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Immune deviation and cervical carcinogenesis

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ABSTRACT

Evidence is emerging that a complex interplay between high-risk human papillomavirus infection, the local microenvironment and the immune system is critical for cervical carcinogenesis. To establish persistence, the virus has to evade or overcome immune control. At the transition from precancer to cancer, however, chronic stromal inflammation and immune deviation build up, which may eventually determine the course of disease. Understanding the molecular basis underlying these pivotal stage-specific changes may help to define new tools for better diagnosis and therapy that are required to efficiently combat human papillomavirus-associated disease.

1. Introduction

In 2018, the International Papillomavirus Society and the WHO "*called to action*" to eliminate cervical cancer, which is an almost exclusively human papillomavirus (HPV)-driven type of cancer. For countries such as Australia with successful implementation of primary and secondary prevention programs, i.e. high coverage gender-neutral HPV vaccination and organized cervical cancer screening including HPV-testing, it is estimated that cervical cancer incidence decreases to less than 1 per 100.000 by app. year 2066 [1]. In many other countries, prevention programs are insufficiently established. They are far away from reaching these goals in the near future and high rates of cervical precancers and eventually also cancers are still expected in the next decades. These may also include "high-income" countries like Germany with prophylactic HPV vaccination rates considerably lower than 50% and opportunistic screening up to now.

Particularly in the latter countries, two major challenges are faced on the path to successfully fighting and eliminating cervical HPV-associated disease: (i) High-grade lesions detected during screening are often overtreated [2] since diagnostic tests are lacking that can reliably discriminate between regressing and "true precancerous" lesions progressing to invasive cancer. (ii) Therapy options for patients with invasive disease are limited and urgently need to be improved.

A clue to overcome both obstacles is seen in a better understanding of the immunological mechanisms underlying HPV-associated cancer development, which might help to improve diagnosis of precancer and to design novel immunotherapeutic approaches.

2. The two faces of the immune system in cervical cancer development

12-15 mucosal HPV types have been characterized as oncogenic high-risk- (HR-) HPV. Undoubtedly, the HPV oncogenes E6 and E7 are essential for host cell transformation. However, it takes years or decades after infection until cancers arise, pointing to additional tumor-promoting steps. Anogenital HPV infections are cleared in up to 90% of individuals within two years [3]. In a smaller percentage of otherwise healthy individuals, however, HPV can overcome immune control and establish persistent infection. Patients with immunosuppression are at particular high risk to develop persistent HPV infection and HPV-related diseases further underlining the importance of the adaptive immune system for the control of HPV infection and associated diseases [4].

While the role of the immune system for HPV control is well accepted, recent studies provide evidence that chronic inflammatory responses initiated by the HPV-transformed cells contribute to deviation of the local immune microenvironment, fuel progression to cancer and critically influence the course of disease (Fig. 1). Thus, a Janus-faced role of the immune system in cervical carcinogenesis is emerging.

2.1. Early immune control and HPV escape

HPV replication in the epithelium is non-cytolytic, involves only low gene expression in basal keratinocytes and lacks a viremic phase thus avoiding to elicit immune responses in an active manner (reviewed in Ref. [5]). In addition, HPV can actively counteract immune recognition and cell-autonomous immune responses by suppressing epithelial inflammatory and interferon responses (reviewed in Refs. [6,7]). As a

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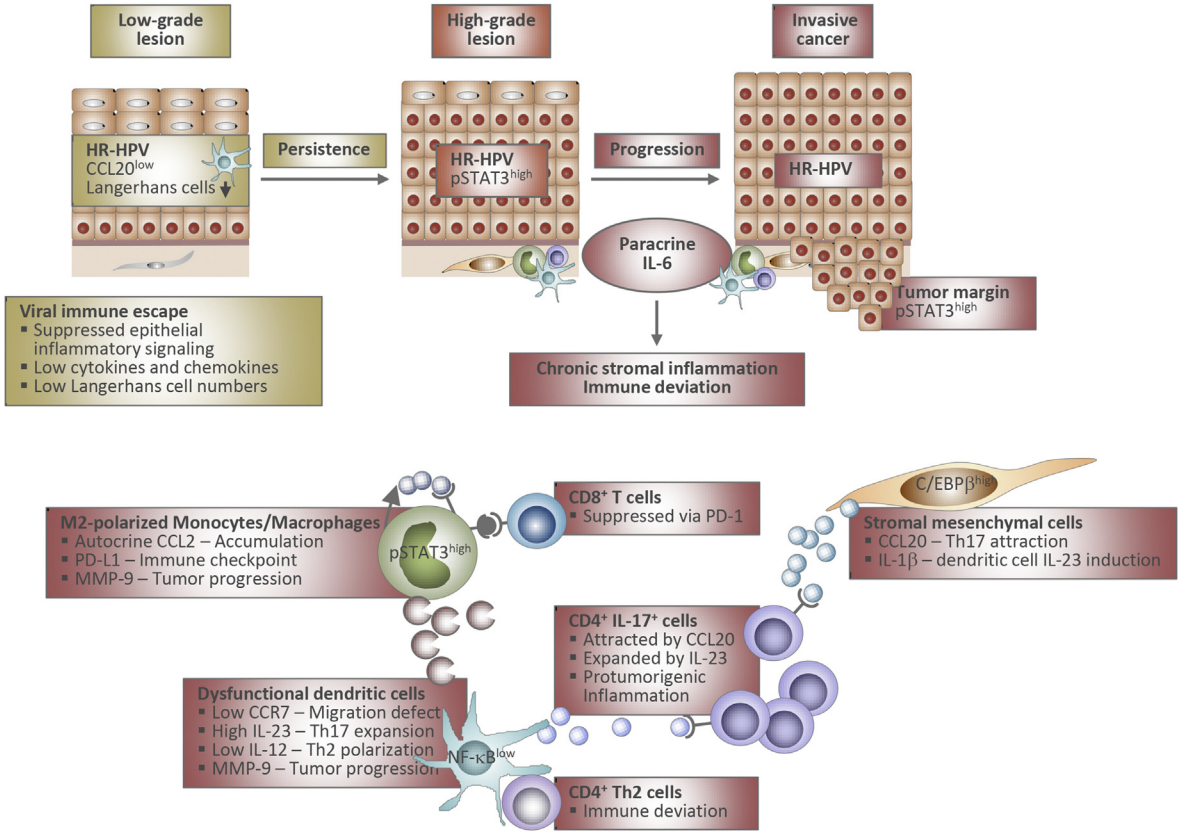

Fig. 1. The interplay between HPV-infected keratinocytes and the local immune microenvironment critically influences cervical carcinogenesis.
HPV suppresses inflammatory signaling in the host keratinocyte. Subsequent low epithelial cytokine and chemokine production prevents Langerhans cell recruitment allowing viral immune escape and persistence. At the transition from precancerous lesions to invasive cancer, the IL-6 cytokine plays a pivotal role for chronic stromal inflammation and immune deviation. Monocytes with an activated IL-6/STAT3 signaling pathway accumulate via autocrine CCL2 chemokine induction. These myeloid cells produce high amounts of MMP-9, a matrix-metalloproteinase promoting tumorigenesis and they can express the immune checkpoint ligand PD-L1 that suppresses cytotoxic T cell activity. On the other hand, stromal fibroblasts respond to IL-6 with C/EBPβ activation. One consequence is the production of CCL20, a chemokine attracting Th17 cells. Another consequence of fibroblast C/EBPβ signaling is the secretion of IL-1β, which induces IL-23 in dendritic cells leading to Th17 expansion further fueling inflammation. At the same time IL-6 suppresses NF-κB activity in dendritic cells, thereby impairing their CCR7-dependent migration to lymph node homing chemokines as well as their IL-12 production, which may contribute to the Th2 shift observed in vivo.

consequence, the recruitment of antigen presenting Langerhans cells to lesional mucosal or cutaneous epithelium is strongly impaired [8], eventually allowing immune escape and persistence.

2.2. Immune deviation and immunopathogenesis in cervical carcinogenesis

2.2.1. Self-reinforcing stromal inflammation with tumor-promoting myeloid cells starting in high-grade lesions

In cervical high-grade lesions, epithelial as well as stromal $CD8^+$ T cell infiltration is greater in regressing than in non-regressing lesions further supporting an important role of adaptive immunity for controlling the disease [9].

In contrast, myeloid cell infiltrates in the stroma of cervical high-grade lesions and cancers [10–12] rather have a negative impact for the patients. HPV potently interferes with inflammatory signaling in the infected epithelium and myeloid cell attracting chemokines are produced only at low levels in HPV-positive epithelial cells [10,11]. Therefore, it was unclear how the myeloid cells are recruited to the precancerous lesions. Notably, it was shown that infiltrating myelomonocytic cells secrete autocrine chemokines in a “self-reinforcing” manner in high-grade lesions. As a consequence, they release the tumor-promoting matrix-metalloproteinase MMP-9 [10], which is associated with poor prognosis for cervical cancer patients.

This “switch” to stromal inflammation starting in high-grade lesions is initiated by HPV-transformed cells activating the janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway in monocytes via a combination of the cytokine interleukin-6 (IL-6) and macrophage colony-stimulating factor [10]. IL-6, which is still expressed in cervical cancer tissue [13,14] together with prostaglandin E2 can promote differentiation of M2 macrophages expressing the immunosuppressive programmed death-ligand 1 (PD-L1), which may then suppress cytotoxic T cell responses [15]. High IL-6 expression, particularly together with low IL-12p40 expression, is associated with a negative prognosis for cervical cancer patients further high-lighting the importance of IL-6 for disease progression [14,16].

2.2.2. Functionally impaired stromal dendritic cells in cervical cancer

Monocytes are able to differentiate into dendritic cells. However, IL-6-instruced phenotypically “mature” dendritic cells are functionally impaired due to suppression of nuclear factor-κB (NF-κB) signaling [17]. In the stroma of cervical cancers, many mature dendritic cells in fact lack the NF-κB-regulated lymph node homing receptor CCR7 [12]. Unable to migrate, they produce pro-tumorigenic MMP-9 locally within the stroma [12]. Moreover, they secrete only low amounts of the Th1-polarizing cytokine IL-12 [18] potentially underlying the Th2 shift observed in cervical carcinogenesis in vivo. This indicates that deviated dendritic cells may rather contribute to tumor progression than to immune control. Notably, treatment with PolyIC, mimicking dsRNA-virus infection, leads to receptor-interacting protein kinase 3 (RIPK3)-dependent necroptosis in cervical cancer cells, and this restores IL-12 production by dendritic cells [19] opening new avenues for therapeutic intervention.

2.2.3. Expansion of stromal Th17 cell infiltrates in cervical cancer

In cervical cancer, IL-6 also up-regulates the CC-chemokine CCL20 in stromal fibroblasts via the CCAAT-enhancer-binding protein β (C/EBPβ) pathway [11]. Stromal CCL20 attracts Th17 cells, an inflammatory subset of the $CD4^+$ T cell lineage. Although their exact role is still unclear, the fact that they accumulate with advanced disease stages indicates a role in cancer progression [11]. The interplay between cervical cancer-instructed fibroblasts and dendritic cells further promotes expansion of Th17 cells via the heterodimeric cytokine IL-23 [18]. Interestingly, the cytokines IL-23 and IL-12 sharing the same IL-12p40 subunit are regulated by IL-6 in an opposing manner. While the IL-12-specific p35 subunit is directly suppressed by IL-6 in the dendritic cells, the IL-23-specific subunit p19 is indirectly induced by IL-6 via C/EBPβ-dependent IL-1β induction in the fibroblasts [18]. This further underscores the importance of IL-6 signaling for the establishment of chronic pro-tumorigenic inflammation and immune deviation in cervical carcinogenesis.

2.2.4. Stage-specific patterns and functional consequences of epithelial STAT3-activation in cervical carcinogenesis

IL-6 inducible signaling pathways such as the JAK/STAT3 or C/EBPβ pathway play a significant role for reprogramming the stromal microenvironment and for local immune deviation in cervical carcinogenesis. Strong STAT3 activation is also characteristic for the epithelium of cervical high-grade lesions [10,20]. In cervical cancer, however, the pattern changes. While epithelial cells remain STAT3-positive at the tumor margin adjacent to the stroma, STAT3 activation strongly declines within the tumor nests of invasive cancer [20]. Due to a loss of the IL-6 binding receptor chain gp80, IL-6 efficiently activates STAT3 in cervical cancer cells only in the presence of soluble gp80 (sgp80) potentially produced by the microenvironment [13,20]. This so-called IL-6 trans-signaling unexpectedly leads to potent induction of the pro-apoptotic interferon regulatory factor (IRF)1 and sensitizes the cancer cells to chemotherapeutic drugs. In line with this observation, inhibition of the STAT3/IRF1-pathway confers resistance to chemotherapy. In vivo, individual epithelial IRF1 expression levels prior to therapy significantly correlate with the responses of cervical cancer patients to chemo- or radiochemotherapy [20].

3. Implications for novel diagnostic and immunotherapy approaches

What can we learn from these observations for better diagnosis and treatment? (1) In contrast to $CD8^+$ T cell infiltration, stromal inflammation particularly with myeloid cells emerges as an important player promoting cervical carcinogenesis. Thus, stage-specific “immunoscores” might help to discriminate between regressing and progressing lesions. (2) Pre-therapeutic epithelial IRF1 expression potently influences (radio-)chemosensitivity of cervical cancers. Therefore, IRF1 may qualify as a novel predictive biomarker for the response of cervical cancer patients to conventional (radio)chemotherapy. (3) Immunotherapy regimens targeting the inflammatory IL-6-driven JAK/STAT3 and/or C/EBPβ pathways may profoundly alter the tumor microenvironment. They might help to correct immune deviation beyond checkpoint inhibition and complement conventional (radio)chemotherapy. (4) However, deliberate combination therapy regimens should consider appropriate timing of adjuvant therapies. Particularly, IL-6/JAK/STAT3-inhibitors might interfere with pro-apoptotic IRF1 expression and should therefore be administered only after (radio) chemotherapy. (5) In cervical cancers expressing high levels of the necroptosis regulator RIPK3, dsRNA-based adjuvants, such as PolyIC-derivatives, or dsRNA oncolytic viruses may be useful to induce necroptosis, which can help to restore IL-12 expression in tumor-resident dendritic cells, thus counteracting immune deviation.

Conflicts of interest

The author declares no conflict of interest.

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Abbreviations

HPV	human papillomavirus
HR	high-risk
MMP	matrix-metalloproteinase
IL	interleukin
PD-L	programmed death-ligand
NF-κB	nuclear factor-κB
CCL	CC-chemokine
CCR	CC-chemokine receptor
C/EBP	CCAAT-enhancer-binding protein
RIPK	receptor-interacting protein kinase
JAK	janus kinase
STAT	signal transducer and activator of transcription
IRF	interferon regulatory factor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pvr.2019.03.006.

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