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ROLE OF PLASMINOGEN ACTIVATOR INHIBITOR -1 (PAI-1) IN CANCER STEM CELLS

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Abstract – *Objective:* *Plasminogen Activator Inhibitor-1 has an important role in the progression of cancer. Although there are many studies about the relation of Plasminogen Activator Inhibitor-1 (PAI-1) with cancer, there exists only a few about showing the relation of PAI-1 with cancer stem cells.*

Materials and Methods: *The purpose of this review is to explain the relation between PAI-1 and carcinogenesis and to at- tract attention to the possible role of this protein in cancer stem cell pathway in the light of literature data.*

Results: *Tumor development harbors various biological processes such as resisting cell death, proliferative signaling, angiogenesis, invasion, and metastasis. Cancer Stem Cells (CSCs), known as subpopulation of tumor cells, are located within the tumor tissue with a great therapeutic resistance, self-renewal capacity, potential of induction of tumor initiation and progression. Processes involved in epithelial mesenchymal transition (EMT) and extracellular matrix (ECM) are important for cancer and CSC development since EMT increases plasticity in tumor cells; therefore, they are separated from other tissues. PAI-1 is the major inhibitor of plasmin and is associated with various diseases such as cardi- ovascular diseases, neuronal cell loss, and progression of hallmarks of cancer. PAI-1, which has high expression levels in most cancer types, has a role in ECM remodeling and regulation of EMT. Recent studies about cancer stem cells reveal the probable importance of PAI-1 in stemness part- way.*

Conclusions: *These studies might be considered as a guide for therapeutic approaches that will be focused in near future.*

KEYWORDS: *Cancer, Cancer stem cell, Plasminogen activator inhibitor-1, Fibrinolytic system, epithelial-mesenchymal transition.*

INTRODUCTION

Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of plasmin being an important regulator of fibrinolytic system. It is associated with various diseases such as depression, Alzheimer's, cardiovascular diseases, diabetes, insulin resistance and neuronal cell loss with remarkable high expression levels[1-6]. PAI-1 is produced as a single-chain glycoprotein containing 379-381 amino acids and with a mass of 45-47 kDa by various cell types such as adipose cells, liver cells, mesenchymal cells, fibroblasts, stromal and endothelial cells. Agonists like hormones, growth factors, endotoxins and cytokines modulate the synthesis and release of PAI-1[7-11]. Besides Transforming Growth Factor-β (TGF-β), interleukin-1 (IL-1), Tumor Necrosis Factor-α (TNF-α), β-fibroblast Growth Factor (FGF) and 4G/5G polymorphism of PAI-1 have important roles in regulation

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of PAI-1 expression[7,10,12,13]. Viewed from another side, in addition to its role in various diseases, PAI-1 stands out with high expression in cancer cells and cancer stem cells. Furthermore, high PAI-1 levels has been associated with cancer development playing roles in angiogenesis, migration, and metastasis[14,15]. Important for cancer and CSC, the activation of Epithelial-Mesenchymal Transition (EMT) and remodeling of the Extracellular Matrix (ECM) can be carried out by PAI-1[16-18]. Therefore, it appears that PAI-1 can play a critical role on cancer stem cell development. This review mainly focuses on the relation of PAI-1 with fibrinolytic system, cancer, EMT, and cancer stem cells depending on the current literature.

Role of epithelial-mesenchymal transition and extracellular matrix in cancer

EMT is a process that plays a role in the development of various cellular outcomes such as normal tissue development, embryogenesis, fibrosis and wound healing. Stimulation of EMT markers results in the transformation of the epithelial cells into mesenchymal cells leading to these processes. EMT markers can be regulated through various pathways including TGF-β, Smad4 and Wnt3. Important EMT markers such as E-cadherin and Vimentin, and several transcriptional factors such as Snai1 and Twist are responsible for deregulating signaling in cancer cells causing resistant phenotype[16-22].

ECM provides structural foundation for tissue function and regulates cytokines. Under normal conditions, components of ECM including fibrin and collagen are degraded remodeling ECM. ECM degrading proteases such as matrix metalloproteinases (MMPs), Cathepsins, Heparanase and Serpins are among ECM remodeling enzymes. Transformation to malignant cells is not only dependent on tumor growth and progression but also on changes in properties of tumor microenvironment. Under pathologic conditions when cancer is initiated, uncontrolled activation of ECM component evokes tumor growth, angiogenesis and metastasis[16-20, 23-25]. The main ECM enzymes involved in degradation of the matrix are MMPs. They can cleave several ECM components. MMP-2, MMP-3 and MMP-9 are shown to have role in invasion and metastasis[23,25-29]. Besides, most signaling pathways such as TGF-β, Hypoxia-inducible factor (HIF1), Cyclooxygenase-2 (COX-2) and Extracellular-signal regulated kinase 1/2 (ERK1/2) function in the regulation of ECM [24] as well as tumor progression. Consequently, it is obvious that tumor progression and tumor spread are widely dependent on ECM and EMT[16, 20, 23, 30-34].

Cancer stem cell

Cancer stem cells (CSCs) are a subpopulation of tumor and are found in tumor tissue with great therapeutic resistance. They have self-renewal capacity, produce pluripotent new daughter cells of different phenotypes and regulate tumor initiation and progression[35-41]. CSCs show high ability of plasticity, the capability to shift between a non-differentiated stemness state and differentiated non-stemness state. The former is characterized by limited tumorigenic potential whereas the latter is characterized by long term tumorigenic potential[42,43]. Processes involved in EMT and ECM are important for cancer and CSC development since EMT increases plasticity in tumor cells; therefore they are separated from other tissues[30, 44, 45]. The rate of metastatic lesions is associated with increase in PAI-1 and Matrix metalloproteinase 2 (MMP2) levels which are involved in remodeling of ECM [28]. Conversion of cancer cells into CSCs occurs via EMT activation, and it is observed that activated EMT markers and signaling pathways such as Wnt and Hedgehog are similar in cancer and CSC, suggesting that similar pathways are shared in these cells[20,35,46,47]. In addition, TGF-β, the main regulator of PAI-1, is also the major promoter of CSC stemness of which is done through the stimulation of EMTs[20,48,49].

Role of PAI-1 in fibrinolytic system

Plasmin in fibrinolytic system remodels ECM either directly through breakage of fibrin and collagen, which provide mechanical stabilization to ECM, blood vessel wall, and basement membrane, or indirectly by activation of MMPs. Moreover, the clot formed in the bloodstream is dissolved by plasmin[9,50-53]. Plasmin is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). The main task of tPA-activated plasmin is to degrade the fibrins in the blood circulatory system, and uPA-activated plasmin is often required for pericellular proteolysis. uPA binds to Urokinase Plasminogen Activator Receptor (uPAR) on the cell surface. This interaction causes intacellular and extracellular changes that increase the affinity of uPAR for Vitronectin (VN), and support its interaction with various integrins and stimulate various signaling pathways including mitogen-activated protein kinase (MAPK), tyrosine kinase, and Ras / ERK[8,10,39,54-56]. Moreover, activation of uPAR receptor upon binding of VN can stimulate migration and survival through intracellular signaling[35, 57]. On the contrary, PAI-1 is the major inactivator of plasmin

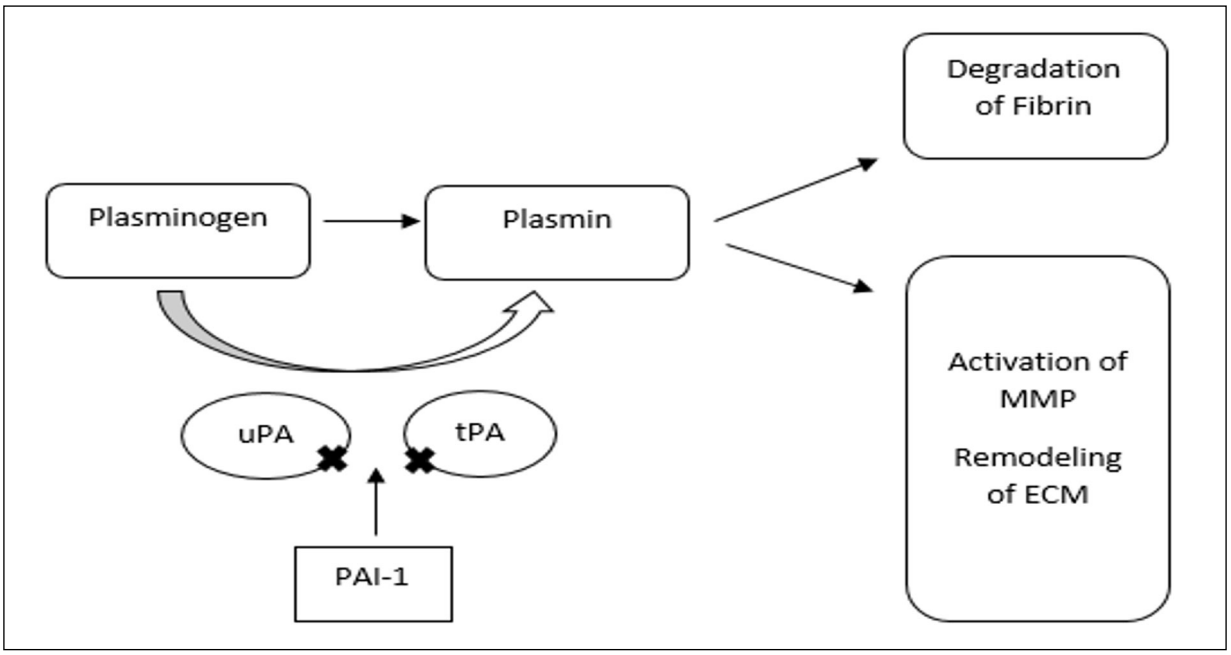

Fig. 1. Regulatory role of Plasminogen activator inhibitor-1 (PAI-1) in the fibrinolytic system. Plasmin is involved in the degradation of fibrin, activation of MMP, and remodeling of extracellular matrix (ECM). Urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA) play a role in the conversion of plasminogen to plasmin in the fibrinolytic system. This function of uPA and tPA can be modulated with PAI-1 resulting in inactivation of plasmin.

in fibrinolytic system. PAI-1 performs inhibitory function by binding covalently to uPA and tPA, which are responsible for the conversion of plasminogen to plasmin (Figure 1). Consequently, the decrease in plasmin levels results in the corruption of fibrinolytic system[7,10,30,56, 58-60].

Relation of PAI-1 with cancer

Tumor development harbors various biological processes such as resisting cell death, proliferative signaling, angiogenesis, invasion and metastasis. PAI-1 is highly expressed in most types of cancers including melanoma, breast, stomach, colorectal, head and neck cancers, and is known to be associated with metastasis, angiogenesis, migration and invasion[9,19,58,61-64]. It is associated with poor prognosis in various types of cancer[38]. Biopsy samples of patients with head and neck cancer have high PAI-1 density creating short progression-free survival compared to that of patients having PAI-1 with medium density and low-density. There appears to be a significant relationship between high PAI-1 levels and cancer recurrence after treatment [64]. Similar to this, another study indicates that PAI-1 has high expression in head and neck squamous cell carcinoma patients[14]. In addition, increased PAI-1 activity is observed in pancreatic cancer. Pancreatic carcinoma especially associated with high thrombosis is dependent on high PAI-1 levels in patients[65,66]. Blockage of PAI-1 in metastatic breast cells was shown to inhibit angiogenesis revealing the significant relation between PAI-1 and angiogenesis[15,35,67,68]. Likewise, down regulation of PAI-1 in melanoma cell lines has anti-migrative and anti-invasive effects[58,61]. Inactivation of PAI-1 has potential to limit tumor angiogenesis in an malignant Pleural Mesothelioma[69].

5' UTR 4G/5G polymorphism of PAI-1 and cancer

PAI-1 gene is located on chromosome 7q21.3-q22. A common polymorphism known as 4G/5G appears in the promoter region of the PAI-1 gene. This polymorphism of PAI-1 is known to modulate PAI-1 levels in colorectal, prostate, lung, breast and ovary cancers[12,70-77]. Many reports indicate that allelic deletion creating 4G/4G genotype acts as an enhancer resulting in higher PAI-1 levels with reduced fibrinolytic activity than insertion 5G/5G polymorphism. Patients with endometrial cancer more commonly have 4G/4G genotype and higher PAI-1 levels. PAI-1 levels correlate with tumor grade, with the 4G polymorphism being more common in stage 2 and 3 tumors than in stage 1 tumors[12,78]. It is revealed that 4G/4G genotype has a higher risk in breast cancer[79]. Breast cancer patients have been shown to have more 4G alleles than 5G alleles and the patients with 4G alleles have increased PAI-1 expression levels.

As with endometrial cancers, PAI-1 levels and 4G alleles were higher in the patients showing histological grade 3 tumors[13,75,80]. Increased PAI-1 levels with the 4G/4G polymorphism causes plasminogen to be in high amounts in oral squamous cell carcinoma and that may be a predictor of the risk for oral cancers[81,82]. The 4G allele has also been shown to play an important role in the early stages of cancer[81]. In another study, 4G/5G and 4G/4G alleles were pointed out as a high risk for patients with polycystic ovary syndrome[83]. Consequently, studies depict the importance of 4G/5G polymorphism in modulating PAI-1 levels in many cancers (Figure 2).

Relation of PAI-1 with EMT

PAI-1 is effective in remodeling of ECM and regulation of EMT markers[16,30,31]. TGF-β has an important role in the control of PAI-1 expression being the main regulator of PAI-1 and affecting the EMT markers[7,10,84]. In a study, A549 (adenocarcinoma of human alveolar basal epithelial cell) cells stimulated by TGF-β1 have decreased E-cadherin and increased N-cadherin and Vimentin expression levels revealing deregulation of mesenchymal markers in carcinogenesis[16,22,85]. mRNA and protein levels of PAI-1 are found to be high in Carboplatin applied A2780 cells (human ovarian cancer cell line) with reduced E-cadherin and increased Vimentin, Snail, and Twist levels. Reversely, PAI-1 inhibition causes increased E-cadherin and decreased Vimentin, Snail, and Twist expressions emphasizing the importance of EMT markers in cancer development[59]. Low PAI-1 levels in PANC-1 (pancreatic cancer cell line) cells result in changed morphology, increased expression of some epithelial and neuronal genes and decreased expression of some mesenchymal genes in these cells. Moreover, increase in the levels of E-cadherin and β-catenin is also observed[86]. In a study performed using MDA-MB-231 breast

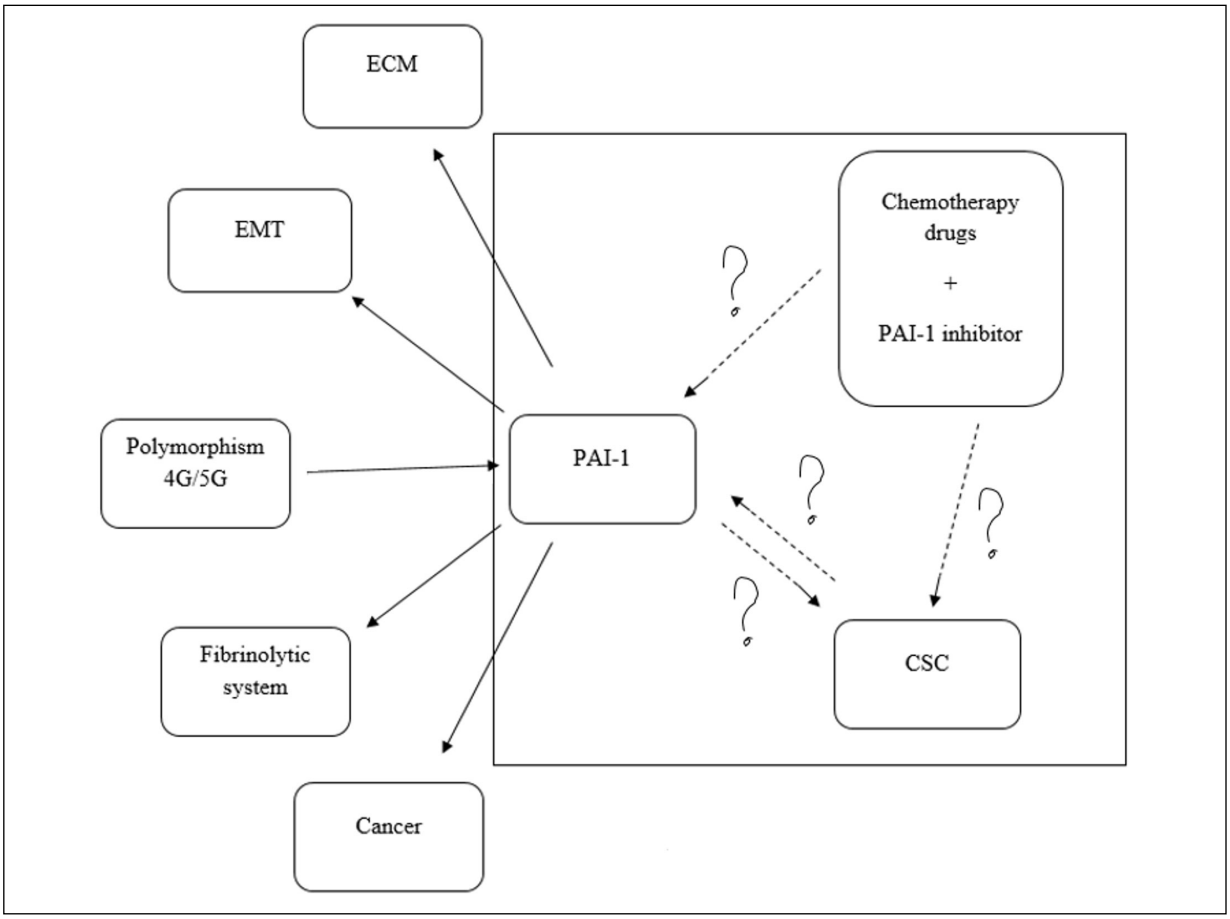

Fig. 2. Relation of Plasminogen activator inhibitor-1 (PAI-1) with cellular events. PAI-1 plays important role in fibrinolytic system, cancer, extracellular matrix (ECM) and epithelial-mesenchymal transition (EMT). It appears that PAI-1 is also important in cancer stem cell (CSC). Whether CSCs induce signaling pathways to increase PAI-1 levels or high PAI-1 levels cause CSC generation has to be determined. While 4G/5G polymorphism has role as a modulator of PAI-1 levels in various cancers, the effect of chemotherapy drugs and PAI-1 inhibitors on PAI-1 levels and CSCs have to be analyzed to improve the understanding of the significance of PAI1 in generation of CSCs.

cancer cell lines, functional blockage of Snai1 caused re-expression of E-cadherin and low levels of PAI-1. These results clearly depict the relation between PAI-1 and Snai1 during tumor progression[38,87]. Loss of E-cadherin through the regulation of TGF-β stimulated EMT markers disrupts adherence junctions and causes cell motility[16, 88,89]. These data suggest PAI-1 as an EMT indicator and PAI-1 is closely connected to the acquisition of stem cell features[30,44,45].

Role of PAI-1 in cancer stem cells

PAI-1, a major inhibitor of plasmin, is a remarkably important protein in CSCs. PAI-1 stimulates EMT markers through activation of AKT and ERK1/2 pathway which is a well-known promoter of survival and metastasis in lung cancer[38,90]. Secreted OPN and PAI-1 via the activation of the AKT pathway, also play an important role in the regulation of cancer stem cell formation in lung cancers[38,67]. Furthermore, fibrocytes regulate CSC-like properties of lung cancer cells by providing OPN, CCL-18, and PAI-1[38,91-93]. Upregulated PAI-1 levels is observed in cells with ALDHL1 which is a marker of Cervical CSCs[39]. The connection between irradiation controlled local tumor and PAI-1 levels is also presented[14,94]. Leptin inhibition in adipose stem cells of obese women resulted in reduction of PAI-1 and MMP2 gene expressions and adipose stem cell-induced metastasis[28]. Differentiation associated Osteosarcoma (OS) is found to be related to PAI-1. Blockage of PAI-1 appears to restrain OS differentiation in cancer stem-like cells through down-regulation of SRY-associated-HMG box (Sox) 2. Conversely, overexpression of Sox2 rescues the suppression phenotype realized by PAI-1 inhibition. Accordingly, PAI-1 consistently increases with OS differentiation. Combination of Doxorubicin and PAI-1 inhibitor (PAI-039) significantly suppresses proliferation of OS cells avoiding differentiation to stem cell phenotype[95]. PAI-1 is one of the important factors for colon cancer. High PAI-1 in blood and tissue of colon cancer patients is associated with poor prognosis[96,97]. Co-culture studies with mesenchymal stem cells and colon cancer cells (HT29 and HCT-116) uncover the reality of higher secretion of PAI-1 from mesenchymal stem cells compared to that of colon cancer cells. This makes it obvious that mesenchymal stem cells are a significant source of PAI-1 for colon tumors[96]. As a result, studies about cancer stem cells reveal the potential importance of Plasminogen Activator Inhibitor-1 in support and development of CSC (Figure 2).

CONCLUSIONS

PAI-1 is a multifunctional protein that is located in the intrinsic and extrinsic cellular signaling network. Knowing that PAI-1 is important in the regulation of EMT, ECM and cancer formation suggests that this protein may also have an important place in the formation of cancer stem cells. There are several limited emerging studies depicting the function of PAI-1 in generation of CSCs as reviewed here. In order to understand the function of PAI-1 in CSC pathway, different types of CSCs should be analyzed. Determination of the function of PAI-1 in tumor plasticity is a wide research area. 4G/4G polymorphism as a modulator of PAI-1 levels causes increment in expression of this gene, and the relation of this polymorphism with key events such as CSC pathway, EMT and ECM regulation should be addressed in cancers. Different chemotherapeutic drugs in combination with PAI-1 inhibitors have to be applied to observe how the response of CSCs and the levels of PAI-1 change upon this application. It is a question to be resolved whether the change in PAI-1 levels results in the formation of CSCs or the development of CSCs causes the changes in PAI-1 levels (Figure 2). When these gaps are fulfilled, these studies will shed light on the relation between PAI-1 and CSC development. Consequently, all of these will also be a guide for therapeutic approaches to inhibit tumor plasticity.

Informed Consent:

Informed Consent is not required for this study.

Ethical Committee:

Ethical Committee is not required for this study.

Conflict of Interest:

The authors have no conflicts of interest to declare.

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