In medulloblastoma, TrkC is a positive prognostic indicator, as the activated receptor can induce tumor cell apoptosis (Kim et al., 1999). Whereas the signaling pathways of neurotrophin induction of cell survival and differentiation are well known, the mechanism by which Trk receptor tyrosine kinases induce cell death in these cancers is far less understood.

In this issue of *Neuron*, Harel et al. (2009) for the first time shed some light on the molecular mechanism underlying the phenomenon of TrkA-induced cell death in pediatric neuroblastic tumors. During a screen for downstream effectors of neurotrophin signaling, the authors identified CCM2, the protein product of the cerebral cavernous malformation 2 gene, as an interactor of TrkA. CCM2 caused extensive cell death when expressed in cells that also express TrkA, and this effect requires the presence of both TrkA and CCM2. Surprisingly, cell death induction was selective for TrkA and was not seen when CCM2 was coexpressed with TrkB or TrkC, even though the cytoplasmic domain of the Trk family is 70% conserved.

How is it possible that CCM2 binds all three Trks but only induces death signaling upon binding TrkA? To address this question, Harel et al. used TrkA deletion mutations to identify the CCM2 interaction site as residues 465–484 in the juxtamembrane domain, a region of the receptor previously reported to be required for recycling and dynein interaction (Chen et al., 2005; Yano et al., 2001). Furthermore, when the juxtamembrane TrkA sequence was swapped into TrkB, this region does confer on TrkB the ability to cause CCM2-dependent death. It is

important to note that although there is no tyrosine in the CCM2-binding TrkA sequence, the kinase activity of TrkA is needed for death induction.

To understand how TrkA-CCM2 interactions induce cell death, Harel et al. analyzed the domain structure of CCM2. The CCM2 protein contains two domains: the N-terminal PTB (phosphotyrosine binding) domain and the C-terminal death domain, designated the Karet domain (for the Hebrew word, which means to sever, describing an unusual form of biblical sanction). While the PTB domain binds the juxtamembrane domain of TrkA, the Karet domain is also required to execute TrkA-CCM2-mediated cell death. Indeed, when the PTB domain was replaced with the Shc-PTB domain (which binds all three Trks), the new construct was able to induce apoptosis when coexpressed with TrkA, TrkB, or TrkC. Together, these data indicate that both regions of CCM2 are required for death signaling: the PTB domain provides specificity for binding to TrkA, and the Karet domain determines death signaling.

During development, TrkA and its cognate ligand NGF (nerve growth factor) promote survival. Pediatric neurogenic tumors are the only clearly identified paradigm wherein TrkA promotes death. In order to determine the biological significance of the CCM2/TrkA interaction, Harel et al. next downregulated endogenous CCM2 in TrkA-expressing medulloblastoma (MB) and neuroblastoma (NB) cell lines. In both cell lines, downregulation of CCM2 by specific shRNA significantly rescued cells from TrkA-induced death. Thus, endogenous CCM2 is a key mediator of TrkA-dependent cell death in pediatric tumors of neural origin.

In order to test the hypothesis that CCM2 is critical for TrkA-mediated apoptosis in tumors, the authors analyzed expression levels from 478 pretreatment neuroblastoma samples from patients at all ages and stages. They found that CCM2 and TrkA are coexpressed in neuroblastoma, highly correlated with one another, and both of them are correlated to improved prognosis, but not independently. No statistically significant correlation was found between expression levels and outcome for TrkB, TrkC, p75, CCM1, and CCM3.

Together these results establish CCM2 as a first molecular link between TrkA activation and cell death induction in pediatric tumors. The importance of this interaction was demonstrated for neuroblastoma biology. It will be very interesting to analyze CCM2 expression data in medulloblastoma and determine whether the same correlations hold true there. If so, another tyrosine kinase might be involved in CCM2-mediated death induction in medulloblastoma, as TrkC is apparently not able to induce death signaling with CCM2.

As the work by Harel et al. clearly shows evidence for CCM2 as a proapoptotic molecule in pediatric tumors, it will be very important to understand the molecular mechanisms whereby the Karet domain leads to death induction. CCM2 can interact with CCM3, another CCM gene widely expressed in the nervous system, and/or CCM1 (Zawistowski et al., 2005; Voss et al., 2007). A ternary complex consisting of CCM2, CCM1, and MEKK3 was shown to activate p38 mitogen-activated protein kinase signaling (Zawistowski et al., 2005). Another recent work by Chen et al. (2009) suggests that CCM3 can also induce apoptosis when overexpressed in endothelial cells. It is intriguing to speculate that CCMs binding to different receptor tyrosine kinases or functioning as scaffolds in complexes could induce cell death in different tumors. As the functional interaction site on TrkA is a specific segment of the juxtamembrane domain implicated in receptor internalization and recycling (Chen et al., 2005), another intriguing speculation is that CCM2 binding to this region could manipulate Trk's endocytic trafficking and thereby switch TrkA signaling from life to death. A full understanding of CCM2 and its role in signaling and trafficking remains to be developed in future experiments.

Investigating the mechanisms that regulate CCM2-dependent death will also be an important extension of the present study. Harel et al. demonstrated that CCM2 binding is unaffected by the state of TrkA phosphorylation, and the juxtamembrane domain does not even have a tyrosine residue. Consistent with this fact, the authors did not see a change in death induction upon the addition of the TrkA ligand NGF. However, death induction still requires an active Trk kinase domain. Taken together, these data indicate that TrkA kinase is necessary for death induction, but does not function in the traditional ligand-dependent manner. Hence, there must be additional steps in pediatric tumor responses that require the kinase and switches TrkA survival signaling to death signaling. As CCM2 has been implicated in the interactions of neural cells with blood vessels, it is intriguing to speculate that some aspect of neurovascular crosstalk may function in this switch.

In summary, Harel et al. provide compelling evidence that CCM2 links TrkA to cell death induction in neuroblastoma. This work is especially valuable, for it is the first explanation of the fact that Trks are good prognostic factors in neurogenic pediatric tumors. CCM2 could be seen as a novel category of tumor suppressors that modulates receptor tyrosine signaling, thereby also modulating the malignancy grade of a given tumor. In this light, it will be very interesting to see if this concept can be transferred to other tumors caused by the activation of receptor tyrosine kinases.

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