PAR-1 and Thrombin: The Ties That Bind the Microenvironment to Melanoma Metastasis

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Abstract

Progression of melanoma is dependent on cross-talk between tumor cells and the adjacent microenvironment. The thrombin receptor, protease-activated receptor-1 (PAR-1), plays a key role in exerting this function during melanoma progression. PAR-1 and its activating factors, which are expressed on tumor cells and the surrounding stroma, induce not only coagulation but also cell signaling, which promotes the metastatic phenotype. Several adhesion molecules, cytokines, growth factors, and proteases have recently been identified as downstream targets of PAR-1 and have been shown to modulate interactions between tumor cells and the microenvironment in the process of melanoma growth and metastasis. Inhibiting such interactions by targeting PAR-1 could potentially be a useful therapeutic modality for melanoma patients. Cancer Res; 71(21); 6561–6.

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Introduction

Tumor cell metastasis is currently the leading cause of cancer-related deaths. The metastatic process depends on both the tumorigenic capacity of the cells and the interactions between these cells and the microenvironment, that is, the "seed and the soil" (1). Signals from neighboring cells are instrumental in promoting tumor growth and metastasis. In melanoma, tumor development relies heavily on remodeling of the microenvironment. The interactions between melanoma cells and the stroma, including fibroblasts, endothelial cells, immune cells, soluble molecules, and the extracellular matrix (ECM), depend not only on cell–cell and cell–matrix contact but also on secretion of cytokines and growth factors. These interactions promote the metastatic process by inducing angiogenesis, invasion, migration, and colonization in a secondary organ (2).

The progression of melanoma from the nonmetastatic to the radial growth phase, vertical growth phase, and metastatic phenotype is accompanied by various alterations in gene expression, including elevated expression of protease-activated receptor-1 (PAR-1), also known as the thrombin receptor. Elevated PAR-1 expression during melanoma progression has been suggested to promote key processes that contribute to melanoma metastasis. Because thrombin is the main activator of PAR-1, it, too, is implicated in promoting melanoma progression. This review describes recent findings regarding the emerging roles of PAR-1 and thrombin in mediating cross-talk between tumor cells and the microenvironment, and the potential use of PAR-1 as a therapeutic target for metastatic melanoma.

Functions of Thrombin and PAR-1

Advanced malignancy often correlates with activation of the coagulation system, termed cancer coagulopathy, which is associated with increased mortality rates (3). Thrombin, a blood-derived serine protease, is the main effector of the coagulation cascade. During the coagulation cascade, tissue factor interacts and forms a complex with circulating factor VIIa. This complex, in turn, activates factor X, producing factor Xa. Factor Xa then converts prothrombin to active thrombin (4). Thrombin activation can subsequently cleave fibronectin into fibrin, increasing both fibrin deposition and thrombin storage in the microenvironment. Degradation of fibrin by plasmin can also release active thrombin (5). Tissue factor is primarily expressed by epithelial cells and macrophages and, to a lesser extent, by circulating monocytes and leukocyte-derived microparticles. Leakage of plasma coagulation factors following disruption of vascular integrity or inflammatory events can promote tissue factor–mediated thrombin activation, leading to coagulation (4). However, the function of thrombin is not limited to promoting coagulation. It also plays a role in promoting tumor growth and metastasis as a result of its involvement in regulating numerous critical cellular events, including cell proliferation, cell adhesion, angiogenesis, and invasion (6–9). Thrombin and other factors involved in the coagulation cascade exert their effects via protease-activated receptors [PAR (4, 9)].

The PARs are a family of G-protein–coupled receptors. The 4 members of this family (PAR-1 to PAR-4) have been implicated in the regulation of various cellular processes, including inflammation and coagulation (4). PAR-1, the prototypic...
member of the PAR family, is activated by thrombin following cleavage of its extracellular amino terminus domain. Other proteases, such as coagulation factor Xa, granzyme A, matrix metalloprotease-1 (MMP-1), and activated protein C (APC), can also activate PAR-1 (4). Cleavage of PAR-1 reveals a new amino terminus domain that functions as a tethered ligand by binding to its receptor and initiating downstream signaling. PAR-1 activation triggers signaling through multiple heterotrimeric G protein subtypes, such as $G_{q}$, $G_{i/o}$, and $G_{12/13}$. Coupling of PAR-1 to these G proteins activates several signaling pathways, including the phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) cascade, Rho kinase, and phospholipase C-$\beta$ (PLC-$\beta$), all of which can promote proliferation, migration, and adhesion (4). Given its function in activating these key cell-signaling processes, it is not surprising that PAR-1 plays an important role in promoting cancer progression in several malignancies, including melanoma, breast, prostate, and lung cancers (5).

**Effect of Thrombin/PAR-1 on Tumor Cells**

Increased expression of PAR-1 is closely associated with melanoma progression. Tissue microarray studies have shown that PAR-1 is highly expressed in malignant melanoma tumors compared with common melanocytic nevi and normal skin. Massi and colleagues (10) observed significantly elevated PAR-1 mRNA and protein expression levels in clinical samples of atypical nevi and melanomas compared with common melanocytic nevi. Moreover, PAR-1 expression in melanoma cell lines has been shown to correlate with the metastatic potential in vivo (11). The role of PAR-1 in promoting melanoma metastasis was initially shown by Nierodzik and colleagues (7), whose work detailed a role for PAR-1 as a rate-limiting factor required for experimental lung metastasis of murine melanoma cells. In accordance with these findings, our group showed that PAR-1 promotes human melanoma metastasis (11). In vivo targeting of PAR-1 using siRNA-encapsulated liposomes resulted in decreased expression of a number of angiogenic and metastatic genes, including VEGF, interleukin-8 (IL-8), and MMP-2. Additionally, these melanoma tumors displayed decreased blood vessel density (CD31), suggesting that PAR-1 regulates melanoma cell growth and metastasis by affecting both invasive and angiogenic factors (11). These factors can act in both an autocrine and paracrine fashion, influencing both melanoma tumor cells and cells in the tumor microenvironment (Fig. 1).

In the past few years, investigators have made significant efforts to elucidate the role of PAR-1 in melanoma progression by examining its interactions with other signaling molecules. Of note, our laboratory established a link between PAR-1 and another proinflammatory G-protein–coupled receptor, platelet-activating factor receptor (PAFR). PAR-1 activation in melanoma cells upregulates the expression of PAFR and the secretion of its ligand PAF. Elevated PAF expression has a number of effects in the melanoma microenvironment.

![Figure 1. Thrombin and PAR-1 promote melanoma progression by inducing cross-talk between tumor cells and the microenvironment. PAR-1 expression on melanoma and stromal cells induces downstream signaling that regulates the expression of genes, such as Cx-43, maspin, VEGF, IL-8, PAFR, and PAF, which in turn affect the metastatic phenotype of melanoma. Cx-43 promotes interaction with vascular endothelial cells, whereas IL-8 serves as a survival factor for vascular endothelial cells and acts in an autocrine fashion to induce MMP-2 secretion by the melanoma cells.](image-url)
First, PAF activates platelets, thereby promoting tumor-platelet aggregation formation. The PAF/PAFR axis also elevates levels of the melanoma cell adhesion molecule (MCAM)/MUC18 (CD146) mRNA and protein in melanoma cells (12). Of interest, enhanced MUC18 expression in melanoma cells promotes adhesion to endothelial cells and increases MMP-2 expression, melanoma cell invasion, and metastasis (12). Our work showed that the effects of PAR-1 on PAFR ultimately result in enhanced melanoma cell adhesion to endothelial cells, increased transendothelial migration, and prolonged lung retention, further suggesting that PAR-1 is essential for promoting cross-talk between metastatic melanoma cells and the microenvironment (ref. 12; Fig. 1).

Recent work highlighted a noncoagulatory role for PAR-1 in melanoma. Indeed, our laboratory has identified two genes that are regulated by PAR-1: connexin 43 (Cx-43) and maspin, both of which are involved in modulating the interactions between melanoma cells and the stroma. We showed that PAR-1 expression in melanoma cells contributes to melanoma metastasis by upregulating the expression of the gap junction communication molecule Cx-43, which enhanced the adherence of melanoma cells to endothelial cells. These results suggest that PAR-1 mediates Cx-43 expression in melanoma cells, thereby promoting adherence of melanoma cells to the vascular endothelium. It may also influence the passage of ions and second messengers, thereby promoting tumor–stromal interaction (13). Moreover, others have shown that thrombin activation of melanoma cells increases their adhesion to aortic and capillary endothelial cells (7), further implicating thrombin signaling through PAR-1 in cell–cell interactions and adherence. PAR-1–mediated Cx-43 expression may contribute to these observed effects.

Although PAR-1 positively regulates the expression of Cx-43, it negatively regulates the expression of the tumor suppressor gene maspin. Maspin is a member of the serine protease inhibitor (serpin) family and functions as a tumor suppressor in melanoma. Metastatic melanoma cells were recently found to have lower levels of maspin expression compared with normal human epidermal melanocytes. We recently showed that in two highly metastatic melanoma cell lines, PAR-1 inhibits maspin expression by promoting the phosphorylation of p38, decreasing the recruitment of CBP/p300, as well as c-Jun and Ets-1 transcription factors, to the maspin promoter (14). This inhibition results in increased melanoma cell invasion. Conversely, PAR-1 silencing in metastatic melanoma cell lines results in a significant increase in maspin expression, decreased MMP-2 activity, and inhibition of melanoma cell invasion. Moreover, silencing of maspin in PAR-1–silenced cells increased tumor growth and metastasis in vivo. Tumors arising from these cells also had elevated levels of MMP-2 and VEGF (14). PAR-1, therefore, is important for transcriptionally regulating various genes involved in the metastatic process in melanoma.

PAR-1 also plays a significant role in promoting melanoma cell migration, motility, and survival. Activation of PAR-1 by either thrombin or a PAR-1 agonist induces the chemokinetic motility of melanoma cells toward fibroblast-conditioned media and fibronectin. Of interest, the mechanism of increased motility was found to involve the activation of another PAR, PAR-2, by PAR-1. These data suggest that PARs can act either alone or in concert to promote melanoma cell motility and migration (15). PAR-1 was also shown to enhance melanoma cell survival and antiapoptotic behavior. Activation of PAR-1 in nonmetastatic melanoma cell lines overexpressing PAR-1 resulted in activation of the Akt/PKB signaling pathway, leading to decreased Bim and Bax expression, as well as cleaved caspase-3 and caspase-9 levels. These apoptosis-related effects were also observed in vivo, as PAR-1 silencing significantly decreased tumor growth, corresponding to increased terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling–positive cells and expression of cleaved caspase-3. Taken together, these data suggest a critical role for PAR-1 in regulating melanoma cell migration, survival, and antiapoptotic behavior (16).

Overexpression of PAR-1 is not the only concern in melanoma progression. In concert, the perpetual activations of one of its ligands, thrombin, also promotes metastatic melanoma because it can function as a growth factor. Moreover, melanoma cells also constitutively express tissue factor, which promotes thrombin activation and its subsequent binding to PAR-1. Plasmin-mediated degradation of fibrin deposits in the tumor microenvironment further stimulates the release of active thrombin (5). Of importance, however, PAR-1 can be activated by proteases other than thrombin. Activation of PAR-1 by MMP-1 in melanoma cells has been shown to induce the expression of growth factors, such as fibroblast growth factor receptor 2 and insulin-like growth factor I, and promote the invasiveness of melanoma cells (17). These data indicate that activation of PAR-1 by MMP-1 results in a disparate gene expression profile compared with PAR-1 activation by thrombin. It is possible that these divergent signaling pathways could have cumulative effects in melanoma progression (17).

In addition to melanoma, PAR-1 has been shown to promote metastasis in several other cancers. In breast cancer, PAR-1 has been linked to both invasion and metastasis (18). In both primary breast tissue specimens and breast carcinoma cell lines, increased expression of PAR-1 positively correlates with tumor cell invasiveness (19). PAR-1 promotes breast carcinoma invasion by inducing extracellular signal-regulated kinase (ERK)1/2 signaling through transactivation of epidermal growth factor receptor and ErbB2 (20). Moreover, activation of PAR-1 by MMP-1 allows apoptotic escape of breast carcinoma cells due to PAR-1–mediated Akt activation (21). In colon cancer, PAR-1 is expressed in colon tumors but not in normal colonic mucosa, and increased PAR-1 gene expression is found in advanced prostate cancer tissues compared with normal prostatic epithelia, implicating PAR-1 in colon and prostate cancer progression. Activation of PAR-1 in colon cancer cells induces cell proliferation and migration (22). A correlation between PAR-1 expression and the metastatic potential of prostate cancer cells was also shown, as bone metastases from prostate cancer cell lines express high levels of PAR-1 (23). Increased MMP-2 and MMP-9 activation in prostate cancer cells is also associated with PAR-1 expression, suggesting that PAR-1 potentially promotes prostate cancer cell metastasis via upregulation of MMPs (24).
Effect of Thrombin/PAR-1 on Stromal Cells

Tumor-stromal interactions are integral to melanoma growth and metastasis. Of interest, PAR-1 plays an important role in modulating these interactions. PAR-1 is not solely expressed on tumor cells; it is also expressed on several types of cells in the tumor microenvironment, such as endothelial cells, platelets, fibroblasts, and macrophages (ref. 20; Fig. 1). Activation of PAR-1 in these cells results in secretion of cytokines, expression of adhesion molecules, and increased vascular permeability, all of which can induce tumor cell proliferation, invasion, and angiogenesis (25). As mentioned above, increased levels of thrombin are found in the tumor microenvironment. This enhanced stromal expression of thrombin is thought to be mediated by both tissue factor and degradation of fibrin (9). Because PAR-1 and thrombin are both expressed within the tumor microenvironment, it is thought that this signaling axis may take part in autocrine or paracrine signaling, thereby contributing to the metastatic cascade (ref. 25; Fig. 1).

Activation of PAR-1 signaling in endothelial cells induces both their proliferation and recruitment, promoting tumor cell angiogenesis by increasing the expression, activation, and secretion of angiogenic mediators. Upregulation of VEGF and its receptor VEGFR2 is induced by thrombin in fibroblasts and endothelial cells (7). Furthermore, thrombin also stimulates the expression of PAF, IL-6, and IL-8 by endothelial cells (4), promoting both endothelial and melanoma cell proliferation, angiogenesis, and recruitment of platelets. Of interest, PAR-1 is also expressed on human endothelial progenitor cells. Thrombin-mediated PAR-1 activation triggers bone marrow–derived cell proliferation, migration, and differentiation into endothelial cells (26). PAR-1 can also be activated by an alternate mechanism through binding of factor VIIa to the endothelial cell protein C receptor, a cellular receptor for protein C. PAR-1 activation through this pathway results in induction of PAR-1–mediated p44/42 (MAPK) activation (27). PAR-1 is also involved in survival signaling in brain endothelial cells. In fact, APC-activated PAR-1 inhibits staurosporine-induced apoptosis in brain endothelial cells (28). Thus, these data show that PAR-1 and thrombin facilitate tumor angiogenesis and progression by inducing endothelial cell proliferation and survival, as well as recruitment and secretion of growth factors and cytokines (Fig. 1).

Platelets have also been shown to play an important role in promoting tumor progression. Depletion of platelets in a variety of mouse models was shown to inhibit metastatic formation, whereas reconstitution of platelets restored metastatic capability (29, 30). Indeed, PAR-1 activation induces both platelet proliferation and aggregation, two events that are critical for tumor cell survival and diapedesis. During vascular dissemination, cooperation between tumor cells and platelets is crucial for the survival and metastasis of tumor cells. It has been shown that thrombin treatment of platelets promotes melanoma cell adhesion to platelets, thereby increasing lung metastasis formation (7). Platelet activation is also involved in promoting tumor cell angiogenesis. Activated platelets secrete proangiogenic and mitogenic factors, such as platelet-derived growth factor, VEGF, and angiopoietin-1 (20). Recently, Trivedi and colleagues (31) showed that activation of PAR-1 by MMP-1 in platelets stimulates Rho-GTP and MAPK signaling, resulting in increased platelet motility and proliferation. Taken together, these data suggest that PAR-1 signaling in platelets plays a key role in regulating various tumor responses, including proliferation, angiogenesis, and metastasis.

In addition to endothelial cells and platelets, PAR-1 is also expressed on various inflammatory cells. D’Andrea and colleagues (25) showed that expression and activation of PAR-1 and PAR-2 are associated with macrophage proliferation and migration. PAR-1 and PAR-2 also stimulate macrophages to synthesize and secrete thrombin, as well as other growth factors, into the tumor microenvironment. Furthermore, stromal fibroblasts surrounding metastatic tumor cells express elevated levels of both PAR-1 and PAR-2. This effect is not observed in stromal fibroblasts surrounding benign, nonmetastatic, or normal epithelial tumor cells (25). It is thought that PAR-1 expression may offer a survival advantage to these tumor-associated stromal fibroblasts. In vitro, fibroblasts treated with thrombin and PAR-1 agonist peptides exhibit activated ERK1/2 and PI3K signaling pathways, resulting in reduced expression of the proapoptotic protein Bim (Fig. 1). These cell-signaling pathways ultimately work to promote the survival of fibroblasts (32). Taken together, these findings show that PAR-1 signaling in stromal and tumor cells is involved in multiple steps during melanoma tumorigenesis, including proliferation, angiogenesis, invasion, and survival.

PAR-1 as a Therapeutic Target in Melanoma Patients

Current treatment options for melanoma patients with metastatic disease are extremely limited and include surgery, chemotherapy (dacarbazine and DTIC), and immunotherapy (IL-2 and IFN-α), with a low response rate of 15%. Other treatment modalities for metastatic melanoma are currently under investigation in a number of clinical trials. These include biochemotherapy (a combination of chemotherapy and cytokine treatment, such as DTIC plus IFN-α), ipilimumab, and targeted therapy [e.g., with the BRAF kinase inhibitor PLX4032, which showed promising results in phase I clinical trials in patients harboring the BRAF V600E mutation (33)]. However, some of these treatments result in resistance, and thus, it is critical to identify new treatment modalities. Because PAR-1 has been shown to be a key player affecting both the tumor and its microenvironment, it is an attractive therapeutic target for treatment of melanoma patients. In the past few years, several groups have shown the effects of targeting PAR-1 in vivo (11, 21, 34).

Our studies showed that targeting PAR-1 with the use of siRNA-incorporated DOPC liposomes inhibited melanoma tumor growth, experimental lung metastases, and angiogenesis in nude mice (11). Targeting of PAR-1 was also employed in a xenograft breast carcinoma mouse model. A specific pepducin inhibitor against PAR-1, P1pal-7, used in combination with docetaxel, significantly decreased tumor growth
and metastasis through inhibition of the Akt signaling pathway (21). The results from these studies suggest that PAR-1 may be useful as a therapeutic target, either alone or in combination with other modalities, such as chemotherapy, antiangiogenic drugs, and proapoptotic drugs. This approach is appealing because it targets both the tumor and the microenvironment in melanoma, and in other cancers where PAR-1 is overexpressed.

Although various investigators have pursued the development of tumor-specific drug delivery systems, it remains a difficult challenge. Serious side effects can be caused by inhibition of PAR-1 in both normal cells and tumor cells. The most likely adverse effect caused by using PAR-1 in targeted therapy is hemorrhage, because PAR-1 expression on platelets functions in aggregation and blood clotting. However, a recent clinical trial of an orally administered PAR-1 inhibitor, SCH530348, did not show any increased bleeding or effects on hemostasis (34). In addition, promising "in vitro" data for the PAR-1 antagonist SCH79797 were recently reported (35). Furthermore, a correlation between thrombosis and cancer patients has been observed in several cancers, including melanoma, and it was shown that anticoagulant therapy prolonged the survival term in cancer patients (7, 36). Decreased melanoma growth and metastasis can be achieved directly by inhibiting PAR-1 on tumor cells (16), indirectly by inhibiting PAR-1 activity on platelets, by facilitating immune cell recognition of tumor cells by blocking tumor-platelet aggregations, or by targeting PAR-1 on vascular endothelial cells and thereby decreasing angiogenesis (37). Taken together, these data suggest that PAR-1 could be a potential therapeutic target for metastatic melanoma patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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