The DARC side of metastasis: Shining a light on KAI1-mediated metastasis suppression in the vascular tunnel

Tumor cell metastasis to distant organs is an inefficient process that is limited in part by recently identified metastasis suppressors. Interactions between tumor cells and the surrounding stroma are thought to control much of cancer progression. In the August issue of *Nature Medicine*, Bandyopadhyay et al. (2006) demonstrate that specific cell surface interactions between the metastasis suppressor KAI1 on tumor cells and the decoy cytokine receptor DARC on adjacent vascular cells triggers senescence in the tumor cells and suppresses metastasis. These new observations demonstrate how metastasis suppressors can relay the restraint imposed by the stroma onto disseminating tumor cells.

It has become apparent that tumor cell metastasis is controlled by molecular processes distinct from those that control tumorigenesis. Progression of metastasis appears to be controlled in part by a unique subset of genes that suppress tumor cell dissemination without affecting development of the primary tumor (Rinker-Schaeffer et al., 2006). While increasing numbers of metastasis suppressor genes are being identified, they are primarily operationally defined and rarely mechanistically understood. It should come as no surprise that, like tumorigenesis, metastasis is strongly influenced by interactions between the tumor and stromal cells. Recent work suggests that paracrine CSF-1 and EGF signaling makes macrophages obligate partners in early steps in metastasis (Condeelis and Pollard, 2006), and that hematopoietic progenitors generate a metastatic niche through SDF-1- and CXCR-4-mediated communication with metastasizing tumor cells (Kaplan et al., 2005). Continuous interaction between the tumor and host cells occurs in a tissue-specific manner, and metastasis suppressors could regulate each of the rate-limiting steps of the metastatic cascade. While some of these interactions occur via soluble ligands, others occur through transmembrane proteins proximal to the cellular surface. Among the latter, the tetraspanins KAI1/CD82 and PETA-3/CD151 have both been demonstrated to regulate metastasis (Hemler, 2005). However, all of their interacting partners have, up to now, been defined as adjacent transmembrane or proximal cytoplasmic proteins expressed within the tumor cell itself. For the first time, researchers in the Watabe lab (Bandyopadhyay et al., 2006) demonstrate that vascular cells can limit tumor cell proliferation and induce senescence by molecular handshaking between the metastasis suppressor KAI1/CD82 on metastasizing tumor cells and the cytokine decoy receptor DARC (*D*uffy *a*ntigen *r*eceptor for *c*ytokines) on the encountered or adjacent vascular cells.

KAI1/CD82 is a member of the tetraspanin family, which consists of 32 members that play critical roles in a myriad of biological processes ranging from sperm-egg fusion to retinal integrity to metastasis. The tetraspanins are functionally defined as organizers of web-like multimolecular membrane complexes and interact with numerous membrane proteins, including integrins, growth factor receptors, and other tetraspanins. However, there is little mechanistic understanding of tetraspanin function (Hemler, 2005). Consequently, although KAI1/CD82 was identified as a metastasis suppressor more than 10 years ago (Dong et al., 1995), a definitive mechanism of action of the suppression has not been forthcoming.

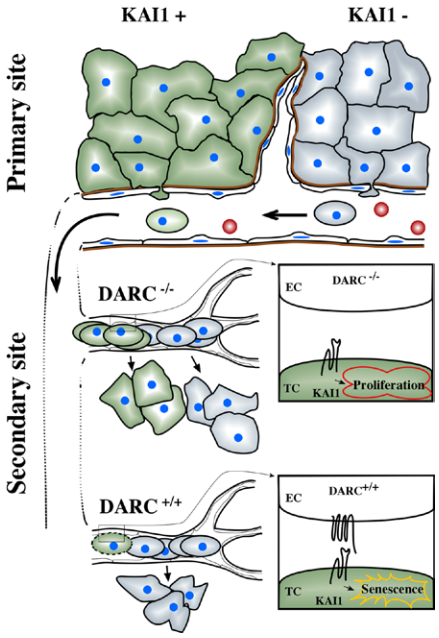

Figure 1. Direct interaction between KAI1-expressing tumor cells and DARC-expressing endothelial cells limits proliferation of metastasizing cells

KAI1-positive (green) and -negative (blue) tumor cells disseminating from the primary tumor are exposed to the vascular cells while intravasating at the primary site as well as during arrest and extravasation at the secondary site. In the absence of DARC (DARC$^{-/-}$), both KAI1-positive and -negative cells proliferate in the circulation and metastasize. In the presence of DARC (DARC$^{+/+}$), handshaking between cell surface KAI1 and DARC on adjacent tumor (TC) and endothelial cells (EC) inhibits proliferation of KAI1-positive tumor cells (green) in the vasculature by inducing senescence (enlarged view). As a consequence, only KAI1-negative cells (blue) proliferate in DARC-expressing vasculature.

Using yeast two-hybrid screening, Bandyopadhyay et al. (2006) surprisingly identified the seven transmembrane protein DARC as a specific KAI1 interacting partner. DARC has been well established as a cytokine decoy receptor with specific ligand interactions and has an accepted role in sequestering soluble cytokines from functionally endowed receptors (Rot, 2005). Interestingly, DARC expression is limited to select cell types, including endothelial cells, erythrocytes, and a few specific epithelial cells. Bandyopadhyay et al. (2006) nicely demonstrate that direct interaction between KAI1-expressing prostate tumor cells and DARC-expressing endothelial cells leads to suppression of proliferation and the induction of senescence in the KAI1-expressing cells. Direct contact between KAI1 and DARC was indicated by coimmunoprecipitation of the KAI1-DARC complex in crosslinking experiments and inhibition of adhesion between KAI1- and DARC-expressing cells using anti-KAI1 antibodies. Yet the most compelling results in this study were from a series of spontaneous and experimental metastasis experiments using metastatic variants of the syngeneic B16 melanoma cell line in wild-type and *Darc*$^{-/-}$ mice. Tumor cells that lacked KAI1 expression metastasized equally well in wild-type and *Darc*$^{-/-}$ mice. However, tumor cell KAI1 expression dramatically suppressed spontaneous and experimental metastasis in wild-type but not *Darc*$^{-/-}$ mice. KAI1 expression does not lead to reduced primary tumor size,

presumably because of limited direct contact between KAI1-positive tumor cells and DARC-positive vascular cells. The metastasis-specific senescence appears to be due to KAI1-DARC interactions occurring between tumor cells and vascular cells during transit of the tumor cells in circulation (Figure 1). The study demonstrates that direct physical contact between stromal cells and "in transit" tumor cells can control the survival of the disseminating metastatic cells—a phenomenon quite distinct from microenvironmental influences at the site of the primary tumor.

Although DARC is a promiscuous cytokine receptor, *Darc*$^{-/-}$ mice exhibit no detectable phenotype deficiency except for a moderate delay in inflammatory cell influx, possibly suggesting DARC involvement in endothelial interactions with another "cell in transit." DARC is also the erythrocyte receptor for the malarial parasite, and it is interesting that, among the human population, 70% of individuals of West African descent lack DARC erythrocyte expression and are resistant to malaria infection. However, men of African descent are not known to exhibit decreased immune function but do exhibit a 60% greater incidence of prostate cancer and a corresponding 2-fold greater mortality (Luo et al., 2000), possibly suggesting a deficient suppressor function. Wang et al. (2006) also demonstrated independently that DARC can act as a negative regulator of metastasis. Upon transfection of metastatic breast cancer cells with DARC, they observed diminished metastasis thought to be due to decreased angiogenesis, which limited tumor size. This correlation is bolstered by clinical evidence from the same study that links low expression of DARC with poor patient survival and metastasis. Could it be possible in these cases that a reversal of roles occurs, and DARC expression on tumor cells limits the proliferation of KAI1-positive angiogenic endothelial cells?

Because KAI1 and DARC have both been clinically demonstrated to be negatively correlated with metastasis and disease progression, it is rewarding to now witness a possible mechanistic connection between these two suppressor genes. However, the structural/biochemical evidence of their physical interaction remains limited. Because of its incorporation into larger molecular complexes of the tetraspanin enriched microdomains (Hemler, 2005), KAI1 is unlikely to act as an isolated player, thus leaving for future investigation the identification of the functional KAI1 membrane complex that interacts with DARC. The results from the study by Bandyopadhyay et al. (2006), although not definitive in terms of the biochemistry of KAI1-DARC interaction, do indeed represent the first indication that the tumor suppressor KAI1 engages a specific and distinct interaction with DARC-expressing vascular cells and initiates a suppression of proliferation of tumor cells "in transit" to a metastatic site.

While it is tempting to assume that suppression of proliferation in the vasculature would limit metastasis, nonproliferative cells might still be capable of escaping the vasculature and subsequently proliferating in the DARC-negative stroma. This suggests that intravascular proliferation of metastasizing cells (Al-Mehdi et al., 2000) may be more critical than previously thought, or that additional KAI1 suppressive mechanism(s) control invasion/expansion of metastatic cells within the stroma of the secondary site (Sridhar and Miranti, 2006).

Hence, until the operational importance of this tetraspanin-DARC interaction in metastasis becomes mechanistically defined, it remains molecularly complex and location obscure.

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