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Matrix Metalloproteinase Modulation in Cervical Cancer

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Abstract

Cervical Cancer (CC) is the fourth most common gynecological cancer and one of the most common causes of mortality in women in developing nations. HPV-related CC is a high spectrum disease with a gradual progression from pre-malignant condition to an aggressive disease. HPV cervical inflammatory process and subsequent pre-malignant lesions development are induced directly or indirectly by a complex system of interaction between oncogenes and host factors, secreted by keratinocytes, immune cells and Extracellular Matrix (ECM). ECM development and modeling can be regulated through the expression of Metalloproteinases (MMPs), which have been associated with several types of tumors, including CC. The present review summarizes how MMPs may be regulated in Tumor Microenvironment (TME) and discusses the role of HPV in modulating MMPs in cervical carcinogenesis.

Keywords: Metalloproteinase; Cervical cancer; HPV; Modulation

Introduction

Cervical Cancer (CC) is the fourth most common gynecological cancer and one of the most common causes of mortality in women in developing nations [1]. There were approximately 604,000 new diagnosed cases and 342,000 deaths from CC worldwide in 2020 [2]. Almost all CC cases are linked to infection with high-risk Human Papillomaviruses (HPV), an extremely common virus transmitted through sexual contact [3].

HPV-related CC is a high spectrum disease with a gradual progression from pre-malignant condition to an aggressive disease, which begins in the cervix, and later spreads towards the lower uterine segment, vagina, para-cervical space, along with the broad and uterosacral ligaments [4]. For the CC development, tumor cells must invade the Extracellular Matrix (ECM) of the primary tumor [5]. For this process, Matrix Metalloproteinases (MMPs) play a deterministic role in tumor cells invasion by ECM cleavage [6]. MMPs, especially MMP-2 and MMP-9, are closely related to cancer cell growth, invasion, angiogenesis, and metastasis [7]. MMP-2 may enhance the invasive ability in cervical tumors cells by facilitating basement membrane and the ECM degradation [8]. MMP-9 expression is up-regulated in tumor and stromal cells of both high-grade Cervical Intraepithelial Neoplasia (CIN) and CC [9]. It is associated with stromal invasion, FIGO stage, lymph node metastasis, and vascular invasion [10].

Tumor infiltrate may change the stromal compartment by modifying the ECM and creating an environment conducive to the invasion of altered cells [5,11]. These modifications can lead to changes in its architecture, regarding the distribution of collagens and other non-cellular components of ECM, which are also important factors for tumor development both in the initial stage and in metastatic sites [12].

Understanding how the Tumor Microenvironment (TME) and all its components interact is essential to improve the cancer knowledge, as well as to develop therapeutic strategies. As one of the main components of the TME, ECM has the function of ensuring the structural balance of the tissue, acting in an orderly manner and being able to regulate cellular functions, in addition to having different physical, biochemical and biomechanical functions. However, when ECM is disrupted and disorganized, cells begin to behave abnormally, leading to a failure in tissue homeostasis and organ function [5]. The current review summarizes how MMP may be regulated in TME and discuss the HPV role in MMP modulation in cervical carcinogenesis.

Extracelullar Matrix as an Actor for Cancer Development

ECM is composed of several biochemical components with different functions, such as: Collagen and elastin, which provide the structural framework and tissue elasticity; fibronectin and

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laminin, related to the cell matrix adhesion; and polysaccharides and proteoglycans, which confer tissue resistance and are responsible for the exchange of nutrients [13]. These components constitute both the basement membrane and the interstitial matrix. The basement membrane is a specialized ECM, more compact and less porous, composed of type IV collagen, laminin, fibronectin, and proteins that connect collagen to other proteins. It is produced together by epithelial, endothelial and stromal cells and separates the epithelium or endothelium from the stroma. The interstitial matrix is rich in fibrillary collagens, proteoglycans, and glycoproteins, ensuring greater tissue resistance to traction, and is preferably composed of stromal cells [14].

Fibroblasts are characteristic cell types in the microenvironment playing a prominent role in the pathology of solid tumors [15]. They have been reported to influence the growth and radiation survival of CC cells [16]. Functional assays indicated that miR-1323 was transferred by CAFs-secreted exosomes and miR-1323 downregulation suppressed cell proliferation, migration, invasion, and increased cell radiosensitivity in cancer cell lineages, such as HeLa, SiHa, CasKi and C33A [17].

Besides, CAFs are the major sources of TGF-β1, an important cytokine that regulate of assembly and remodeling of EMC during cancer progression. TGF-β1 is the key growth factor involved in driving Epithelial-Mesenchymal Transition (EMT), a process which an epithelial cell alters its phenotype to that of a mesenchymal cell in response to external stressors or specific growth factors [18,19]. It involves the down regulation of epithelial markers as cytokeratin and E-cadherin, and the up regulation of mesenchymal markers including vimentin, fibronectin and α-Smooth Muscle Actin (α-SMA) [20]. Cancer cells have plasticity and can continuously adapt to the constantly changing TME, and this process is mediated by EMT [21]. In some cancer cells in primary tumors, epithelial cells lose their characteristic polarity and adherence because of EMT and attain a mesenchymal phenotype that enables invasion and metastasis, and these transformed cells exhibit molecular alterations, as confirmed by reduced E-cadherin expression and increased N-cadherin and vimentin expression [22].

EMT is considered the pre-step of cancer cell metastasis. Meanwhile, studies have been performed in an attempt to reverse the EMT process and inhibits the CC progression. The Enhancer of Zeste Homolog 2 (EZH2) is a positive upstream regulator of the EMT program. It may combine with the CDH1 (encoding E-cadherin) promoter to decrease the expression of E-cadherin and promote the metastasis and invasion of gastric cancer cells [23]. Chen and collaborators [24] demonstrated that miR-138 suppressed tumor progression by targeting EZH2 in CC and uncovered the role of DNA methylation in the miR-138 promoter in its downregulation. Upon miR-138 overexpression, cell proliferation, metastasis, invasion and EMT were suppressed. These findings demonstrated the potential of miR-138 to predict disease metastasis and/or function as a therapeutic target in CC.

In addition to miRNAs, several long non-coding RNA (lncRNA) impact CC advancement via modulating the EMT process. lncRNAs affects the apoptosis, invasion and metastasis of tumor cells and have a significant influence on tumor development [25]. The altered lncRNAs in tumors are expected to be used as diagnostic markers in multiple tumors [26]. Studies suggested that lncRNA may be crucial regulator of CC progression. For example, lncRNA FBXL19-AS1 promotes the proliferation and metastasis of CC cells by sponging miR-193a-5p and up-regulating Collagen type I Alpha 1 (COL1A1), a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon [27]. LncRNA LIPE-AS1 was over-expressed in CC tissues, related to tumor volume and declined survival rate. *In vitro*, LIPE-AS1 accelerated CC cell proliferation, migration and EMT, inhibited apoptosis [28].

Structure and Function of MMPs

The ECM degradation, covering all its components, is dependent on the action of proteolytic enzymes [29], such as MMPs, also called Matrixins. MMPs belong to a family of zinc-dependent enzymes, classified according to their structure and substrate specificity, as collagenases, gelatinases, stromelysins, Matrilysins and Membrane-Bound Metalloproteinases (MT-MMP) [30]. Currently, 24 MMPs are known, of which 23 in humans [29].

MMP family members are homologous in structure, containing five typical distinct functional domains: The signal peptide, N-terminal, with variable length, responsible for the MMPs secretion; the pro-peptide (~80 aa), which has a cysteine switch, which chelates the active site of Zn^{2+}, ensuring the latent form of MMP (pro-MMP); the catalytic domain (~170 aa), which contains the Zn^{2+} binding motif, two Zn^{2+} ions, and Sn' pockets, which confer substrate specificity, in addition to Ca^{2+} ions, providing stability and thus, responsible for proteolytic activity; a variable length linker (~15-65 aa), which links the catalytic domain to the hemopexin-like domain, also called the hinge region; and the hemopexin-like domain (~200 aa), C-terminal, which together with the S1' pocket are determinant for substrate specificity, being essential for the recognition and catalytic degradation of fibrillar collagen [29,31].

However, some MMPs differ with respect to these structures. For example, gelatinases have three type II fibronectin repeats in the catalytic domain; matrilysins lack the hinge region and hemopexin-like domain; secreted MMPs (MMP-11, -21 and -28) have a Furin-like proprotein convertase recognition sequence in the C-terminal chain of the propeptide [31]; and Membrane-Type MMPs (MT-MMPs) have MT-MMPs can be linked to the membrane in two ways: Through the transmembrane domain, followed by a short cytoplasmic tail, or anchored to the GPI [32].

It was seen that different MMPs have specific characteristics in their structure, differentiating them from the typical structure of an MMP (Figure 1). The S1' site, a hydrophobic pocket of well-defined depth, is the main difference between MMPs, which is a determining factor for the specific interaction with the substrate [33].

MMPs are classified as the main responsible for the renewal of the ECM through the proteolytic degradation of its components [34]. Each type of MMP has a different biological function: Collagenases (MMP-1, -8, -13 and -18) participate in the degradation of fibrillar collagen, which is essential in bones and ligaments; gelatinases (MMP-2 and -9) are involved in angiogenesis and neurogenesis, being able to modify the molecules of the basal lamina, leading to cell death; stromelysins (MMP-3, -10 and -11) degrade ECM segments;

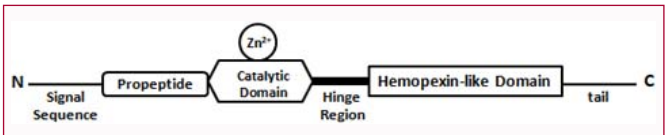

Figure 1: Typical structure of an MMP [31].

matrilysins (MMP-7 and -26) cleave cell surface molecules and digest ECM components; and Membrane Metalloproteinases (MT-MMPs) are able to activate some proteases and cell surface components [34].

Because MMPs disintegrate physical tissue barriers and contribute to the migration of tumor cells, initially these enzymes were known to initiate the metastasis process [35]. However, MMPs, in fact, may participate in all stages of tumor progression, since they are capable of modifying signaling pathways, regulating cytokines involved in tumor immune response, and inducing angiogenesis [36]. These roles will be further discussed in the next topics.

Cell surface receptors act in the conversion of extracellular messages into intracellular signals, and the proteolysis of these receptors can affect their activation, half-life and signal transduction [37]. MMP-1, for example, is capable of cleaving PAR1 (Protease-Activated Receptor), which is part of the family of G Protein-Coupled Receptors (GPCRs), inducing signaling, changes in the morphology of the p38 protein, in addition to activation by an alternative pathway and phosphorylation of this protein [38]. MMP-7 is also capable of inducing immune evasion mechanisms, through the cleavage of FasL and CD95 (Fas) [39,40]. This was observed by Strand et al. [41] who showed that MMP-7 cleaved the recombinant CD95 protein on the surface of colon tumor cells (HT-29), preventing apoptosis, whereas treatment with a broad-ranging MMP inhibitor (TIMP) spectrum, increased cellular sensitivity to CD95-mediated apoptosis. It was also seen that MT1-MMP cleaves CD44, a transmembrane receptor molecule that helps in cell migration inducing changes in the ECM, being, therefore, intimately involved in the processes of tumor invasion and migration [41]. Kajita et al. [42] observed the co-expression of MT1-MMP and CD44 in human breast carcinoma cells, generating 3 different fragments of CD44. Zarrabi et al. also demonstrated that one of the blades of the helix of the hemopexin domain of MT1-MMP interacts with CD44 leading to phosphorylation of EGFR, and this interaction is blocked by peptides that mimic the helix of the hemopexin domain of MT1-MMP, resulting on decreasing cancer cell metastasis in a murine model of breast cancer. In contrast, studies have shown that Death Receptor 6 (DR6), widely expressed on the surface of prostate and breast cancer tumor cells, is a substrate of MT1-MMP [43]. Cleavage of the Death Receptor 6 (DR6) ectodomain by this metalloproteinase shifted T cell differentiation away from Th1, induced monocyte cell death, and affected cytokine profiles of immature dendritic cells [44,45]. The interaction between DR6 and MT1-MMP suggests an increase in innate and adaptive immune response in antitumor therapy [37].

Studies have also shown that MMPs are important regulators of immune cell recruitment in inflammatory processes. Some MMPs can cleave chemokines, altering their function and resulting in the formation of a chemical gradient that directs cell migration [46]. It was seen that MT6-MMP is able to cleave about 14 chemokines that are related to the recruitment of macrophages and monocytes, such as CXCL2 and CXCL5, during an inflammatory process [37,46], increasing its chemotactic activity [37,46]. Chemokines CCL15 and CCL23, when cleaved by MMPs, increase their binding to Glycosaminoglycans (GAGs), present in the ECM and on the surface of endothelial cells [47] and induce chemotaxis, facilitating the migration of inflammatory cells in tissues. In contrast, MMP-2 cleaves CCL7, preventing the recruitment of macrophages and lymphocytes, thus decreasing the immune response [48]. McQuibban et al. [48] demonstrated that mice treated with cleaved CCL-7, compared to whole CCL-7, experienced a decrease in mononuclear inflammatory cell infiltration.

MMPs can both promote inflammation and act in anti-inflammatory regulation, through the processing of some cytokines, such as TNF-α, IL-1β and TGF-β. It has been shown that multiple MMPs (-1, -2, -3, -7, -9, -12, -14 and -17) are able to cleave active TNF-α at the cell surface, reinforcing their role as signaling influencers cellular [46]. Furthermore, MMPs can regulate IL-1β activity by cleaving soluble type II decoy receptor from IL-1 (sIL-1R II) [49]. MMPs2, -3 and -9 cleave IL-1β to reach a biologically mature form [50]. These same MMPs, plus MT1-MMP, also showed the ability to cleave the latent TGF-β complex, separating it from the cell surface, releasing the mature TGF-β [51]; moreover, MMPs can also trigger the release of TGF-β through the degradation of decorin, a collagen-associated proteoglycan that acts as a TGF-β depot in the ECM [52]. Briefly, MMPs are capable of triggering a signaling cascade in various types of systems and tissues; however, this mechanism needs to be better elucidated with regard to its *in vivo* effects and its association with the disease [37].

These data show us that MMPs have many other substrates in addition to those related to the ECM, having a wide range of functions in several cellular processes, both in normal conditions and in pathologies, mainly in tumorigenesis.

MMPs Modulators

In healthy tissues, under normal physiological conditions, the proteolytic activity of MMPs is low, and their expression is transcriptionally regulated by inflammatory cytokines, such as Tumor Necrosis Factor (TNF-α), growth factors, such as Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF-β), hormones, cell-cell and cell-matrix interaction [53]. Most members of the MMP family have a cis element in their promoter region, which allows for strong control of their expression by cells, thus allowing them to be co-expressed and co-repressed in response to the stimuli [54]. In the tumor microenvironment, the pro-inflammatory cytokine TNF-α induces increased expression of MMP-2, -3, -7 and -9, facilitating the invasion of malignant cells [55,56]. EGF induces MMP-1 expression in skin fibroblasts; when it binds to its EGR-1 receptor, and it is able to suppress the transcriptional MMP-9 activation in stromal cells [57]. PDGF (Platelet-Derived Growth Factor) leads to increased MMP-1 expression and when in conjunction with TGF-β, it increases the MMP-3 and Tissue Metalloproteinase inhibitor (TIMP-1) expression [58]. Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF-2) act as an angiogenic factors inducing MMP expression and facilitating metastasis [59].

Some proteases may be regulated post-transcriptionally. MMP transcripts have specific sequences in their 5'-UTR and 3'-UTR regions, which are targets of proteins that can bind and destabilize the mRNA [32,60]. An example of this post-transcriptional modulation is through miRNAs, which are small sequences of non-coding RNA that regulate gene expression, repressing translation or by degradation of their mRNA targets. miR21, which leads to the suppression of the Phosphatase and Tensin Homologue (PTEN), a tumor suppressor, induced a strong regulation of MMP-2, in an experimental model of myocardial infarction in mice [61]. This miRNA was also capable of negatively regulate the TIMP-3, and consequently leading to the activation of MMPs 2 and 9, as clinically relevant integral components of STAT3 signaling and are responsible

for maintaining activated state of STAT3 in HPV-infected cells during cervical carcinogenesis [62]. Furthermore, MMP-9 expression can be regulated by proteins like Siglec-15, a protein found in Tumor-Infiltrating Macrophages (TIMs). Recently, studies reported that Siglec-15 has immunosuppressive function [63] and related the migration of tumor cells in liver cancer and osteosarcomas [64,65]. In osteosarcoma cells suppression of Siglec-15 leaded to a decrease in the MMP-9 expression and in the opposite ways, when Siglec-15 protein was expressed, the MMP-9 expression was upregulated [64].

Experimental studies have already shown that MMP expression is transient after exposure to an external stimulus, leading to the belief that the genes of most of these proteases are inducible. However, in cancer cases, tumor cells start to express them constitutively at high levels, indicating that other types of mechanisms may be collaborating for the regulation of MMPs. In cases of methylation, the promoter region of several MMP genes have CpG islands that can be methylated, leading to gene silencing [60,66].

MMPs are secreted in their inactive form as pro-MMP (zymogen) and are activated in the extracellular space, with the exception of MT-MMPs, MMP-11, -23 and -28, which are activated in the intracellular space by a convertase protein, such as Furin [67]. Once mature, the MMPs activity is regulated by general protease inhibitors, such as α2-macroglobulin in plasma and blood fluids and TIMPs [68]; in addition to reactive oxygen, hypochlorous acid originating from leukocytes during the inflammatory process, MMPs and other proteases [68].

The active MMPs function is controlled by endogenous inhibitors, which include serum globulins and the tissue TIMPs [69,70]. They belong to a family of four homologous members (TIMP-1, -2, -3 and -4) and may be expressed constitutively in variety of cell types, induced or tissue-specific, being regulated at the transcriptional level by cytokines and growth factors [71]. The first evidence that these inhibitors play an important role in the ECM degradation, arose from the observation that TIMPs are capable of inhibiting several MMPs *in vitro* and the increased TIMP expression was associated with matrix accumulation [72]. All TIMPs are capable of inhibiting all MMPs with variations in their effectiveness [68]. TIMP-1 is a weak inhibitor of MT1-MMP, MT3-MMP, MT5-MMP and MMP-19, while TIMP-2 is the only one that, in addition to inhibiting, is able to activate pro-MMP-2, through interaction with MT1-MMP [68], where pro-MMP-2 is cleaved, and its active form released. Based on this process, it was suggested that MT1-MMP should act as a surface receptor by which TIMP-2 could influence cell growth; however, there is no evidence that this same mechanism can lead to suppression of the proliferation of endothelial cells [73]. TIMP-3 can promote apoptosis in tumor cell lines and smooth muscle cells, however, involving the modulation of MMP activity [71]. They have anti-angiogenic activity when acting as a Vascular Endothelial Growth Factor (VEGFR)-2 antagonist, independently of MMP inhibition [74]. TIMP-4, although mechanisms have not been described, it is suggested to increase or inhibit the growth of tumor grafts *in vivo* [75].

The Role of MMPs in Cancer

Tumor invasion process is orchestrated by a large set of cells including the tumor cells themselves, the adjacent stromal cells and the intratumor inflammatory cells, and it is believed that they are all capable of expressing a variety of MMPs [76]. However, non-malignant stromal cells are the main sources of MMP production, as they are induced by tumor cells through the secretion of cytokines and growth factors [77].

During a carcinogenic process, MMPs are able to degrade adhesion molecules that mediate cell-cell or cell-ECM interaction (*e.g.*, cadherins and integrins), causing the tumor cell to separate from adjacent cells and ECM. In addition, they degrade the basement membrane and ECM facilitating the locomotion and invasion of tumor cells, allowing them to reach and penetrate in blood or lymph vessels, leading to metastasis [78]. During this process, MMPs can modulate the bioavailability of growth factors and function/activation of surface receptors, release precursors to some growth factors linked to the cell membrane, activate signaling pathways to promote survival and degrade apoptosis mediators or mediators. Antitumor immunity, thus supporting tumor cells and promoting cell proliferation [78,79].

In angiogenesis, a fundamental stage in tumor development, MMPs play an important role, since they can degrade the basement membrane of blood vessels and recruit precursors of bone marrow endothelial cells to newly formed vessels [80]. MMP-9, one of the main regulators of this process, can both activate pro-angiogenic factors, such as VEGF, FGF and TGF-β, and promote the migration of endothelial cells [81], as well as generate angiogenesis inhibitors, such as endostatin and tumstatin, through proteolysis of the ECM and basement membrane of the vessels [79].

Another mechanism that contributes to tumor expansion is the escape of the immune response. MMPs produced by tumor cells can interfere with the chemotaxis of inflammatory cells to tissues, by cleaving some chemokines [48]. CCL/Monocyte Chemoattractant Protein (MCP) family of chemokines are cleaved by MMPs, which specifically renders them into non-activating receptor antagonists with inflammation-dampening effects [82]. In the melanoma model, the proteolytic cleavage of CCL8 by MMP-1 and MMP-3 may inhibit the antitumor capacity of this chemokine, demonstrating that the chemokine proteolytic cleavage can strongly affect a clinically relevant scenario of tumor development [83].

Several studies have focused on the association of MMPs with the development of various types of cancer. The expression of MMP-9, for example, was positively correlated with the expression of VEGFR-1 in patients with Hepatocellular Carcinoma (HCC), where they exhibited the worst clinical outcome of the disease, suggesting that MMP-9 as a prognostic marker of HCC [84]. In oral squamous cell carcinoma, Shrestha et al. [85], observed a positive correlation between MMP-2 and TIMP-2 with the degree, stage and metastatic capacity of the tumor, the highest expression of MMP-2 being associated with a reduced survival in cancer patients. The authors suggested that the expression of this enzyme may be an intrinsic biological characteristic of tumors and may indicate aggressive tumor behavior, regardless of the stage, while TIMP-2 demonstrated a relationship with the stage of the disease. Chang et al. [86] observed in patients with gastric cancer, an increase in serum levels of MMP-3, -7 and -11, and MMP-9, -12 and -21 in a tumor sample, and associated with the reduced survival of these patients. Likewise, a study evaluating the immunoexpression of MMP-9 demonstrated a strong association between the presence of this enzyme in patients with breast cancer metastasis, who had died [87].

The Role of MMPs in Cervical Cancer

CC is a major health problem due to its late diagnosis and poor prognosis, especially among women in underdeveloped countries worldwide. The initial establishment of cervical lesions and their progression to CC are closely associated with HPV E6 and E7 oncogenes, which are constitutively expressed leading to

tumorigenesis. The genome organization and protein structure of E6 and E7 have been discussed followed by their mechanism to establish the six major cancer hallmarks in cervical tissues for tumor propagation.

In cervical carcinogenesis, invasion process begins with the disruption of intracellular junctions of carcinoma cells *in situ* and their adherence to the basement membrane. The E6 proteins from high-risk HPV are characterized by the presence of a PDZ (PSD95/Dlg/ZO-1) binding motif in their extreme carboxy termini, through which they interact with several cellular PDZ domain-containing substrates leading a loss of tight-junction integrity [88]. This interaction with E6 protein results in the proteasomal degradation and/or mislocalization of the PDZ proteins, hence disrupting cell polarity, a common characteristic of malignant cells [89].

Cellular invasion takes place when tumor cells start to cross-talk with stromal cells, leading to cooperative enzymatic degradation of the Basement Membrane (BM) and the subjacent ECM, which allows the tumor to access vascularization to grow and metastasize [90]. The BM and ECM contain several proteins, such as collagens, fibronectin, and laminin [91], and so, there are several proteolytic enzymes that are involved in invasion [92], including MMPs, that are essential in the ECM degradation process and also promote cellular migration, regulate growth factors and cytokines, influence apoptosis and collaborate in neovascularization.

In addition to destabilizing the junction's integrity, E6 and E7 HPV oncoproteins can destabilize the interaction of MMPs with their regulators, affecting cell migration and invasion [93]. *In vitro* studies demonstrate that C33a (HPV-negative keratinocytes) cell lines transfected with E6 and E7 showed greater expression of MMP-9, MMP-2 and MT1-MMP, while the co-expression of E6/E7 led decrease in TIMP-2. However, their silencing led to a decrease in the levels of these MMPs [93]. Likewise, positive HPV16 cell lines showed high expression of MMP-2, MMP-9 and MT1-MMP, and once E6 and E7 were silenced, there was a decrease in both protein and mRNA levels of these MMPs [93]. In all studies, the silencing of these oncoproteins led to a reduction in the migratory capacity of these cells. A proteomic analysis study of the secretome of CC lines C33A (HPV negative), HeLa (HPV-18+) and SiHa (HPV-16+) demonstrated that TIMPs were over-regulated in these cell lineages when compared to a normal HCKT1 cell line, where MMPs-2 and -9 were not identified in its secretome. In addition, through zymography analysis confirmed the increased TIMPs regulation, leading to a decrease or absence of catalytic activity of MMP-2 and -9 in the secretome of these cell lines [94].

In the clinical context, studies have demonstrated the association of the MMP expression with the development and stage of CC. Some groups highlighted the involvement of MMPs-2, -9 and MT1-MMP in the development and progression of cervical tumors based on increased mRNA expression and protein in CIN 2/3 and CC [95]. In CIN 1 or normal cervical tissue the presence of these MMPs was decreased or absent [96]. In addition, these MMPs were also correlated with angiogenesis during the evolution from a high-grade lesion to cancer, and with the vascular density of tumors, and can be considered a good prognostic marker [97].

Guo et al. [98] observed an association between increased expression of MMP-7 and -9 with lymph node tumor metastasis in patients with early CC, suggesting that there was a positive correlation between these MMPs and the invasive potential of cervical tumors. The same was observed by Wu and collaborators [99], when they reported an increase in MMP-7 mRNA, as well as the protein, in CC tissues and metastatic lymph node tumors. These results suggested that these enzymes may contribute to the increase in the invasive potential of the tumor.

Furtado et al. [100] analyzed the presence of TIMP-2 methylation in Low and High Squamous Intraepithelial Lesions (LSIL and HSIL, respectively), invasive cancer and normal cervical samples, and their relationship with the presence of HPV DNA. Methylation was frequently detected in the lesion and CC groups compared to normal control samples. Although they did not find a statistically significant relationship between TIMP-2 methylation and the presence of HPV DNA, this association was more frequent in patients with an unfavorable clinical outcome. The group suggests that TIMP-2 methylation inactivates certain regions of the gene, reducing protein expression and impairing its role in tumor suppression, which may infer that TIMP-2 methylation may be a prognostic biomarker for unfavorable lesion evolution.

Understanding the complex mechanism of cervical carcinogenesis is essential to search for biomarkers that can be used as prognostics tools, and thus improve the clinical follow up and early identification and treatment of selected patients. The topics discussed in this review are essential for the understanding of the complex mechanisms involved in HPV-related cervical lesions and the action of MMPs in the tumor microenvironment.

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