OPEN ACCESS

Saudi Journal of Oral and Dental Research

Abbreviated Key Title: Saudi J Oral Dent Res
ISSN 2518-1300 (Print) | ISSN 2518-1297 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Review Article **Oncology**

Role of Plasminogen Activator Inhibitor-1 (PAI-1) in Oral Squamous Cell Carcinoma

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DOI: https://doi.org/10.36348/sjodr.2025.v10i02.002 | **Received:** 14.12.2024 | **Accepted:** 17.01.2025 | **Published:** 12.02.2025

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Abstract

***Background*:** Squamous cell carcinoma (SCC) accounts for around 90% of malignant neoplasms of the oral cavity and is a serious public health problem. The 5-year survival rate of oral and pharyngeal SCC is estimated to be around 63%, despite advances in the treatments. Particularly SCCs affecting the oral and mobile portion of the tongue (OTSCCs) show a high pitfall of recurrence and lymph node metastasis. Within this context, biomarker studies are essential for better understanding of the pathogenesis of the disease, better prognostication and better therapeutic strategies. Proteins of the plasminogen activator system (PAS) have been correlated with the prognosis and clinical behavior of several types of cancer, such as breast, lung, esophageal, gastric, and oral cancers. The PAS consists of a set of molecules that integrates extracellular matrix (ECM) changes. Within this system, conversion of the pro-enzyme plasminogen into plasmin occurs, cleavage of ECM, and stimulation of other proteolytic enzymes as matrix metalloproteinases (MMPs). In malignant neoplasms, the conversion of plasminogen to plasmin is intermediated principally by urokinase-type plasminogen activator (uPA). The formation of plasmin is blocked basically by plasminogen activator inhibitor-1 (PAI-1, also called SERPINE-1). Although, the main function of PAI-1 is the regulation of the PAS, it also shares in alternate biological processes implicated in tumorigenesis. ***Aim of the Study*:** This review discusses the role of PAI-1 in inhibiting fibrinolysis in oral cancer and its potential as a biomarker and therapeutic target.

Keywords: PAI-1, Oral Squamous Cell Carcinoma, Fibrinolysis, Tumor Metastasis.

INTRODUCTION

Squamous cell carcinoma (SCC) accounts for around 90% of malignant neoplasms of the oral cavity and is a serious public health problem. The 5-year survival rate of oral and pharyngeal SCC is estimated to be around 63% , despite advances in the treatments (Chi, 2015). Particularly SCCs affecting the oral and mobile portion of the tongue (OTSCCs) show a high pitfall of recurrence and lymph node metastasis (Nóbrega *et al*., 2018). Within this context, biomarker studies are essential for better understanding of the pathogenesis of the disease, for better prognostication and better therapeutic strategies (Hussein *et al*., 2018). Proteins of the plasminogen activator system (PAS) has been correlated with the prognosis and clinical behavior of several types of cancer, such as breast, lung (Lin *et al*., 2017), esophageal (Tian *et al*., 2017), gastric (Brungs *et al*., 2017), and oral (Serpa *et al*., 2018)cancers. The PAS consists of a set of molecules that play critical pathophysiological roles in extracellular matrix (ECM) change. Within this system, conversion of the pro-enzyme plasminogen into plasmin occurs, cleavage of ECM, and stimulation of other proteolytic enzymes as matrix metalloproteinases (MMPs) (Santibanez *et al.*, 2018). In malignant neoplasms, the conversion of plasminogen to plasmin is intermediated principally by urokinase-type plasminogen activator (uPA) through reaction with its receptor (uPAR). The formation of plasmin is blocked basically by plasminogen activator inhibitor-1 (PAI-1, also called SERPINE-1) (Brungs *et al.*, 2017). Although the main function of PAI-1 is the regulation of the PAS, it is also shared in other biological processes. PAI-1 combines with affinity to vitronectin

and decreases the adhesion between neoplastic cells and ECM factors. Also, it activates biochemical signaling pathways, as PI3K/AKT and EKT to regulate cell proliferation, migration, and invasion (Mahmood *et al*., 2018). As well, in vitro, and in vivo studies showed that PAI-1 stimulates progression of cell cycle (Gomes Henriques Á *et al*., 2014) and suppress apoptosis (Gomes-Giacoia *et al*., 2013). Still, PAI-1 impact on the cell cycle of head and neck SCC remains uncertain with many studies suggesting several roles (Pavón *et al*., 2015);*(Arroyo-Solera et al.*, 2019).

Structure and Function of PAI-1

PAI-1 is considered as a single chain molecule that composed of two special interactive domains; a reactive center loop (RCL), and a flexible joint region with three helices; helix D (hD), helix E (hE), and helix F (hF) binding domains (Placencio & DeClerck, 2015)(Figure 1). The RCL domain is the primary site for uPA or tissue PA (tPA) binding and contains a P1-P10 peptide bond that interacts with these proteases. When uPA/tPA binds to PAI-1, over cleavage of the P1-P10 bond occurs, PAI-1/PA molecules form an irreversible complex leading to inhibition of PA, as well as partial internalization of the RCL domain (Boudier *et al*., 2005). Additionally, uPA and tPA split PAI-1 without irreversible complex formation. PAI-1 prohibits uPA/tPA by forming the complex. PAI-1 would still be capable to combine to other proteins through its helix sites, although its ability to prohibit uPA/tPA would then be decreased (Dupont *et al*., 2009).

Attachment of vitronectin to the flexible domain of PAI-1 (hD, hE, and hF sites), result in the prevention of vitronectin binding with integrin and holds PAI-1 in its active version while rising its binding with uPA/tPA (Figure 1) (Schroeck *et al*., 2002; Wind *et al*., 2002). Moreover, PAI-1 can separate cells through the interconnection with uPA bound to its receptor (uPAR) leading to decrease adhesion between neoplastic cells and ECM factors. Without PAI-1, uPA bound to uPAR leads to a conformational change that increases uPAR reaction with vitronectin or other integrins, also, attain cell adhesion to ECM (Kjøller, 2002; Wei *et al*., 2001). However, PAI-1 binding to uPA-uPAR can disrupt this interaction with vitronectin or integrin, reducing cell adhesion to ECM and induce migration (Chapman & Wei, 2001; Stefansson & Lawrence, 2003).

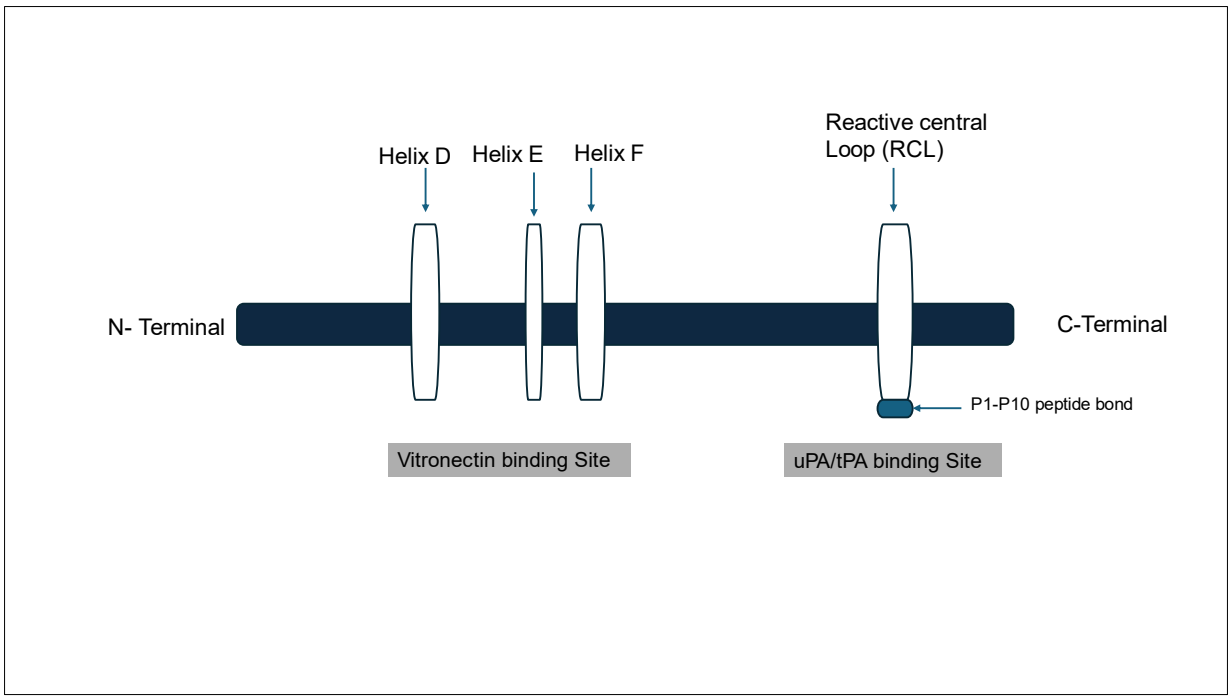

Fig. 1: Structure of PAI-1

It describes the structure of PAI-1 is a single chain molecule that consists of two specific interactive domains; a reactive center loop (RCL), and a flexible joint region with three helices; helix D (hD), helix E (hE), and helix F (hF) binding domains.

Also, PAI-1 react directly with the somatomedin B domain on vitronectin than uPAR, thus, further limiting cell adhesion to ECM (Deng *et al*., 1996). However, PAI-1 attached to the uPA-uPAR complex, can react with lipoprotein receptor-related protein 1 (LRP1) that found on the cell membrane through its hD and hE domains, as well enhance endocytosis of the uPAR complex, resulting in reduced levels of uPA and uPAR (Cubellis *et al*., 1990; Schroeck *et al*., 2002). The absence of uPA/uPAR activity, and decreased cell adhesion may increase apoptosis of cells (Figure 2) (Al-Fakhri *et al*., 2003).

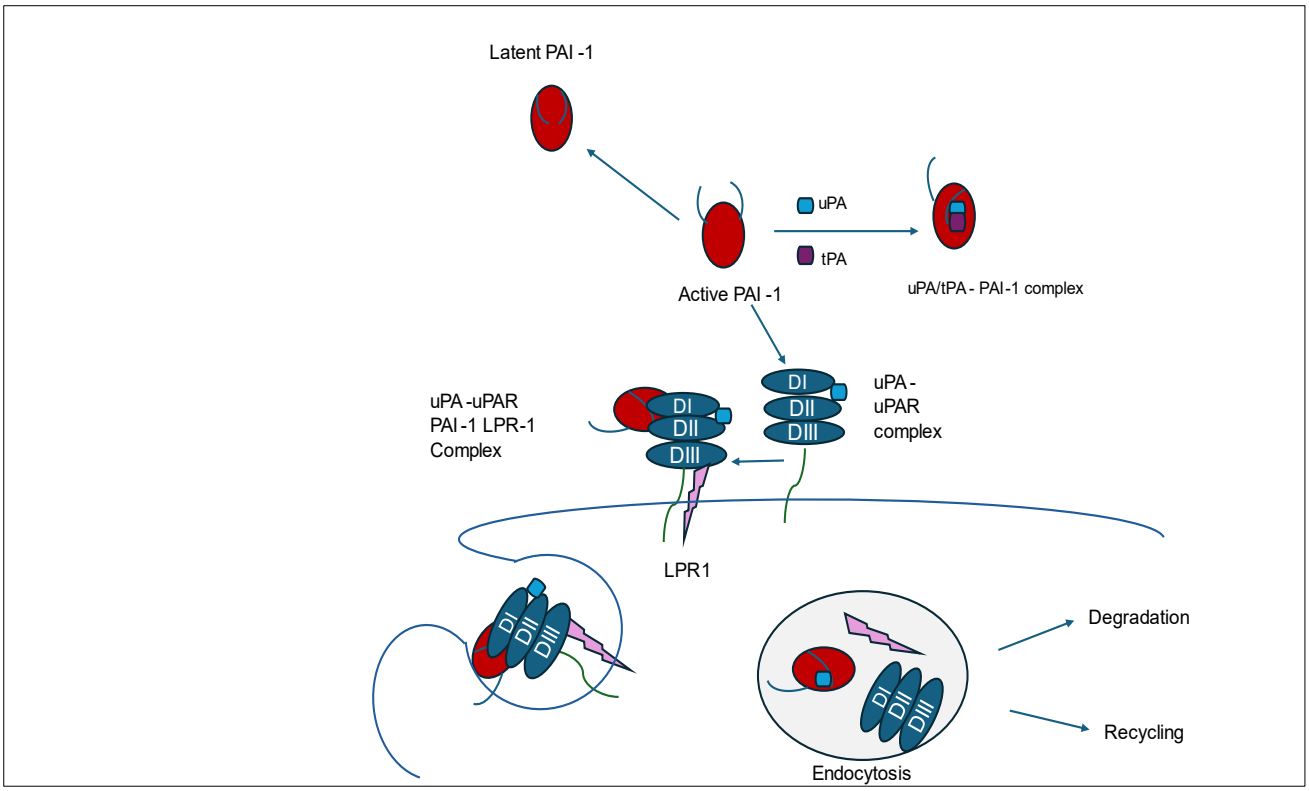

Fig. 2: Regulation and function of PAI-1

Vitronectin-bound PAI-1 prevents its premature conversion to its latent state and improves its binding affinity to uPA/tPA. PAI-1 may also be internalized by the cell, through its interaction with lipoprotein receptor-related protein 1 (LRP1) and uPA/uPAR, ultimately leading to its degradation or recycling.

Studies of PAI-1 structure have detected that this protein is released from cells in its active form, moreover this form is short lived. The ideal half-life of active PAI-1 is among 1–2 hours prior it is immediately changed to its highly stable inherent (partially inactive) shape (Fjellström *et al*., 2013). Similar to the cleavage of the P1-P10 peptide bond by plasminogen activators this may act as a regulatory technique to prohibit prolonged anti-fibrinolytic activity of PAI-1(Dupont *et al*., 2009). However, the latent shape can be reactivated by denaturing and refolding, this action may not be physiologically pertinent as well (Vaughan *et al*., 1990). Latent PAI-1 can react with cell surface receptors or ECM factors through its helix domains, like the divided form of PAI-1 or it may interact directly to fibrin to suppress tPA-induced degradation (Reilly & Hutzelmann, 1992).

While the main regulator of the plasminogen system, PAI-1 has an essential role in ECM remodeling through the modification of MMP activity. However PAI-1 does not react with MMP directly, its upstream restrained function on plasmin activation decreases the splitting-mediated activation of pro-MMP (Ghosh & Vaughan, 2012). Moreover, Plasmin activates elevated MMP production whereas its zymogen (i.e., plasminogen) activates increased secretion of PAI-1. By this way, PAI-1 activation may act as a negative-feedback mechanism to limit plasmin- and MMP-mediated ECM degeneration (Figure 3) (Lee *et al*., 1996). PAI-1 are typically expressed concurrently with tissue inhibitors of metalloproteinases (TIMP)s *(Ahmed et al*., 2011; Leivonen *et al*., 2013). For example, fibrogenic signaling cascades resulting in elevated levels of PAI-1 and TIMPs together, TIMPs directly inhibit MMPs, that way restricting ECM degradation (Qureshi *et al*., 2005).

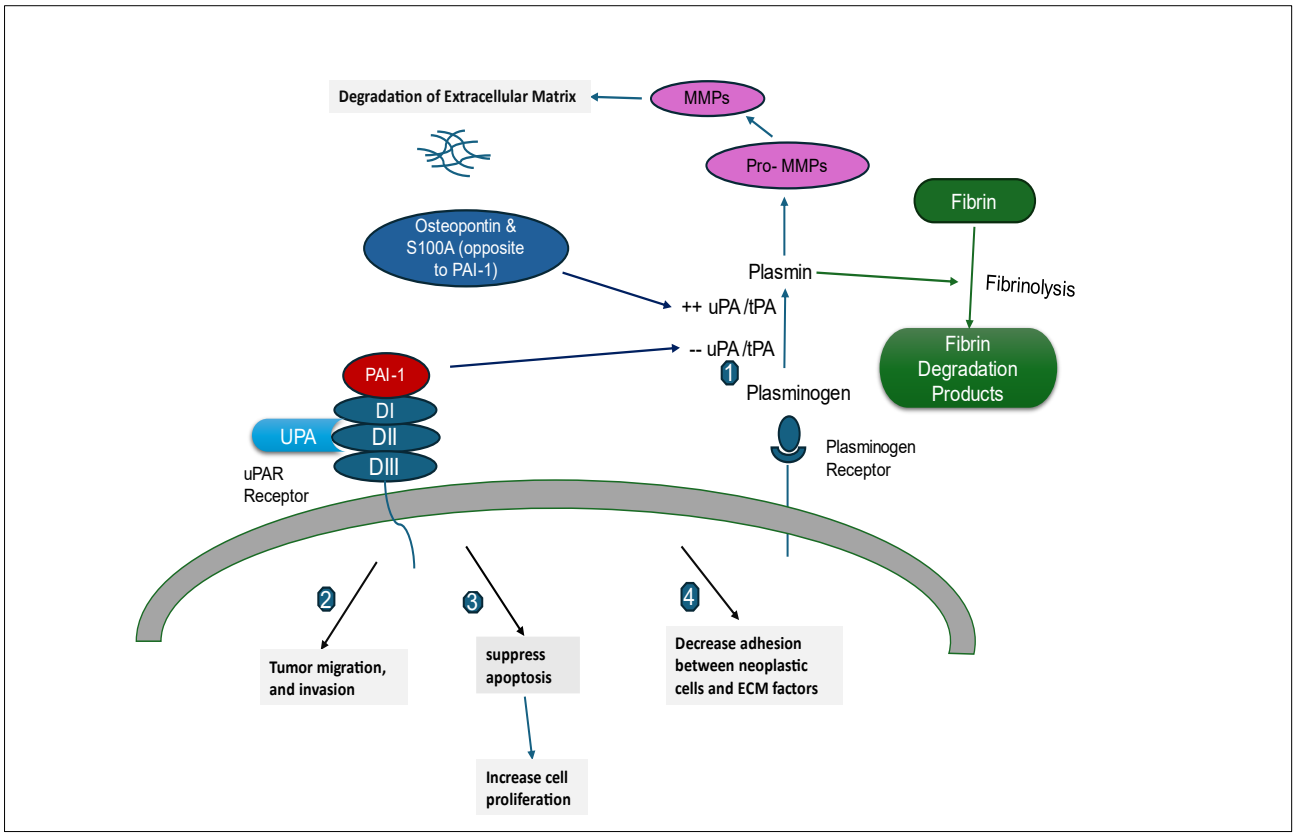

Fig. 3: Plasminogen - PAI-1 interaction on cancer cell and ECM remodeling

PAI-1 inhibits urokinase plasminogen activator (uPA) and plasminogen activator (tPA). PAI-1 inhibits plasmin activation and fibrinolysis, MMPS activation and ECM degradation, and promotes tumor cell invasion. PAI-1 suppresses apoptosis, increases cell proliferation and decreases adhesion between neoplastic cells and ECM factors. Osteopontin & S100A have opposite effects on uPA and ECM in comparison to PAI-1.

Transcriptional Regulation of PAI-1

Multiple signaling cascades can lead to rapid synthesis and secretion of PAI-1. PAI-1 is expressed in endothelial cells, and smooth muscle cells, immune cells, heart, liver, kidney, adipose tissue, and some cancer cells (Li *et al*., 2018; Oishi, 2009). Also, Skeletal muscles express PAI-1 during regeneration, indicating that PAI-1 has a role in modification of skeletal muscle ECM (Dadgar *et al*., 2014; Rahman *et al*., 2020; Zhao *et al*., 2002) (GEO dataset: GDS234; Reference series GSE469). Gene encoding for PAI-1 (PAI-1 transduction) is the same across most tissue types. The major contributors to PAI-1 transduction are three types: (1) pro-fibrotic signaling, (2) pro-inflammatory signaling, and (3) hormonal signaling.

Pro-fibrotic signaling of transforming growth factor-β (TGF-β) is a main contributor to transduction of PAI-1. The canonical TGF-β binding to its receptor leading to activated SMAD2/3, which can interacts with SMAD4 and translocate to the nucleus then binding to PAI-1 promoter, with other pro-fibrotic promoter sites (Keski-Oja *et al*., 1988). The TGF-β cascade has multiple non-canonical pathways as well, including increase in mitochondrial and cytosolic reactive oxygen species (ROS), leading to stimulation of mitogen-associated protein kinase (MAPK), and nuclear factor kappa B (NF-B)(Kwon *et al*., 2017; You *et al*., 2019). ROS production is one of the main mediators of TGF- β mediated PAI-1 transcription (Jiang *et al*., 2003). ROS Elevations also signal the transduction of cell cycle regulator p53, that beside SMAD2/3/4, can assist in the transcription of PAI-1 (Higgins *et al*., 2018; Samarakoon *et al*., 2011). ROS signaling can also stimulate activator protein-1 (AP-1), and hypoxia-inducing factor-1 (HIF-1) (Fink *et al*., 2002; Liao *et al*., 2007). As well as its regulation of PAI-1, TGF-signaling raise the half-life of PAI-1 mRNA, leading to increased PAI-1 protein translation (Westerhausen *et al*., 1991).

Characteristic inflammatory signaling including tumor necrosis factors (TNF) and interleukins (IL) can induce PAI-1 expression. Attachment of TNF to its respective receptor can lead to the direct stimulation of NF-β and following PAI-1 transcription (Dimova & Kietzmann, 2008; Hou *et al*., 2004). Moreover, the suppression of TNF-β via adiponectin has also been demonstrated to decrease expression of PAI-1 (Chen *et al*., 2017). Both IL-signaling and TNF show similar transduction of PAI-1. Attachment of IL receptors can immediately stimulate NF-β (e.g., IL-1R), or increase MAPK levels (e.g., IL-6R) within cells, still leading to

Serpine1 transcription (Dimova & Kietzmann, 2008; Kocić *et al*., 2013).

Numerous hormones affect PAI-1 transcription, involving insulin, glucagon, and glucocorticoids (GC). Both proinsulin, insulin, and insulin-like growth factor-1 (IGF-1) have been stated to enhance PAI-1 expression (Dimova & Kietzmann, 2008; Nordt *et al*., 2001). This was shown to be a consequence of AKT stimulation, leading to suppression of glycogen synthase kinase-3 (GSK inactivation prevents the inhibitory phosphorylation of HIF, leading to its translocation to the nucleus and PAI-1 transcription (Dimova & Kietzmann, 2008). Glucagon binding to its G protein coupled receptor enhances adenylate cyclase leading to increased cytosolic concentration of cyclic AMP (cAMP), following stimulating protein kinase A (PKA). Activated PKA phosphorylated cAMP-response element binding protein-1 (CREB1), that translocate to the nucleus resulting in PAI-1 transcription. Eventually, PAI-1 transcription can be mediated by the translocation of lipid soluble GC into the nucleus as well as their reaction with nuclear receptors (Heaton *et al*., 1989).

PAI-1 Promotes Tumorigenesis in HNSCC

Mafra *et al*., 2022 concluded that PAI-1 stimulated cell proliferation, migration, and invasion of OTSCC in vitro and Expression of PAI-1 was associated with tumor budding in OTSCC tumor sections. Immunohistochemical analysis of PAI-1 showed a high percentage of positive staining in neoplastic cells, in one line with previous studies (Magnussen *et al*., 2014; Pavón *et al*., 2015). Membrane immunopositivity for PAI-1 detect the time of reaction of this protein with uPA or uPAR, accompanied by internalization of the uPA/ uPAR/PAI-1 complex. This resulting in loss of attachment between the neoplastic cell and vitronectin (an ECM component), favoring cell migration. Meanwhile, cytoplasmic immunopositivity for PAI-1 showed its accumulation in the intracellular medium, where the molecule is subject to inhibition or degradation (Milenkovic *et al*., 2017).

The effect of PAI-1, PLAU and ACTA1 in controlling the initiation and progression of HNSCC was demonstrated by **(K. Yang *et al.*, 2019)**. They used in this study 39 HNSCC, 52 paraffin embedded cases, and 13 oral epithelial dysplasia. They concluded that a total of 80 down-regulated and 52 up-regulated differentially expressed genes (DEGs) in HNSCC were demonstrated, that were mainly correlated with ECM–receptor reaction and focal adhesion signaling mechanisms. Group of prognostic signatures involving PAI-1, PLAU and ACTA1 were detected by DEGs, that predict overall survival in HNSCC patients from TCGA cohort. Clinical samples experiment supported that these three signature genes were abnormally expressed in the oral epithelial dysplasia and HNSCC and related with HNSCC patients 'aggressiveness, demonstrating critical roles in controlling HNSCC initiation and progression. They could serve as essential biomarkers for accurate diagnosis and prognosis of HNSCC, that will supply potential goals for clinical treatment.

PAI-1 related with overall survival, through RNA-seq and miRNAs sequencing data from the Cancer Genome Atlas (TCGA) demonstrating the dysfunctional miRNAs microenvironment and providing useful targets for miRNAs treatment. Seven miRNAs were shown to be independent prognostic factors in the training cohort of HNSCC patients with 60 target genes prognosticated. They demonstrated that nine of these target genes (CDCA4, CXCL14, FLNC, KLF7, NBEAL2, P4HA1, PFKM, PFN2 and PAI-1) were associated with patient's overall survival consequence. The target genes involved in cancer metabolism and might be beneficial biomarkers in cancer treatment **(Wu *et al*., 2021)**.

The plasminogen activator (PA) system components can be used as prognostic markers for OSCC prognosis in comparison to the commonly used biomarker Ki-67. A tissue-micro-array (TMA) based immunohistochemical analysis of primary tumor tissue obtained from a North Norwegian cohort of 115 patients diagnosed with OSCC in the period 1986–2002 was conducted. The statistical analyses revealed that low expression of uPAR in the tumor cells was significantly associated with low disease specific death ($p=0.031$) and ($p=0.021$) respectively, in patients with small tumors and no lymph node metastasis (T1N0). The commonly used biomarker, Ki-67, was not associated with disease specific death in any of the groups of patients examined. While uPAR and PAI-1 are predictive biomarkers in early-stage tumors, also further studies on a larger cohort of patients are recommended **(Magnussen *et al*., 2014)**.

Immunohistochemistry was used to analyze the expressions of these proteins in 52 tumoral tissue samples of patients with OSCC, were obtained from the Head and Neck Genome Project, surgically treated, and followed up for 24 months after surgery. They showed that positive PAI-1 membrane expression was remarkably correlated with local disease relapses (P = .027). The positive PAI-1 membrane expression is beneficial marker for local disease relapses as shown by multivariate analysis, with approximately 14-fold increased risk compared to negative expression (OR = 14.49; CI = 1.40-150.01, P = .025). The less differentiation grade was greatly associated with strong PAI-1 cytoplasmic expression (P = .027), the positive PAI-1 membrane expression, showed to increase the risk for local disease relapse by 14-fold **(Peterle *et al*., 2018)**.

PAI-1 can prohibit apoptosis by different ways, involving the prevention of caspase-3 activation, interruption of the Fas/Fas-L pathway, (Fang *et al*., 2012), and stimulation of the PI3K/AKT pathway using in vitro studies (Kang *et al*., 2016). Furthermore, PAI-1 can stimulate synthesis of the antiapoptotic proteins Bcl-2 and Bcl-xL (Balsara & Ploplis, 2008). (Pavón *et al*.,

2015) initially evaluated the influence of PAI-1 on apoptosis in HNSCCs. In vitro, after exposure to cisplatin, cells overexpressing PAI-1 presented great decrease in the number of apoptotic bodies followed by the p-AKT activation. Finally, (Arroyo-Solera *et al*., 2019) showed in vivo a remarkable decrease of apoptotic bodies and reduced caspase-3 activation in HNSCCs overexpressing PAI-1, indicating the involvement of this protein in the resistance to apoptosis.

PAI-1 Correlated With Metastasis and Proliferation in HNSCC

(Arroyo-Solera *et al*., 2019) studied the effect of PAI-1 overexpression on primary tumor growth, lymph node dissemination, and distant metastasis in a HNSCC orthotopic xenograft mouse model. They examined the phenotypic characteristics and metastasis of HNSCC cells overexpressing PAI-1, they used 74BserpE1up by transduction of the 74BGFPLuc HNSCC cell line with the serpinE1-expressing lentiviral vector (pFUGW_serpinE1). PAI-1 overexpression increased tumor budding, proliferation, and the stromal component, as well as suppressing apoptosis in primary tumors. It increased the influence and metastasis in lymph nodes, also the dispersal and growth of metastatic foci in the lung. High PAI-1 expression was correlated with larger tumor size, undifferentiated tumors, lymph node metastasis, extracapsular spread, the perineural and angiolymphatic invasion, and lung metastasis along with its correlation with poor prognosis in HNSCC patients. In agreement with their results, the level of PAI-1 expression progressively increased in HNSCC tumor areas when they progress from normal mucosa to dysplasia, carcinoma in situ, and finally to invasive carcinoma(Lindberg *et al*., 2006). Therefore, they stated the relation between higher PAI-1 expression and perineural or angiolymphatic invasion in the HNSCC patient cohort involved in TCGA database. So, taken together, their results showed that PAI-1 enhances tumor invasiveness in HNSCC.

The role of PAI-1 and SMA for the detection of extracapsular spread (ECS) in cervical lymph nodes of OSCC patients, compared with MRI. 102 OSCC cases are used for this study, they showed that Both, in combination and separately, PAI-1 and SMA were supreme, to MRI for extracapsular spread detection (sensitivity: PAI-1: 95%; SMA: 82%; combination: 81%). MRI demonstrated poor sensitivity for detection of ECS (7%) and nodal metastasis (56%). Both PAI-1 and SMA immunohistochemical expressions at the tumor-advancing front were remarkably correlated with extracapsular spread ($P<0.001$). ECS was associated with the expression of either or both proteins in all cases. SMA+/ PAI-1+ expression in combination was highly significantly associated with poor survival ($P<0.001$). A combination of SMA and PAI-1 IHC offers potential as prognostic biomarkers in OSCC. These findings suggested that biomarkers at the invasive front are likely to be necessary in prediction of ECS or in therapeutic stratification **(Dhanda *et al*., 2014)**.

Both uPA/uPAR and PAI-1 are directly related to the stimulation of epithelial-to-mesenchymal transition, the acquisition of stem cell characteristics and antitumor agents 'resistance. Associated overexpression of uPA/uPAR and PAI-1 correlated with a high metastasis and could be utilized to identify patients who benefit from an adjuvant treatment. The specific inhibitors of uPA/uPAR and PAI-1 could be used to fabricate new therapeutic strategies in HNSCCs. It would therefore be expected that PAI-1, as the main regulator of uPA, plays an antitumor role **(Pavón e*t al*., 2016)**. However, in vitro and in vivo studies suggested an important role of PAI-1 in the motility and dissemination of neoplastic cells in different types of cancer (Humphries *et al*., 2019), involving oral SCC (Pavón *et al*., 2015), (Arroyo-Solera *et al*., 2019). Epithelial-mesenchymal transition (EMT) is phenomenon in which epithelial cells obtain the phenotypic properties of mesenchymal cells, elevating their migration and invasion ability (Williams *et al*., 2019). In a study using lung carcinoma cell lines(Lin *et al*., 2017), PAI-1knockdown decreased proteins expression accompanied with the mesenchymal phenotype. Another study used breast carcinoma cell lines(Xu *et al*., 2018), cell culture with rhPAI-1 resulted in mesenchymal markers upregulation and increased cell invasion and migration. Based on this principle and considering the association between tumor budding and high membrane immunoexpression of PAI-1, so the role of this protein in the induction of EMT can be suggested. In agreement with these findings,(Arroyo-Solera *et al*., 2019) observed a significant increase of tumor budding in OTSCC with overexpression of PAI-1.

Regulation of PAI-1 in OSCC

miR-30e-3p had tumor-suppressive functions in HNSCC cells, that was demonstrated by ectopic expression analysis. Fifty-three genes were detected as potential miR-30e-3p targets in HNSCC cells. Between these targets, high expression of PAI-1 among other genes (ADA, CPNE8, C14orf126, ERGIC2, HMGA2, PLS3, PSMD10, RALB, PAI-1, SFXN1, and TMEM87B) remarkably foreseen the short survival of HNSCC patients, according to The Cancer Genome Atlas (TCGA). protein expression levels of PAI-1 as well as mRNA were suppressed in miR-30e-3p-transfected HNSCC cells. The effects of overexpression of PAI-1 in HNSCC cells, were showed using Gene set enrichment analysis (GSEA) that stated the most enrichment mechanism was "epithelial–mesenchymal transition". The study presented that the malignant characteristics (e.g., proliferation, migration, and invasion abilities) of HNSCC cells were prohibited by the silencing of PAI-1 expression. miR-30e-3p expression level was considerably suppressed in HNSCC clinical specimens using TCGA database analysis. Ectopic expression assays presented that miR-30e-3p prohibited HNSCC

cells' aggressiveness, suggesting that miR-30e-3p represents a tumor suppressor. Additionally, some of the putative targets monitored by miR-30e-3p closely anticipated the prognosis of HNSCC patients. miR-30e-3p identification (the passenger strand of pre-miR-30e) in HNSCC molecular pathogenesis may lead to improved treatments of HNSCC oncogenesis ***(Minemura et al., 2022)***.

The effects circ_0058063 exerted on oral squamous cell carcinoma (OSCC) and its downstream mechanism correlated with miR-145-5p and PAI-1. The relevant contents of miR-145-5p, circ 0058063, and PAI-1 mRNAs in OSCC were examined in vivo to detect the biological impacts on OSCC cells. Dual-luciferase reporter, RIP, and RNA pull-down assays were used and the direct binding correlation between miR-145-5p, circ 0058063, and PAI-1, SMAD3, CYR61, and IGF1R mRNAs were proved. They used human OSCC cell lines (H157, SJG-1, and HSC-2), and normal human immortalized oral epithelial cell line (HIOEC) provided by ATCC. They detected that Circ 0058063 was greatly roverexpressed in OSCC. circ_0058063 downregulation inhibited OSCC cell migration and proliferation, but increased cell apoptosis. Functionally and mechanistically, circ_0058063 could specifically attach with miR-145-5p and thus upregulated expression of downstream target PAI-1, that together led to the OSCC progression. Therefore, Circ_0058063 activate OSCC malignant behavior through upregulating PAI-1 by sponging miR-145-5p **(Yu *et al*., 2022)**.

MicroRNA (miRNA)-mediated PAI-1 gene repression in OSCC, as posttranscriptional regulation upregulated by miRNAs is well certified in carcinogenesis of pan-cancers, involving OSCC. GEO database records correlated to OSCC were analyzed and PAI-1 was identified as one of the most remarkable molecules controlling OSCC progression and its oncogenicity was confirmed through a group of in vitro functional assays. Also, Bioinformatics analysis was used to screen critical miRNAs with dysregulated expression in response to PAI-1 upregulation in OSCC. miRNA 617 (miR-617) target PAI-1 in OSCC and was suppressed in OSCC tissues. These findings clarify OSCC pathogenesis and explore new lines for clinical treatment of OSCC. mRNA expression profiles were downloaded from the GEO Datasets database and PE/CA-PJ41 and SCC-090 cell lines were used. The cells were transfected with pcDNA 3.1-SERPINE1 vector or PAI-1 siRNA (si- SERPINE1), miR-617 mimic, miR-617 inhibitor or corresponding negative controls (NC). RNA pulldown and luciferase reporter assays stated that miR-617 included a response for PAI-1 overexpression. As well as, miR-617 was downregulated in OSCC cell lines and tissues, and it had a negative relation with progressive stages. Unless miR-617 mimic or inhibitor transfection could inhibit or increase PAI-1 expression. PAI-1 is a proproliferative oncogenic agent which is partly limited by miR-167 suppression in OSCC cells. So, the miR-617/ PAI-1 axis is a valuable therapeutic target for OSCC **(Zhao & Liu, 2021)**.

The tumor suppressive role of NOTCH1 in OSCC by ETV7-mediated suppression of PAI-1 and the NICD/TEL2/ PAI-1 regulatory axis as a stratification and prognostic tool in OSCC. Comparative gene expression profiling detected downregulation of PAI-1 on NICD overexpression and foreseen reaction between PAI-1 and genes involved in cell proliferation and migration. Overexpression of NICD leads to upregulation of ETV7/TEL2, that negatively controls PAI-1 expression. They showed that Notch signaling negatively controls expression of PAI-1 via ETV7 to regulate cell behavior. Knockdown of PAI-1 phenocopied the influence of NICD overexpression in culture. According to in vitro results, there were inverse relations between ETV7 and PAI-1 expression and survival in OSCC primary tumors. Their findings stated that the tumor suppressive role of NOTCH1 in OSCC is intermediated, at least in part, by suppression of PAI-1 via ETV7 **(Salameti *et al.*, 2019)**.

(Pavón *et al*., 2015) showed the prognostic value of PAI-1 expression, investigating a retrospective (n = 80) and a prospective (n = 190) cohort of HNSCC patients. In a third patient cohort from the Cancer Genome Atlas database (n = 507), they investigated PAI-1 expression. They also examined the influence of PAI-1 expression on proliferation, migration, and apoptosis induction in HNSCC cell lines. They stated that, in a retrospective study (n = 80), cancer-specific ($p = 0.040$) survival and poor progression-free ($p = 0.022$) was related with high expression of PAI-1. In a prospective study (n = 190), poor local recurrence-free ($p = 0.022$), progression-free ($p = 0.002$) and cancer-specific ($p = 0.006$) survival was correlated with high PAI-1 expression. Patients treated with chemo-radiotherapy or radiotherapy ($p = 0.043$), PAI-1 expression was considered as an independent risk factor for progression-free survival. In both patient cohorts, increased the risk of metastasis was related with high PAI-1 expression ($p = 0.045$; $p = 0.029$). Using the HNSCC cohort involved in The Cancer Genome Atlas project, The correlation between PAI-1 expression and survival was proved (n = 507). Patients with high expressions had poorer survival ($p < 0.001$). PAI-1 over-expression in HNSCC cells diminished cell proliferation and increased migration. It protected cells from cisplatin-induced apoptosis, which was mediated by PI3K/AKT pathway. There was opposite effect of downregulation of PAI-1 expression. So PAI-1 expression is a prognostic marker that might be used to classify HNSCC patients according to their risk of recurrence.

PAI-1 as Therapeutic Target

A recent in vivo study **(Arroyo-Solera *et al*., 2019)** demonstrated higher tumor budding and a larger number of metastatic foci in OTSCCs that overexpressed PAI-1 as previously described. TCGA analysis showed

that HPV-negative tumors had higher PAI-1 expression than HPV positive tumors. PAI-1 expression lost its statistical correlation with lymph node metastasis and angiolymphatic invasion if they included HPV-positive tumors. These results indicate that PAI-1 may not be causally implicated in the progression of HPV positive HNSCC; however, further studies are necessary to validate these findings.

Twenty oral squamous cell carcinomas patients with and fifteen healthy volunteers were examined regarding PAI-1 expressing cell types by immunohistochemistry, PAI-1 mRNA levels by RT-PCR, and PAI-1 gene methylation to demonstrate mechanisms for increased expression in oral cancers. Bisulfite sequencing was used to detect DNA methylation of 25 CpG sites within 960 bp around the transcription initiation site of the PAI-1 gene, they showed that both tumors and tumor-adjacent normal tissue had a higher level of methylation, while very little methylation was detected in tissue from healthy volunteers, indicating that tumor-adjacent normal tissue already had transformation-associated epigenetic changes. Although, general inverse relation between PAI-1 mRNA levels and PAI-1 gene methylation in all tissues, suggesting that CpG methylation is not the main determinant of the PAI-1 expression level in oral tissue **(Gao *et al*., 2010)**.

(Chen *et al*., 2015) examined if PAI-1 could act as a target in antitumor and antimetastasis therapies of colorectal cancer (CRC). This study undertaken to detect plasma PAI-1 and MMP-9 levels in Chinese patients with hepatic metastasis from CRC and investigate the biologic consequences of PAI-1 silencing in colon cancer cell lines and CRC bearing nude mice. They showed PAI-1 plasma level in CRC patients and its association with the clinicopathologic features was detected. Proliferation, invasion, and migration of CRC cells that transfected with lentivirus expressing PAI-1 small interfering RNA were significantly decreased. Fewer metastatic nodules in the liver and smaller tumor volumes were detected in nude mice inoculated with PAI-1 knockdown cells. They concluded that plasma PAI-1 level was increased in CRC patients with liver metastasis, and PAI-1 silencing may significantly compromise the malignant behaviors of CRC cells in vitro and in vivo. These results give evidence for PAI-1 targeted treatment of CRC.

TEL2 has a critical role in nasopharyngeal carcinoma (NPC) metastasis by directly down-regulating PAI-1, and that this novel axis of TEL2 / PAI-1 may be essential to provide new strategies for therapy of NPC patients with metastasis. They explored the roles of the transcriptional factors and the known markers for EMT in NPC metastasis by generating the special gene microarray containing these elements. They used this microarray to make screenings, for the pair of primary tumor tissues of NPC and its lymph node metastatic tissues, and for two cell lines of S18 and S26 derived from the NPC cell line CNE2 with higher and lower metastatic abilities and to explore the downstream targets of TEL2 that inhibit NPC metastasis, they performed the whole genomic expression profiles by the stable TEL2 shRNAs in both S26 and 6–10B cell lines. The luciferase vector cloned to three variable promoter sites of PAI-1, they concluded that TEL2 was downregulated in highly metastatic NPC cells and the metastatic tissues in lymph node. TEL2 suppresses metastasis in vivo and the cell migration and invasion in vitro through its direct inhibition on the PAI-1 promoter in NPC. Moreover, an inverse relation was detected between the protein levels of TEL2 and PAI-1 by clinical NPC samples. Collectively, they have given the first evidence that TEL2 has a critical role in NPC metastasis by directly down-regulating PAI-1, and that this novel axis of TEL2 / PAI-1 may be essential to provide new strategies for treating NPC patients with metastasis **(Sang *et al*., 2015)**.

(Zhang *et al*., 2020) examined the function and mechanism of PAI-1 in the development of paclitaxel (PTX) resistance in triple-negative breast cancer (TNBC) cells. Bioinformatics analysis showed that PAI-1 was greatly correlated with PTX resistance. Moreover, PAI-1 mRNA levels and protein were more in PTX-resistant cells compared to PTX-sensitive parent cells. PAI-1 Knockdown markedly suppressed cell survival and stimulated cell apoptosis in vitro. As well as PAI-1 silencing resulted in downregulation of the key angiogenetic vascular endothelial growth factor A (VEGFA). Moreover, PAI-1 suppression significantly attenuated tumor growth in vivo. These results showed that PAI-1 significantly had a role in the proliferation and apoptosis of TNBC cells by controlling VEGFA expression. The study showed PAI-1 as an oncogene in PTX drug resistance of breast cancer, and it may act as a possible target for therapy of BC.

The prognostic role and regulatory mechanism of PAI-1 in colon cancer was examined using bioinformatics analysis. This study used 15 patients with colon cancer that underwent one-stage operation surgery. All patients involved in the study were chemotherapy or radiotherapy naive the influence of high and low expression of PAI-1 on the survival of patients with different clinical features was examined using the survival curve. Gene Set Enrichment Analysis (GSEA) was performed on the results of LinkFinder calculation by LinkInterpreter module, that was associated with Pearson correlation analysis to get the kinase targets and miRNA targets, transcription factor targets, and signaling pathways correlated with PAI-1. Depending on TIMER, starBase, and UALCAN databases, PAI-1 was detected to be significantly highly expressed in colon cancer patients, that was further analyzed by clinical tissue. Also, it correlated with various clinical features (nodal metastasis status, stages, subtypes). As well as survival analysis detected those patients with low expression of PAI-1 had a longer survival time,

indicating that PAI-1 was a prognostic risk factor for colon cancer. Pearson correlation showed that the expression of Integrin Alpha 5, Matrix Metallopeptidase 19, and ADAM Metallopeptidase with Thrombospondin Type 1 Motif, 4 had the highest association with that of PAI-1. The findings of GSEA showed that these genes were significantly enriched in the pathways of RNA expression and kinases**(Wang *et al.*, 2023)**.

PAI-1 as Soluble Biomarker in Peripheral Blood

(Kim *et al.*, 2022) examined the influence of antiplatelet therapy directly on cancer cells in the absence of platelets to mimic the effects of long-term therapy. If four antiplatelet factors (aspirin, prasugrel, clopidogrel, and ticagrelor) were used to colon cancer cells, cancer cell proliferation was inhibited. However, if cells were treated with a purinergic P2Y12 inhibitor (purinergic antiplatelet agent), cancer cells migration was greatly elevated. Also, gene expression profiles were used to investigate the influence of P2Y12 inhibitors on cell migration, and PAI-1 was common gene associated with cell motility and cell death in three groups. Antiplatelet treatment elevated PAI-1 levels in cancer cells and induced PAI-1 secretion into the medium. Increased PAI-1 was detected to induce MMP1 so, thus, increase cell motility. Additionally, using the serum of patients who received these antiplatelet drugs confirmed an increase in PAI-1. Additionally, PAI-1 might be a novel target gene to restrict the metastasis and initiation of cancer in long-term antiplatelet therapy patients.

Detection of PAI-1 levels in plasma, PAI-1 and uPA have been detected as biomarkers for breast or prostate cancers in the clinical uses of level of evidence studies by the American Cancer Society(Duffy *et al*., 2014). As well as, increased plasma PAI-1 levels have also been correlated with multiple pathological lesions, e.g., septic disease course(Chi *et al*., 2015), hypertension in American Indians(Peng *et al*., 2017), obesity adiposis (Barnard *et al.*, 2016), and type II diabetes mellitus(Wang *et al.*, 2018). Several ELISA kits were commercially used for determination of plasma PAI-1 levels. However, the findings of these kits detected poor consistency, due to the results measured by seven different kits varied by 4- to 6-fold (Longstaff, 2018). One cause is that PAI-1 is found in four different shapes, and the various kits measured the concentration of different forms of PAI-1. In ELISA, the target proteins are normally combined by monoclonal antibodies that recognize the exosites in state the active site (RCL) of PAI-1 and thus fail to distinguish the active form of PAI-1 from other forms. They stated an ELISA assay for the precise determination of active-form PAI-1 based on PAItrap(H37R)-HSA (Shang *et al.*, 2019). PAItrap(H37R)-HSA greatly distinguished active PAI-1 through exposed RCL but did not attach other forms of PAI-1 while the RCL loop was either divided or embedded.

PAI-1 and the Tumor Microenvironment:
PAI-1 and the Tumor Immune Cells

Cancer cells effect on the differentiation of monocytes to Tumor-Associated Macrophages (TAM) subsets, including CD163+, CD204+, and CD206+ cells, in OSCC was examined by immunohistochemistry, flow cytometry, and a cytokine array. Interleukin-8 (IL-8) concentrations and PAI-1 in the conditioned media of OSCC cell lines (n = 10 for each group) were examined by ELISA. High concentrations of PAI-1 and IL-8 were detected in OSCC cell lines conditioned medium. PAI-1 and IL-8 enhanced CD14+ cells to express CD206. Additionally, there was a positive association between the CD206+ numbers, PAI-1+, and IL-8+ cells in OSCC cells. OSCC produce PAI-1 and IL-8 that acted in differentiation of monocytes to CD206+ TAMs**(Kai *et al.*, 2021)**.

However, PAI-1 influence macrophage mobility and polarization as part of its pro-tumorigenic activity within the tumor microenvironment (TME) was examined by **(Kubala *et al.*, 2018)**. They showed that PAI-1 stimulates the recruitment and M2 polarization of monocytes/macrophages through various structural domains. Its LRP1 interacting domain-controlled macrophage migration, whereas its C-terminal uPA interacting domain stimulated M2 macrophage polarization via stimulation of p38MAPK and NF-κB and induction of an autocrine interleukin (IL)-6/STAT3 activation pathway. Then they demonstrated in different experiments in mice that PAI-1 expression is correlated with increased tumorigenicity, increased presence of M2 macrophages, higher levels of IL-6, and increased STAT3 phosphorylation in macrophages. Meta-analysis of transcriptome data detected strong positive relation between IL-6, PAI-1, and CD163 (M2 marker) expressions in different human cancers. So, these findings give evidence for mechanism suggesting the paradoxical pro-tumorigenic function of PAI-1 in cancer.

(Zhou *et al.*, 2022) detected a novel panel of important prognostic markers that involved HPV-related inflammatory TME remodeling and immune evasion. Their results could give a basis for more demonstration of the clinical importance and molecular mechanism and for favoring immunotherapy for HNSCCs using full analysis of the RNA sequencing (RNA-Seq) data collected from healthy tissue of tumor tissue of 502 HNSCC patients and 44 control samples. Then, they showed 4,237 differentially expressed genes, including 2,062 upregulated and 2,175 downregulated genes. They showed that biomarker candidates were remarkably correlated with unfavorable patient prognosis: ITGA5, PLAU, PLAUR, PAI-1, TGFB1, and VEGFC. They showed that these genes expression was negatively controlled by DNA methylation. These potential biomarkers are significantly included in epithelial–mesenchymal transition (EMT) stimulation in HNSCCs. Additionally, these targets were shown to be positively associated with the immune invasion levels of CD4+ T

cells, macrophages, neutrophils, and dendritic cells, however negatively associated with B-cell infiltration and CD8 + T-cell invasion. Significantly, their results revealed expression levels in HPV-positive HNSCCs in comparison to normal controls, suggesting the role of these biomarkers as transformation and malignant progression markers for HNSCCs in HPV patients. Notably, the findings stated ITGA5, PLAU, PLAUR, PAI-1, and TGFB1 as potential prognostic biomarkers for HNSCCs, that might be included in the HPV-related TME remodeling of HNSCC. These give significant indications for the development and improvement of patient stratification and immunotherapies in HNSCC.

Dysregulated genes correlated with nicotine-related oral cancer and screen key genes was examined by data mining. So, the expression and function of the key genes were verified via big data analysis and experiments. PAI-1 was showed as the beneficial gene upregulated in nicotine-treated oral cells and played a role an independent prognostic factor for oral cancer. PAI-1 was involved in different pathways, as the tumor necrosis factor and apelin pathways, and was associated to macrophages infiltration, CD4+T cells, and CD8+T cells. PAI-1 overexpression was correlated with N staging and may be included in hypoxia, angiogenesis, and metastasis. PAI-1 Knockdown in oral cancer led to decreased cell proliferation and invasion as well as increased sensitivity to bleomycin and docetaxel. This study stated PAI-1 as a main gene for nicotine-related oral cancer, suggesting that PAI-1 may be a novel prognostic indicator and therapeutic target for oral carcinoma**(Guo *et al*., 2023)**.

PAI-1 and Angiogenesis

PAI-1 Upregulation supports angiogenesis, metastasis, and poor prognosis, however, when used in very high concentrations it prohibits angiogenesis and tumor growth, the phenomenon is called PAI-1 paradox. PAI-1 can be proangiogenic or anti-angiogenic depending on its concentration (Devy *et al*., 2002; Lambert *et al*., 2003). In experimental animal tumor models, physiological levels of PAI-1 are required for tumors vascularization, however in PAI-1 knockout (PAI-1-/-) mice angiogenesis was diminished. adenoviral PAI-1 gene transfer to cancer cells resulted in increased PAI-1 levels that increases tumor angiogenesis (Bajou *et al*., 2001). Elevated PAI-1 levels diminish angiogenesis as in chicken chorioallantoic membrane angiogenesis model and some animal models demanding supraphysiological levels of PAI-1. High PAI-1 concentration prohibits uPA on the angiogenic vessel restricting its progression into the tumor body(Nishioka *et al*., 2012; Stefansson *et al*., 2003; Zheng *et al*., 2013).

It was first stated in 2004, that PAI-1 effect for tumor angiogenesis was dose-dependent in vivo, indicating PAI-1 is pro-angiogenic at physiological levels and anti-angiogenic at higher levels (Bajou *et al*., 2004). Studies approved that normal level of PAI-1 was necessary for tumor angiogenesis, as this was damaged in PAI-1 knockout mice (Kwaan *et al*., 2013). Otherwise, PAI-1 overexpression in human prostate carcinoma cells prohibits tumor-related angiogenesis in an athymic mouse model (Soff *et al*., 1995). On contrary, in most other cancers, PAI-1 has a critical role in tumor angiogenesis. like, targeting PAI-1 by tiplaxtinin led to suppression of angiogenesis in a T24 xenograft model (Gomes-Giacoia *et al*., 2013). Regardless of angiogenic stimuli in malignant pleural mesothelioma, PAI-1 suppression restricted tumor vascularization (Takayama *et al*., 2016). In hepatocellular carcinoma, HIF-2α dependent PAI-1 induction stimulated angiogenesis by lowering concentrations of active plasmin(Geis *et al*., 2015). Otherwise, PAI-1 favoring angiogenesis of tumor was explained in breast cancer, clear cell renal cell carcinoma and non-small cell lung cancer through clinical specimen analysis(Zubac *et al*., 2010), while the detailed mechanisms remain largely uncertain except that PAI-1 was found to facilitate endothelial cell migration from vitronectin to fibronectin mediated by different integrins and protect endothelial cell from apoptosis mediated by FasL through controlling the activity of plasmin(Bajou *et al*., 2008). As well as uPA-uPAR-PAI-1 systems might be included in tumor vascularization by the influence on endothelial cell migration and fibrin deposition in thrombus (Binder *et al*., 2007)

Localization studies have showed that PAI-1 is strongly expressed in endothelial cells in a number of tumor types (Pepper *et al*., 1996). Studies in PAI-1–null mice have showed that PAI-1 was absolutely required for tumor-induced angiogenesis. In one model, separation of tumor and stromal cells by the interposition of a type I collagen gel, that allows independent evaluation of exogenously added tumor cell (Bajou *et al*., 2001; Bajou *et al*., 1998). Absence of PAI-1 from the host (but not of uPA, uPAR, or tPA) greatly destroyed tumor cell invasion and host-derived neovascularization. Inversion of this phenotype was acquired via adenovirally delivered human PAI-1 to PAI-1–null mice. In wild-type mice, PAI-1 expression in the stroma could be localized to endothelial and non-endothelial cells. Although, in wild-type mice, uPA was localized to newly formed blood vessels, uPA was replaced by tPA in mice deficient in uPA, indicating the ability for compensation between the PAs. Also, The requirement for host PAI-1 (but not uPA) for tumor angiogenesis has been showed in a fibrosarcoma model in gene inactivation mice (Gutierrez *et al*., 2000). Basic FGF (bFGF)-stimulated corneal angiogenesis was similarly blocked in PAI-1– but not in uPA-deficient mice.

Two potential mechanisms demonstrate why PAI-1 is necessary for angiogenesis. Firstly, by preventing the ECM from excessive degradation, PAI-1 act to preserve the matrix scaffold necessary for endothelial cell migration and tube formation (Pepper & Montesano, 1990). Secondly, a complex groups of

reactions have been detected between PAI-1, uPAR, vitronectin and integrins(Preissner *et al.*, 2000). So, Modifications in PAI-1 expression and activity would be expected to change the adhesive, migratory, and growth characteristics of endothelial cells, that in turn would control angiogenesis. So, adenovirus was used to transport two mutant shapes of PAI-1 to PAI-1–deficient mice in the collagen gel interposition model demonstrated above (Bajou *et al.*, 2001). The PAI-1 mutants either (1) bound vitronectin normally however failed to suppress uPA or tPA or (2) suppressed PAs normally but had negligible attachment to vitronectin. Angiogenesis was obtained by the PAI-1 mutant that maintained PA inhibitory activity, not by the mutant that bound vitronectin but failed to inhibit the PAs, suggesting that the requirement for PAI-1 in this model is because of suppression of excessive proteolysis instead of inhibiting cellular adhesion to vitronectin. In a different study, exogenously added PAI-1 ("at therapeutic concentrations") was detected to prohibit bFGF-stimulated angiogenesis in the chicken chorioallantoic membrane (CAM) (Stefansson *et al.*, 2001). Using a variety of PAI-1 mutants, this activity could be associated both with PAI-1's antiprotease activity and its vitronectin-binding ability.

PAI-1 and Fibroblast

(Masuda *et al.*, 2019) stated whether downregulation of PAI-1 inhibited myofibroblast (MF) properties of cancer-associated fibroblasts (CAFs) and decreased chemotherapy resistance in lung cancer. PAI-1 inhibition could elevate the chemotherapy influence on lung cancer by prohibiting the MF criteria of CAFs. They concluded that PAI-1inhibition increased the chemotherapeutic efficacy on lung cancer through limiting the MF characteristics of CAFs. So, PAI-1 might be a promising therapeutic goal for patients with chemotherapeutic-resistant lung cancer with CAFs. Otherwise, α-SMA expression of CAFs was diminished by PAI-1 suppression, and apoptosis of CAFs was elevated.

(Sakamoto *et al.*, 2021) showed an indirect coculture assay between human bone marrow-derived mesenchymal stem cells (MSCs) and ESCC (esophageal squamous cell carcinoma) cells. Compared to monocultured MSCs, Cocultured MSCs presented more fibroblast activation protein, which is one of CAFs markers. So, they identified cocultured MSCs as CAF-like cells. To clarify molecules associated with the ESCC progression in CAFs, they used a cDNA microarray analysis on monocultured MSCs and CAF-like cells and compare their gene expression profiles. They stated that PAI-1, that encodes PAI-1, was more numerous in CAF-like cells than in monocultured MSCs, and the PAI-1 released from CAF-like cells activated migration and invasion in both ESCC cells and macrophages through Akt and Erk1/2 signaling pathways using low-density lipoprotein receptor-related protein 1 (LRP1), that is a PAI-1 receptor. Immunohistochemistry of ESCC tissues showed that higher expression levels of PAI-1 and LRP1 were correlated with poor prognosis in ESCC patients. They showed that the PAI-1/LRP1 axis leads to ESCC progression, making it a potential goal for ESCC therapy.

OSTEOPONTIN AND S100A ALTERNATE MECHANISM OF TUMORIGENESIS COMPARED TO PAI-1

Osteopontin Promotes uPA

Osteopontin (OPN) is one of the TME components that is released from various cells, including tumor cells, endothelial cells, immune cells, as well as fibroblast cells, within the TME, also has a central role in tumor progression. OPN induces tumor cell invasion, tumor growth, metastasis, EMT, drug-resistance, angiogenesis, stemness and immune suppression via cell surface receptors like CD44 and integrins. OPN increases the secretion of MMPs and uPA in cancer cells. inducing cancer cell invasion and extracellular matrix degeneration (Napoli *et al.*, 2020).

PAI-1 & Osteopontin have opposite effects on uPA(Das *et al.*, 2005). The functional molecular association between OPN and the uPA system in the context of tumor cell motility was detected in that OPN transfection led to activation of uPA expression by induction of NFkappaB and activation of the PI 3'-kinase/Akt/IKK pathway, subsequently upregulating promatrix metalloproteinase-2 that in turn enhances tumor cell invasiveness and extracellular matrix degradation. Silencing breast cancer metastasis suppressor gene 1 (BRMS1), for example, lead to a massive upregulation of both OPN and uPA expression that stimulate migration and invasion of breast cancer cells (Sheng *et al.*,2014).

S100A Stabilizes tPA

The plasminogen receptor S100A10 is one member of the S100 family of proteins, and its expression has been correlated to enhanced plasminogen activation and invasion of cancer cells. The production and localization broad-spectrum proteinase to the cell surface' plasmin' is through the plasminogen receptors. S100A10 regulate mainly the cellular plasmin production, acting around 50% of cellular plasmin production (Bydoun & Waisman, 2014). The S100A10-null mouse model showed the major role of S100A10 in fibrinolysis and oncogenesis regulation. S100A10 plays two essential roles in oncogenesis, firstly in control of cancer cell invasion and metastasis, secondly in control of tumor-associated cells movement to the tumor site, like macrophages. There is a reverse relation between PAI-1 and S100A10 (Madureira *et al.*, 2012).

S100A10 combines tPA and plasminogen and co-occur with the uPA/uPAR complex, that induces the transformation of plasminogen to the broad specificity protease, plasmin. Plasmin also binds to S100A10 which protects the newly generated plasmin from inactivation

by its inhibitor, α_2-antiplasmin, and serves to focus the proteolytic activity of plasmin to the cell surface. tPA and plasmin from PAI-1 and alpha2-antiplasmin have been conserved by S100A10 (Kassam G, *et al*.,1998). S100A10 has two carboxyl-terminal lysine residues that have been shown to combine with both tPA and plasminogen and to play a critical role in the conversion of plasminogen to plasmin by the plasminogen activators (Figure.4). S100A10 has a significant role in physiological processes, such as fibrinolysis and inflammation, suggesting a different axis of tumorigenesis compared to PAI-1 (Fogg DK *et al*., 2002).

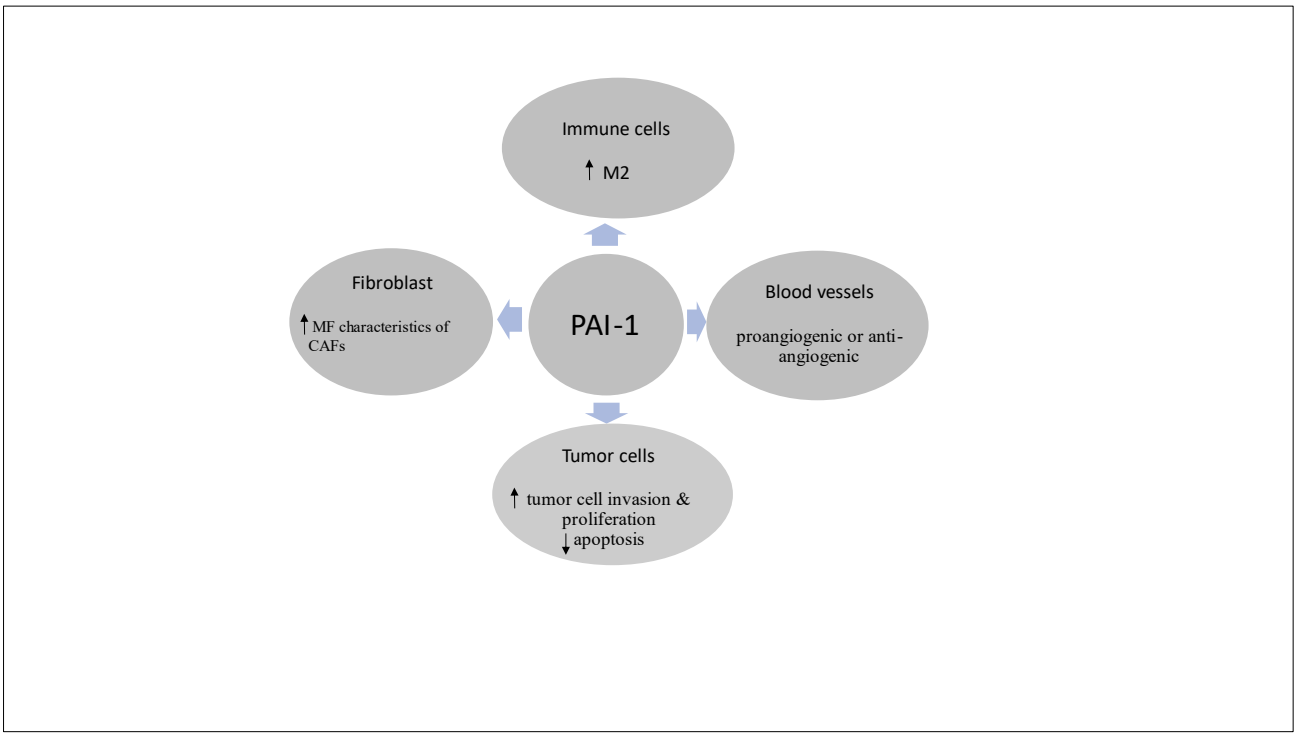

Fig. 4: Effect of PAI-1 on different parts of tumor microenvironment

- **Fibroblast:** PAI-1 promotes myofibroblast (MF) characteristics of cancer-associated fibroblasts (CAFs) and limited chemotherapy resistance in cancer.
- **Tumor cells:** PAI-1 inhibits ECM degradation, by inhibiting fibrinolysis and MMPs. PAI-1 cause tumor cell invasion, suppress apoptosis, increase cell proliferation and decrease adhesion between neoplastic cells and ECM factors.
- **Immune cells:** PAI-1 stimulates the recruitment and polarization of M2 monocytes/macrophages through various structural domains.
- **Blood vessels:** PAI-1 is pro-angiogenic at physiological levels and anti-angiogenic at higher levels

Summary:

PAI-1, also called SERPINE1, is one of the serine proteinase inhibitors (serpin) superfamily. It functions as the main inhibitor of uPA and the tissue plasminogen activator (tPA). It has an important role in the homeostasis of fibrinolysis and coagulation (Ghosh & Vaughan, 2012). Studies have shown the aberrant expression of PAI-1 in several cancers and specified its tumorigenic roles (Li *et al*., 2018). PAI-1 was stated to be highly expressed in gastric adenocarcinoma (GAC), where it has a role in the proliferation and migration of GAC cells (J. D. Yang *et al*., 2019). PAI-1 over-expression has also been shown to stimulate breast cancer metastasis (Zhang *et al*., 2018), ovarian cancer(Koensgen *et al*., 2018), and lung cancer(Zekanowska *et al*., 2004). High expression of PAI-1 in OSCC has also been reported (Hu *et al*., 2019). PAI-1, one of the major inhibitors of fibrinolysis (Ghosh & Vaughan, 2012), was shown to not only regulate the intravascular fibrin deposition but also share in several pathological processes, such as tumorigenesis (McMahon & Kwaan, 2008). It affects tumor growth and metastasis through regulating cell migration, immune cell polarization and angiogenesis (Caiolfa *et al*., 2007). Studies on OSCC also demonstrated that PAI-1 was overexpressed in the serum and tissues of OSCC patients, suggesting PAI-1 role as a potential soluble biomarker and therapeutic target for in OSCC(Huang *et al*., 2014).

Funding Statement: Self-Funding - No other source of specific funding was obtained for this work.

Ethical Declaration:

This study will be carried out after ethical approval of the ethical committee, School of Dentistry, University of Maryland, Baltimore, USA.

Conflict of Interest: All authors declare that there is no conflict of interest.

Author Contribution Statement: All authors contributed equally to this study.

Availability of Data: Data is available upon request.

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