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.

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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 In USA the traditional three tier CIN grading system used for the reporting of cervical cytology specimens has been replaced by a two tier grading system.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-
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). HPV's 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).
3. Matrix metalloproteinases in the progression of uterine cervical carcinoma

Matrix metalloproteinases. MMPs are a family of metalloendopeptidases 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
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 microinvasive 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>
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<tr>
<th>Diagnosis (no. of cases)</th>
<th>% of cases expressing MMPs</th>
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<tbody>
<tr>
<td>SCC (31)</td>
<td>90 87 (14)</td>
</tr>
<tr>
<td>HSIL (23)</td>
<td>83 83 (14)</td>
</tr>
<tr>
<td>LSIL (8)</td>
<td>13 13 (14)</td>
</tr>
<tr>
<td>SCC (80)</td>
<td>46 n.d. (15)</td>
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<tr>
<td>SCC (160)</td>
<td>42 31 (57)</td>
</tr>
<tr>
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</tr>
<tr>
<td>HSIL (18)</td>
<td>61 n.d. (60)</td>
</tr>
<tr>
<td>LSIL (11)</td>
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</tr>
<tr>
<td>SCC (49)</td>
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</tr>
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<td>LSIL (13)</td>
<td>n.d. 54 (65)</td>
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Table I. Expression of MMP-2 and MMP-9 in uterine cervical neoplasms.

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 suggest 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 1β, 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 neoplasia. 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 women between the ages of 9 and 26. This vaccine should improve diagnostic and therapeutic strategies.

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References


