A putative link between exocytosis and tumor development

Desmoplastic small round cell tumor (DSRCT) is characterized by a t(11;22)(p13;q12) translocation breakpoint. In this issue of *Cancer Cell*, Palmer et al. (2002) now show that the resulting EWS-WT1 gene-fusion product leads to overexpression of BAIAP3, a protein implicated in regulated exocytosis.

Nonrandom chromosomal translocations associated with soft tissue sarcomas, similar to those found in hematological malignancies, have drawn much attention. The most striking feature of these genetic aberrations is the tight association of a particular translocation breakpoint to a certain tumor type. The first glimpse at the underlying molecular mechanism of human soft tissue sarcomas came from the cloning of the t(11;22)(q24;q12) translocation breakpoint gene associated with Ewing's sarcoma (Delattre et al., 1992). Sequence analysis has revealed a gene fusion event of the *EWS* gene on chromosome 22q12 to the DNA binding domain of the transcription factor FLI1 located on chromosome 11q24. Similar fusion products have subsequently been isolated from various other soft tissue tumors, including desmoplastic small round cell tumor (DSRCT). Many of these translocations involve the fusion of the *EWS* gene and members of the growing family of ETS transcription factors (Arvand and Denny, 2001).

It is clear that these tumor-associated fusion products are bona fide oncoproteins. For example, it has been shown that some of these chimeric molecules can promote anchorage-independent growth and enhance tumorigenicity in animals. Furthermore, site-directed mutagenesis of the EWS and ETS coding sequences and antisense oligonucleotide-based experiments strongly suggest that EWS/ETS fusion products are required for the initiation and perhaps the maintenance of malignant phenotypes.

Intuitively, these chimeric transcription factors are expected to regulate the transactivation of genes important for cell growth and differentiation. In DSRCT, it has been shown previously that EWS-WT1 increased transcription of interleukin (IL)-2/15 receptor-β and PDGF-A (Lee et al., 1997; Wong et al., 2002). Palmer and colleagues (Palmer et al., 2002) now add an additional target to the mix. By expressing the EWS-WT1 fusion gene under a tetracycline-repressible promoter in U2OS osteosarcoma, they have isolated a gene, BAIAP3 (Brain-specific angiogenesis inhibitor 1 [BAI1] associated protein 3) (Shiratsuchi et al., 1998), which is upregulated by more than 100-fold (Figure 1). Importantly, BAIAP3-transcripts could also be detected in tumor but not stromal cells of DSRCT specimens, supporting a role of BAIAP3 in tumor promotion. In addition, ectopic expression of BAIAP3 in a melanoma cell line (MC) enhanced cell proliferation under low serum conditions, promoted anchorage-independent growth, and augmented tumor formation in animals.

Interestingly, BAIAP3 is a member of a family of proteins whose founding member, the *C. elegans* protein *Unc13*, has been implicated in the regulation of exocytosis (Brose et al., 2000; Koch et al., 2000). Indeed, it has been demonstrated that Munc13-1 (the mammalian homolog of *Unc13*) is directly involved in a process called priming of large dense-core vesicles in chromaffin cells (Ashery et al., 2000; Brose et al., 2000).

Fusion of large dense-core vesicles (and other vesicles such as synaptic vesicles at the neuronal synapse) is a multistep, highly regulated process culminating in the assembly of SNARE protein complexes that drive membrane fusion and release of vesicle contents into the extracellular space. Morphological docking of the vesicles to the plasma membrane is followed by a series of events, one of which is called priming, before Ca^{2+}-triggered exocytosis takes place. Munc13-1 is an essential player in the process of priming (Ashery et al., 2000; Brose et al., 2000). Largely based on the homology to Munc13-1, it has been suggested that members of this family of proteins, such as BAIAP3, play important roles in regulated exocytosis (Brose et al., 2000; Koch et al., 2000; Palmer et al., 2002; Shiratsuchi et al., 1998).

Perhaps it should come as no surprise that exocytosis and tumor growth might be linked. There is ample evidence in the past demonstrating that increased production and resulting secretion of certain growth factors such as PDGF, FGF, and others can result in oncogenic transformation. What is exciting about the work of Haber and colleagues is that it opens the possibility that modulation of exocytosis per se might play a role in tumor development.

It is interesting to speculate how BAIAP3 overexpression, by virtue of modulating secretion, could transform a cell. One attractive model is that since EWS-WT1 has previously been shown to increase the expression of several growth-promoting soluble factors, including PDGF-A and IGF-1, the simultaneous upregulation of BAIAP3 will lead to their overall increased secretion to the extracellular compartment. On the other hand, the activity of BAIAP3 is likely subjected to modulation itself. In this model, overexpression of BAIAP3 could make secretion of growth-enhancing factors independent from an otherwise necessary, exocytosis-promoting signal. In DSRCT, these mitogenic factors can potentially act in either an autocrine or a paracrine fashion. The dense stroma, which is characteristic of DSRCT, is certainly consistent with a role of paracrine stimulation in tumor growth.

The literature is filled with examples of tumors where growth factors and cytokines play an important role in tumor progression (Aaronson, 1991). For example, PDGF and its receptors are coexpressed in a high fraction of sarcomas and malignant gliomas. The existence of a functional autocrine loop in these cases has been implicated in promoting tumor proliferation. In addition, increases in the production of soluble factors, such as EGF, PDGF, IL-1, and VEGFs, can act in a paracrine fashion to modulate tumor growth and metastasis by acting on stromal cells and stimulating the formation of new vasculature (angiogenesis) or lymph vessels (lymphangiogenesis).

Usually, this is associated with increased transcription and expression of these soluble factors. Consequently, so far, little if any attention has been paid to a possible role of regulation of the exocytic machinery in the pathogenic development of these tumors. Clearly, the data provided by Palmer et al. (2002) provide a strong impetus to revisit these and similar cases with an increased focus on exocytosis.

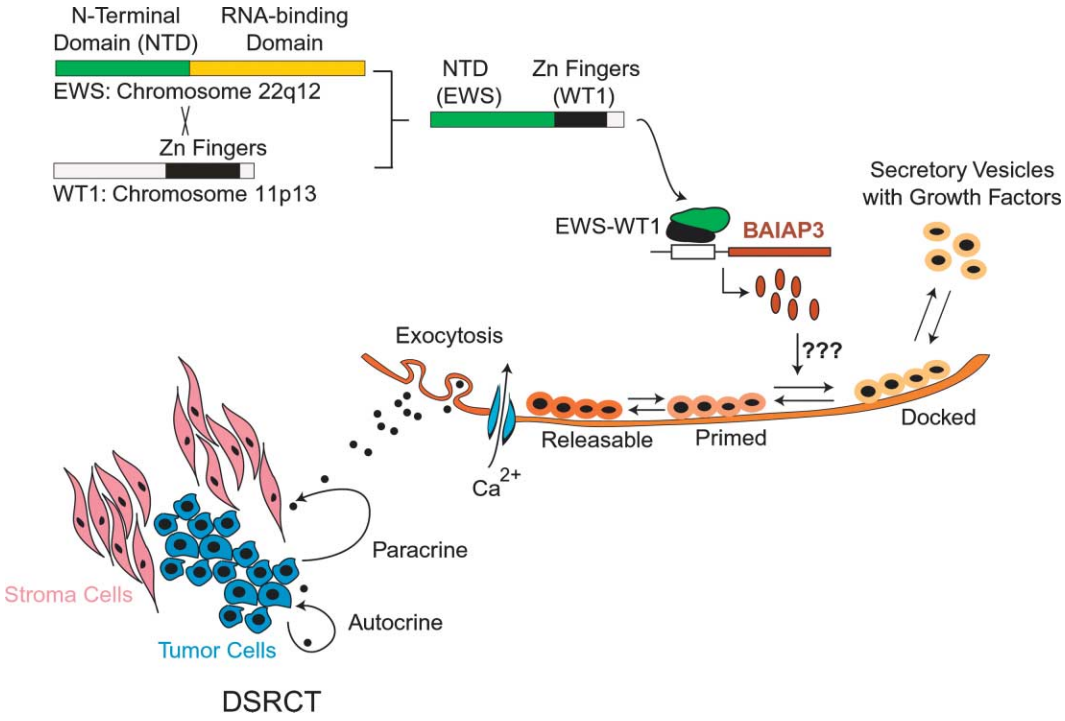

Figure 1. Possible mechanism for stimulation of tumor progression by transcriptional activation of BAIAP3

Translocation of the N-terminal domain of the Ewing's Sarcoma (EWS) gene on to the C-terminal domain of the Wilms' Tumor 1 (WT1) gene results in the expression of an EWS-WT1 fusion protein. EWS-WT1 has now been shown to transactivate BAIAP3. BAIAP3, similar to a homologous protein Munc13-1, might be involved in the regulation of exocytosis. One possibility is that, like Munc13-1, BAIAP3 might stimulate a process called priming. Priming has been reported to be a necessary step in the secretion of vesicular contents from large dense core vesicles. Alternatively, BAIAP3 might influence other steps in the exocytic pathway. The increased secretion of growth factors or cytokines could then stimulate tumor progression of desmoplastic small round cell tumor (DSRCT) in an autocrine and/or paracrine fashion.

The link of BAIAP3 to tumorigenesis also raises the possibility that other proteins involved in exocytosis could play a role in cancer progression. Growth factor receptors have become attractive targets for cancer therapy. For instance, herceptin, which is an antibody against HER2/neu, has become an important tool in the treatment of breast cancer. The inhibition of signaling molecules, such as kinases, has become an important strategy in cancer therapy. As an example, the kinase inhibitor STI571 (Glivec) aimed at the fusion product Bcr-Abl has been shown to be highly effective in the treatment of chronic myelogenous leukemia. Similarly, BAIAP3—and in the future, perhaps other proteins involved in exocytosis—has now become a potential therapeutic target. The importance of new avenues of treating cancer by discovering new pathways of tumor progression cannot be overestimated.

The study of Palmer et al. represents the first—albeit still tentative—link between cancer and exocytosis. While much remains to be learned about the mechanism and the precise role of BAIAP3 in tumorigenesis, the publication of Haber and colleagues (Palmer et al., 2002) is the first page of a new chapter in cancer research.

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