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Human immunodeficiency virus interaction with oral and genital mucosal epithelia may lead to epithelial–mesenchymal transition and sequestration of virions in the endosomal compartments

Sharof M. Tugizov

Department of Medicine, University of California–San Francisco, San Francisco, CA, USA

Abstract

Oral and genital mucosal epithelia are multistratified epithelial barriers with well-developed tight and adherens junctions. These barriers serve as the first line of defense against many pathogens, including human immunodeficiency virus (HIV). HIV interaction with the surface of mucosal epithelial cells, however, may activate transforming growth factor-beta (TGF-β) and mitogen-activated protein kinase signaling pathways. When activated, these pathways may lead to the disruption of epithelial junctions and epithelial–mesenchymal transition (EMT). HIV-induced impairment of the mucosal barrier may facilitate the spread of pathogenic viral, bacterial, fungal, and other infectious agents. HIV-induced EMT promotes highly motile/migratory cells. In oral and genital mucosa, if EMT occurs within a human papillomavirus (HPV)-infected premalignant or malignant cell environment, the HPV-associated neoplastic process could be accelerated by promoting viral invasion of malignant cells. HIV also internalizes into oral and genital mucosal epithelial cells. The majority (90%) of internalized virions do not cross the epithelium, but are retained in endosomal compartments for several days. These sequestered virions are infectious. Upon interaction with activated peripheral blood mononuclear cells and CD4+ T lymphocytes, epithelial cells containing the virus can be transferred. The induction of HIV-1 release and the cell-to-cell spread of virus from epithelial cells to lymphocytes is mediated by interaction of lymphocyte receptor function-associated antigen-1 with the epithelial cell receptor intercellular adhesion molecule-1. Thus, mucosal epithelial cells may serve as a transient reservoir for HIV, which could play a critical role in viral transmission.

Keywords

endosomal sequestration; epithelial-mesenchymal transition; human immunodeficiency virus

Correspondence: Sharof M. Tugizov, Department of Medicine, University of California–San Francisco, San Francisco, CA 94143-0512, USA. sharof.tugizov@ucsf.edu.

AUTHORS' CONTRIBUTIONS

This paper was written by Sharof Tugizov, who has conceived and designed the experiments.

CONFLICT OF INTEREST

This paper has only one author, Sharof Tugizov, and he has no conflict of interest. He did not receive grants or speaker fees from any commercial body within the past two years.

1 | INTRODUCTION

Human immunodeficiency virus (HIV) interaction with oral and genital mucosal epithelia may occur upon primary HIV contact and during systemic HIV/AIDS disease. Oral and genital epithelial cells may express one or more of the following proteins, which may facilitate HIV binding and entry: C-X-C chemokine receptor type 4 (CXCR4), C-C chemokine receptor type 5 (CCR5), *galactosylceramide* (GalCer), heparan sulfate proteoglycans (HSPG), mannose receptor, and T-cell immunoglobulin and mucin domain 1 (TIM-1) (Bobardt et al., 2007; Dwinell, Eckmann, Leopard, Varki, & Kagnoff, 1999; Herrera et al., 2016; Howell, Asin, Yeaman, & Wira, 2005; Liu et al., 2003; Tugizov et al., 2011, 2012; Yasen, Herrera, Rosbe, Lien, & Tugizov, 2018). HIV interaction with the epithelial surface may cause the disruption of tight and adherens junctions, leading to epithelial–mesenchymal transition (EMT) (Lien, Mayer, Herrera, & Rosbe, 2019). After HIV internalization into epithelial cells, virions can be sequestered in vesicular–endosomal compartments to be released and transferred into activated lymphocytes upon cell-to-cell interactions (Yasen, Herrera, Rosbe, Lien, & Tugizov, 2017; Yasen et al., 2018).

2 | HIV-INDUCED EMT OF ORAL AND GENITAL EPITHELIAL CELLS

2.1 | Cell-free HIV virions, HIV proteins gp120 and Tat induce EMT in oral and genital epithelial cells

HIV-1 interaction with oral and genital mucosal epithelial cells may depolarize epithelia and disrupt their tight and adherens junctions (Sufiawati & Tugizov, 2014, 2018), leading to emergence of cells with the EMT phenotype (Lien et al., 2019) (Figure 1). During embryonic development, EMT is a normal multistep epigenetic process that coordinates and regulates the differentiation of cell lineage identity (Moustakas & Heldin, 2007). The EMT phenotype, however, also plays a role in neoplastic processes, facilitating growth, migration, invasion, and metastasis of tumor cells (Moustakas & Heldin, 2007). During cancer-associated EMT, epithelial cells lose cell–cell junctions and baso-apical polarity and become apoptosis-resistant, proliferative, mobile, and invasive (Talbot, Bhattacharya, & Kuo, 2012).

Epigenetic reprogramming to facilitate EMT involves multiple signaling pathways. The dominant canonical regulatory network for cancer-associated EMT, however, is the transforming growth factor-beta (TGF-β) signaling pathway (Gordon & Blobe, 2008). TGF-β signaling is activated by binding of mature TGF-β to TGF-β-R2 leading to recruitment and activation of downstream molecules, including Smad family transcription factor complexes. These complexes activate the transcriptional regulators Snail, Slug, and Twist1, which lead to downregulation of E-cadherin transcription and upregulation of vimentin, N-cadherin, and fibronectin (Meulmeester & Ten Dijke, 2011; Wendt, Tian, & Schiemann, 2012). Upon inhibition of downregulation of E-cadherin and upregulation of vimentin, the EMT phenotype is reversible; epithelial morphology normalizes, a process known as mesenchymal–epithelial transition (MET) (Takenouchi, Yoshioka, Yamanaka, & Kitani, 2010).

The EMT phenotype in tonsil, cervical, and foreskin epithelial cells is induced upon prolonged interaction with cell-free HIV-1 virions, and viral envelope and transactivator

proteins gp120 and Tat, respectively (Lien et al., 2019). Characteristic of the EMT phenotype, E-cadherin expression is reduced, while the expression of vimentin and N-cadherin is upregulated. HIV gp120- and Tat-induced EMT is mediated by activation of TGF-β and mitogen-activated protein kinase (MAPK) signaling, SMAD2 phosphorylation, and upregulation of transcription factors Slug, Snail, Twist1, and ZEB1, promoting characteristic mesenchymal cells that are highly migratory on collagen-coated membranes.

To induce HIV-1-specific EMT, the source of TGF-β may be the elevated blood levels in HIV-infected individuals (Amarnath, Dong, Li, Wu, & Chen, 2007; Elrefaei et al., 2010). TGF-β expression is upregulated by the AP-1 transcription factor (Birchenall-Roberts et al., 1990), which is induced by MAPK signaling (Glauser & Schlegel, 2007). HIV is a strong activator of MAPK signaling and two viral proteins gp120 and Tat independently activate MAPK. HIV Tat is a transactivator protein that binds to α5β1, α5β3, and αvβ3 integrins (Barillari, Sgadari, Fiorelli, et al., 1999; Barillari, Sgadari, Palladino, et al., 1999; Urbinati et al., 2005) and induces Ras-dependent activation of MAPK (Toschi et al., 2006). Viral envelope gp120 binds to the chemokine receptors CXCR4 and CCR5, also activating MAPK (Del Corno et al., 2001; Freedman, Liu, Del Corno, & Collman, 2003; Lee et al., 2003). HIV gp120 also binds to HSPG (Bobardt et al., 2007; Herrera et al., 2016; Howell et al., 2005; Liu et al., 2003; Tugizov et al., 2011, 2012), which binds TGF-β superfamily proteins, and leads to activation of TGF-β signaling (Rider & Mulloy, 2017).

HIV-1 gp120 and Tat may also activate expression of proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) (Leghmari, Bennasser, Tkaczuk, & Bahraoui, 2008; Planes, Serrero, Leghmari, BenMohamed, & Bahraoui, 2018), which may also activate MAPK and TGF-β to stimulate EMT (Bates & Mercurio, 2003; Lv et al., 2015).

2.2 | Human papillomavirus-associated orogenital cancer is significantly increased in HIV-infected individuals

The incidence of Human papillomavirus (HPV)-associated oropharyngeal cancer is about sixfold greater in HIV-infected individuals than in HIV-negative individuals (Engels et al., 2008; Grulich, van Leeuwen, Falster, & Vajdic, 2007; Powles et al., 2009). In addition to oral cancer, the incidence of HPV-associated anal and cervical cancer is 80 and 22 times greater, respectively, in HIV-infected individuals than in HIV-negative individuals (Denny et al., 2012; Mallari et al., 2012; Palefsky, 2012). These observations lead to the question of how HIV-1 predisposes to an increased incidence of HPV-associated cancers?

2.3 | HIV-induced EMT may promote the progression of HPV-associated neoplasia

Epithelial–mesenchymal transition occurs in most epithelial cancers, including HPV-associated neoplastic processes (Hsu et al., 2007; Lee & Shen, 2012). In HPV 16-infected cervical cancers, reduction of E-cadherin and induction of vimentin expression are associated with the acquisition of the mesenchymal phenotype (Gilles et al., 1996; Lee, Chou, Tang, & Shen, 2008). Reduced E-cadherin expression and increased expression of vimentin and fibronectin appear to be driven by expression of the *HPV oncoproteins E6/E7 in normal foreskin keratinocytes, which acquired a* spindle-like morphology (Hellner, Mar,

Fang, Quackenbush, & Munger, 2009). HPV E6/E7 may synergize with TGF-β -treated HPV 16-immortalized human cervical cancer cells (SiHa) to promote EMT and increased invasion (Yi et al., 2002).

While HIV may induce the EMT phenotype in oral and genital epithelia, HIV-induced EMT alone does not lead to malignancy. As described above, HPV E6/E7 proteins induce the EMT phenotype. Although the incidence of HPV-associated cancer is clearly increased in HIV-infected individuals, little is known about the mechanisms by which HIV potentiates the development of HPV-associated oral and anogenital cancer. EMT may be induced by both HIV and HPV, but through different mechanisms, suggesting that HIV and HPV may induce EMT additively or synergistically, with important consequences for the development of HPV-associated neoplasia in HIV-infected individuals (Figure 1).

There are two scenarios in which HIV and HPV might interact in the oral and anogenital epithelia. (a) If an individual has a prior, persisting HPV-infected epithelial lesion, subsequent acquisition of HIV infection and HIV proteins in the microenvironment might accelerate disease progression because of synergy in the induction of EMT. Conversely, HIV proteins may inhibit MET, which might otherwise have occurred, increasing the risk of disease persistence and progression. (b) If an individual acquires HIV and associated EMT followed by HPV infection, the synergistic induction of EMT by HPV E6 and E7 proteins would increase the risk of carcinoma incidence, persistence, and progression.

3 | HIV INTERNALIZES INTO OR AL AND GENITAL EPITHELIAL CELLS TO FORM INTR A-EPITHELIAL RESERVOIRS

3.1 | HIV endocytosis and macropinocytosis into oral and genital epithelial cells lead to viral sequestration in the vesicles

The interaction of HIV-1 with epithelial surface proteins HSPG, GalCer, and TIM-1, and internalization of virus by clathrin- and caveolin/lipid raft-associated endocytosis and macropinocytosis, leads to viral sequestration in vesicles (Yasen et al., 2017, 2018) (Figure 2). Of initially inoculated virions, about 20% attach to the cell surface and ~4% internalize into cells. About 0.01% of the initial inoculum undergoes transcytosis, indicating that >95% of internalized virions sequester in the cells (Yasen et al., 2018).

Although GalCer and HSPG are well known to contribute to HIV endocytosis, TIM-1 stimulates HIV internalization by macropinocytosis as we reported (Yasen et al., 2018). TIM-1 is a phosphatidylserine (PS) receptor; their binding induces micropinocytosis, which is an actin-dependent process induced by membrane ruffling and the formation of large vacuoles, that is, macropinosomes (Mercer & Helenius, 2009; Tugizov, Herrera, & Palefsky, 2013). The outer leaflet of the HIV-1 envelope contains PS (Aloia, Jensen, Curtain, Mobley, & Gordon, 1988; Aloia, Tian, & Jensen, 1993; Callahan et al., 2003; Gekonge, Schiralli, Schlegel, & Henderson, 2006), and binding of HIV-1-associated PS with TIM-1 of oral and genital epithelial cells facilitates viral micropinocytosis (Yasen et al., 2018).

Thus, GalCer/HSPG-mediated HIV endocytosis and TIM-1-induced micropinocytosis deliver virions into early endosomes, which subsequently mature and form multivesicular

bodies (MVB), where HIV-1 becomes sequestered (Yasen et al., 2017, 2018). Virions may also sequester in vacuoles formed by homophilic fusion of macropinosomes (Araki, Hamasaki, Egami, & Hatae, 2006; Falcone et al., 2006; Hamasaki, Araki, & Hatae, 2004; Hewlett, Prescott, & Watts, 1994; Racoosin & Swanson, 1993; Schnatwinkel et al., 2004).

HIV-1 intra-epithelial sequestration without substantial HIV-1 release is common in tonsil, cervical, and foreskin epithelial cells isolated from different donors (Yasen et al., 2017, 2018), suggesting that this phenomenon may be relevant to the biological functions of both oral and genital mucosal epithelia. Although these epithelia are found at different anatomical sites, they have similar morphological features—squamous epithelial morphology and stratified organization—and serve as portals of entry for HIV-1 (Bouschbacher et al., 2008; Carias et al., 2013; Dinh et al., 2015; Tugizov et al., 2011, 2012; Zhou et al., 2011) (Kohli et al., 2014; Moyes, Islam, Kohli, & Naglik, 2016). Squamous epithelia from different anatomical locations may also have similar mechanisms for HIV-1 sequestration in their endosomal/vesicular compartments (Yasen et al., 2017, 2018).

3.2 | Activated lymphocytes induce release and uptake of intra-epithelial virions

HIV-infected epithelial cells release of trapped virions into co-cultivated activated lymphocytes (Yasen et al., 2017, 2018). During the interaction of lymphocyte receptor function-associated antigen-1 (LFA-1) with the epithelial cell receptor intercellular adhesion molecule-1 (ICAM-1), epithelial cells depolarize and the disruption of cortical actin contributes to viral release. Furthermore, LFA-1/ICAM-1 promotes formation of virological synapses and cell-to-cell spread of HIV-1 from epithelial cells directly to activated peripheral blood mononuclear cells (PBMC) and CD4+ T lymphocytes. Thus, the ICAM-1/LFA-1-induced depolarization of epithelial cells and reorganization of the actin network are critical for (a) viral exocytosis from epithelial cells and (b) cell-to-cell viral spread from epithelial cells to lymphocytes, potentially initiating HIV mucosal transmission.

3.3 | Inflammation of mucosal epithelium may induce the release of sequestrated HIV from endosomes of epithelial cells

We showed that proinflammatory cytokines TNF-α and IFN-γ induce the disruption of oral epithelial tight junctions (Tugizov, 2016), reorganization of cortical actin, and release of intravesicular virus from infant tonsil epithelial cells (Yasen et al., 2017), suggesting that inflammation of mucosal epithelium can induce the release of sequestered virus and its paracellular spread. Induction of HIV release from epithelial cells by activated PBMC (Yasen et al., 2017) suggests that proinflammatory cytokines secreted by activated immune cells may play a critical role in the reorganization of cortical actin and release of intravesicular virus from epithelial cells (see above). Thus, the activation of immune cells due to inflammation of the mucosal environment could be one of the critical factors for HIV mucosal transmission.

4 | SUMMARY

Over the last several years, there have been significant advances in our understanding of the interaction of HIV with oral and genital mucosal epithelia. The two distinct features of HIV

interaction with mucosal epithelial cells are as follows: (a) interaction of HIV with the mucosal surface disrupts epithelial junctions and induces the EMT phenotype and (b) internalization of HIV into epithelial cells establishes virus sequestration in the endosomes. Thus, our future goals should focus on identifying the critical molecular targets for EMT termination and MET induction. These targets could lead to the development of drugs that terminate EMT and induce MET and substantially reduce the development of HPV-associated high-grade lesions and invasive cancer. Future research should also focus on (a) investigating molecular mechanisms of HIV vesicular sequestration and (b) designing new antiviral drugs that would inhibit viral sequestration and/or inactivate endosomal virions.

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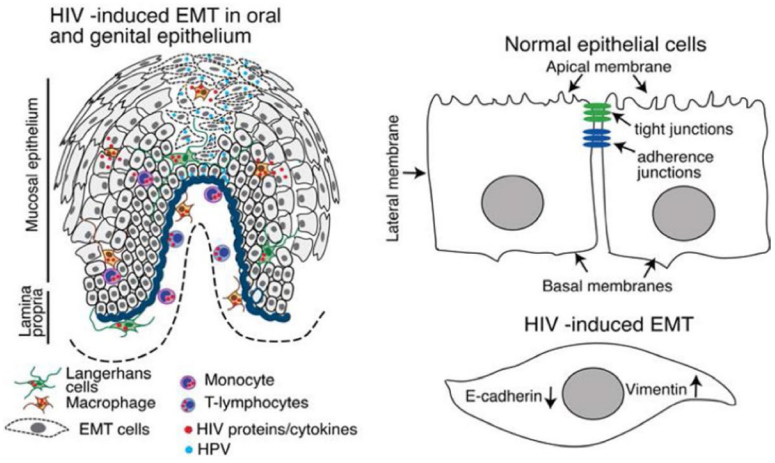
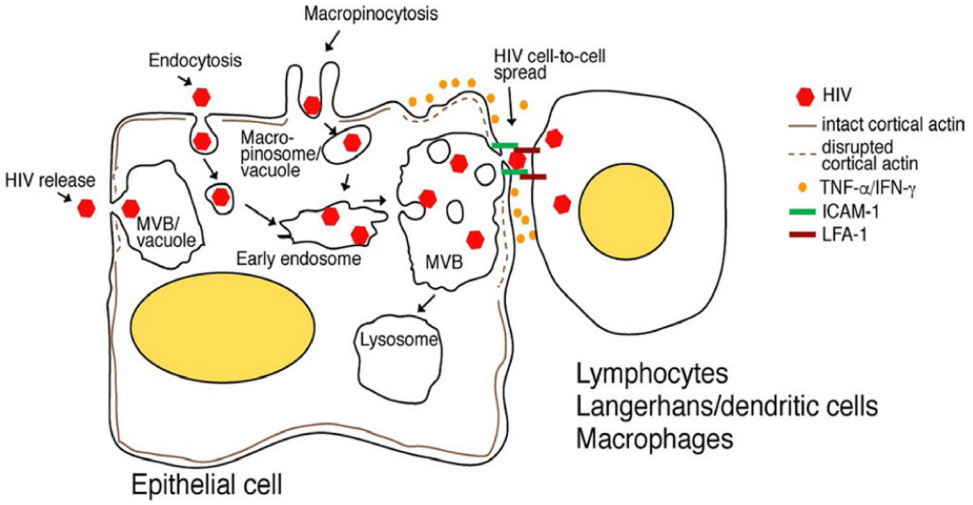


F I G U R E 1.

Model of HIV-mediated EMT in the progression of HPV-associated cancer. In HIV-infected individuals, HIV-infected CD4 + lymphocytes, monocyte/macrophages, and Langerhans/dendritic cells (LC/DCs) migrate into oropharyngeal and genital epithelia, secreting virions, the HIV proteins Tat and gp120, and/or cytokines, including TNF-α and IFN-γ, within the mucosal environment (A). The interaction of cell-free HIV virions and proteins/cytokines with epithelial cells activates MAPK and TGF-β, leading to the disruption of epithelial junctions and induction of the EMT phenotype (A and B). Within the HPV-associated premalignant or malignant cell environment of oral and genital mucosa, HIV-induced EMT cells are highly invasive. If EMT occurs, the neoplastic process could be accelerated by promoting the invasion of malignant cells. Inhibition of EMT or induction of MET may normalize the epithelial morphology and physiological functions. Induction of MET in the HIV/HPV-coinfected mucosal epithelia could significantly reduce the progression of HPV-associated neoplasia



F I G U R E 2.

Model of HIV sequestration in oral and genital epithelia. HIV-1 internalization into oropharyngeal and genital epithelia by endocytosis and macropinocytosis subsequently delivers virus to the early endosomes, which maturate and form MVB-containing virions. Some macropinosomes containing virus may also fuse with one another and form vacuoles containing HIV. The lymphocytes, macrophages, and LC/DCs infiltrating the mucosal epithelia bind to epithelial cells through lymphocyte LFA-1 and epithelial ICAM-1. Infiltrating immune cells under activation/inflammatory conditions secrete TNF-α and IFN-γ, which induce the disruption of cortical actin, leading to the induction of HIV exocytosis. Adhesion of immune cells to epithelial cells may form a virological synapse and facilitate HIV spread from epithelia into CD4+ lymphocytes, macrophages, and LC/DCs. Inactivation of intravesicular HIV may inhibit transmucosal viral transmission