The Neurotrophic Receptor TrkB in Anoikis Resistance and Metastasis: A Perspective

Thomas R. Geiger and Daniel S. Peeper

Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, the Netherlands

Abstract

Resistance to anoikis ("detachment-induced apoptosis") has been suggested to be a prerequisite for cancer cells to metastasize. In a functional screen for suppressors of anoikis, we identified the neurotrophic receptor TrkB. Upon s.c. inoculation in mice, TrkB-expressing cells formed highly invasive and metastatic tumors. Here, we discuss our findings within the context of the proposed role of TrkB in human malignancies and address the question of its feasibility as a target for cancer therapy. (Cancer Res 2005; 65(16): 7033-6)

Background

Metastasis comprises a multitude of complex events, including tumor cell invasion of the surrounding tissue, invasavation of lymphatics or the vasculature, transport through, and extravasation from, these vessels, and "seeding" at a distant site (1). In order for tumor cells to successfully metastasize, they must sustain a number of genetic or epigenetic alterations that allow them to complete the various steps in the process. For example, metastasizing tumor cells must acquire the ability to survive in foreign environments (2).

Denial of their extracellular matrix causes normal epithelial cells to undergo programmed cell death called anoikis, a term derived from the ancient Greek word for homelessness (3, 4). By disseminating away from normal tissue sites through the vascular or lymphatic compartment, tumor cells are confronted by abnormal environments where they are deprived of physiologic adhesion signals. In this manner, anoikis may act as a barrier to cancer progression and metastasis, due to the absence of survival signals that are needed to support localized cancer cells (3, 4). Additionally, a large fraction of the tumor cells in a primary tumor may also face pro-anoikis signals as they lose interaction with the basement membrane (5).

TrkB Is a Suppressor of Anoikis

We hypothesized that genes suppressing anoikis may also confer increased metastatic capacity. Therefore, we did a functional screen for genes that could confer anoikis resistance. To mimic anoikis in vitro, we used cell culture dishes to which cells cannot attach, resulting in massive death of normal (anoikis-sensitive) cells within days. Out of several epithelial cell lines tested, we selected rat intestinal epithelial cells (6), as they were highly sensitive to anoikis, with only minimal background. Another important advantage of these cells is that they are completely benign, failing to produce tumors when transplanted in nude, immunodeficient mice. This allowed us to address both the in vitro characteristics and the oncogenic and/or metastatic activity of any hit obtained in the screen within the context of a single cell system.

Using this screen, we identified the full-length wild-type neurotrophic receptor TrkB, which allowed epithelial cells to survive as large aggregates in suspension (7). TrkB is a receptor tyrosine kinase that has brain-derived neurotrophic factor as one of its primary ligands. It plays an essential role in the development and function of the neuronal system, including promotion of neuronal survival (8). We found that TrkB protects cells against anoikis, in different epithelial cell lines and across species, by a mechanism that required activation of the phosphoinositide-3-kinase/protein kinase B pathway. Remarkably, TrkB completely failed to rescue programmed cell death by another proapoptotic insult, namely serum withdrawal of MYC-overexpressing fibroblasts, indicating that it does not act as a general pro-survival factor (7).

To address a potential role in oncogenicity and metastasis, epithelial cells expressing TrkB were injected i.v. into nude mice. These cells were able to survive in the bloodstream and at distant sites, leading to tumor formation in the lungs and other tissues, with the first signs of morbidity already apparent after a week. The tumors were undifferentiated and exhibited high proliferative indices. Importantly, very few tumor cells underwent apoptosis, whether they were present in large tumor masses or in small metastases, consistent with the anoikis-resistant nature of these cells in vitro. They not only invaded blood and lymphatic vessels in various tissues but also infiltrated and destroyed bone tissues.

As the i.v. experimental metastasis system bypasses the initial invasion and invasavation steps of metastasis, we also implanted TrkB-expressing cells s.c. In this setting, they produced rapidly growing tumors that were highly invasive and metastatic. Immunohistochemical analysis revealed the presence of TrkB-expressing cells in muscle tissue within a few days of implantation, and subsequently in lymphatics and in the lymph node draining the tumor site. Within 2 weeks of introduction, the tumor cells produced large secondary tumors in the lungs.

An important question arising from our work concerns the issue of how specific these effects are for TrkB. Although the screen was carried out under saturated conditions, and eventually with three different (genome-wide) cDNA expression libraries, only TrkB-expressing clones were recovered. Although this might suggest at least some level of specificity, clearly, it doesn’t rule out the possibility that overexpression of other (proto-)oncogenes would similarly diminish the sensitivity to anoikis. Indeed, a number of other genes have been implicated in anoikis resistance, including activated phosphoinositide-3-kinase (9), Bel-2 (4), activated focal adhesion kinase (10), and integrin-linked kinase (11). The implication of each of these genes in regulating cell-cell and cell-matrix interactions is consistent with the idea that...
anoikis occurs as a result of loss of adhesion signaling. However, mutant oncogenes (like activated Ras123) or relatively large cDNAs (e.g., encoding other receptor tyrosine kinases) were not present or may have been underrepresented in the libraries used. In addition, although specific downstream, prosurvival effectors of TrkB (including phosphoinositide-3-kinase) also protect cells against anoikis, they do so only in their activated forms, be it on appropriate physiologic stimulation or by (oncogenic) mutation. And last but not least, although other genes may suppress anoikis, it is important to realize that for identification in our screen, their activities had to be sufficiently dominant to allow a single cell to survive in suspension amidst hundreds of thousands of dying cells.

To address this important issue of specificity in anoikis resistance, standardized assays of (mutant) receptor tyrosine kinases expressed in untransformed cell systems will be required to identify the full spectrum of cellular players and their signaling pathways. Furthermore, within this context, genetic and proteomic analyses will be instructive to illuminate the cellular profile that anoikis resistance and metastasis have in common.

TrkB Is Overexpressed in Neuroblastoma and Other Cancers

TrkB is often overexpressed in neuroblastoma, the most common solid tumor in childhood, arising from primitive cells of the sympathetic nervous system (12, 13). Patients can be divided into low-risk and high-risk groups, often with either spontaneous regression of primary tumors or metastases, respectively. One of the most reliable markers for poor outcome is amplification of the MYCN locus. MYCN amplification is strongly associated with, but likely not responsible for, TrkB overexpression (14). As increased levels of brain-derived neurotrophic factor could be detected in some of the neuroblastomas with high TrkB expression, it is plausible that an autocrine or paracrine loop promoting cell survival exists in these tumors (15). Additionally, TrkB has been shown to protect neuroblastoma cell lines from chemotherapypinduced apoptosis (16, 17). Taken together, these data suggest that TrkB might drive one or more facets of tumor formation and metastasis, consistent with our finding that TrkB overexpression is sufficient to transform nonmalignant cells into invasive and metastatic cells.

Unlike its relatives TrkA and TrkC, which can be activated in cancer by fusion with multimerizing proteins or by aberrant alternate splicing, TrkB has not been observed to be activated in cancer by these mechanisms (18–20). Therefore, the primary mechanism of TrkB activation in human tumors seems to be through overexpression of the full-length protein (12). In addition to a potential role in neuroblastoma, an increasing number of reports show overexpression of TrkB in human cancers. For example, elevated TrkB levels have been shown in a significant fraction of pancreatic adenocarcinomas, which produce liver metastases and display a shorter time to local recurrence after therapy (21). Furthermore, the increased metastatic potential of a pancreatic cancer cell line during multiple in vivo selection cycles was found to correlate with the expression level of TrkB. In prostate cancer, TrkB overexpression was reported in 70% of 32 cases examined (22). In Wilms’ tumor, TrkB overexpression has been associated with an increased mortality risk (23). TrkB overexpression in Hodgkin lymphomas and multiple myeloma can promote survival in conjunction with brain-derived neurotrophic factor (24, 25). Anoikis is normally thought to play a critical role in tissue homeostasis of colonic epithelium. Interestingly, recent sequence analysis of the tyrosine kinase in colorectal cancers has revealed two point mutations in the TrkB gene, both within its kinase-encoding domain (26). However, the functional impact of these mutations remains to be determined. Further large-scale sequencing analysis for mutated forms of TrkB in tumors would be desirable and might lead to the identification of more cancer-associated mutants.

TrkB: A Therapeutic Target?

The prevalence of TrkB elevation in human cancer has prompted interest in this receptor as a therapeutic target (27). However, whereas we have provided evidence that TrkB overexpression is sufficient to drive tumorigenesis, invasion, and metastasis in experimental models, an important question remains as to whether TrkB activity is essential to maintain these malignant properties in human tumors. Various preclinical xenograft studies with Trk-inhibitory compounds suggest negative effects on tumor growth (27). However, the interpretation of these observations is not always straightforward, as much of this work was done in transplanted tumor cell lines and because these inhibitors also interfere with targets other than Trk receptors, including FLT3 (27, 28).

Furthermore, the suitability of TrkB as a therapeutic target is largely unknown because of an incomplete picture of the precise requirements for TrkB in normal physiology. TrkB is widely expressed in the peripheral and central nervous systems (29), where knock-out studies in the mouse have revealed that it, like brain-derived neurotrophic factor, has an important function during development (8, 30, 31). However, extrapolating these observations to the clinical applicability of TrkB-inhibitory drugs may yield a too pessimistic picture: the side effects of such drugs to the patient may be less severe than TrkB and brain-derived neurotrophic factor knock-out mice might predict, given that many tissues normally expressing TrkB lie behind the blood-brain barrier (which could help reduce side effects by achieving tissue specificity). Although phase I trials with patients suffering from mainly solid tumors indicate that Trk-inhibitors are well-tolerated and have acceptable toxicities, they failed as yet to reveal a tumor response (32, 33).

Is it necessary to develop inhibitors that target the Trk family members individually? Interestingly, neuroblastomas from patients with favorable prognosis often express high levels of TrkA instead of TrkB (13), suggesting that these receptors differentially regulate a small set of genes involved in apoptosis, invasion, and therapy resistance (34). Although it remains to be established whether increased TrkA levels contribute to a favorable prognosis (e.g., by inducing differentiation or apoptosis, as suggested by ref. 35), this illustrates how different Trk family members could affect tumor aggressiveness in radically different ways. This issue is important because it could influence whether tumors should be treated with “Pan”-Trk inhibitors (which are currently being developed) versus TrkB-specific agents.

Our findings add to the growing evidence that TrkB overexpression could drive anoikis resistance, tumorigenesis, invasion, and metastatic capability in cancer cells (Fig. 1). The tractability of TrkB as well as its ability to drive malignant pathophysiology at several levels makes it an attractive therapeutic target to consider. As discussed, a key question that remains is whether inactivation of TrkB in TrkB-overexpressing cancer cells is sufficient to limit cell survival or progression. It will also be important to dissect the
signaling pathways downstream of TrkB in tumor cell survival and other aspects of tumorigenicity, not only to increase the understanding of the mechanism by which TrkB exerts its diverse actions, but also to define additional targets for therapeutic intervention.

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References

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