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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. Inhibition of these interactions by targeting PAR-1 could be utilized as a potential therapeutic modality for melanoma patients.

Keywords

PAR-1; Thrombin; Melanoma metastasis; Microenvironment

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, i.e. the “seed and the soil” (1). These 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 extra cellular 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 non-metastatic melanoma phase, radial growth phase, to the metastatic phase, vertical growth phase, 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 which contribute to melanoma metastasis. Since thrombin is the main activator of PAR-1, it too is implicated in

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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 usage 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 (TF) 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). TF 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 TF-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, due to its involvement in regulating numerous critical cellular events including cell proliferation, cell adhesion, angiogenesis and invasion (6–8). Thrombin, as well as other factors involved in the coagulation cascade, exert their effects via the Protease Activated Receptors (4, 8).

PARs are a family of G-protein-coupled receptors (GPCR), comprised of 4 members (PAR 1–4), which are 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 which 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 PAR-1 to these G proteins activates several signaling pathways including the phosphatidylinositol 3-kinase (PI3-K), mitogen-activated protein kinase (MAPK) cascade, Rho kinase and phospholipase C- β (PLC- β), all of which can promote proliferation, migration, and adhesion (4). Due to 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 such as breast, prostate, lung and melanoma (5).

The Effect of Thrombin/PAR-1 on Tumor Cells

Increased expression of PAR-1 is closely associated with melanoma progression. Tissue microarray studies demonstrate that PAR-1 is highly expressed in malignant melanoma tumors as compared to common melanocytic nevi and normal skin. Massi et al. observed significantly elevated PAR-1 mRNA and protein expression levels in clinical samples of atypical nevi and melanomas compared to common melanocytic nevi (9). Moreover, in melanoma cell lines, PAR-1 expression correlates with their metastatic potential *in vivo* (10). The role of PAR-1 in promoting melanoma metastasis was initially demonstrated by Nierodzik et al. whose work detailed a role for PAR-1 as a rate-limiting factor required for experimental lung metastasis of murine melanoma cells (7). In accordance with these findings, our group demonstrated that PAR-1 promotes human melanoma metastasis (10). *In*

vivo targeting of PAR-1 using siRNA- encapsulated liposomes resulted in decreased expression of a number of angiogenic and metastatic genes, including vascular endothelial growth factor (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 (10). These factors can act in both an autocrine and paracrine fashion, influencing both melanoma tumor cells, as well as cells in the tumor microenvironment (Fig. 1).

In the past few years, significant research efforts have focused on elucidating the role of PAR-1 in melanoma progression by examining its interactions with other signaling molecules. Notably, our laboratory established a link between PAR-1 and another pro-inflammatory 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. 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 (11). Interestingly, enhanced MUC18 expression in melanoma cells promotes adhesion to endothelial cells and increases MMP-2 expression, melanoma cell invasion and metastasis (11). Importantly, our work demonstrated that the effects of PAR-1 on PAFR ultimately results in enhanced melanoma cell adhesion to endothelial cells, increased transendothelial migration and prolonged lung retention, further suggesting that PAR-1 is essential in promoting cross-talk between metastatic melanoma cells and the microenvironment (11) (Fig. 1).

Recent work highlights a non-coagulatory 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 involved in modulating the interactions between melanoma cells and the stroma. We have demonstrated 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, as well as possibly influencing the passage of ions and second messengers, thus promoting tumor cell-stromal interaction (12). Moreover, others have demonstrated 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, PAR-1 actually 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 to normal human epidermal melanocytes. We recently demonstrated 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 (13). 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 (13). PAR-1, therefore, is important in 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 chemokinetic motility of melanoma cells toward fibroblasts conditioned medium and fibronectin. Interestingly, 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 (14). PAR-1 was also shown to enhance melanoma cell survival and anti-apoptotic behavior. Activation of PAR-1 in non-metastatic 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. *In vivo*, these apoptosis-related effects were also observed, as PAR-1 silencing significantly decreased tumor growth, corresponding to increased TUNEL 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 anti-apoptotic behavior (15).

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 as it can function as a growth factor. Moreover, melanoma cells also constitutively express TF, 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). Importantly, 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 FGFR2 and IGF1, as well as promote the invasiveness of melanoma cells (16). These data indicate that activation of PAR-1 by MMP-1 results in a disparate gene expression profile, compared to PAR-1 activation by thrombin. It is possible that these divergent signaling pathways could have cumulative effects in melanoma progression (16).

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 (17). In both primary breast tissue specimens and breast carcinoma cell lines, increased expression of PAR-1 positively correlates with tumor cell invasiveness (18). PAR-1 promotes breast carcinoma invasion by inducing ERK1/2 signaling through transactivation of EGFR and ErbB2 (19). Moreover, activation of PAR-1 by MMP-1 allows apoptotic escape of breast carcinoma cells due to PAR-1 mediated Akt activation (20). In colon cancer, PAR-1 is expressed in colon tumors, but not in normal colonic mucosa, and increased PAR-1 gene expression was found in advanced prostate cancer tissues, compared to 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 (21). A correlation between PAR-1 expression and metastatic potential of prostate cancer cells was also demonstrated, as bone metastases from prostate cancer cell lines express high levels of PAR-1 (22). Increased MMP-2 and MMP-9 activation in prostate cancer cells also associates with PAR-1 expression, suggesting that PAR-1 potentially promotes prostate cancer cell metastasis via upregulation of MMPs (23).

The Effect of Thrombin/PAR-1 on Stromal Cells

Tumor-stromal interactions are integral to melanoma growth and metastasis. Interestingly, PAR-1 plays an important role in modulating these interactions. PAR-1 is not solely expressed on tumor cells but is also expressed on several cell types in the tumor microenvironment, such as endothelial cells, platelets, fibroblasts and macrophages (19) (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 (24). As mentioned previously, increased levels of thrombin are found in the tumor microenvironment. This enhanced stromal expression of thrombin is thought to be mediated by both TF and degradation of fibrin (8). Since PAR-1 and thrombin are both expressed within the tumor microenvironment, it is thought that this signaling axis may take part either in autocrine or paracrine signaling, together contributing to the metastatic cascade (24) (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 in 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. Interestingly, 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 (25). PAR-1 can also be activated by an alternate mechanism through binding of factor VIIa to the endothelial cell protein C receptor (EPCR), a cellular receptor for protein C. PAR-1 activation, through this pathway, results in induction of PAR-1-mediated p44/42 (MAPK) activation (26). PAR-1 is also involved in survival signaling in brain endothelial cells. In fact, APC activated PAR-1 inhibits staurosporin-induced apoptosis in brain endothelial cells (27). Thus, these data demonstrate 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, while reconstitution of platelets restored metastatic capability (28, 29). Interestingly, PAR-1 activation induces both platelet proliferation and aggregation, two events critical to tumor cell survival and diapedesis. During vascular dissemination, cooperation between tumor cells and platelets is crucial for the survival and metastasis of tumor cells. Indeed, thrombin treatment of platelets promotes melanoma cell adhesion to platelets, increasing lung metastasis formation (7). Platelet activation is also involved in promoting tumor cell angiogenesis. Activated platelets secrete pro-angiogenic and mitogenic factors, such as PDGF, VEGF, and angiopoietin-1 (19). Recently, Trivedi et al. demonstrated that activation of PAR-1 by MMP-1 in platelets stimulates Rho-GTP and MAPK signaling, resulting in increased platelet motility and proliferation (30). 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 et al. demonstrate 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 (24). 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, non-metastatic, or normal epithelial tumor cells (24). 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 PI3-K signaling pathways, resulting in reduced expression of the pro-apoptotic protein Bim (Fig. 1). These cell signaling pathways ultimately work to promote survival of fibroblasts (31). Taken together, 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; they include surgery, chemotherapy (Dacarbazine, 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, such as a BRAF kinase inhibitor (PLX4032), which showed promising results in phase I clinical trials in patients harboring the BRAF V600E mutation (32). As some of these treatments results with resistant, it is critical to identify new treatment modalities. Since 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 demonstrated the effect of targeting PAR-1 *in vivo*.

Our studies demonstrate that targeting PAR-1 utilizing siRNA incorporated DOPC liposomes inhibited melanoma tumor growth, experimental lung metastases, as well as angiogenesis, in nude mice (10). Targeting PAR-1 was also demonstrated in a xenograft breast carcinoma mouse model. A specific peptidic inhibitor against PAR-1, P1pal-7, used in combination with taxotere, significantly decreased tumor growth and metastasis through inhibition of the Akt signaling pathway (20). These studies suggest that PAR-1 can be used as a possible therapeutic target, either alone or in combination with other modalities, such as chemotherapy, anti-angiogenic drugs, and proapoptotic drugs. This approach is appealing since it targets both the tumor and the microenvironment in melanoma, and other cancers where PAR-1 is overexpressed.

Although tumor-specific drug delivery systems have been pursued, it remains a difficult challenge. The most apprehensive side effect can be caused by inhibiting PAR-1 on both normal cells and tumor cells. The most likely adverse effect caused by using PAR-1 in targeted therapy is hemorrhage, as PAR-1 expression on platelets functions in aggregation and blood clotting. However, a recent clinical trial utilizing an orally administered PAR-1 inhibitor, SCH530348, did not show any increase bleeding or effects on hemostasis (33). In addition, a promising *in vitro* data for the PAR-1 antagonist, SCH79797, has been recently reported (34). Furthermore, a correlation between thrombosis and cancer patients has been reported in several cancers including melanoma, and it was demonstrated that anti-coagulant therapy prolonged the survival term in cancer patients (7, 35). Nevertheless, platelet activation can be induced by another PAR, at high thrombin concentrations (5). Thus, the possible anti-platelet effect of inhibition of PAR-1 activity holds potential promise for melanoma patients.

Decreased melanoma growth and metastasis can be achieved directly by inhibiting PAR-1 on tumor cells (15), 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 thereby decreasing angiogenesis (36). Taken together, these data suggest that PAR-1 could be a potential therapeutic target for metastatic melanoma patients.

References

1. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer*. 2003; 3:453–458. [PubMed: 12778135]

2. Villanueva J, Herlyn M. Melanoma and the tumor microenvironment. *Curr Oncol Rep*. 2008; 10:439–446. [PubMed: 18706274]
3. Milsom C, Yu J, May L, Meehan B, Magnus N, Al-Nedawi K, et al. The role of tumor-and host-related tissue factor pools in oncogene-driven tumor progression. *Thromb Res*. 2007; 120 Suppl 2:S82–S91. [PubMed: 18023719]
4. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature*. 2000; 407:258–264. [PubMed: 11001069]
5. Villares GJ, Zigler M, Bar-Eli M. The emerging role of the thrombin receptor (PAR-1) in melanoma metastasis--a possible therapeutic target. *Oncotarget*. 2011; 2:8–17. [PubMed: 21378407]
6. Hu L, Roth JM, Brooks P, Luty J, Karparkin S. Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. *Cancer Res*. 2008; 68:4666–4673. [PubMed: 18559512]
7. Nierodzik ML, Chen K, Takeshita K, Li JJ, Huang YQ, Feng XS, et al. Protease-activated receptor 1 (PAR-1) is required and rate-limiting for thrombin-enhanced experimental pulmonary metastasis. *Blood*. 1998; 92:3694–3700. [PubMed: 9808563] Thrombin induces tumor growth, metastasis, and angiogenesis: Evidence for a thrombin-regulated dormant tumor phenotype. *Cancer Cell*. 2006; 10:355–362. [PubMed: 17097558]
8. Rickles FR, Patierno S, Fernandez PM. Tissue factor, thrombin, and cancer. *Chest*. 2003; 124:58S–68S. [PubMed: 12970125]
9. Massi D, Naldini A, Ardinghi C, Carraro F, Franchi A, Paglierani M, et al. Expression of protease-activated receptors 1 and 2 in melanocytic nevi and malignant melanoma. *Hum Pathol*. 2005; 36:676–685. [PubMed: 16021575]
10. Villares GJ, Zigler M, Wang H, Melnikova VO, Wu H, Friedman R, et al. Targeting melanoma growth and metastasis with systemic delivery of liposome-incorporated protease-activated receptor-1 small interfering RNA. *Cancer Res*. 2008; 68:9078–9086. [PubMed: 18974154]
11. Melnikova VO, Balasubramanian K, Villares GJ, Dobroff AS, Zigler M, Wang H, et al. Crosstalk between protease-activated receptor 1 and platelet-activating factor receptor regulates melanoma cell adhesion molecule (MCAM/MUC18) expression and melanoma metastasis. *J Biol Chem*. 2009; 284:28845–28855. [PubMed: 19703903]
12. Villares GJ, Dobroff AS, Wang H, Zigler M, Melnikova VO, Huang L, et al. Overexpression of protease-activated receptor-1 contributes to melanoma metastasis via regulation of connexin 43. *Cancer Res*. 2009; 69:6730–6737. [PubMed: 19679555]
13. Villares GJ, Zigler M, Dobroff AS, Wang H, Song R, Melnikova VO, et al. Protease activated receptor-1 inhibits the Maspin tumor-suppressor gene to determine the melanoma metastatic phenotype. *Proc Natl Acad Sci U S A*. 2011; 108:626–631. [PubMed: 21187389]
14. Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM. Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. *Mol Cancer Res*. 2004; 2:395–402. [PubMed: 15280447]
15. Salah Z, Maoz M, Pokroy E, Lotem M, Bar-Shavit R, Uziely B. Protease-activated receptor-1 (hPar1), a survival factor eliciting tumor progression. *Mol Cancer Res*. 2007; 5:229–240. [PubMed: 17374729]
16. Blackburn JS, Liu I, Coon CI, Brinckerhoff CE. A matrix metalloproteinase-1/protease activated receptor-1 signaling axis promotes melanoma invasion and metastasis. *Oncogene*. 2009; 28:4237–4248. [PubMed: 19734937]
17. Arora P, Ricks TK, Trejo J. Protease-activated receptor signalling, endocytic sorting and dysregulation in cancer. *J Cell Sci*. 2007; 120:921–928. [PubMed: 17344429]
18. Booden MA, Eckert LB, Der CJ, Trejo J. Persistent signaling by dysregulated thrombin receptor trafficking promotes breast carcinoma cell invasion. *Mol Cell Biol*. 2004; 24:1990–1999. [PubMed: 14966279]
19. Garcia-Lopez MT, Gutierrez-Rodriguez M, Herranz R. Thrombin-activated receptors: promising targets for cancer therapy? *Curr Med Chem*. 2010; 17:109–128. [PubMed: 19941475]
20. Yang E, Boire A, Agarwal A, Nguyen N, O'Callaghan K, Tu P, et al. Blockade of PAR1 signaling with cell-penetrating pepducins inhibits Akt survival pathways in breast cancer cells and suppresses tumor survival and metastasis. *Cancer Res*. 2009; 69:6223–6231. [PubMed: 19622769]

21. Darmoul D, Gratio V, Devaud H, Lehy T, Laburthe M. Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells. *Am J Pathol.* 2003; 162:1503–1513. [PubMed: 12707033]
22. Chay CH, Cooper CR, Gendernalik JD, Dhanasekaran SM, Chinnaiyan AM, Rubin MA, et al. A functional thrombin receptor (PAR1) is expressed on bone-derived prostate cancer cell lines. *Urology.* 2002; 60:760–765. [PubMed: 12429291]
23. Wilson SR, Gallagher S, Warpeha K, Hawthorne SJ. Amplification of MMP-2 and MMP-9 production by prostate cancer cell lines via activation of protease-activated receptors. *Prostate.* 2004; 60:168–174. [PubMed: 15162383]
24. D'Andrea MR, Derian CK, Santulli RJ, Andrade-Gordon P. Differential expression of protease-activated receptors-1 and -2 in stromal fibroblasts of normal, benign, and malignant human tissues. *Am J Pathol.* 2001; 158:2031–2041. [PubMed: 11395381]
25. Tarzami ST, Wang G, Li W, Green L, Singh JP. Thrombin and PAR-1 stimulate differentiation of bone marrow-derived endothelial progenitor cells. *J Thromb Haemost.* 2006; 4:656–663. [PubMed: 16460448]
26. Sen P, Gopalakrishnan R, Kothari H, Keshava S, Clark CA, Esmon CT, et al. Factor VIIa bound to endothelial cell protein C receptor activates protease activated receptor-1 and mediates cell signaling and barrier protection. *Blood.* 2011; 117:3199–3208. [PubMed: 21252088]
27. Cheng T, Liu D, Griffin JH, Fernandez JA, Castellino F, Rosen ED, et al. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med.* 2003; 9:338–342. [PubMed: 12563316]
28. Camerer E, Qazi AA, Duong DN, Cornelissen I, Advincula R, Coughlin SR. Platelets, protease-activated receptors, and fibrinogen in hematogenous metastasis. *Blood.* 2004; 104:397–401. [PubMed: 15031212]
29. Karparkin S, Pearlstein E, Ambrogio C, Collier BS. Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. *J Clin Invest.* 1988; 81:1012–1019. [PubMed: 3280598]
30. Trivedi V, Boire A, Tchernychev B, Kaneider NC, Leger AJ, O'Callaghan K, et al. Platelet matrix metalloprotease-1 mediates thrombogenesis by activating PAR1 at a cryptic ligand site. *Cell.* 2009; 137:332–343. [PubMed: 19379698]
31. Chalmers CJ, Balmanno K, Hadfield K, Ley R, Cook SJ. Thrombin inhibits Bim (Bcl-2-interacting mediator of cell death) expression and prevents serum-withdrawal-induced apoptosis via protease-activated receptor 1. *Biochem J.* 2003; 375:99–109. [PubMed: 12844349]
32. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med.* 2010; 363:809–819. [PubMed: 20818844]
33. Macaulay TE, Allen C, Ziada KM. Thrombin receptor antagonism -the potential of antiplatelet medication SCH 530348. *Expert Opin Pharmacother.* 2010; 11:1015–1022. [PubMed: 20307225]
34. Silini A, Ghilardi C, Ardinghi C, Bernasconi S, Oliva P, Carraro F, et al. Protease-activated receptor-1 (PAR-1) promotes the motility of human melanomas and is associated to their metastatic phenotype. *Clin Exp Metastasis.* 2010; 27(1):43–53. [PubMed: 20084489]
35. Sparsa A, Durox H, Doffoel-Hantz V, Munyangango EM, Bedane C, Cendras J, et al. High prevalence and risk factors of thromboembolism in stage IV melanoma. *J Eur Acad Dermatol Venereol.* 2011; 25:340–344. [PubMed: 20629849]
36. Tsopanoglou NE, Maragoudakis ME. Thrombin's central role in angiogenesis and pathophysiological processes. *Eur Cytokine Netw.* 2009; 20:171–179. [PubMed: 20167556]

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 regulate 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, while IL-8 serves as a survival factor for vascular endothelial cells and acts in a autocrine fashion to induce MMP-2 secretion by the melanoma cells.