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Review

Initial steps and mechanisms of HPV infection

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Abstract

Human papillomaviruses (HPV) are small, non-enveloped viruses. They have circular double-stranded DNA in an icosahedral capsid. Structural proteins of viral capsid, play important role in efficient virus infectivity. The viral L1 protein binds to the exposed basement membrane via heparan sulphate proteoglycans (primary receptor) and α6 integrin (secondary receptor). That binding triggers receptor mediated endocytosis of HPV. Most HPV types use clathrin dependent endocytic pathway. Clathrin coated vesicles become uncoated after endocytosis and fuse with early endosomes. HPV can avoid lysosomal degradation and escape from the endosomes to the cytosol, with various mechanisms, including membrane disruption, transmembrane pore formation and pH decline. Finally, the complex of HPV genome and L2 protein enters the nucleus. Then, HPV transcription and replication occur in association with promyelocytic leukemia (PML) nuclear bodies. During latent infection, HPV genome maintain as autonomous replicating episome in the proliferating basal cells of the squamous epithelium.

Key words: molecular biology; HPV; infection; receptors; endocytosis; intracellular trafficking; nuclear entry

The infectious cycle of human papilloma virus (HPV) is entirely carried out in a fully differentiated squamous epithelium[1]. It is essential that the virus particles gain access to the basal layer of the epithelium and enter to the dividing basal cells[2]. For this purpose, a micro - abrasion of the epithelial surface is necessary, which removes the epithelium but retains intact with its basement membrane. Metaplastic epithelium is thinner, more fragile and may be more susceptible to the micro - abrasion process and the HPV infection[1].

Viral structure

HPVs are small, non - enveloped viruses[1]. They have circular double - stranded DNA in an icosahedral capsid[2]. They are completely species and tissue specific. Their genome usually contains around 8,000 bp and encodes 8 or 9 ORFs (open reading frames)[2]. They have very complex molecular biology, despite their small size[3]. Viral capsid is composed by 2 structural proteins (L1 and L2)[3-5]. Moreover, structural proteins play important role in efficient virus infectivity[2]. Also, HPVs have 3 oncogenes (E5, E6 and E7) and 2 regulatory proteins (E1 and E2). Oncogenes modulate the transformation process. Regulatory proteins modulate transcription and replication[3,4].

Binding on cell surface receptors

The viral L1 protein binds to the exposed basement membrane probably via heparan sulphate proteogly-

cans (HSPGs) (primary receptor)[1,2,6-10]. This binding results in conformational changes and a distortion of the virus capsid[1,6-8]. Exposed basal keratinocytes (by minor trauma or abrasion) during wound healing, overexpress syndecan - 1 and increase their ability to bind and internalize HPV in vivo[9,11]. This distortion exposes the N - terminus of viral L2 protein to cleavage by furin or by proprotein convertase 5/61[6-8,12,13]. N - terminus of viral L2 protein, is essential for its correct conformation in the assembled capsid[6,14]. Moreover, the cleavage site is absolutely conserved in all HPV types[1,12-14]. Proteolytic cleavage by furin, is necessary for successful infection[6,8,12-14]. Subsequently the viral L2 protein binds to the cell surface and triggers a second conformational change in the virus capsid. This conformational change either exposes the binding site on the viral L1 protein for the secondary receptor or lowers the affinity for the primary receptor[1,6,8,13,14]. Viral L1 protein binds on the cell surface to α6 integrin (secondary receptor). That binding to the secondary receptor, triggers receptor mediated endocytosis of HPV[1,6,7,10,13-15].

Endocytic pathways

Several endocytic pathways have been described for HPV16. However, most HPV types use clathrin dependent endocytic pathway[6,8,13,14,17,18]. That pathway is triggered by HPV binding to cell surface receptors. Clathrin coated vesicles become uncoated after endocytosis and fuse with early endosomes[10,19,20]. Nevertheless, some HPV types use alternative endocytic pathways, including caveolae dependent or clathrin and caveolae independent endocytic pathways[6,8,13,14,17,21,22]. Caveolae dependent endocytic pathway is also triggered by HPV binding to cell surface receptors. After endocytosis, grape like multicaveolar complexes (caveosomes) appear in the cytoplasm[10,23]. Clathrin and caveolae independent endocytic pathway, involves tetraspanin enriched microdomains as a platform for viral internalization[6,13,14,22].

Intracellular trafficking

Clathrin coated vesicles progress to early endosomes. Early endosomes progress to late endosomes or lysosomes. Alternatively, early endosomes can recycle back to the cell surface[24]. Molecules in early endosomes, experience a fast decline in pH from neutral to a pH of approximately[6]. Subsequently, they move to late endosomes and are ultimately degraded in lysosomes, with a pH of approximately[5,10,25]. Nevertheless, HPV can avoid lysosomal degradation. It can escape from the endosomes to the cytosol, with fusion independent mechanisms including membrane disruption and transmembrane pore formation. Moreover, the decline in pH in early endosomes results in conformational changes of HPV capsid which trigger the escape of the HPV genome or the complex of HPV genome and L2 protein from the endosomes[10,18,25].

Viral L2 protein is necessary for egress of viral genomes from endosomes, but not for initial uptake and uncoating. Specifically, C - terminus of viral L2 protein is necessary for this function. This feature is conserved among HPV types[8,26-28]. Moreover, viral L2 protein interacts with the microtubule network via the motor protein dynein. This interaction mediates the transport of the complex of HPV genome and L2 protein along microtubules towards the nucleus[13,26]. Caveolae dependent endocytic pathway performs internalization at a lower speed. Moreover, internalization via caveolae is not a constitutive process and occurs only upon cell stimulation[10,24,29]. Molecules in caveosomes fail to become acidized. Subsequently, they bypass endosomes and move to the Golgi body and / or the endoplasmic reticulum[10,24,30]. For many years it was believed that these endocytic pathways were parallel and separate. Recently, it has become evident, that there are complex interactions and cross talk between them[10,23,31].

Nuclear entry

HPV L2 protein has nuclear localization signals (NLSs), in the n and the c terminus (nNLS, cNLS). These NLSs interact with a network of karyopherins and mediate nuclear entry of the complex of HPV genome and L2 protein via several pathways (karyopherin α2β1 heterodimers, karyopherin β2 and

karyopherin β3). Binding of Ran - GTP to the karyopherins, causes dissociation of the import complexes and release the complex of HPV genome and L2 protein in the nucleus[32-34]. Moreover, cell cycle progression through early stages of mitosis is critical for successful HPV infection. Perhaps nuclear entry of the complex of HPV genome and L2 protein may follow nuclear membrane breakdown during early mitosis, rather than active transport via karyopherins[13,32,35,36]. Finally, the complex of HPV genome and L2 protein enters the nucleus and subsequently the complex localizes at punctate nuclear structures responsible for transcriptional processes including promyelocytic leukemia (PML) nuclear bodies, promyelotic oncogenic domains, and nuclear domain[10]. HPV transcription and replication occur in association with PML nuclear bodies[13,36,40-42]. During latent infection, HPV genome maintain as autonomous replicating episome in the proliferating basal cells of the squamous epithelium[43,44].

Conclusion

HPV has very complex molecular biology. Despite significant advances regarding initial steps and molecular biology of HPV infection, there are many questions to be answered.

Conflict of interest

All authors declare no conflict of interest.

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