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Sex differences in molecular pathways of human papillomavirus (HPV) and HPV-related diseases: do sex and gender matter?

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Summary. Human papilloma viruses (HPVs) are responsible for transient or persistent infections affecting human epithelia and mucosa and have been recognized as causal agents of 5% of malignancies in both sexes worldwide. Cultural factors, lifestyles, and social and relational behaviours play an important role in the different exposure and transmission of viruses between men and women, but the stages concerning HPV infection are deeply influenced by biological sex. Genetic, hormonal and epigenetic factors are the key components underlying the molecular dialogue between viruses and the host cells, in a sex-biased manner. Sex steroids are the most investigated ones, because of their receptor-dependent regulatory activity on the mechanisms of viral recognition and acquisition, on the morpho-functional phenotype of immune cells, and on the molecular processes of HPV-related persistence and carcinogenesis. Furthermore, the gonadal hormones seem to synergize with the complex microenvironment surrounding the HPV infection site, which is rich in resident cells, extracellular molecules and bacterial components of the microbiota. The in-depth knowledge of gender-specific molecular pathways involved in HPV infection, in viral persistence and in carcinogenetic processes could lead to the identification of more adequate therapeutic targets for men and women and of gender-oriented preventive strategies in HPV infections.

Key words: human papillomavirus, gender differences, sex-steroids.

Le differenze sesso-specifiche nei meccanismi molecolari del papilloma virus umano (HPV) e delle malattie HPV correlate: sesso e genere contano?

Riassunto. I papilloma virus sono responsabili di infezioni transitorie o persistenti che colpiscono uomini e donne in vari distretti cutanei e mucosi e sono stati riconosciuti come agenti causali del 5% dei carcinomi maligni in entrambi i sessi. Fattori culturali, stili di vita, comportamenti sociali e relazionali rivestono un ruolo importante nella diversa esposizione e trasmissione dei virus tra uomini e donne, tuttavia le diverse fasi dell'infezione sono profondamente influenzate dal sesso biologico. Fattori genetici, ormonali ed epigenetici rivestono un ruolo chiave nel dialogo molecolare tra virus e cellule ospiti, con modalità declinate al sesso del paziente. Gli steroidi sessuali sono i più studiati, principalmente a causa della loro attività regolatoria recettore-dipendente sui meccanismi di riconoscimento e acquisizione dei virus, sul fenotipo morfo-funzionale delle cellule immunitarie e sui processi molecolari di persistenza e carcinogenesi correlate all'HPV. Inoltre, gli ormoni gonadici sembrano essere sinergici con il complesso microambiente che circonda il sito di infezione da HPV, ricco di cellule residenti, molecole extracellulari e componenti batteriche del microbiota. La conoscenza approfondita dei percorsi molecolari genere-specifici coinvolti nell'infezione da HPV, nei processi di persistenza virale e di carcinogenesi potrebbe portare all'identificazione di obiettivi terapeutici più adeguati per uomini e donne e di strategie preventive orientate al genere nelle infezioni da HPV.

Parole chiave: papilloma virus umano, differenze di genere, steroidi sessuali.

Introduction

Human papilloma viruses (HPVs) are a heterogeneous group of the *Papillomaviridae* family of pathogens formed by a non-enveloped icosahedral capsid with circular double-stranded DNA (dsDNA) that show a preferential tropism for squamous epithelia and mucosa, principally for the metaplastic transforming zone, more rarely for the glandular cells and probably for the epithelial stem cells[1-2].

The small HPV genome includes 8,000 base pairs with three "functional" regions based on the protein expression during its lifecycle. The early region (E) has six genes coding for non-structural proteins involved in viral transcription, replication and transformation (E1, E2, E4, E5 and the oncogenes E6 and E7); the late region (L) contains genes coding for the structural capsidic proteins L1 and L2 and the upstream regulatory region (URR) is a non-coding long control region with promoter and enhancer sequences that regulate transcription and viral replication[1] (Figure 1).

HPV has been phylogenetically classified into 5 genera (alpha, beta, gamma, mu, nu) and more than 200 genotypes have been described in humans, based on the homology between nucleotide sequences of the L1 capsid protein. About 60 types of HPV belong to Alphapapillomaviruses and infect the ano-genital tissues or the skin. Betapapillomaviruses are involved in the develop-

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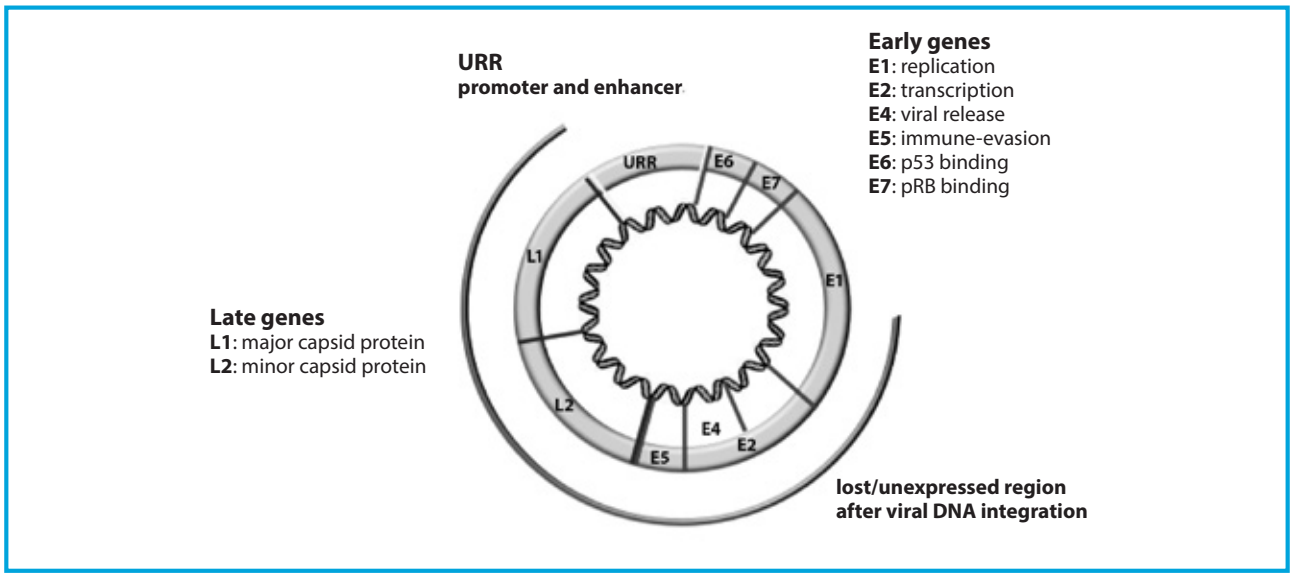

Figure 1. The organization of HPV genome.

ment of rare non-melanoma skin cancers (NMSC) and in the actinic keratoses that can preceed, or progress to, squamous cell carcinoma. The viruses are not the direct cause of skin cancers and do not even maintain the neoplastic phenotype, but they are important cofactors of UV in promoting cancer, by allowing the evasion of apoptosis in UV-damaged cells only at an early stage, with a "hit and run" mechanism[3]. The gamma genera include HPV types associated with the development of benign papillomas, such as genital and skin warts[2]. The Mu HPV types have only three members, while only one Nu type is known (HPV41)[4].

HPV infection is one of the most common sexually transmitted diseases, acquired through direct contact of any nature (genital-genital, anal-genital, oral-genital, manual-genital, through objects, self-inoculation, vertical transmission during childbirth) and also causing inapparent infections: the clue of HPV transmission is that a long-term shedding of virus particles can occur from apparently healthy surface layers[5]. It seems that the virions can also survive outside the human cells for a long time: persons with plantar warts can transmit HPV from one to another simply walking barefoot [6]. In all cases, the presence of microlesions in cutaneous or mucosal tissues is the indispensable condition for HPV infection[5].

The biology of the virus still remains incompletely defined, and the model studying the acquisition and the clearance of HPV has been analyzed in women. The virus lifecycle and the pattern of expression of the viral proteins are tightly associated with the maturative status of the keratocytes in the multilayered epithelium. The virus is acquired on the cell surface of the basal epithelial layer, via interaction of the L1 capsid protein with heparan sulphate proteoglycans (HSGPs), principally with syndecan-1, laminin-5 and alpha6-integrin. This interaction produces conformational changes in capsid proteins, leading to the exposure and proteolysis of the L2 protein, that allows virions to bind to specific receptors. Keratinocytes initiate the process of HPV internalization, principally mediated by transmembrane tetraspanins CD151, that interact laterally with each other to form specific tetraspanin webs (tetraspanin-enriched microdomains – TEMs) that modulate the attachment and endocytosis of particles in a HPVtype-specific manner[7].

Virions are disassembled in the endosomes and viral DNA (about 10-100 episomal non-integrated copies per cell) is transported into the nucleus through the protein L2, where it is integrated in the DNA of the host cell. The gene expression of E1 and E2 activates the replication of the viral genome, using the transcriptional machinery of the host cell. In the intermediate epithelial layers, E2 proteins also allow the expression of E6 and E7 proteins that interfere with the proliferation and differentiation of the host cell. In the mid-upper layers, viral DNA replication stops and the synthesis of E4 and E5 proteins begins, activating the expression of L1 and L2 late genes, whose products are involved in the encapsidation of viral DNA. After the assembly of new virions, the viral progeny is released through the exfoliation of the superficial cells and not through cytolysis. Now HPV can begin a new cellular contamination[8].

The incubation period ranges from 3-4 weeks to months/years[9]. About 90% of infections can be cleared in a few months through the host immune response, sometimes the infections can persist into the host cell in episomal form, can reactivate many years later and more rarely can lead to cancer[1].

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Immune response to HPV: sex differences

Gender impacts on the natural story of infectious diseases and immune responses[10]. Several biological factors contribute to this bias: (i) the effects of sex hormones on immune cell subsets, (ii) the chromosome Y influence on immune gene expression[11], (iii) the higher transcription of X-linked immune-related genes and immune-associated microRNA in women due to X chromosome escape inactivation[12], and (iv) the dimorphic regulation of the infiltration of certain immune cells by sex-influenced autosomal loci[13].

Many specific steps concerning HPV infection are influenced by sex.

HPVs complete their lifecycle only into human epidermal keratinocytes, thus the absence of viremia, the distance of complete virions from submucoseal germinative centers, the transcription of the antigenic products of capsid only at the upper epithelial layers and the lack of cellular lysis promote viral immune evasion as a necessary mechanism for successful infection[14].

However, the immune response plays an important role in clearing the viral infections. To generate an effective immune response, the virus should come into contact with the dendritic cells (DCs), the Langherans cells (LCs), the natural killer cells (NK), myeloid-derived suppressor cells (MDSCs), mast cells (MCs) and the mucose-associated lymphoid tissue (MALT) of the stroma[9]. This opportunity is restricted both to the microenvironment surrounding the microinjuries in the infected cells before HPV internalization, and to the keratinocytes, after infiltration of the immune cells into the epithelium (Figure 2). The resident cells are the first efficient line of defence to counter the productive infection, principally through an innate response with a very low adaptive response and immune memory[9].

Innate immunity

The epithelia represent the primary defense barrier against pathogens and keratinocytes have been considered immune sentinels[15]. Like innate immune cells, they are provided with an efficient antimicrobial system, expressing three classes of pattern-recognition receptors (PRRs): the toll-like receptors (TLRs) located both on the cell surface and on the endosome membrane, the RNA-helicases and the NOD-like receptors (NLRs)[16]. Different members of these three systems detect specific pathogen-associated molecular patterns (PAMPs) and trigger distinct signal transductions, risulting in increased production of different cytokines, interleukins and chemokines[17].

The preponderant role of TLRs in the activation of the immune responses was a revolutionary finding by the 2011 Nobel prize winners for medicine, Bruce Beutler and Jules Hoffman.

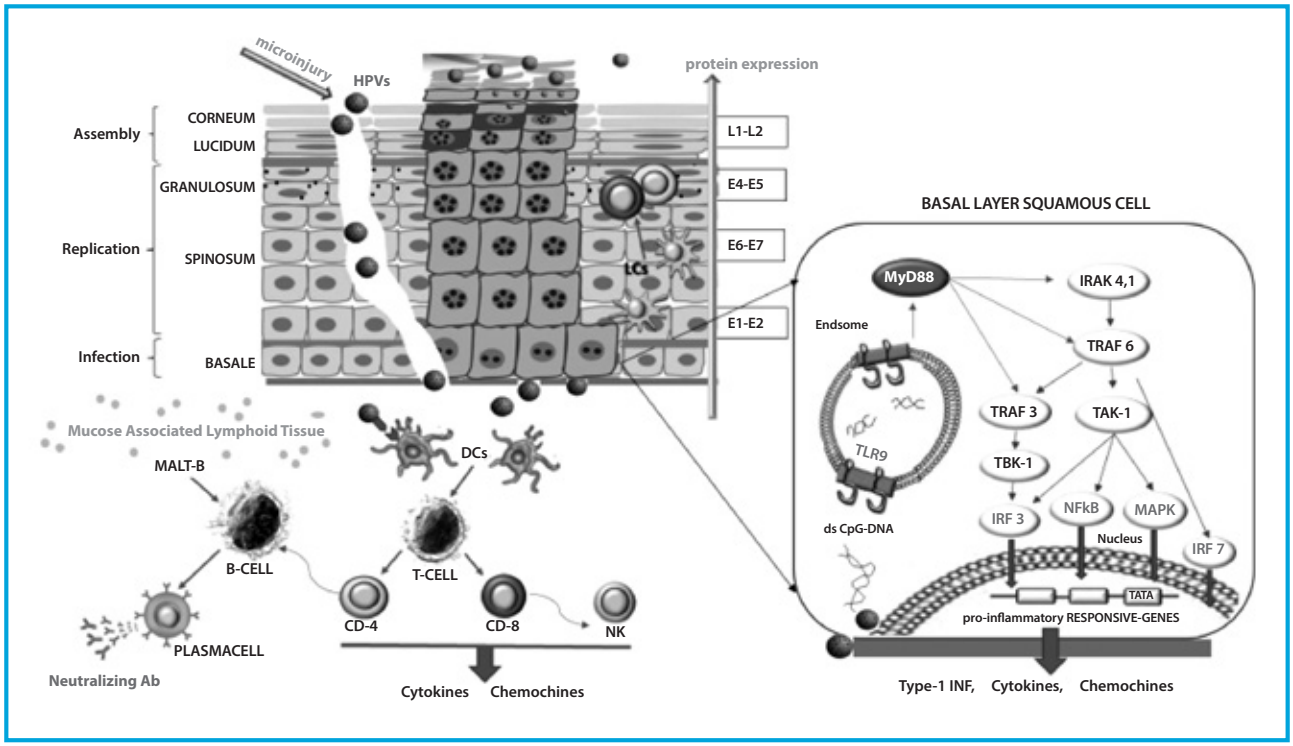

Figure 2. The immune response of the keratinocyte and of the local cellular microenviroment surrounding the HPV-infected epithelia.

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Viruses are recognized by epithelial TLRs mainly through the specificity of their nucleic acids. The Baltimore Classification grouped viruses according to their genome and mechanism of replication. (Figure 3): HPVs are dsDNA viruses belonging to group I of the Baltimore classification and unmethylated citosine-guanine DNA (CpG-DNA) is the ligand that can be specifically recognized by the endosomal TLR9 of both keratinocytes and immune cells.

HPV internalization represents a crucial step promoting the viral clearance. After the TLR9 has bound viral CpG-DNA, the complex recruits the adaptor protein MyD88, triggering a specific downstream signalling cascade that leads to the activation of transcription nuclear factor-kappaB (NFkB), mitogen-activated-phospho-kinase (MAPK) and interferon transcriptional factors (IRF3, IRF7). The MyD88-dependent pathway results in controlling the gene transcription of pro-inflammatory cytokines (CKs), interleukins (ILs) chemochines (CCLs) and type-I interferons (IFNs-1), leading to inflammation, cell-cycle entry and antiapoptotic effects[18,19] (Figure 2). NFkB activity seems to be the central mechanism underlying the molecular responses of the keratinocytes to contrast HPV infection.

Endosomial TLR9 are also expressed in the resident cells of the surrounding microenvironment, as NKs, pDCs, LCs, MDSCs. CpG-DNA is recognized by innate pathogen recognition machinery and the TLR9-MyD88 downstream promotes the molecular processes of maturation, recruitment, activation and functions of the resident cells[20]. Once again, NFkB activity is the central mechanism underlying these actions, whose regulation modulates the trend of innate response[21].

Both keratocytes and innate immune cells express sex steroid receptors (SSRs): sex hormones activate multiple transductional signals and are crucial regulators of NFkB activity. According to a non-genomic pathway, estrogen may exert both anti- and pro-inflammatory effects through direct interaction of receptor alpha (ERα) with various adaptor proteins or kinases, forming multiprotein active complexes[22].

Upon a high estrogen stimulation, ERα is quickly methylated at arginine 260 by arginine-methyl transferase (PRMT1) and metERα interacts with the adaptor protein MyD88. The multiprotein active complex metERα-MyD88 cannot be recruited by TLR9-dsDNA: estrogen-dependent methylation of ERα exerts an inhibitory effect on the MyD88 downstream signaling cascade, leading to the failure of NFkb activation[23]. The estrogenic anti-inflammatory effect is potentiated by the ERα-dependent displacement of several regulators and coregulators from NFkB binding site[24].

On the contrary, when estrogens are low, ERα is demethylated at arginine 260, and TLR9-dsDNA can now recruit the multiprotein complex demethERα-MyD88, triggering NF-kb transcriptional activity, with a pro-inflammatory effect[23].

Progesterone may inhibit NFkB activation in human myometrial cells, resulting in an anti-inflammatory action[25].

Studies conducted in mice models indicate that orchidectomy can decrease the expression of MyD88 and IL-6, suggesting an inflammatory role of testosterone[26].

SSRs cooperate with TLR9 also to modulate the morpho-functional phenotype of innate immune cells. The inhibition of NFkB by high estrogen could have anti-inflammatory effects by inducing a shift in DCs towards the production of type-2 cytokines (IL10, IL4, TGFβ) and by decreasing the production of neutrophil chemoattractans, oxidative metabolism, and the adhesion to endothelial cells via up-regulation of annexinA1. On the contrary, the activation of NFkB signaling by low estrogen increases the pro-inflammatory response by type-1 cytokines (TNFalpha, IL1beta, IL6, IFNγ, IL2) by promoting DC cell differentiation and the expression of the major histocompatibility complex (MCHII)[27].

Progesterone may inhibit NFkB signal and testosterone seems to activate it: thus, the specific TLR9-dependent molecular pathways make the sex-specific immune response against HPVs differ at times from the general data, which vice versa show that progesterone promotes the activation of NK[28], macrophages[29] and DCs[30], while testosterone inhibits NK cells[31].

Adaptive immunity

Adaptive immunity is still partially unclear in HPVs infection. It seems to be based on the activation of the regulatory T-cell (Treg) suppressive response by morpho-functional subsets of skin and mucosal professional DCs. DCs migrate to lymphoid organs, where they provide for the optimal processing and presentation of the L1 antigen to naïve T-cells, through receptor-mediated binding. The up-regulation of co-stimulary molecules contributes to cytotoxic T-cell selection, engagement and activation, resulting in their recruitment to the infectious site and in the production of pro-inflammatory and immune-mediating cytokines[32].

Besides, L1 and E7 are able to make LCs migrate, to induce the overexpression of chemochines and T_{H1} cytokines and to stimulate a specific CD8+ response (Figure 3).

In a natural infection, the capsidic L1 protein also provides humoral immunity by activating a potent neutralizing immunoglobulin G response: such specific-type antibodies appear between 6 and 24 months after infection only in 50-70% of infected individuals, while other viral proteins (E1, E2 and E6) do not induce any ef-

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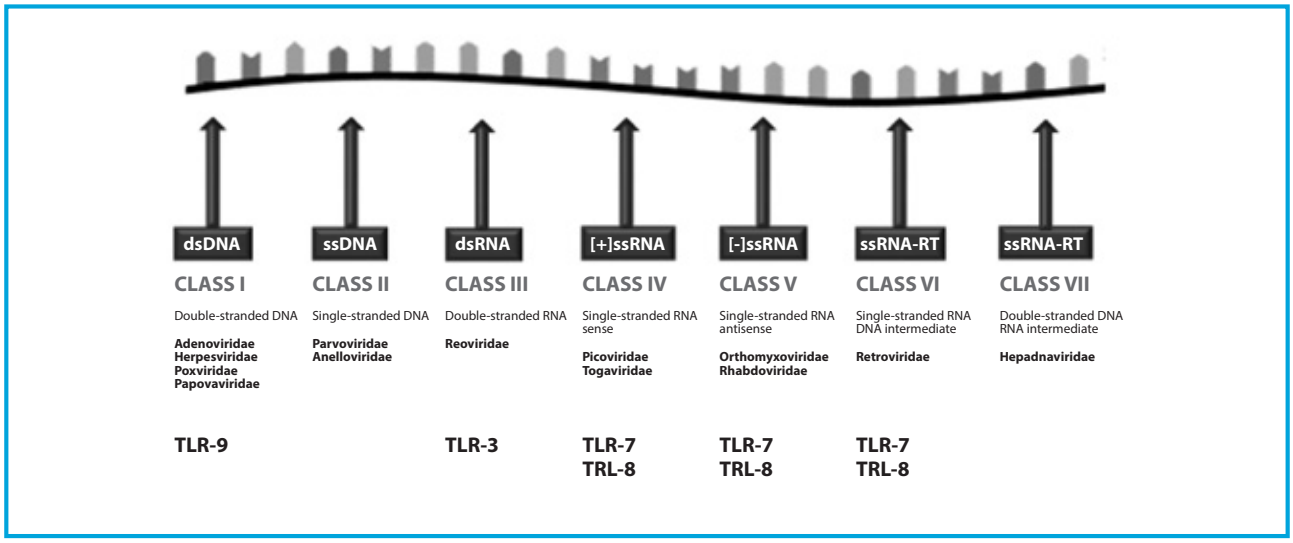

Figure 3. The Baltimore classification of viruses.

fective immunitary response[33]. Recent studies have shown that some women and men develop low levels of antibodies 8-9 months after infection[1,6,34].

Sex hormones regulate the proliferation and the morphofunctional phenotype of T-cells through interaction with SSRs. A "cytotoxic phenotype" favors a Th1/Th17 paradigm leading to the production of type-1 pro-inflammatory cytokines and a cytotoxic T-response, while a "tolerogenic phenotype" favors the Th2/Treg paradigm, with an increase in the type-2 anti-inflammatory cytokines and immunosuppressive T-response.

Data in the literature has shown that testosterone generally plays a potential immunosuppressive role as it decreases the proliferation and differentiation of lymphocytes[35], inhibits Th1 differentiation[36] and suppresses immunoglobulin synthesis, especially IgA[37]. Progesterone has marked anti-inflammatory and immunosuppressive effects, decreasing antibody production[27], and inducing the differentiation to T-reg[28]. Estrogens generally exert both pro-inflammatory/immunostimulatory effects and anti-inflammatory/immunosuppressive effects. High estrogen generally promotes the expansion, development and activity of Treg[38], while low estrogen directly interacts with the expression and transcription of INFγ by Th1-lymphocytes. Once again, the TLR-9-NFkB signals play a pivotal role: low levels of estrogens promote a cytotoxic phenotype, mainly through Akt/mTOR pathway and NFkB pathway, while high levels of estrogens promote a tolerogenic phenotype, mainly through the repression of NFkB pathway and c-Jun pathway[27].

As regards humoral immunity, female sex hormones stimulate B-cell mediated responses.

Persistence

The very rare cases of persistent infection lasting over 24 months reflects the fractured balance between viruses and the host, mainly supported by high-oncogenic-risk HPV subtypes, which have acquired a great ability to subvert, suppress or paradoxally use the host defense system to survive, grow, ensure efficient replication and preserve the infection for a longer time[39].

The deregulation of the host biological recognition machinery, mainly through the action of E6-E7 proteins, can represent, for HPVs, the primary efficient means to become invisible to immune surveillance and make the host response weak and unsuccessful.

The oncoproteins down-regulate the pro-inflammatory microenvironment both in infected cells and neighboring cells. They avoid antigen presentation decreasing the number and activation of LCs, DCs T-cells, and disrupting their migration, adhesion and infiltration through the down-regulation of chemochines CCL20, adherence molecules and E-cadherin expression[40].

Further, E6-E7 proteins (i) directly inhibit the production of IRFα, (ii) dump the expression of TLRs and their multiple downstream cascade, lowering the NF-kB pathway, the inflammatory response and the viral clearance[41], (iii) subvert the expression of citokines from a Th1 to a Th2 paradigm, and (iv) modulate the adaptive immune cells to their own advantage: in fact, the oncoproteins down-regulate the activity of cytotoxic T-cells, the humoral antibody response NK-receptors involved in cytotoxic activity[40] and recruit T-regs and Th2 cells.

HPVs are also able to deregulate the host cell cycle and to control apoptosis of infected cells[42].

Finally, papillomaviruses recruit the DNA damage repair (DDR) machinery of the host cells to avoid the

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removal of episomal viral genome by the nucleotide excision repair (NER) pathway and to efficiently replicate[42].

In addition to the self-advantageous deregulation of the immune response, HPVs can persist also through a direct interference on several physiological pathways of the infected cells.

The oncoproteins affect the mitochondrial morphology and functions, leading to a deep alteration of proliferative, anabolic and bioenergetic capacities of mature keratinocytes. They (i) deregulate cellular bioenergetics through interference with several proteins associated with the inner mitochondrial membrane (IMM) or to the matrix, (ii) increase the production of reactive oxygen species (mROS), (iii) influence signal transduction pathways by lowering the threshold to open the permeability transition pore (PTP) and changing Ca^{2+} uptake, (iv) directly inhibit apoptosis and (v) interfere with proteins associated with the outer mitochondrial membrane (OMM) that controls innate immunity[43].

The influence of sex steroids on specific HPVs persistence has been little explored. However, HPV impact on immunity and the estrogen-induced tolerogenic immune phenotype seem to overlap functionally, suggesting a biological synergism between the two systems.

Carcinogenesis

HPV is well established as an oncogenic virus, but only the very rare persistent infections can progress to cancer, also when high-risk genotypes are involved: the persistence of high-risk viral subtypes in keratinocytes has been defined a "necessary", but "not sufficient" condition to induce tumourigenesis[41].

The multistep process of neoplastic transformation needs the presence of genetic and epigenetic cofactors linked to the papillomavirus (HPV genotype, high viral load, multiple infections), to the host (genic arrangement, congenital or acquired immunodepression, immunosuppressive therapies, contemporary infections by sexual transmitted diseases, such as clamydia or HSV2), and to lifestyles such as habitual tobacco smoking, oral contraceptives, deficit of vitamins, early sex activity and multiple sex partners[44].

The whole mechanism whereby HPV-infected cells disarm antiviral defence promoting carcinogenesis is not fully understood. The random integration of the viral DNA into the host genome is a key event in HPV tumourigenesis[45]. Some regions of the viral DNA are deleted, such as those included within E1-E2 tract, while others are integrated (E6-E7) and are transcriptionally active. The loss of the E2 gene sequence, that normally controls the regulated expression of E6 and E7, involves the uncontrolled transcription of viral oncoproteins: the host cells containing the E6 and E7 sequences acquire an elective growth advantage compared to the one containing viral DNA in episomal form.

The oncoproteins E6 and E7 interfere with the host immune system and the anti-tumoural regulatory system, evading immune surveillance, affecting the course of the cell cycle and apoptosis, and making the cell more susceptible to carcinogenic mutations[2].

HPV proteins play a key role in several molecular processes promoting angiogenesis, cell immortalization, tumour growth and progression, but the complexity of their biology cannot be entirely described. However, a synthetic summary of some of the biological properties shows that E6 and E7 (i) abrogate the cell cycle checkpoint and induce genomic instability[46], (ii) deregulate the expression of suppressors of oncogenesis p53 and pRB[47], respectively, (iii) activate the IL6/STAT3 pathway[48], (iv) up-regulate the expression of several proteins inhibiting apoptosis[49], (v) lead to the loss of LCs, (vi) up-regulate programmed death-ligand 1 (PD-L1), a transmebrane protein that suppresses the immune system[32], (vii) down-regulate pro-inflammatory cytokines, chemokine CCL20, interleukin (IL)-1β40, and pro-apoptotic factors[50], (viii) interfere with TLR signaling pathways[9] and (ix) induce high levels of IL-17 and IL-8[43].

The viral E6 induces telomerase activation through many post-transcriptional mechanisms mantaining telomere length and leading to cell immortalization[51].

In particular, the interplay between HPVs and the host TLR signaling has a pivotal role in establishing a favorable microenvironment for cancer growth and the invasiveness of cancerous cells. HPV infection activates TLRs, triggering viral clearance and a resolution phase where tissue repair takes place. The viruses can contrast this pathway, inducing a dampened TLR9 gene expression, that promotes immune evasion and viral persistence, as previously seen[41]. One could therefore infer that the progressive severity of the cellular damage that underlies the possibility of cancer transformation was proportional to a chronic down-regulation of TLR9 by persistent HPVs. On the contrary, the oncoproteins E6 and E7 have been shown to up-regulate the expression of TLR9 and of various components of TLR-NF-kB signaling in cervical cancer keratinocytes[52] and in HPV-related oropharyngeal carcinoma[53].

It seems that the inappropriate and continuous stimulation by hr-HPVs could increase the overexpression of these receptors and an excessive immune response, driving the inflammation to a chronic state that leads to the activation of transcriptional factors for pro-cancerous effectors involved in mutation, angiogenesis and resistance to apoptosis[54-55].

Some authors have demonstrated that the increased stimulation of TLR9-expressing cancer cells and mesenchymal stem cells also activates their migration and in-

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vasion via up-regulation of the matrix metalloproteinase 2,9,13 (MMP2, MPP9, MPP13)[56].

To date, the explanation of the coexisting protumour/antitumour mechanisms governing the expression of TLR9 in cancer cells and their downstream cascades remains still unclear[57].

Over the past 25 years, the contribution of partially known local factors has emerged: HPV oncogenes alter gene expression of stromal cells, promote an aberrant epithelial-stromal cross-talk and sustained levels of extracellular molecules in the tumour microenvironment (TME) that are recognized as critical mediators of immune-suppression or tumour progression, angiogenesis, cell invasion and metastasis, as a variety of cells (Treg, M2 macrophages, MMP-9, myeloid-derived suppressors cells, fibroblasts)[32], hypoxia, nitric oxide (NO)[58] and microbiota[59].

The synergistic effect between vaginal microbiota patterns and (hr)HPV infection has become increasingly apparent. Many studies have examined the link between the vaginal microbiota, hrHPV infection and cervical diseases, reporting that progression is associated with increased vaginal microbiome diversity, probably due to the transition to community state type IV, leading to microbial dysbiosis and increasing the risk of cervical disease. On the other hand, other studies have investigated how the infecting HPV type could influence the variation of cervicovaginal microbiota composition. Recent studies have revealed that the acquired type of hrHPV may not be caused by a group of common cervicovaginal microbiota but rather by the changes in the species proportions of these bacteria and by some pathogenic agents that are specific to each SIL, regardless of the abundance[60].

Tumour cells express SSRs receptors that mediate the genomic transcriptional disregulation of several genes involved in cell survival and proliferation, and the non-genomic dialogue with several growth factor pathways. Sex hormones also play an important role in the development, growth, progression and invasion of cancer, although their contribution to intratumoural signaling is still controversial[61].

The influence of sex steroids on specific HPV-mediated cancerogenesis has been little explored.

Reports indicate that high ERα expression is associated with low expression of TLR9, which vice versa is up-regulated in several cancer cells in the presence of testosterone[62].

Non-cancerous TME cells are recognized as critical mediators of tumour progression. Estrogens enhance a greater aberrant amount of protumoural responses within TME: they shift the balance toward Th2 responses that favor the production of tumour-promoting cytokines (IL-6, IL-4, IL-17A, TNFα) and the infiltration of M2 tumour-associated macrophages, and promote immune-evasion through proliferation of Treg, MDSC, inhibition of T-CD8+ and NK apoptosis[63]. Moreover, cancer-associated fibroblasts have been shown to mediate estrogenic proliferative signaling in cervical cancer[64]: estrogens drive an aberrant epithelial-stromal crosstalk leading to a strong pro-inflammatory and immune-related gene-expression signature, specifically for chemokine and cytokine activity. HPV oncogenes and estrogens interact across these tissue compartments driving the progression and maintenance of cervical cancer[65].

These poor data could suggest that female cells would be more prone to HPV-related carcinogenesis, according to the role of estrogen in promoting rapid growth of tumour cells, angiogenesis and remodeling of the local microenvironment to facilitate invasion[66].

Conclusions

Sex can differently impact HPV infections and related diseases between men and women.

The probability of acquiring the virus could be biologically higher in women with high estrogen, because of ERalpha dependent up-regulation of syndecan1, as demonstrated in other epithelial tissues. Once the virus has been acquired, its clearance seems to be faster in men and in women with low estrogen, due to a more powerful pro-inflammatory and immunostimulatory response, mainly due to a dimorphic regulation of TLR9-NFkB pathway into infected keratinocytes and immune cells. Female cells could be more prone to HPV-induced immune tolerance, virus persistence, cellular transformation, tumour progression, angiogenesis and metastatis, in a steroid receptor-dependent way.

These results are often conflicting with general findings on the immunosuppressive role of testosterone and the pro-inflammatory effects of estrogens, which classically give women better protection against infections and their complications than men. However, the rich body of data in the literature has been obtained from many physiological and pathological tissues, not specifically in HPV-infected cells, and cannot always be passively translated into "specific" HPV diseases.

Gender assessment of the aspects concerning HPV infection is further burdened by several considerations.

Above all, the impact of the sex is quite variegated in both sexes, since the effects of sex steroids are not univocal, but depend on a dynamic cross-talk between the share of circulating hormones, the receptor system, the genomic or non-genomic transductional patterns and the cellular enzymatic-metabolic profile, which continuously vary with age, hormonal phases, target-tissue specificity, different organ microenvironment and microbioma.

Moreover, sex hormones affect the dialogue between the host and pathogen in a different way, depending on

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the nature of the stimulus: the response to bacteria is quite different from that to viruses and the diverse subtypes of hrHPV show different biological effects on the same molecular pathways.

Moreover, the antitumour or protumour role of TLR receptors is still controversial, as the human TLR9 gene has five isoforms that are generated through alternative splicing in normal tissues, inducing different responses, but little is known about their presence in cancer cells, specifically in HPV-related cancers.

Finally, biological "sex" has to intersect with "gender": social, relational and health-seeking behaviours, lifestyles, cultural factors, and access to health care can deeply influence both the exposure to HPVs and the transmission, severity and duration of infection, possibly changing the basic dimorphism of the biological profile.

Further research is required to clarify the peculiar sex-specific behaviour of immune cells and keratocytes during specific HPV infections; the biological data could represent the starting point for future gender-oriented studies on HPV-related diseases, which could lead to personalize medical management in both men and women.

New biomarkers of persistence and early carcinogenesis are still required for effective gender-specific screening.

In consideration of the pivotal role of TLRs-mediated pathways in HPV infection and of the influence of sex steroid on their biology, the use of endogenous regulators of TLR9 signaling molecules could be therapeutic agents in HPV-related cancers, from a gender perspective.

Key messages

- HPV is a common virus in both sexes, has a tropism for ano-genital regions and oral sites, as well as the skin and mucosa, and features a latent, subclinical and clinical form.
- The persistence of high-risk virus infection may trigger the mechanism of transformation in the host cell towards carcinogenesis.
- Gender differences in the incidence and prevalence of the infection, the HPV-related diseases and cancers have emerged.
- Cultural factors, life styles, social and relational behaviours and peculiar host biological factors (sex steroids, immune responses, genetic and epigenethic molecular mechanisms) play an important role in gender bias.
- The gender perspective should be a strategic goal for medical education, more adeguate programs of prevention, vaccination, health policies and more targeted plan of treatments.

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