

Context is everything for dependence receptors in colorectal cancer

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Cancer is a consequence of the accumulation of mutations and epigenetic alterations in oncogenes and tumor-suppressor genes. Recent advances in high-throughput sequencing methods and methylation array technology has led to genome-wide analyses of gene alterations in a wide variety of cancers, including colorectal cancer (1). These studies have revealed that there are hundreds to thousands of DNA alterations in the average colorectal cancer genome, and that there are roughly 80–100 genes that are commonly altered by nonsynonymous mutations and roughly 15 mutations in candidate “driver” genes per genome. (“Driver genes” are those genes that induce the formation of cancer.) These studies have also revealed considerable heterogeneity between colorectal cancers and that the majority of gene mutations are likely passenger events, which are bystanders in cancer formation. Thus, one of the major challenges facing cancer biologists now is how to identify the mutated genes that are functionally relevant in the tumorigenesis process. The traditional approach to deciphering which genes are functionally relevant assumes mutations in genes will lead to constitutive tumor-promoting or tumor-suppressing biological effects. Thus, mutations in genes for the tyrosine kinases (e.g., *EGFR*, *BRAF*, and so forth) have typically been classified as oncogenic because they often induce a constant state of inappropriate proliferation, whereas other genes, such as *BRCA2*, are most often classified as tumor-suppressor genes because these mutations impair DNA fidelity.

In this context of deciphering the functional consequences of altered genes in the colorectal cancer genome, in PNAS Genevois et al. (2) have identified *TrkC* (also called *NTRK3*) as a tumor-suppressor gene in colorectal cancer that is commonly silenced by aberrant DNA methylation (“The dependence receptor *TrkC* is a putative colon cancer tumor suppressor”). *TrkC* is a member of the neurotro-

phin receptor family and is the receptor for the neurotrophin NT-3 (Fig. 1). This family of receptors was identified following the discovery of the neurotrophins, NGF, BDNF, NT-3, and NT-4/5, and consists of *TrkA*, *TrkB*, *TrkC*, and *p75^{NTR}* (also known as *NTRK1*, *NTRK2*, *NTRK3*, and *NGFR*, respectively). These receptors are single-pass transmembrane tyrosine kinases that were initially found to mediate neurotrophin-induced cell survival in the nervous system. Although initially identified in the nervous system, the kinases are ubiquitously expressed and are involved in a variety of diseases, including cancer (3, 4).

With regards to *TrkC* and colorectal cancer, one of the first insights into the potential role of neurotrophin receptors in colorectal cancer came when Bardelli et al. found that *NTRK3* is a commonly mutated kinase in colorectal cancer (5). Given the classic view of tyrosine kinases as being oncogenic, the authors proposed that the *NTRK3* mutations (i.e., G608S, I695V, and L760I) were likely tumor promoting. However, subsequently it has been shown that the neurotrophin receptors, and *TrkC* in particular, can function as both tumor-suppressor genes and oncogenes (4, 6). This contradiction led Genevois et al. (2) to carry out a series of careful studies to define the role of *TrkC* in colorectal cancer. This group chose to study *TrkC* in colorectal cancer because they had earlier shown that *TrkC* can function as a “dependence receptor” and because other dependence receptors, such as Deleted in Colon Cancer (*DCC*) and *UNC5H*, can act as tumor-suppressor genes in colorectal cancer (7, 8). It is the dependence-receptor aspect of the studies by Genevois et al. that make their findings particularly interesting and of importance to field of cancer biology. The concept of dependence receptors is a controversial one that was first introduced by Rabizadeh and Bredesen to explain neuron death induced by the absence of NGF (9, 10). Unlike with classic receptor biology, in which a receptor is in the “off” position

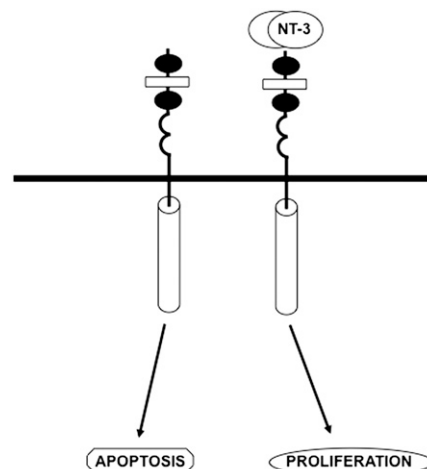


Fig. 1. Schematic representation of the biological effects induced when *TrkC* is bound and not bound by NT-3. In the unbound state, *TrkC* can induce apoptosis through caspase-mediated cleavage. The receptor has two cysteine-rich domains (black ovals), a leucine-rich domain (rectangle), two Ig like domains (half-circles), and cytoplasmic tyrosine kinase.

when not bound with a ligand, dependence receptors are biologically active in both the ligand-bound (on) and -unbound (off) state (11). Initially, this phenomenon was demonstrated for the *p75^{NTR}* neurotrophin receptor, but several investigators, including Patrick Mehlen, have provided evidence for other receptors functioning as dependence receptors (e.g., *DCC*, *RET*, *Patched*, *UNC5H*, *IR*) (12–14). The concept of dependence receptors is not universally accepted because it is plausible that the effects attributed to the ligand-free receptor are actually a default program intrinsic to the cell. In the case of the neurotrophins, it is well established that these ligands enhance cell survival in neurons, but it is less clear how the absence of ligand induces cell death. Mehlen’s research group has produced a series of well-designed studies that support the mechanism being based on dependence-receptor function (8, 15, 16).

After providing support for the dependence-receptor concept in the nervous

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system, for the last decade Mehlen's team has been assessing the role of this class of receptors in cancer. In studies from over a decade ago, they resolved the mystery behind the role of DCC in colorectal cancer. Although DCC was one of the first putative tumor-suppressor genes found in colon cancer, mouse-model studies and other functional studies failed to reveal tumor-suppressor activity (17). Mehlen's group revealed that the reason for this discrepancy was because DCC is a dependence receptor and that it had opposing effects on intestinal epithelial cells, depending on whether its ligand, netrin-1, was present (18). Genevois et al. and other investigators have further demonstrated a role for other dependence receptors in the pathogenesis of colorectal cancer, including *RET* and *UNC5H* (6, 19, 20).

In PNAS, Genevois et al. (2) have now demonstrated that TrkC has tumor-suppressor activity in the colon and, perhaps more importantly, they have provided evidence that TrkC is a conditional tumor suppressor that mediates its effects through its dependence-receptor activities (21). The authors found that the majority of colorectal cancers silence *NTRK3* through aberrant DNA methylation and that reconstitution of TrkC in colorectal cancer cell lines suppressed hallmark behaviors of cancer (2). Having established that *NTRK3* acts as a tumor-suppressor gene in the colon, they then showed that the addition of the ligand for TrkC, NT-3, rescues the cells from the tumor-suppressor effects of TrkC. The authors also showed that a naturally occurring *NTRK3* mutation, *E543D*, lacks the proapoptotic activity of TrkC. These last sets of experiments demonstrated the conditional nature of TrkC's tumor-suppressor activity and its role as a dependence receptor in colorectal cancer.

The significance of these findings is twofold. Genevois et al. (2) have found a unique tumor-suppressor gene for colorectal cancer

and have provided support for the dependence receptor concept in cancer biology. Their results also extend a context-dependence model of mutations in cancers by reinforcing the idea that tumor-suppressor genes can be conditional tumor-suppressor genes, the effects of which vary depending on not only the state of other mutant genes in the cell, but also on the presence of ligands in the case of dependence receptors.

Although the studies by Genevois et al. (2) provide strong evidence that TrkC is inactivated in colon cancer and that it functions as a tumor-suppressor gene, their findings also raise a number of questions. Given that TrkC is a member of a family of receptors, it would help to know the expression levels of the other neurotrophins and their receptors in colorectal cancer. The expression of p75^{NTR} and sortilin is particularly germane, as these

receptors can bind the unprocessed form of neurotrophins, called proneurotrophins, and can induce cell death (22). It is possible that some of the ligand-independent effects of TrkC are actually secondary to p75^{NTR} activity. In addition, the expression of TrkC and NT-3 in the normal colon and in colon adenomas remains to be defined. This information would provide a richer understanding of the consequences of TrkC deregulation in colorectal cancer formation and would address how NT-3 and TrkC interact to create a selective advantage for TrkC inactivation in cancer cells. The studies by Genevois et al. (2) have provided compelling evidence to justify further study of the neurotrophins in colorectal cancer. These additional studies will further define the potential for neurotrophins and their receptors to be the targets of anticancer therapies.

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