Molecular Mechanisms of HIV Protease Inhibitors Against HPV-Associated Cervical Cancer: Restoration of TP53 Tumour Suppressor Activities

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Cervical cancer is a Human Papilloma virus-related disease, which is on the rise in a number of countries, globally. Two essential oncogenes, E6 and E7, drive cell transformation and cancer development. These two oncoproteins target two of the most important tumour suppressors, p53 and pRB, for degradation through the ubiquitin ligase pathway, thus, blocking apoptosis activation and deregulation of cell cycle. This pathway can be exploited for anticancer therapeutic interventions, and Human Immunodeficiency Virus Protease Inhibitors (HIV-PIs) have attracted a lot of attention for this anticancer drug development. HIV-PIs have proven effective in treating HPV-positive cervical cancers and shown to restore impaired or deregulated p53 in HPV-associated cervical cancers by inhibiting the 26S proteasome. This review will evaluate the role players, such as HPV oncoproteins involved cervical cancer development and how they are targeted in HIV protease inhibitors-induced p53 restoration in cervical cancer. This review also covers the therapeutic potential of HIV protease inhibitors and molecular mechanisms behind the HIV protease inhibitors-induced p53-dependent anticancer activities against cervical cancer.

Keywords: cervical cancer, human papilloma virus, p53, HIV protease inhibitors, E6, anti-HIV drugs, PDZ proteins

INTRODUCTION

Besides the current SARS-CoV-2 virus-related pandemic, cancer remains an alarming threat to human kind, not only in South Africa, but globally. Cancer, a cluster of neoplastic disorders caused by deregulated cell growth, is due to mutations in key regulatory genes, resulting in deregulated cell proliferation, resistance to apoptosis, and amplified cell survival signals (Sinha, 2018). According to Sung et al. (2021), cancer is one of the leading causes of death worldwide, contributing to an estimated 10 million deaths by 2020. In 2018, cancer claimed 9.6 million lives, 70% of which occurred in low and middle income countries (Dalton et al., 2019). The rising burden of cancer in low and middle-income countries is attributed to aging populations, lifestyle changes and adoption of Western habits, which include smoking and alcohol consumption (List and O’Connor, 2020). Furthermore, there are over 200 different types of cancers with different metastatic nature and unique characteristics, making the fight against cancer far more complex. Therefore, cancer remains a major public health concern exerting enormous pressure on healthcare systems. Additionally,
oncoviruses play crucial roles in the carcinogenesis process, leading to the manifestation of different types of cancers, including cervical cancer (Bray et al., 2018).

Cervical cancer is the second most common cancer among women in South Africa, and an estimated 5,743 new cases and over 3,000 deaths are reported annually (Akokuwebe et al., 2021). Globally, cervical cancer is the fourth most diagnosed cancer in women with 342,000 deaths recorded in 2020 (Sung et al., 2021). Cancer of the cervix is characterized by abnormal proliferation of glandular or squamous cells lining the cervix, mostly due to infection with high risk Human papillomavirus (HPV) (Jalil and Karevskiy, 2020). Biological and epidemiological evidence exists, which confirmed HPV, a sexually transmitted virus, as a primary causative agent of the pre-cancerous lesions that lead to full blown invasive cervical cancer (Li et al., 2011; Guan et al., 2012; de Martel et al., 2017; Jalil and Karevskiy, 2020). As demonstrated in Figure 1, pre-cancerous changes can occur in cervix cells infected with high-risk HPV, and most of these changes resolve over time, but some do progress to cervical cancer. HPV is responsible for almost 100% of cervical cancers (de Martel et al., 2017).

The genome of HPV encodes gynaecological cancer-related oncogenes, which include E5, E6 and E7 (Jiang and Yue, 2014). These oncogenes encode proteins that are implicated in carcinogenesis, a process that has been demonstrated in both in vitro and in vivo models (Hoppe-Seyler et al., 2018). The HPV prevents apoptosis in E6 expressing cells by inactivating tumour suppressors, such as p53 (Jiang and Yue, 2014). E5 protein protects cells from apoptosis triggered either by tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) or by Fas ligand (FasL). This is achieved through downregulating the Fas receptor and impairing the assembly of TRAIL-induced death-inducing signalling complex (DISC) (Kabsch and Alonso, 2002). On the other hand, E6 oncoprotein predominantly inhibits apoptosis by regulating p53 turnover (Hengstermann et al., 2001; Murray-Zmijewski et al., 2008; Abboodi et al., 2021), thus inhibiting p53-mediated apoptosis pathways; and lastly, E7 oncoprotein is involved in both apoptosis activation and inhibition by targeting pRb (McLaughlin-Drubin and Münger, 2009; Zimmermann et al., 2011). Clearly, these HPV proteins function by targeting key pathways that are necessary for sustaining cellular homeostasis. The most studied and well-understood HPV oncoproteins, E6 and E7, aim their effect on deregulating cell survival signalling pathways, thus, promoting viral replication; however, these effects also upset cell homeostasis, consequently leading to carcinogenesis.

Most of HPV infections are asymptomatic and partners can unknowingly pass it on to one another through sexual contact (Botha and Dochez, 2012). Majority of HPV-related cancers are prevented by using commercial HPV vaccines; however, these vaccines are ineffective in eradicating persistent HPV infections, and have not been demonstrated to slow down the HPV-related progression of malignant tumours (Cheng et al., 2020). Although cervical cancer can be prevented and managed at its early stages, most women continue to lose their lives due to ineffective pre-cancer screening and treatment programs, especially in developing countries, which include South Africa (SA).

Studies have shown that through protein-protein interactions, HPV affect the functioning of different cellular proteins, which include pRB, p53 and PDZ-containing proteins (Nagasaka et al., 2013; Wang et al., 2017). HPV targets cellular proteins containing the PDZ (PSD-95/Dlg-A/ZO-1) domain, and these proteins are involved in cell signalling, epithelial polarity and membrane trafficking (Nagasaka et al., 2013). Examples of PDZ proteins regulated by HPV in cervical cancer include the NHERF-2 (NHERF-2) (Saidu et al., 2019), a tumour suppressor that regulates endothelial proliferation. To regulate NHERF-2, E6 oncoproteins of high risk HPV bind to it and target it for proteasome-mediated degradation (Saidu et al., 2019). Drugs such HIV protease inhibitors have been shown to disturb HPV interactions with proteins involved in cell cycle and apoptosis such as p53 (Park et al., 2021), however, there is no information on the effect of HIV protease inhibitors on HPV-PDZ protein interactions.

To promote cervical cancer pathogenesis, HPV interrupts cellular regulatory machinery by inducing the expression of viral proteins, which promote cellular transformation by...
modulating the expression of p53 (Cobzeanu et al., 2019). Since p53 expression varies according to the stage of cervical cancer (Pandey et al., 2016), identifying new role players in the regulation of p53 is pivotal. One of these key role players are the HIV protease inhibitors, which have been implicated in enhancing the expression of p53, thus, inhibiting the progression of cervical cancer (Xia et al., 2017). Under normal cell homeostasis, p53 regulates the strict compliance of individual cells to maintain cell homeostasis, thus impaired p53 leads to cancer (Mehta et al., 2021).

Epidemiology of Human Papillomavirus Infection

The Human Papillomaviruses are double-stranded DNA viruses characterised by circular DNA with no envelope (Yilmaz et al., 2018). HPV infects humans and different animal species; zur Hausen, 1977 revealed that HPV is the main cause of cervical cancer. Since HPV is sexually transmissible, it was previously estimated that about half of sexually active adults would contract HPV in their lifetime (Chesson et al., 2014). HPVs are classified into five different major genera, which include alpha (α), beta (β), gamma (γ), mu (µ), and nu (ν) (De Villiers et al., 2004; Brancaccio et al., 2018). Amongst these HPV genera, α, β and γ are implicated in the development of different diseases, including cervical cancer (alpha), skin cancer (beta) and head and neck squamous cell carcinoma (gamma) (De Villiers et al., 2004; Agalliu et al., 2016; Tommasino, 2017; Mirbahari and Sadeghi, 2018; Becerril et al., 2021). HPV is most prevalent among adolescents and young adults between ages 15 and 25 and declines significantly after this age (Baussano et al., 2013). Alpha, beta and gamma HPV genera are most implicated in this age groups (Sias et al., 2019). HPVs can be categorised into high-risk and low-risk, with the former mostly associated with carcinogenesis, and these include HPV 16 and 18, while the latter are barely linked to carcinogenesis, and are exemplified by HPV 6 and 11 (Li et al., 2011; Guan et al., 2012; Cornall et al., 2013). High-risk HPVs account for 5% of all cancers, worldwide, with cervical cancer being the most prevalent (de Martel et al., 2017; de Sanjosé et al., 2018).

HPV Oncoproteins Promote p53 Proteasome Degradation

The TP53 is a tumour suppressor gene, which is localised on chromosome 17p13.1, and is transcribed into 15 transcripts due to the use of alternative promoters (P1 and P2), alternative splicing (Δ40p53β and Δ40p53γ) and alternative initiation sites of translation (Δ40p53, Δ133p53a, Δ133p53β, and Δ133p53γ) (Marcel and Hainaut, 2009; Khoury and Bourdon, 2011; Joruiz and Bourdon, 2016). These transcripts are translated into 12 different protein isoforms, which differ by domain arrangements (Figure 2). The findings from clinical studies suggest that the expression patterns of specific p53 isoforms could predict tumour progression, clinical response, and prognosis (Kim and An, 2016). Since TP53 variants are implicated in cancer, it is possible that HPV modulate these variants to inhibit and degrade p53 in order to abolish its cancer prevention effects (Sousa et al., 2011). Although studies on the interaction between p53 isoforms and HPV are limited, research in this field will contribute significantly to finding specific HPV-associated cancer biomarkers for diagnostic and therapeutic purpose.

TP53 mutations mostly occur in its DNA-binding domain coding region, interfering with p53’s ability to bind to DNA and transactivate downstream genes, thus promoting different cancers, including cervical cancer (Kato et al., 2012).
2003; Banister et al., 2017; He et al., 2019a). The DNA-binding domain is not only affected by mutations, it is also implicated in the interactions between HPV oncoproteins and p53 (Bernard et al., 2011). In response to various stress signals (such as DNA damage, oncogene activation, hypoxia, and nutrient depletion), p53 is stabilized and activated by
post-translational modifications such as the activation of ataxia telangiectasia mutated (ATM) (Parrales and Iwakuma, 2015). p53 then binds to p53 response elements on different genes, either activating DNA damage, cell cycle, or apoptosis (Figure 3). Binding of p53 to these genes triggers their expression, thus, regulating cell cycle, apoptosis, autophagy and DNA repair. The effect of p53 interaction with these genes is summarized in Table 1. By activating genes, such as Bax, CDKN1A and DDB21, p53 is able to orchestrate a variety of mechanisms; namely, apoptosis, cell cycle and DNA-damage-response mechanisms to avoid cancer development (Table 1).

E6 oncoproteins of high-risk HPVs interfere with the transcriptional activity of p53. Studies showed that E6 promote p53 degradation in the presence of E6-associated protein (E6AP) (Figure 4A) (Talis et al., 1998; Li et al., 2019). It is not only the HPV that targets p53 for degradation to subdue its tumour suppressive effects (Trave and Zanier, 2016), but other proteins regulate p53 through the 26S proteasome and ubiquitination pathway. These include Retinoblastoma Binding Protein 6 (RBBP6) (Li et al., 2007), Tripartite motif protein 25 (TRIM25) (Zhang et al., 2015), F-box and WD repeat domain-containing 7a (FXBW7a) (Tripathi et al., 2019), and the list will continue lengthening as more studies emerge towards understanding the role of p53.

Martinez-Zapien et al. (2016) demonstrated the interactions between p53 and HPV-16 oncoprotein, E6 (Figure 4B). The structure showed that the interaction is governed by the LxxLL (L is leucine and X is any amino acid) motif of the E6AP. In the cellular ubiquitin ligase E6AP, E6 binds to a short consensus sequence in the LxxLL motif, and the LxxLL motif is sufficient to render E6 liable to interact with p53. Bernard et al. (2011) also showed that the core DNA binding domain of p53 is required for the interaction with E6/E6AP. Since p53 is mostly mutated in cancerous cells, especially in its DNA-binding domain, understanding the E6-mediated degradation of the p53 mutants is vital. Interestingly, Bernard et al. (2011) found that unlike the wild type p53, mutant p53 was resistant to HPV16 E6-mediated degradation. Li et al. (2019) showed similar effects on the mutant p53 where the HPV E6 failed to promote the degradation of a mutant p53; the study highlighted that it is possible to re-establish the tumour suppressor function of wild type p53 by using a p53 mutant. However, earlier findings indicated that p53 mutations occur rarely in HPV-associated cervical cancers (Denk et al., 2001) and these mutations are more common in HPV-negative tumours than HPV-positive tumours (Banister et al., 2017).

E6 oncoprotein also interacts with other cellular proteins which are linked to p53, stimulating their ubiquitination and their subsequent degradation (White et al., 2012; Mesri et al., 2014). These proteins include tumour suppressor, p300, which is implicated in p53 acetylation and activation (Xie et al., 2014), and Bak protein, which is involved in apoptosis induction (Underbrink et al., 2008). These results show the importance of targeting E6-mediated mechanisms for anticancer therapeutic interventions. A number of compounds, which include arsenic trioxide (Wen et al., 2012) and rutin (Pandey et al., 2021), demonstrated therapeutic potential for the treatment of HPV-associated cervical cancer. This is due to their capacity to downregulate the expression of E6 and E7 proteins, thus restoring p53 expression and function (Wen et al., 2012; Pandey et al., 2021). Identifying new drugs to disturb p53 and HPV-16 E6 interactions can be a promising strategy to eradicate HPV-associated cancer; some of these drugs include anti-HIV drugs because they have demonstrated anticancer properties (Chow et al., 2009; Subeha and Telleria, 2020). Anti-HIV drugs are also implicated in resolving deregulated cell cycle arrest and resistance to apoptosis during carcinogenesis (Maksimovic-Ivanic et al., 2017; Marima et al., 2020). The antitumour effect of anti-HIV drugs have been shown in a wide range of cancer types including skin cancer (Paskas et al., 2019), lymphoma (Kariya et al., 2014), glioblastoma (Gratton et al., 2018), thyroid cancer (Kushchayeva et al., 2014), Kaposi’s sarcoma (Gantt et al., 2011) and cervical cancer (Qiu et al., 2020).

**The Effect of anti-HIV Drugs on HPV Infection**

Cancer is a non-communicable disease but communicable oncogenic viruses, such as Human immunodeficiency virus (HIV) (Hernández-Ramírez et al., 2017; Shiels and Engels, 2017; Stelzle et al., 2021), Epstein-Barr virus (EBV) (Cameron et al., 2018), HPV (De Martel et al., 2017) and Hepatitis B virus (HBV) (Feng et al., 2021) drive the rate of certain cancers, including cervical cancer (Hernández-Ramírez et al., 2017; Shiels and Engels, 2017). The launch of highly active antiretroviral therapy (HAART) has significantly decreased mortality rates associated with HIV, and has improved life expectancy of HIV/AIDS patients (Shiels and Engels, 2017). Despite the use of HAART, HIV patients are still vulnerable to life threatening diseases such as virus-induced cancers (Shmakova et al., 2020).

Anti-HIV drugs have a controversial impact on the natural history of squamous intraepithelial lesions (SIL), the true effect of HAART on HPV is poorly understood because researchers tackle this topic using different study designs, outcomes, and timing of HAART (Blitz et al., 2013; Kelly et al., 2018). In the early days of HAART, Heard et al. (1998) found a non-significant reduction in SIL cases when compared with women who did not receive the treatment. Other reports suggested that HAART has a minimal impact on the incidence of cervical lesions (Heard et al., 2006; Adler et al., 2012). On the other hand, other studies argued by showing that long-term use of HAART is associated with reduced HPV persistence in high-grade cervical intraepithelial neoplasia (Shrestha et al., 2010; Kelly et al., 2017). Additionally, there has been some evidence that non-HPV 16 genotypes have a greater relative prevalence in women with severely compromised immune systems as well as in those without access to HAART (Sahasrabuddhe et al., 2007; McKenzie et al., 2010), further suggesting that HAART duration has a positive impact on reducing the prevalence of other HR-HPVs. Although the effect of HAART on HPV infection is inconclusive, HIV protease...
inhibitors as one of the components that makes up HAART have shown interesting antitumour effects against HPV-associated cervical cancer (Lin et al., 2021; Park et al., 2021).

**Therapeutic Potential of HIV Protease Inhibitors**

Since the discovery of HIV-PIs in the early 1990s, HIV-PIs have been used as components of combination antiretroviral regimens to control HIV viral load in people living with HIV (Roberts et al., 1990; Voshavar, 2019). HIV-PIs are required in the final stage of viral life cycle to block the activity of HIV protease enzyme, which is utilised by HIV to split precursors of gag and gag-pol polyproteins required to produce infectious viruses (Yang et al., 2012; Lv et al., 2015). For more than 20 years, HIV-PIs have been the gold standard as therapeutic agents that inhibit HIV protease. Saquinavir was the first HIV-PI to be developed and used as HIV suppressive drug (Vella, 1994). Currently, there are 10 HIV-PIs approved by the FDA, namely, saquinavir, nelfinavir, indinavir, darunavir, amprenavir, atazanavir, fosamprenavir, lopinavir, tipranavir and ritonavir (Lv et al., 2015). The FDA-approved HIV protease inhibitors share similar binding pattern and structural similarities, suggesting that they bind to the HIV protease in a similar manner (Lv et al., 2015). In addition to their antiretroviral properties, HIV-PIs have pleiotropic pharmacological effects, including anticanter effects (Chow et al., 2009; Toschi et al., 2011). As anticanter drugs, HIV-PIs have generally low potency, requiring concentrations above 10 μM to exert anticanter effect (Bernstein and Dennis, 2008), but in both preclinical and clinical trials, chemotherapeutic drugs are being tested in combination with HIV-PIs (Sombogaard et al., 2018) to determine whether the combination of cancer chemotherapy and HAART can improve response rates compared to antineoplastic therapy alone.

HIV-PIs possess direct antitumour properties independent of their antiviral properties, and this was shown in several independent studies, which demonstrated that HIV-PIs exhibited antitumour and antiangiogenic effects independent of viral load and CD4 cell count (Barillari et al., 2003; Monini et al., 2003; Sgadari et al., 2003; Sgadari et al., 2011; Patton et al., 2013). The direct antitumour effects of HIV-PIs have been shown in cervical cancer (Barillari et al., 2012; Qiu et al., 2020), leukemia (Piccinini et al., 2005; Meier-Stephenson et al., 2017), lung cancer (Yang et al., 2006; Rengan et al., 2019), breast cancer (Srirangam et al., 2006; Soprano et al., 2016), glioblastoma (Pajonk et al., 2002; Rauschenbach et al., 2020), multiple myeloma (Ikezoe et al., 2004; Mendez-Lopez et al., 2019), melanoma (Jiang et al., 2007; Paskas et al., 2019), and ovarian cancer (Kumar et al., 2009; Perna et al., 2017).

HIV-PIs such as indinavir and saquinavir have shown antineoplastic potential in human tumours, such as hepatic, colon, lung and breast adenocarcinomas by blocking matrix metalloproteinases (MMPs) activity, which facilitate angiogenesis by degrading basement membranes, permitting endothelial cell invasion (Spugnini et al., 2006; Kumar et al., 2009; Toschi et al., 2011). In addition, these protease inhibitors impaired cellular proteasome by targeting its chymotrypsin activity which affects the rate of protein break down (Gaedicke et al., 2002; Chow et al., 2009; Toschi et al., 2011). Interestingly, proteasome inhibition by HIV-PIs prevented E6 induced p53 degradation in cervical cancer cells (Hampson et al., 2006). Through these mechanisms, HIV protease inhibitors increased the levels of growth-suppressive proteins in tumour cells, thus arresting tumour cell growth and/or stimulating apoptosis in tumour cells by inducing the expression of cell cycle and apoptosis regulators such as reactive oxygen species (ROS) and p21 (Gaedicke et al., 2002; Hampson et al., 2006; Chow et al., 2009; Toschi et al., 2011, Xiang et al., 2015). The common mechanisms of HIV protease inhibitors for preventing cervical cancer development and progression are summarised in Table 2. The table highlights cancer processes that are modulated by HIV protease inhibitors; these processes include cell invasion, apoptosis and cell cycle. To regulate cancer related processes, HIV protease inhibitors augment the expression of p53 (Park et al., 2021).

**HIV-PIs Restore p53 Expression in HPV-Associated Cervical Cancer**

Since the pathogenicity of HPV-associated cancer involves function of viral E6 oncoprotein, HPV-associated neoplasms may be treated effectively by selectively blocking E6-dependent degradation of tumour suppressors and other cell homeostasis-related role players (Stuqui et al., 2016; Sharma and Munger, 2020; Gusko and Laimins, 2021). Previous studies have shown that there are inhibitors of E6/E6AP-mediated p53 degradation such as Pitx2 (Wei, 2005) and RITA (Zhao et al., 2010). These inhibitors induce the destruction of the E6/E6AP complex to restore the transcriptional function of p53, they do so by binding to E6 protein and blocking it to access E6AP and p53 thus preventing E6 mediated p53 proteosomal degradation (Wei, 2005; Zhao et al., 2010). As potential anticancer drugs, HIV protease inhibitors have also been shown to cause selective inhibition of the 26S proteasome (Piccinini et al., 2002; Lv et al., 2015). A foundation has been laid to demonstrate that some HIV-PIs can inhibit HPV-mediated degradation of p53 and induce apoptosis in HPV-associated cervical cancer cells (Hampson et al., 2006). Park et al. (2021) showed that a subset of HIV-1 protease inhibitors (nelfinavir, saquinavir, lopinavir and ritonavir) reduced the levels of HPV16 E6 and E7 oncoproteins in both CaSki and NIKS16 cells. The reduction of E6 and E7 oncoproteins correlated with increased levels of wild type p53 and HPV-positive cervical cancer cell death. Restored p53 resulted with enhanced apoptosis and accumulation of cells arrested in the G1 phase (Park et al., 2021).

However, different mechanisms on how HIV protease inhibitors restore the expression of p53 in HPV-associated cervical cancer remains unclear because studies in this field are limited. This has risen more questions, especially on the mechanisms used by HIV protease inhibitors to modulate p53 expression in cancer and the role players involved. Therefore, more studies are required both in vitro and in vivo to shed light on the clear mechanisms and role players involved in the restoration
of p53 by HIV protease inhibitors. Previously, Hampson et al. (2006) attempted to answer these questions by examining whether the wild-type p53 that accumulates in cervical cancer cells after treatment with lopinavir is functional by examining the expression of the p53-transactivated gene, p21. p21 is an inhibitor of cyclin-dependent kinases, which are required to permit cell cycle progression. Surprisingly, there was no increase in the levels of p21 protein (Hampson et al., 2006). However, other studies showed that in some cell lines, E6 is able to downregulate p21, independently of p53 by inactivating p150Sal2, a p53-independent positive regulator of p21 transcription (Burkhart et al., 1999; Fan et al., 2021). Additionally, McFarlane et al. (2015) indicated that there are other ways to regulate p53 in HPV-associated cervical cells by targeting serine/arginine-rich splicing factors (SRSFs). The study showed that the depletion of SRSF2 resulted in a marked reduction in E6 and E7 transcript levels leading to induction of p53 expression, activity and stability.

Molecular Mechanisms of p53-dependent Anticancer Activities Induced by HIV Protease Inhibitors

To promote cancer, HPV oncoprotein E6 through E6AP ubiquitinates p53 labelling it for degradation by the 26S proteasome (White et al., 2012; Yuan et al., 2012; Mesri et al., 2014). These effects remove the p53 and Rb-mediated G1 cell cycle checkpoint arrest (Lomazzi et al., 2002), thus promoting carcinogenesis. HIV protease inhibitors restore cell-cycle arrest and apoptosis, which are the most prominent outcomes of p53 activation (Qiu et al., 2020). In addition to their direct tumouricidal effects against cervical cancer cells, HIV-PIs have also been shown to curb the growth of lung cancer (Sirirangam et al., 2011), breast cancer (Shim et al., 2012), colon cancer (Subeha and Telleria, 2020), and hepatic origin adenocarcinomas (Sun et al., 2012) by inhibiting the growth of new blood vessels that support the growth of tumours and their spread to other organs (Toschi et al., 2011).

Xiang et al. (2015) found that nelfinavir did not only promote apoptosis in HPV-positive cervical cancer cells, but also induced G1 cell cycle arrest. According to Xiang et al. (2015), nelfinavir induced apoptosis and cell cycle arrest by stimulating the production of ROS, which trigger cytochrome c release from mitochondria during apoptosis. Based on the discussion above, HIV-PIs have valuable therapeutic effects in haematological and solid cancers due to their inhibitory effects on tumour cell growth, invasion and angiogenesis and their ability to induce apoptosis and cell cycle arrest (Figure 5). Therefore, since the suppression of cancer cell growth can be caused either by the induction of apoptosis or cell cycle arrest mechanisms, the ability of HIV protease inhibitors to fine-tune p53 apoptosis and cell-cycle arrest activities should be explored further for therapeutic purposes.

Other HPV-Mediated Deregulated p53-Related Mechanism During Carcinogenesis

Through its oncoproteins, HPV can evade the immune response of the host in different ways (Bonab et al., 2021). HPV oncoproteins also influence cell survival pathways, which target p53. HPV oncoproteins, E5 and E6, have been shown to regulate the activation and augmentation of epidermal growth factor receptor (EGFR), which is associated with poor prognosis in cervical cancer (Akerman et al., 2001; Iida et al., 2011; Ilahi and Bhatti, 2020). EGFR activates downstream oncogenesis-stimulating pathways such as PI3K and MAPK pathways (Zhou et al., 2015; Wee and Wang, 2017). Cancer cells rely on these pathways for differentiation, mitogenesis, survival and mobility (Wee and Wang, 2017). p53-dependent anticancer mechanisms are counteracted by aberrant activation of PI3K and MAPK signalling pathways, because these signalling pathways are involved in promoting cell survival (Hanel et al., 2013; Martini et al., 2014; Guo et al., 2020). There is evidence

### Table 2: The mechanisms of different HIV protease inhibitors in cervical cancer.

| HIV protease inhibitor | Mode of action | References |
|------------------------|---------------|------------|
| **Apoptosis inducers**  |               |            |
| Lopinavir              | Induces apoptosis by stimulating the production of ROS | Xiang et al. (2015) |
| Indinavir              | Induces apoptosis by stimulating the production of ROS | Xiang et al. (2015) |
| Ritonavir              | Induces apoptosis by stimulating the production of ROS | Xiang et al. (2015) |
| Saquinavir             | Protect p53 from degradation | Xiang et al. (2015) |
| **Cell cycle arrest inducers** | Protect p53 from degradation | Xiang et al. (2015) |
| **Cell invasion inhibitors** | Protect p53 from degradation | Xiang et al. (2015) |
| Lopinavir              | Inhibits E6-mediated proteosomal degradation of p53 | Park et al. (2021) |
| Indinavir              | Inhibits E6-mediated proteosomal degradation of p53 | Hampson et al. (2006) |
| Ritonavir              | Inhibits E6-mediated proteosomal degradation of p53 by reducing E6 and E7 protein levels | Barillari et al. (2012); Park et al. (2021) |
| Saquinavir             | Inhibits E6-mediated proteosomal degradation of p53 by reducing E6 and E7 protein levels | Bandiera et al. (2016); Park et al. (2021) |
| **Other HPV-Mediated Deregulated p53-Related Mechanism During Carcinogenesis** | Protect p53 from degradation | Xiang et al. (2015) |
indicating that cells harbouring high levels of the mutant protein p53 have elevated PI3K/Akt activity (Hanel et al., 2013); therefore targeting these pathways can be a promising strategy to halt the progression of HPV positive cancer cells. PI3K/Akt signalling pathways are activated by HPV infection accompanied by E6/E7 expression, which affects multiple cellular events that contribute to cancer development (Rodon et al., 2013; Manzo-Merino et al., 2014). Rodon et al. (2013) showed that HPV-associated laryngeal papillomas exhibited significant high activated levels of PI3K, which was accompanied by an increase in EGFR and subsequent activation of MAPK/ERK pathways. To promote cancer, HPV oncoproteins do not only affect survival pathways, they also targets other proteins whose defects cause cancer and other diseases (Bonab et al., 2021).

**HPV E6 Targets PDZ Proteins for Degradation**

Contrary to low-risk HPVs, the cancer-causing high-risk HPVs’ E6s possess the PDZ-domain binding motif (PBM) at their C-termini (Banks et al., 2003; Subbaiah et al., 2011; Morgan et al., 2021), which mediate the interaction between HPV E6 and PDZ binding-domain containing proteins, such as NHERF-2 (Saidu et al., 2019), MAGI-1 (Araujo-Arcos et al., 2022), MAGI-2 (Thomas et al., 2002) and MAGI-3 (Ainsworth et al., 2008). The HPV E6 targets these PDZ domain containing proteins for degradation through the 26S proteasome (Massimi et al., 2008). PBM-mediated E6 interactions with PDZ-domain containing proteins play a role in both viral life cycles and the ability of the viruses to cause cell transformations and contribute to cancer development in transgenic mice (Banks et al., 2012; Yoshimatsu et al., 2017; Morgan et al., 2021). Additionally, the PBM also contains a phospho-acceptor site that has been demonstrated to negatively regulate the interaction of E6 with its PDZ domain-containing substrates (Boon and Banks, 2013). This phospho-acceptor site is also important for a feedback loop regulation between p53 and E6 (Vats et al., 2022). This finding suggests the complexity of the relation between p53 and HPV E6, and this is further complicated by E6 interaction with PDZ domain-containing proteins and the phosphorylation of E6.

Phosphorylated E6 shifts from interacting with PDZ domain-containing proteins to interact with proteins in the 14-3-3 family (Boon and Banks, 2013). In light of the knowledge that certain 14-3-3 isoforms are required for optimum p53 activity, this was particularly intriguing because there is evidence that 14-3-3ε and 14-3-3σ are involved in stimulating the binding of p53 to p53 regulated promoters (Lee and Lozano, 2006; Rajagopalan et al., 2010; Falcicchio et al., 2020). A positive outcome of p53 and 14-3-3 protein interactions is also the protection of p53 from MDM2-mediated proteosomal degradation, which leads to an increase in p53 transcriptional activity and cell cycle arrest, thus restoring the tumour-suppressing properties of p53 (Yang et al., 2003; Rajagopalan et al., 2008; Falcicchio et al., 2020). To promote cancer, E6 targets other isoforms of the 14-3-3 such as 14-3-3ζ which regulates multiple biological pathways that are involved in cancer progression (Boon and Banks, 2013). Overexpression of 14-3-3ζ has been detected in several human cancers including cervical cancer (Higareda-Almaraz et al., 2011), suggesting that it may be an oncogene. The complexity of the interaction between HPV E6 and other proteins emerged further when studies showed that HPV also regulates p53-associated lncRNAs (Mattick and Makunin, 2006; Sharma and Munger, 2020).

**HPV Regulate p53-Associated lncRNAs**

To date, only a limited number of lncRNAs have been found to be associated with cervical cancer (Zhu et al., 2017; Wu et al., 2018).
and some have been linked to HPV oncoproteins, E6 (Sharma and Munger, 2018; Barr et al., 2019) or E7 (Sharma et al., 2015; He et al., 2019b). As a consequence of HPV infection, various lncRNAs are altered (Ma et al., 2017) and lncRNAs act as a bridge between HPV infection and downstream signalling pathways involved in cell death and cell survival (Cao et al., 2014; Jiang et al., 2015; de Carvalho Galvão and Coimbra, 2020).

In some reports, HPV have been shown to modulate the expression of lncRNAs in cervical cancer, independent of the known targets of HR-HPV oncoproteins, p53/E6AP, the altered lncRNAs in cervical cancer include Fanconi anemia complementation group-2 (FANCi-2) (Liu et al., 2021), Family With Sequence Similarity 83 Member H antisense RNA 1 (FAM83H-AS1) (Barr et al., 2019) and Thymopoietin pseudogene 2 (TMP0P2) (He et al., 2019b). The expression of these lncRNAs are associated with increased cell proliferation suggesting a crucial role in cervical cancer progression (Barr et al., 2019; He et al., 2019b).

Since HPV elude immune response of the host in different ways, it is possible that it does so by altering the expression of p53-associated lncRNAs. Sharma and Munger (2020) showed that in HPV E6/E7-expressing-human foreskin keratinocytes (HFKs), DNA damage induced noncoding (DINO) lncRNA expression was lower than in control HFKs. The decrease in DINO expression is understandable because DINO is a p53 transcriptional target, amplifying p53-mediated signalling (Schmitt et al., 2016). Therefore, its depletion renders the cells more resistant to cell death caused by metabolic stress or chemotherapy drugs (Sharma and Munger, 2020). HPV E6/E7 also decreased the expression of maternally expressed gene 3 (MEG3) lncRNA (Harden et al., 2017). MEG3 acts as an activator of TP53 (Zhou et al., 2007) and its expression in cervical cancer cells inhibited proliferation, enhanced apoptosis, and reduced tumorigenesis (Zhang and Gao, 2019). Together these results show the potential of lncRNAs as an option that can be considered to interrupt HPV mediated p53 degradation.

**CONCLUDING REMARKS**

HPV infection is involved in the ongoing burden of cancer in low and middle-income countries. HPV encode proteins which promote cancer by degrading the tumour suppressor p53 and new anticanter drugs can be developed to target p53 restoration. Therefore, there is a need to identify new key role players to rescue p53 from HPV degradation. Some of these key role players is HIV protease inhibitors, which showed strong promise for therapeutic purposes. However, studies on the effect of HIV protease inhibitors in the restoration of p53 in HPV-associated cervical cancer are limited with inconsistent results. Therefore, more research is required in this field to bridge this gap. Additionally, lncRNAs, splicing factors, PDZ and 14-3-3 proteins hold potential to serve as alternatives that can be targeted to restore p53 expression in HPV-associated cancers.

**AUTHOR CONTRIBUTIONS**

ZM and SM contributed to conceptualization and LM interpreted relevant literature.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2022.875208/full#supplementary-material
