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Cruising the cellular highways: How human papillomavirus travels from the surface to the nucleus

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ABSTRACT

The non-enveloped human papillomaviruses (HPVs) specifically target epithelial cells of the skin and mucosa. Successful infection requires a lesion in the stratified tissue for access to the basal cells. Herein, we discuss our recent progress in understanding binding, internalization, uncoating, and intracellular trafficking of HPV particles. Our focus will be on HPV type 16, which is the most common HPV type associated with various anogenital and oropharyngeal carcinomas. The study of HPV entry has revealed a number of novel cellular pathways utilized during infection. These include but are not restricted to the following: a previously uncharacterized form of endocytosis, membrane penetration by a capsid protein, the use of retromer complexes for trafficking to the trans-Golgi network, the requirement for nuclear envelope breakdown and microtubule-mediated transport during mitosis for nuclear entry, the existence of membrane-bound intranuclear vesicles harboring HPV genome, and the requirement of PML protein for efficient transcription of incoming viral genome. The continued study of these pathways may reveal new roles in basic biological cellular processes.

Contents

1. Introduction

In this article, we are reviewing the binding and entry process of human papillomaviruses (HPVs) with a focus on HPV type 16. This is the best-studied type due to its association with the majority of HPV-induced cancers. Even though we will cover every aspect of binding, internalization, and intracellular trafficking, our focus is on the most recent findings regarding the late trafficking events of HPV16. Over the years, HPV entry has been very contentious and many conflicting reports have been published. However, more recently a consensus view has emerged. We are refraining from covering all articles disputing this consensus view and redirect those interested to a number of excellent recent reviews (Day

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and Schelhaas, 2014; Raff et al., 2013; Sapp and Bienkowska-Haba, 2009; Sapp and Day, 2009).

2. HPV capsid

The HPV capsid is comprised of two proteins, the major capsid protein L1 and the minor capsid protein L2. There are 360 molecules of L1 monomers arranged into 72 pentamers, also called capsomeres, forming a $T = 7$ icosahedral lattice (Baker et al., 1991; Finch and Klug, 1965; Liddington et al., 1991). Computer reconstructions of cryo-electron micrographs demonstrated that twelve capsomeres are pentavalent, which means they contact five other capsomeres whereas the remaining sixty capsomeres are hexavalent contacting six other capsomeres (Baker et al., 1991; Trus et al., 1997). Crystal structure determined by X-ray crystallography demonstrated that L1 folds into a 'jelly roll' β sandwich (Chen et al., 2000). The intimate contacts between adjacent L1 monomers makes the pentamer form a tightly packed donut-like shape with a conical hollow opening on the top. Protruding from the exterior surface are a number of poorly conserved exposed loop domains (Chen et al., 2000). The very C-terminus of L1 is α-helical in nature, which makes it both disordered and flexible. This allows for the C-terminus of the L1 protein to act as an "invading arm" that invades neighboring capsomeres (Modis et al., 2002; Wolf et al., 2010). These intercapsomeric interactions are further stabilized by L1 disulfide bonds that covalently link highly conserved cysteine residues resulting in the formation of L1 dimers and trimers (Buck et al., 2005; Fligge et al., 2001; Li et al., 1997; Sapp et al., 1998, 1995; Volpers et al., 1994). The minor capsid protein, L2, is present in an undetermined number of copies, but it is estimated that each capsid can accommodate up to 72 molecules (Buck et al., 2008; Doorbar and Gallimore, 1987; Komly et al., 1986). Conservative estimates for the number of L2 molecules per capsid range between 12 and 36 copies per capsid (Roden et al., 1996; Volpers et al., 1994). However, the exact conformation of the L2 protein in the capsid still remains mostly a mystery. It has been shown that the majority of the L2 protein is hidden within the mature capsid, whereas only a portion of the N-terminus at residues 60–120 is surface exposed (Liu et al., 1997). A C-terminal peptide of L2 (residues 384–460 for BPV-1 or 396–439 for HPV11) has been shown to interact with the C-terminus of L1 via mostly hydrophobic interactions (Finnen et al., 2003; Okun et al., 2001). Upon infectious entry, the L2 protein undergoes conformational changes and emerges (Day et al., 2008; Richards et al., 2006).

3. Binding

In the past, the study of HPV entry was hindered due to the lack of a system to generate efficient quantities of infectious viral particles. The development of pseudoviral vectors, also termed "pseudoviruses" (PsVs), has been used to overcome this roadblock (Buck et al., 2004, 2005; Kawana et al., 1998; Rossi et al., 2000; Touze and Coursaget, 1998; Unckell et al., 1997). PsVs are generally considered indistinguishable from native virions with only few reported differences with regard to entry (Biryukov and Meyers, 2015). PsVs are generated by co-transfecting expression plasmids that encode codon-optimized L1 and L2 genes that allow for high-level expression (Leder et al., 2001; Zhou et al., 1999) together with a reporter plasmid. The pseudoviral capsids are composed of both structural proteins exhibiting the proper disulfide bondages (Buck and Thompson, 2007; Buck et al., 2005) and encapsidate the plasmid vector as a pseudogenome. This system is very flexible as there is no specific packaging sequence required. Packaging of the pseudogenome seems to only be limited to abundance and size exclusion, about 8 kb (see review by Cerqueira and Schiller in this issue). Successful delivery of the pseudogenome to the nucleus offers an easily measurable readout for infectivity using a chosen reporter. The pseudoviral system also offers the ability to utilize reverse genetics while generating efficient quantities of mutant pseudoviruses used for entry assays; a mutational approach is very restricted using native virions. Furthermore, the availability of monoclonal antibodies and DNA-labeling techniques has allowed us to investigate the trafficking of each individual component of the HPV capsid. Therefore, PsVs have been critical for our success in the last decade to tease apart how HPV virions bind to and enter keratinocytes during a primary infection.

During a primary infection in cell culture, it was (Fig. 1) demonstrated that HPV capsids preferentially bind to components of the extracellular matrix (ECM). The ECM is a network of secreted molecules that support the cell in adhesion, cell-to-cell communication, differentiation, and structure (reviewed in (Mouw et al., 2014)). In cell culture models, the ECM mimics the basement membrane, which separates the dermis from the epidermis. The ECM is rich in proteoglycans, particularly heparan sulfate proteoglycans (HSPGs), which are glycoproteins that contain one or more covalently attached heparan sulfate chains (Esko and Lindahl, 2001). The HPV capsid directly engages these molecules on the ECM and cell surface (Giroglou et al., 2001; Johnson et al., 2009; Joyce et al., 1999; Knappe et al., 2007; Selinka et al., 2007). This engagement is largely attributed to the L1 protein involving the sequential engagement of three heparan sulfate-binding sites, but triggers specific conformational changes in both L1 and L2 proteins (Dasgupta et al., 2011; Richards et al., 2013). In addition, several groups have shown that ECM-resident laminin 332 (also known as laminin 5) can also function as an additional attachment receptor for HPV11 and HPV16 but not HPV18 and related types of species 7 and may even contribute to anatomical-site specificity (Culp et al., 2006; Richards et al., 2014; Selinka et al., 2007). After this engagement, cell surface-resident host cell cyclophilin B, a peptidyl-prolyl *cis-trans* isomerase, facilitates the exposure of the very N-terminus of the L2 protein (Bienkowska-Haba et al., 2009). This finding was recently challenged by Campos and coworkers, who claimed that exposure of L2 does not depend on cyclophin B (Bronnimann et al., 2016). However, the authors of this study used a L2 protein harboring a large tag at the N-terminus for their studies, which can be expected to alter the conformation of L2 protein within the capsid. More subtle alterations, such as the introduction of point mutations to the N-terminus already pre-expose the L2 protein (Bienkowska-Haba et al., 2009, 2012). Exposure of the N-terminus, which can be measured by accessibility to monoclonal antibodies, is followed by proteolytic processing by the pro-convertase enzyme furin or closely related proteases, which cleaves off the first 12 amino acids of the L2 protein at a highly conserved cleavage motif site (R-X-K/R-R) (Richards et al., 2006). These events occur on the cell surface and are essential downstream for uncoating in the endocytic compartment and subsequent trafficking. Following these specific conformational changes, which likely reduce the affinity to heparan sulfate, the virion associates with subsequent non-HSPG uptake receptor(s) (Day et al., 2008). Numerous candidates of the non-HSPG receptors have been identified including: integrins, tetraspanins, growth factor receptors, and annexin A2, (Abban et al., 2008; Dziduszko and Ozbun, 2013; Evander et al., 1997; Scheffer et al., 2013; Spoden et al., 2008, 2013; Surviladze et al. 2016; Woodham et al., 2012). Among these, tetraspanins are the best studied. They form tetraspanin-enriched microdomains (TEM) in the plasma membrane, which may serve as an entry platform where many of the other putative uptake receptors have been shown to localize (reviewed in (Scheffer et al., 2014)). The ECM and cell surface events are mainly mediated by L1 protein and do not require L2. However, the L2 protein is absolutely required for infection, as post-internalization events seem to rely heavily on