Uterine cervical carcinoma: Role of matrix metalloproteinases (Review)

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Abstract. Epidemiological and experimental studies have provided evidence that human papillomavirus (HPV) infection is a main player in the development of uterine cervical neoplasms. Migration of cancer cells from the origin tissue to surrounding or distant organs is essential for tumor progression. Many studies of tumor invasion and metastases have focused on the degradation of the extracellular matrix where matrix metalloproteinases (MMPs) play a central role. Two of these enzymes, MMP-2 and MMP-9, have been correlated with the processes of tumor cell invasion and metastasis in human cancers, including uterine neoplasms. It has been shown that the up-regulation of MMPs is associated with progression of cervical uterine neoplasms. This review describes the current understanding of MMP-2 and MMP-9 expression and activity in pre-cancer and cancer lesions of cervical uterine, which may open new strategies for diagnostic and therapeutic interventions.

Contents

1. Introduction

Uterine cervical carcinoma is the second most common malignant tumor among women in the developing countries (1). Cervical intraepithelial neoplasia (CIN) is the preferred designation for the range of squamous intraepithelial abnormalities of the cervix that are associated with an increased risk of subsequent development of invasive squamous carcinoma. Traditionally, intraepithelial abnormalities are graded as CIN I, CIN II or CIN III depending on the degree of differentiation. Many cases of CIN are associated with infection by human papillomavirus (HPV) (2), confirmed by koilocytosis that may be identified in cervical biopsy specimens with or without CIN. In USA the traditional three tier CIN grading system used for the reporting of cervical cytology specimens has been replaced by a two tier grading system. This system, known as the Bethesda system, was developed in 1988 following a workshop sponsored by the National Cancer Institute which addressed the standardisation of cervical cytopathology reports (3). Premalignant cervical squamous abnormalities were divided into low grade squamous intraepithelial lesions (LSIL), which include features of HPV infection and CIN I (confined to the basal 1/3 of the epithelium), and high grade squamous intraepithelial lesions (HSIL), which include CIN II (confined to the basal 2/3 of the epithelium) and CIN III (2/3 of the entire epithelium) that may involve the full thickness (carcinoma *in situ*) (4).

The Bethesda system and the histological diagnosis of CIN are relevant for the management of the disease. The severity of CIN is expressed by its microscopic grade, which influences treatment of the patient. This is understandable in view of the regression, persistence and progression figures of different CIN grades. An inadequacy of the grading by microscopic pathology is that it assesses exclusively epithelial features and usually only those visible with the standard hematoxylin-eosin staining, thereby not taking into account other possibly valuable information. Another serious disadvantage is due to the three distinct grades used in CIN (or two in SIL) that can easily give a faulty static impression of a solidified sculpture as if CIN or SIL were a static event, whereas in reality a CIN lesion is a dynamic process that can progress and persist but also regress. Compounding the above are the well-known issues of intraobserver and interobserver reproducibility, which, for grading of CIN, is far from perfect (5,6). It is also difficult to distinguish CIN reliably from non-neoplastic lesions, resulting in either over-

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treatment or undertreatment (7,8). These points emphasize the need for adjuvant methods to interpret the actual morphological impression of a CIN lesion in dynamic terms rather than in static morphological grades. Such adjunctive methods are also important for better distinction of CIN from non-neoplastic lesions and to predict accurately the risk for progression of low-grade and regression of high-grade CIN lesions. Even small improvements in prognostic accuracy will enormously reduce the number of patients erroneously or unnecessarily treated, as shown for CIN (2).

The cause of cervical cancer is still unknown. However, HPV infection represents the primary risk factor in cervical and vulvar cancer. Evidence of HPV is found in nearly 80% of cervical carcinomas (9). Many other risk factors have been demonstrated to influence the pathogenesis of cervical carcinoma, such as an early onset of sexual activity, parity, pregnancy, immunosuppression, smoking and a large number of life-time or recent sexual partners. Another well established risk factor for HPV infection is the mode of contraception, *i.e.* non-use of condom and long-term usage of oral contraception. Human immunodeficiency virus (HIV) infection reduces the immune system's ability to fight infection, including HPV, and increases the likelihood that precancerous cells will develop into frank cancer.

In the last ten years several studies have suggested that one of the mechanisms of cervical carcinoma progression is the increased expression of matrix-metalloproteinases (MMPs) in cervical tumor tissue and its association with tumor growth, metastasis, and recurrence (10-15) as a result of the degradation of extracellular matrix (ECM) components (16,17).

2. HPV infection in uterine cervical carcinoma

Basic virology of HPV. The HPV virus is a small non-enveloped particle 55 nm in diameter. It has an icosahedral capsid composed of 72 capsomers, which contains at least two capsid proteins, L1 and L2. The virus is said to resemble a golf ball when viewed by electron microscopy. The HPV genome consists of a single molecule of double-stranded, circular DNA (18,19). The genome is functionally divided into three regions. The first is a non-coding upstream regulatory region of 400-1,000 bp, which contains the long control region (LCR), or the upper regulatory region that regulate DNA replication by controlling the transcription of the open reading frames (OFRs). The second is an early region, consisting of ORFs E1, E2, E4, E5, E6, and E7, which are involved in viral replication. The third is a late region, which encodes the L1 and L2 structural proteins for the viral capsid. High-risk HPVs have different biological and biochemical properties important in cancer risk. Many lines of evidence suggest that once inside the host cell HPV DNA start replicating by employing host cell factors to regulate viral transcription and replication. The interaction of host cell factors with LCR region leads to the transcription of the viral E6 and E7 genes whose products deregulate the host cell growth cycle by binding and inactivating tumor suppressor proteins (p53 and pRB), cell cyclins, and cyclin-dependent kinases (20). The binding of HPV E7 to the cellular transcription factor E2F-1 resulting in the liberation of the factor allows the cell to enter S phase of cell cycle. The outcome is stimulation of cellular DNA synthesis and cell proliferation. The E7 protein from low-risk HPV types binds pRB with decreased affinity (21). In addition other potential mechanisms contribute to the transformation such as methylation of viral and cellular DNA, telomerase activation and hormonal and immunogenetic factors. Progression to cancer generally takes place over a period of 10-20 years. Some lesions become cancerous more rapidly, sometimes within a year or two (22).

HPV infection in the development of uterine cervical carcinoma. During the last decade, research on cervical cancer has elucidated the role of HPV in the pathogenesis of this malignancy (23). The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. The magnitude of the association between HPV and cervical cancer is higher than that one between smoking and lung cancer (24). Squamous cell carcinoma (SCC) is the most frequent cancer representing almost 90% of cervical malignancies. Accumulating evidence suggests that SCC is related to HPV infection, which is estimated to be the most common sexually transmitted disease, with a lifetime risk of 75% (25,26). Of 120 different HPV genotypes which have been discovered only 40 can infect the genital mucosa. Recurrent microtrauma allow HPVs to infect basal epithelial cells of the skin or inner lining of tissues. Following infection, HPV genes E1, E2, E4, E5, E6, and E7 are expressed and the viral DNA replicates from episomal DNA. The progression from pre-cancerous lesions to invasive cancer is associated with the integration of the HPV genome into the host chromosomes with loss of E2 and upregulation of E6 and E7 oncogene expression (Fig. 1). HPVs can be divided into high-risk and low-risk HPV types. Low-risk HPV types include types 6, 11, 42, 43, and 44. High-risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Infections with low-risk types can cause benign or low-grade cervical cell changes, genital warts, and recurrent respiratory papillomatosis (27,28). High-risk HPV types act as carcinogens in the development of high grade cell abnormalities, cervical cancer and other anogenital cancers (29). Among these, four are most often found within the malignant cells of cervical cancers, with type 16 accounting for about half of the cases in the United States and Europe and types 18, 31, and 45 accounting for an additional 25-30% of cases (30). Many studies have shown that also adenocarcinoma of the cervix is related to HPV although the correlation is less pronounced and age-dependent (31).

HPV infection is extremely common among young sexually active women. Mostly the infection remains sub-clinical and self-limited (32), undergoing spontaneous clearance in a relatively short time, i.e., within months to two years (19,33). Many studies have shown that only 5% of these infections lead to squamous intraepithelial lesions (SILs) and even fewer develop to invasive cancer. The fact that only a small percentage of young women (<25 years) develop cervical cancer is explained by a necessary 10-year interval between the infection and cancer progression. Women with persistent (>6 months) oncogenic HPV infection are at risk for progression to high-grade SIL (34).

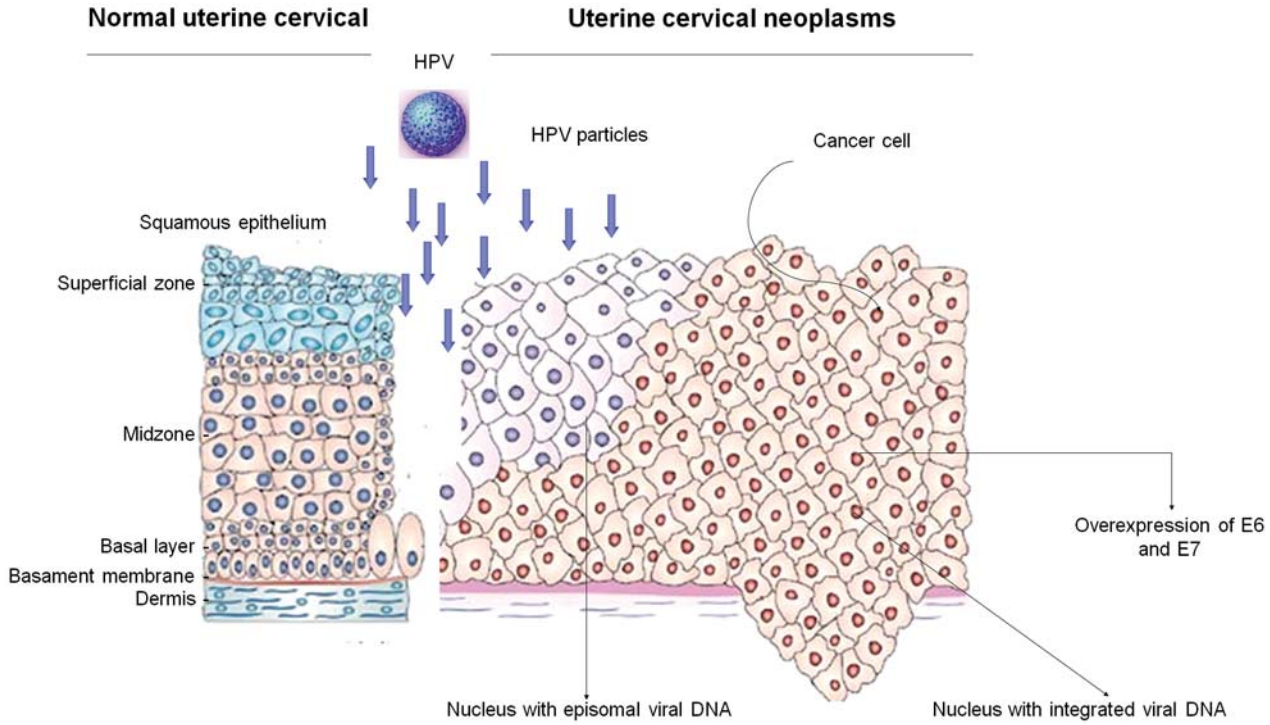

Figure 1. After human papillomavirus (HPV) infection, the early HPV genes (E1, E2, E4, E5, E6, E7) are expressed and the viral DNA replicates from episomal DNA. The progression from pre-cancerous lesions to invasive cancer is associated with the integration of the HPV genome into the host chromosomes with loss of E2 and upregulation of E6 and E7 oncogene expression.

3. Matrix metalloproteinases in the progression of uterine cervical carcinoma

Matrix metalloproteinases. MMPs are a family of metallo-endopeptidases that cleave the protein components of the extracellular matrix (ECM) and thereby play a central role in tissue remodelling and degradation (35). In physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, of activation of the pro-MMP precursor zymogens and of inhibition by endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (36,37). Most of the MMPs are synthesized as inactive latent enzymes and conversion to the active enzyme is generally mediated by activator systems that include plasminogen activator or the pro-hormone convertase, furin (38).

For many years MMPs were thought to function as regulators of ECM composition and to facilitate cell migration simply by removing barriers such as collagen. Many studies have shown that MMPs are implicated in the functional regulation of non-ECM molecules that include growth factors and their receptors, cytokines and chemokines, adhesion receptors and cell surface proteoglycans, and a variety of enzymes. Moreover MMPs play a significant role in the control of cellular interactions and response to their environment in physiological conditions that promote tissue turnover, such as normal development, or pathological, such as inflammation and cancer (39).

MMPs and cancer. The ability of cancer cells to migrate from the tissue of origin and metastasize to surrounding or distant organs is essential for tumor progression. Many studies of tumor invasion and metastases have focused on the degradation of the extracellular matrix and endothelial cell basement membrane (16,40,41). It is becoming increasingly clear the central role of MMPs in the degradation of ECM (Fig. 2). Two of these enzymes, designated MMP-2 and MMP-9, are potent gelatinases and have been correlated with the processes of tumor cell invasion and metastasis. MMP-2 (gelatinase A) (42) and MMP-9 (gelatinase B), have been found in large quantities in cancer tissues (43,44). Currently there is a growing evidence of their role in tumor progression (43,45,46). Many different processes are involved in cancer cell invasion and wide spreading such as the transcriptional control of the genes encoding MMPs, their activation, and the production of their natural inhibitors TIMP-1 and TIMP-2 (40,43). Substantial evidence suggests the importance of the MMPs/TIMPs ratio in tumor tissues e.g., the inhibition of tumor cell invasion and metastasis in animal models has been demonstrated using *in vivo* injections of TIMPs (47). During the many years following their discovery, MMPs have been revealed to have other significant functions in addition to the proteolytic activity. There are increasing data on their contribution to the tumor angiogenesis (48,49) and their impact on cytokines regulation. Both inflammation and angiogenesis are exacerbated by increased production of chemokines/cytokines, growth factors, proteolytic enzymes, proteoglycans, lipid mediators and prostaglandins. It has been observed that approximately 15-20% of all malignancies are initiated or exacerbated by inflammation. The process of angiogenesis requires degradation and remodeling of ECM, cell migration and proliferation, and tube formation (50) and it can explain the relevant role played by MMPs in tumor growth and progression. The recruitment and infiltration of macrophages, called tumor-associated

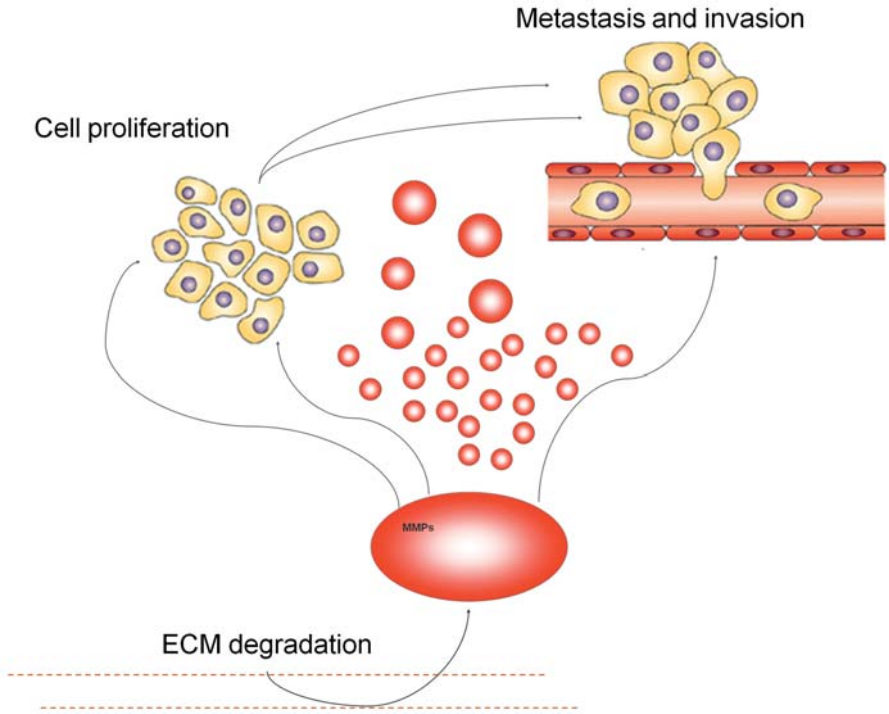

Figure 2. Matrix metalloproteinases (MMPs) promote cancer cell proliferation and invasion through the structural degradation of extracellular matrix (ECM) components.

macrophages, in the tumor microenvironment that activates them to support the malignant progression of cancer cells have also been reported.

MMPs in uterine cervical neoplasm. Overexpression of MMP-2 and MMP-9 has been observed in pre-cancer and cancer lesions of the cervical uterine (Table I). During the last decades progress in research on enzyme activities showed the potential significance of MMP-2 and MMP-9 in the progression of cervical uterine cancer suggesting their prognostic value (14,15,51-65). Sheu *et al* demonstrated that MMP-2 and MMP-9 were overexpressed in >90% of squamous cell carcinomas (SCC) and 83-100% of HSIL, but were less frequently expressed in LSIL and normal squamous epithelium (13%). MMP-1, MMP-14, and MMP-15 were detected in 55-81% of SCC cases, and MMP-1 was detected in 39% of HSIL (14). Many lines of evidence show that the activity of MMP-9 tends to increase from normal cervix to HSIL and SCC, and more advanced stages (64-66). Cervical cancer provides a useful model to study the relationship of MMPs and TIMPs (67-69) to tumor behavior. First of all the well characterized microinvasive carcinoma of the cervix, with its excellent outcome, can be compared with more deeply invasive tumors which invariably evolve from the micro-invasive state, and have a poorer prognosis. Many studies have shown a 1:1 ratio of MMPs:TIMPs in early cervical cancers (11). It is explained by the fact that tumor progression may select for cells expressing MMPs and do not express TIMP messages by promoting tumor cell growth. HPV infection is an essential step in the development of cervical cancers and transfection with the E6 and E7 HPV ORFs is sufficient to induce malignant transformation in normal squamous cells *in vitro*, but it is not known if these or other

Table I. Expression of MMP-2 and MMP-9 in uterine cervical neoplasms.

Diagnosis (no. of cases)	% of cases expressing MMPs		Refs.
	MMP-2	MMP-9	
SCC (31)	90	87	(14)
HSIL (23)	83	83	(14)
LSIL (8)	13	13	(14)
SCC (80)	46	n.d.	(15)
SCC (160)	42	31	(57)
SCC (15)	100	n.d.	(60)
HSIL (18)	61	n.d.	(60)
LSIL (11)	0	n.d.	(60)
SCC (49)	84	n.d.	(61)
HSIL (10)	100	n.d.	(61)
SCC (18)	n.d.	72	(62)
HSIL (5)	n.d.	50	(62)
Adenocarcinoma (18)	94	94	(63)
SCC (24)	n.d.	50	(64)
HSIL (30)	n.d.	86	(65)
LSIL (13)	n.d.	54	(65)

Abbreviations: SCC, squamous cell carcinoma; LSIL, low grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesions; n.d., not determined.

HPV proteins can influence MMP or TIMPs transcripts or enzyme activity. Recent findings suggests that HPV E6 and E7 transcription correlates with MMPs and TIMPs transcription (11,70). Although early studies obtained contrary outcomes, recent research has shown that EBV proteins may up-regulate MMP-1 expression in nasopharyngeal carcinoma, suggesting that other viral proteins may also regulate MMP expression (71).

Many studies have also observed that MMPs, and in particular MMP-2 and MMP-9, are expressed in stromal cells and inflammatory cells around tumors (72). These findings suggest the importance of these proteases in the pathogenesis of cancer. Of various inflammatory cell types infiltrating the tumor area in response to inflammatory stimuli, tumor-supporting macrophages, and tumor-associated macrophages (TAM), are thought to play key roles in further production of various growth factors, angiogenic factors, proteinases, chemokines and cytokines, through cross-talk with cancer cells and other tumor stromal cells (73). These factors stimulate cell migration/motility, proliferation, survival, angiogenesis and metastasis, resulting in a dynamic environment that favors the progression of cancer (48,49,74,75). For example it has been recently observed that MMP-9 plays a central role in the cleavage of certain cytokine receptors (i.e., interleukin 2Rα) on tumor-infiltrating lymphocytes derived from human cervical cancer (76,77). Other evidence shows that MMPs activate tumor necrosis factor α or inactivate interleukin 1ß, which may potentiate tumor progression by regulating the activity of these immunoregulatory cytokines at the site of tumor invasion (78).

4. Conclusions

Several studies have provided evidence that HPV infection plays an important role in the development of uterine cervical neoplasm. In June of 2006, the Food and Drug Administration (FDA) approved a cervical cancer vaccine for girls and women between the ages of 9 and 26. This vaccine should dramatically decrease the risk of uterine cervical cancer in the near future. Numerous studies support the possibility that overexpression of MMPs promotes tumor growth and metastasis in uterine cervical carcinoma. In particular, over-expression and activity of MMP-2 and MMP-9 has been observed and their prognostic significance in the progression of cervical uterine cancer has been proven. An appreciation of how these enzymes are linked to progression of this disease may improve diagnostic and therapeutic strategies.

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