Interaction of Duffy Antigen Receptor for Chemokines and KAI1: A Critical Step in Metastasis Suppression

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Abstract

Tumor metastases suppressor protein KAI1/CD82 is capable of blocking the tumor metastases without affecting the primary tumor formation, and its expression is significantly down-regulated in many types of human cancers. However, the exact molecular mechanism of the suppressor function of KAI1 remains elusive. Evidence from our laboratory supports a model in which tumor cells dislodge from the primary tumor and intravasate into the blood or lymphatic vessels followed by attachment to the endothelial cell surface whereby KAI1 interacts with the Duffy antigen receptor for chemokines (DARC) protein. This interaction transmits a senescent signal to cancer cells expressing KAI1, whereas cells that lost KAI1 expression can proliferate, potentially giving rise to metastases. Our model of the mechanism of action of KAI1 shows that metastasis suppressor activity can be dependent on interaction with host tissue and explains how KAI1 suppresses metastasis without affecting primary tumor formation. Taken together, in vitro and in vivo studies identify the KAI1-DARC interaction as a potential target for cancer therapy. [Cancer Res 2007;67(4):1411–4]

KAI1 Blocks Metastases without Affecting Primary Tumor Formation

When cancer is diagnosed, the most critical question is whether the disease is localized or has it already disseminated to other parts of the body. Unfortunately, the majority of patients already have a clinically undetectable metastatic disease at the time of a visit to the clinic, and >90% of cancer patients ultimately succumb to sequelae of metastatic disease. Following primary tumor formation, a population of tumor cells can acquire molecular and cellular changes, which enable cancer to spread to distant sites. These include invasive phenotype that results in the loss of cell-cell adhesion and cell-extracellular matrix adhesion followed by proteolytic degradation of the matrix. Additional changes are needed in order for cells to intravasate into neighboring blood and lymphatic vessels and disseminate through the circulation. Those cells that survive in the circulation are arrested at distant organs sites, extravasate, and lodge at the secondary sites, where the cells must also proliferate and colonize for successful metastasis. The molecular mechanism(s) regulating acquisition of metastatic ability remains poorly understood despite the urgent need for development of novel treatment options for patients with metastatic disease. The discovery of a series of metastasis suppressor genes in the past decade has shed new light on many crucial aspects of this intricate biological process. The metastases suppressor genes and their encoded proteins, by definition, suppress the process of metastasis without affecting tumorigenesis. To date, more than a dozen of these genes have been identified and include nm23, KAI1, Kiss1, BRMS1, MKK4, RhoGD12, RKIP, Drg-1, CRSP3, SSeCKs, TXNIP/VUP1, Claudin-4, and RRM1 (1).

The KAI1 gene was originally isolated as a prostate-specific tumor metastasis suppressor gene using the microcell-mediated chromosome transfer method followed by Alu-PCR. It is located in the p11.2 region of human chromosome 11 (2, 3). When the KAI1 gene was transferred into highly metastatic Dunning rat prostate cancer cells, KAI1-expressing cancer cells were suppressed in their metastatic ability in mice, whereas their primary tumor growth was not affected (2). The DNA sequencing analysis of the KAI1 gene revealed that it is identical to CD82, a surface glycoprotein of leukocytes (3). The protein has four hydrophobic and presumably transmembrane domains, two extracellular domains, and three short intracellular domains and belongs to the family of tetraspanins. Immunohistochemical analysis of human prostate tumor samples revealed that the KAI1 expression was down-regulated in >70% of the primary tumors (4). Similar results were also observed in other types of tumors, including lung, breast, pancreatic, colon, bladder, ovarian, hepatocellular carcinoma, and melanoma (1, 5). KAI1 gene expression is correlated with poor survival in patients with these types of cancers. Therefore, the KAI1 is a bona fide metastasis suppressor protein in multiple cancer types. This raises the intriguing question of how the KAI1 gene suppresses the metastasis process.

Duffy Antigen Receptor for Chemokines on Endothelial Cell Plays a Key Role in the Suppressor Function of KAI1

To understand the mechanism of KAI1 in metastasis suppression, a yeast two-hybrid system was used to systematically screen interacting proteins of KAI1 and found that KAI1 was physically associated with the Duffy antigen receptor for chemokines (DARC; ref. 6). DARC is a promiscuous CXC chemokine receptor that is strongly expressed on the endothelial cells of lymphatic and blood vessels as well as on RBCs. Immunohistochemical analyses confirmed that DARC is highly expressed in the endothelium, particularly in the small veins and venules as well as lymphatic vessels in both normal and tumor tissue of the prostate and breast, whereas its expression is undetectable in the epithelial cells or stroma. On the other hand, KAI1 is highly expressed in the normal epithelial cells in these organs, and the expression is significantly reduced in carcinoma. Based on this spatial localization, we hypothesized that KAI1 on epithelial cells interacts with the DARC when cancer cells expressing KAI1 intravasate and encounter the endothelial lining of small blood vessels. A series of experiments...
done to test this putative interaction using human endothelial cells found that tumor cells expressing KAI1 indeed bound to endothelial cells and this binding was blocked by KAI1 antibody. These results prompted the question "what is the physiologic outcome of the interaction between KAI1 expression tumor cells and DARC on endothelial cells?" Results from recent studies on T-cell activation provided clues crucial to answering this question. KAI1/CD82 is barely detectable on resting peripheral T and B lymphocytes, whereas its expression is highly up-regulated on activation of these cells. This up-regulation is associated with morphologic changes and expression of activation markers, such as CD82 and MHC II antigens. Lebel-Binay et al. described that the coengagement of KAI1/CD82 and T-cell receptor by anti-CD82 monoclonal antibody (mAb) and anti-CD3 mAb, respectively, was able to activate T cells in vitro. Specifically, when T cells are stimulated in vitro by anti-KAI1/CD82 mAb, KAI1/CD82 seems to transmit a signal that results in tyrosine phosphorylation, a rapid increase in intracellular Ca\(^{2+}\) level, and interleukin-2 production (7). Interestingly, this activation was associated with a change in cellular morphology and inhibition of cell proliferation (8). Therefore, we hypothesized that engagement of KAI1/CD82 on cancer cells may also activate a similar signal pathway, which results in growth arrest of tumor cells. Consistent with this hypothesis, the addition of anti-CD82 antibody in the culture of KAI\(^+\) cells resulted in significant growth suppression of tumor cells, which was also observed when the tumor cell was cocultured with human endothelial cells. Therefore, our data strongly suggest that growth suppression is determined by dynamic and reciprocal interaction of KAI1 on cancer cells and DARC on endothelial cells in the vasculature (Fig. 1).

To further corroborate these findings, melanoma cells with or without expression of KAI1 were transplanted into DARC knockout as well as wild-type mice and resultant overt lung metastases were quantitated. Primary tumors developed in all mice without significant changes of growth rate regardless of the KAI1 level in the tumor cells and DARC status of the mice. However, the KAI1-positive cell lines developed significant number of pulmonary metastases in the DARC knockout mice, whereas metastasis was almost completely abrogated when the same cell lines were injected into the heterozygote and wild-type littermates. Thus, in the absence of DARC, tumor cells expressing high level of KAI1 formed metastases, supporting our model that the metastasis suppressor function of KAI1 is dependent on the interaction of KAI1 and DARC on endothelial cells. The biochemical nature of this growth suppression through the KAI1-DARC interaction is of significant interest. To explore this, green fluorescent protein–labeled tumor cells were cocultured with endothelial cells and found that KAI1-positive cells became senescent without a sign of apoptosis. Further, senescence was associated with down-regulation of

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**Figure 1.** KAI1-DARC interaction blocks tumor metastasis.
TBX2 and up-regulation of the cyclin-dependent kinase inhibitor p21. These studies suggest that growth suppression induced by the KAI1-DARC interaction is due to the activation of p21 followed by cellular senescence.

**DARC Signal for Cancer Therapy?**

Our model of the mechanism of action of KAI1 explains how KAI1 suppresses metastasis without affecting primary tumor formation. However, it has also provoked many critical questions about (a) whether KAI1 function requires other "cofactors," (b) what cellular signal is induced by DARC and KAI1 interaction, and (c) whether KAI1-DARC signal be targeted for cancer therapy.

KAI1 has been reported to be associated with many different membrane proteins, including integrins, Kitenin, epidermal growth factor receptor (EGFR), CD63, CD9, EW12, and c-Met (9–14). KAI1 belongs to the transmembrane 4 superfamily, which is known to form a multimeric complex referred to as tetraspanin web (9) that also interacts with integrins. KAI1 was indeed found to be associated with various integrins, including α5β1, αvβ3, α5β1, and αvβ3, and the complex of integrin α5β1 and KAI1 was reported to suppress fibroblast/α5β1-induced cell invasion through inhibition of the cytoskeletal system (10). KAI1 was also found to be associated with other tetraspanins, including CD9 and CD63 (11). The functions of these molecules are not well understood; however, the loss of expression of CD9 and CD63 correlates with poor prognosis and increased metastasis (12). Therefore, these two tetraspanins may also play an important role in the KAI1 function.

More recently, Lee et al. (13) found that KAI1 is associated with another tetraspanin molecule, Kitenin, whose overexpression promoted increased tumorigenicity and metastasis in vivo. The exact molecular function of Kitenin is yet to be understood; however, it was proposed that Kitenin decreases the metastasis suppressor functions of KAI1 and/or cytoplasmic signaling pathway that shifts the invasive/anti-invasive balance toward invasion. In addition to integrins and tetraspanins, Odintsova et al. (14) recently found that KAI1 physically associates with the EGF and rapidly desensitizes the EGF-induced signal, which could lead to suppression of cell migration, although it is as yet unclear whether this mechanism indeed accounts for the metastasis suppression in vivo. Nevertheless, KAI1 seems to be able to interact with various proteins on the membrane. It is yet to be determined whether all these components indeed form a "multiprotein complex" and whether they are all necessary or sufficient for the metastasis suppressor function of KAI1 because these proteins associated with KAI1 have been identified in different systems.

DARC-KAI1 interaction seems to transduce cytoplasmic signal to nucleus to modulate TBX2 and p21 expression and induce senescence. The signal transduction mechanism involved in this senescence pathway is a crucial question. Thus far, however, little information is available about the signal related to the KAI1 function. Zhang et al. (10) recently reported that protein kinase C (PKC) is associated with various tetraspanins, including KAI1, and that these tetraspanins act as linker molecules to recruit PKC into proximity with specific integrins. It was also shown that only those integrins (αvβ3 and α5β1) that strongly associated with tetraspanins, such as KAI1, were in association with PKC.

Therefore, KAI1 may act as a modulator of the PKC signal, which plays a crucial role in cell cycle progression, migration, and invasiveness as well as in cell cycle arrest (15, 16). Interestingly, PKC was found to be able to directly modulate p21 expression and activity (15), which is the hallmark of KAI1/DARC-induced senescence. More recently, KAI1 has been shown to suppress integrin-dependent activation of the receptor kinase c-Met (17). The ligand of c-Met is hepatocyte growth factor, which is capable of both stimulating and arresting cell cycle, and these effects in either case are mediated through the up-regulation or down-regulation of p21. Therefore, PKC and c-Met pathway may play key roles in the KAI1/DARC-induced signaling, although this possibility needs to be tested directly.

DARC is the receptor of the malaria parasite *Plasmodium vivax*. Approximately 70% of West African descendants have lack of expression of DARC on erythrocytes, thereby resistant to malaria infection. Interestingly, the same population showed significantly higher incidence of both prostate and breast cancer as well as higher rate of metastatic disease than white (18). DARC also serves as promiscuous receptor for both C-C and CXC chemokines, which is believed to function as "decoy" of excess chemokines. Therefore, DARC is proposed to play a role as antimetastatic molecule by clearing angiogenic CXC chemokines. In fact, Shen et al. (19) recently have shown that growth of prostate tumor was significantly augmented in DARC-deficient mice compared with the wild-type.

More recently, it was also reported that overexpression of DARC in breast cancer cells significantly suppressed the spontaneous pulmonary metastasis (20). Therefore, DARC may function as a metastases suppressor by two different mechanisms (i.e., by inducing KAI1 signal in tumor cells and by sequestering angiogenic factors). This raises an attractive possibility of developing antimetastatic drugs that mimic the action of DARC. Further understanding of the biochemistry of the interaction of KAI1-DARC and their cofactors as well as the downstream signal should lead to effective therapeutic strategy against metastatic cancer.

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