The Effects of HPV Oncoproteins on Host Communication Networks: Therapeutic Connotations

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Abstract

Human Papillomavirus (HPV) infections are a leading cause of viral-induced malignancies worldwide, with a prominent association with cervical and head and neck cancers. The pivotal role of HPV oncoproteins, E5, E6, and E7, in manipulating cellular events, which contribute to viral pathogenesis in various ways, has been extensively documented. This article reviews the influence of HPV oncoproteins on cellular signaling pathways within the host cell, shedding light on the underlying molecular mechanisms. A comprehensive understanding of these molecular alterations is essential for the development of targeted therapies and strategies to combat HPV-induced premalignancies and prevent their progress to cancer. Furthermore, this review underscores the intricate interplay between HPV oncoproteins and some of the most important cellular signaling pathways: Notch, Wnt/β-catenin, MAPK, JAK/STAT, and PI3K AKT/mTOR. The treatment efficacies of the currently available inhibitors on these pathways in an HPV-positive context are also discussed. This review also highlights the importance of continued research to advance our knowledge and enhance therapeutic interventions for HPV-associated diseases.

Introduction

Human papillomaviruses (HPV) are ubiquitously present, small double-stranded DNA viruses responsible for several malignancies that arise at different anatomical sites. Virtually all individuals are susceptible to HPV infection at some point in their lifetime[1]. HPV infection does not necessarily lead to cancer. Among over 450 different HPV types identified, only a small proportion of them have been demonstrated to play a role in promoting the development of cancer[2,3]. HPV infection can be cleared by the host immune system. However, persistent infection with certain HPV subtypes is considered the major risk factor for the development of HPV-induced cancers [4,5]. Among HPV-induced cancers, cervical cancer (CC) stands out as of particular importance. It ranks the fourth most common cancer among women, affecting more than 600,000 individuals worldwide across all age groups[6]. Even though extensive efforts from governments and non-governmental organizations (NGOs) to promote HPV screening and vaccination programs, CC remains a major global health concern.

In addition to CC, there is a growing global alarm over the increasing incidence of head and neck cancers (HNCs). Most HNCs are categorized as squamous cell carcinomas, a type of cancer that arises in squamous cells lining the oral cavity. HNC ranks the seventh most common cancer globally, responsible for more than 660,000 new cases and 330,000 deaths. Interestingly, the increasing rate of HNC in developed countries is attributed to a rise in oropharyngeal cancer, which is a subtype of HNC[7,8]. Unlike the common cancer risk factors, such as excessive tobacco and alcohol consumption, HPV-positive (HPV+) oropharyngeal cancers are often diagnosed in young adults, non or light smokers, with

low alcohol consumption and multiple sexual partners[9]. Patients with HPV+ oropharyngeal cancers have higher survival rates and better therapeutic responses[9,10]. In the future, it would be important to distinguish between HPV+ and HPV-negative (HPV-) HNCs, during diagnosis to reduce side effects and treatment toxicities.

HPVs are classified into five distinct genera based on the DNA sequence homology in the L1 gene. The five genera are alpha (α), beta (β), gamma (γ), mu (μ), and nu (ν), with the α types being the most extensively studied due to their significant clinical implication[11,12]. This review focuses mainly on the α-HPV types due to their ability to cause significant ailments in humans. The α-HPVs infect human mucosal epithelia and, depending on their potential to cause malignant transformation, are divided into low-risk (LR) and high-risk (HR) types. Infections with LR-HPV types, including 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81, result in self-limiting, benign anogenital warts. In contrast, cancer-causing or the so-called "HR"-HPVs, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are involved in malignant transformation[13]. Nearly all CCs are caused by HPV infections, notably, HPV-16 and -18 are highly involved in approximately 70% of all cases. In addition, HR mucosal α-HPVs were reported to cause approximately 70% of other anogenital cancers and nearly half of HNC, especially HPV-16, which is detected in over 80% of oropharyngeal cancer specimens[14–16].

It is now generally accepted that α-HPV infection takes place when the virus penetrates the squamous epithelium via microinjuries, subsequently infecting the basal cells. The viral life cycle is entirely dependent on keratinocyte differentiation in the stratified epithelium, making it the primary target for the virus. In normal differentiating epithelium, the cells undergo division in the basal layer, in which the basal cells are the only proliferating cells. After the division, these cells then move to the suprabasal layers, where they achieve terminal differentiation and exit the cell cycle[17]. In the scenario of HPV infections, the thickness of suprabasal layers is increased. The cell cycle of this population of cells is perturbed by HPV E6 and E7 oncoproteins, which target the major cell cycle regulators p53 and pRB, respectively[18,19]. E7 interacts with and degrades the retinoblastoma (RB) protein family, including pRB, p130, and p107, leading to the release and activation of the E2F transcription factor, which then signals the transactivation of genes involved in regulating the S-phase of the cell cycle[20]. Additionally, the E7 oncoprotein can stabilize p53 and activate the p53 tumor suppressor pathway resulting in apoptosis[21,22]. To evade this, E6 has evolved to efficiently degrade p53 in a proteasome-dependent manner[18]. All these events lead to shortened G1/S transit, prolonged S-phase that favors viral gene transcription and replication, and delayed G2/M exit[17]. The collaborative effect of E6 and E7 oncoproteins is a well-defined process, however in certain circumstances, mostly during persistent infection, viral DNA gets randomly integrated into the host genome. Most viral genes are lost during this process, while E6 and E7 become continuously and uncontrollably upregulated, driving cellular

immortalization and transformation, and, ultimately, resulting in HPV-induced malignancies[17,23–25]. This viral integration is an exclusive feature of α-HPVs.

To date, there are four HPV prophylactic vaccines available on the pharmaceutical market: Cervarix, Gardasil, Gardasil-9, and Cecolin[26]. Many reports have shown the high effectiveness of these vaccines against the development of HPV-associated cancers. Unfortunately, it is important to note that access to these HPV vaccines remains limited, for various logistic and economic reasons, in low- and middle-income countries, where the burden of HPV-associated cancers, particularly cervical cancer, remains high. Additionally, as the name indicates, these vaccines are merely prophylactic and have no therapeutic potential. Currently, there are no specific therapies for treating HPV-mediated cancers available, except the commonly used clinical approaches and procedures for cancer treatment. Hence, a better understanding of underlying molecular mechanisms on how E6 and E7 contribute to disease progression is a pressing need. Uncovering these mechanistic details and host signaling pathways altered by HPV oncoproteins could be of significant aid in developing antiviral therapies against HPV-induced malignancies, as well as more accurate markers that could predict disease development at early stages at atypical sites of viral infection. Taken together, these factors are likely to result in a positive impact on patient survival rates and quality of life.

Wnt/β-catenin signaling

Wnt signaling pathways are divided into canonical and non-canonical pathways. The canonical Wnt pathway plays a pivotal role in controlling cell proliferation, self-renewal, migration, and differentiation, whereas the non-canonical Wnt pathway is important for regulating cell polarity and migration[27,28]. The major difference between canonical and non-canonical Wnt signaling is that the activation of the non-canonical pathway occurs in a β-catenin-independent manner[28], whilst the canonical Wnt signaling pathway involves β-catenin. The activation of Wnt is regulated by Dickkopf-1 (DDK1) and Axin. DDK1 binds to low-density lipoprotein receptor-related proteins (LRP) 5/6, signaling for the Wnt pathway to be switched off. Another mode of negative Wnt regulation is when Axin forms a complex with glycogen synthase kinase-3β (GSK-3β) and adenomatous polyposis coli (APC) proteins, which then lower the level of β-catenin[29]. Upon activation of the canonical Wnt pathway, or designated as Wnt/β-catenin (WBC), the Wnt ligand binds to the Frizzled (FZD) and low-density lipoprotein (LRP) 5/6 receptors. This, then, activates Disheveled (DVL), allowing the recruitment of the Axin, GSK-3β, CK1, APC, and phosphorylated β-catenin to form a complex[30]. This, in turn, prevents proteomic degradation of β-catenin. The accumulation of phosphorylated β-catenin signals the translocation of β-catenin into the nucleus, leading to transcriptional activation of the Wnt target genes, which are mostly tissue or developmental stage-specific, modulated by T-cell factor/lymphoid enhancer-binding

factor (TCF/LEF)[31] (Figure 1A). The cytoplasmic-nuclear trafficking of β-catenin links to the Hippo pathway. When this pathway is activated, cytoplasmic phosphorylated Yes-associated protein (YAP) of the Hippo pathway binds to β-catenin, impeding the nuclear translocation of β-catenin and its subsequent transcriptional transactivation of Wnt target genes[32]. In contrast, when the Hippo pathway is inactivated, WBC is active. Therefore, the Hippo pathway is a negative regulator of the canonical WBC pathway.

HPV and Wnt/β-catenin

Wnt genes are aberrantly expressed in cancers, including HPV-associated cervical and head and neck cancers[33,34]. FZD, DVL, Wnt4, and Wnt8A genes are upregulated in HPV16+ CC compared to normal epithelia[35,36]. The upregulation of WBC genes can occur even in the absence of active HPV infection[37]. This, perhaps, indicates that the upregulation of the WBC pathway is a sequential and accumulative effect exerted by E6 and E7. These two HPV oncoproteins collaboratively target WBC to enhance cell proliferation and transformation, as shown in *in vitro* and *in vivo* studies. In a double transgenic mouse model, expression of 16E7 and β-catenin constitutively (K14-E7/ΔN87βcat) drives E6AP-mediated[38] degradation of Na(+)/(H+) exchange regulatory factor 1 (NHERF1), thereby withdrawing inhibition on WBC[39] and promoting cervical carcinogenesis[40]. Another mechanism of how E7 instigates the degradation of NHERF1 is that E7 activates cyclin-dependent kinase (CDK) complexes to promote phosphorylation of NHERF1, which then stimulates the degradation of NHERF1 by E6. The degradation of NHERF1 mediated by E6/E7 in an E6AP-dependent manner is a common feature shared by both LR- and HR-HPV types[41].

E7 also strategies to target WBC through PTPN14 and the Hippo signaling pathway. Tumor suppressor PTPN14 associates with YAP and negatively regulates YAP function by keeping YAP in the cytoplasm[42,43]. E7 binds and degrades PTPN14 in a proteasome-dependent manner[44,45]. This abolishes PTPN14 control over YAP, allowing the trafficking of YAP[46] and β-catenin into the nucleus. In synergy with E7, E6 can also target the Wnt-Hippo pathway[47,48]. HR-HPV E6, through its PDZ binding motif (PBM), retains nuclear YAP/β-catenin[47]. This can also occur through the degradation of NHERF1 by E6[41]. E6 encoded by cutaneous HPVs, albeit at a lower strength than HR HPVs, can interact with YAP and Transcriptional Enhanced Associated Domain 1 (TEAD1)[49], and enhance Wnt/β-catenin/TCF transcription[50]. In the nucleus, YAP/β-catenin forms a complex with TCF. The YAP/β-catenin/TCF transcriptional complex then mediates the transcription of genes linked to cell proliferation and apoptosis[51]. Meanwhile, E6 can also bind to and activate β-catenin. The activation of β-catenin stabilizes TCF-4 protein, thereby activating TCF-4 transcriptional activity[52]. The activation of WBC directly or through the Hippo pathway, leads to the enhancement of cell proliferation[40,52] (Figure 1A) and migration, displacement of neighboring normal cells, overriding contact inhibition and differentiation into the suprabasal layer[41,53].

E6 also bridges cross-talk between Wnt and epidermal growth factor-like receptor (EGFR) pathway. Activation of EGFR by E6 incites nuclear localization of β-catenin[54] (Figure 1A). Despite the connection between E6/E7 in augmenting WBC appears to be plausible, other studies show that WBC genes and proteins are upregulated in both HPV+, including those HPV+ CCs that do not express HPV transcripts, and HPV- formalin-fixed paraffin-embedded (FFPE) HNC samples[36]. Indeed, the root of cancer formation and progression is multifactorial, and the augmentation of WBC signaling is unexclusive for HPV-associated cancers. This may depend on the epigenetic modification and mutation of members of the WBC signaling pathway. For instance, epigenetic silencing of DKK1 can result in activation of Wnt, as observed in CC cells[55].

Wnt/β-catenin inhibitors in therapy

To date, numerous Wnt-receptor complex inhibitors are undergoing clinical trials, mainly to test their safety, immunogenicity, and anti-tumor efficacy. These inhibitors target Porcupine, FZD, R-spondin, and TCF-CBP protein complexes. However, none of these trials are specifically for HPV-associated cancers, but a few of them were designed for CC and HNC treatment. Porcupine-specific inhibitor, LGK974 or Wnt974, was in clinical trials for both cancers. Porcupine is a membrane-bound O-acyltransferase (MBOAT) that plays a crucial role in palmitoylation of Wnt during Wnt ligand secretion[56]. The preclinical findings showed that LGK974 is effective in reducing cancer formation of the HNCHPV mouse xenograft model[57]. There were 3 clinical trials on Wnt974 (NCT02278133, NCT02649530, NCT01351103). Wnt974 mono-treatment is well-tolerated, and specifically downregulates the Axin2 gene, revealing its target specificity. Nonetheless, Grade 1/2 adverse effects were inevitable and treatment-related death was also reported[58]. The lipophilic properties of Wnt974 may render its poor absorption and limit its anti-tumor efficacy. Moreover, the cross-talks of Wnt with other tumor-related signaling pathways may suggest that a combination of Wnt974 with other anti-tumor agents could pose a better treatment modality. Another Phase I trial on the use of Wnt974, encorafenib (MAP/ERK kinase inhibitor), and cetuximab (EGFR inhibitor) was conducted. However, the safety and anti-tumor efficacy of the treatment regimen is of concern. Grade 3/4 adverse effects were reported among 80% of the subjects[59].

Besides Wnt974, other Wnt inhibitors used in Phase I or II clinical trials include ^{90}γ-OTSA-101, Ipafricept, Vantictumab, OMP-18R5 and OMP-54F28 (targeting FZD receptor); BC2059 or Tegavivint, CWP232291 and E7386 (targeting β-catenin); PRI-724 (CBP/β-catenin antagonist); Rosmantuzumab (R-spondin-3); BHQ880 and DKN-01 (Anti-DDK1 monoclonal antibody); and LY2090314 and 9-ING-41 (GSK-3 inhibitor)[60,61]. These therapeutics have not been on trial for CC and HNC. Drug repurposing has become a fast-track mode to the discovery of novel and alternative therapeutic options. Intriguingly, studies have been conducted to repurpose aspirin, celecoxib, and diclofenac (NSAIDs); niclosamide

(anti-parasitic); curcumin and resveratrol (natural compounds), to target the Wnt signaling pathway in clinical trials[61]. Despite many studies suggesting that Wnt is a promising therapeutic target, many Wnt inhibitors have not been in trials for HPV-associated cancers. Given the significant function of the Wnt signaling pathway in maintaining self-renewal, differentiation, and proliferation of normal cells and crosstalk with other cellular signaling pathways, the Wnt signaling pathway may not be an "easy" target. The target specificity, bioavailability, anti-tumor efficacy, and treatment-related adverse effects of the currently available Wnt inhibitors remain to be of concern. As far as HPV-associated cancers are concerned, with the unique genotypic and phenotypic characteristics of the cancer cells, designing a treatment modality targeting the presence of genes and proteins exclusive to HPV-containing cells could be a better approach.

MAPK signaling

The Mitogen-activated protein kinase (MAPK) signaling pathway is another major cascade that connects signals from the cell surface receptor to the nucleus to regulate diverse cellular events, including cell proliferation, transformation, differentiation, and survival[62]. MAPK are serine-threonine kinases that are activated by diverse extracellular and intracellular stimuli, including growth factors, cytokines, hormones, and cellular stress. In mammalian cells, fourteen MAPK enzymes exist, the most well-characterized being extracellular signal-regulated kinase 1 and 2(ERK1/2), c-Jun N-terminal kinase/stress-activated protein kinase 1 to 3 (JNK1-3/ SAPK), p38 kinase (α, β, γ, and δ) and ERK5. Each cascade pathway consists of at least three enzymes that are activated in a series of binary interactions: a MAPK kinase kinase (MAPKKK/MAP3K), a MAPK kinase (MAPKK/MAP2K), and a MAPK[62,63]. In addition, the signaling cascade can be guided by scaffolding proteins such as kinase suppressor of Ras (KSR1/2), IQGAP1, β-arrestin 1/2, MORG1, MP1 (MEK partner-1), and paxillin[64–66]. ERKs act as the core kinase of the MAPK signaling pathway and are involved extensively in different cellular processes (Figure 1B). ERK is also a serine-threonine protein kinase and acts as a signal transduction protein to transmit mitogen signals. It generally locates in the cytoplasm and it migrates to the cell nucleus upon activation by proteins like Ras or Raf. Once there, ERK binds to transcription factors to imitate gene expression. Given its vital role, it is not surprising that the dysfunction of the ERK/MAPK pathway is linked to numerous diseases, including autoimmune disorders, neurodegenerative diseases, and particularly cancers[63]. In fact, activation of the mutant ERK cascades is the most common oncogenic factor across all cancer types. For instance, Ras (mainly K-ras) mutations are detected in nearly 30% of all cancer types and Ras mutations appear in 10% of all cancers, making them a significant oncogenic factor[67]. Activation of Ras increases p38γ and PTPN3 expression[68]. PTPN3 can then stabilize the active complex between p38γ and itself to allow efficient dephosphorylation of p38γ[69], relaying the "off" signal to MAPK.

HPV and MAPK signaling

In the context of HPV infection, the virus targets the MEK/ERK signaling pathway to regulate its productive replicative cycle. It does this by activating the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK) that is notably influential in the growth and differentiation of keratinocytes, and the formation of epithelium. Keratinocytes dividing in the basal epithelial layer exhibit the highest levels of EGFR expression and activity to sustain its proliferation. During epithelial differentiation, EGFR activity is downregulated, leading to the blockage of proliferation and induction of early termination of the epidermal keratinocytes[70]. The interaction between HPV E6/E7 oncoproteins and the EGFR or the MAK/ERK is complicated and seems to differ across different cell lines. For example, in HPV-16 positive CC cell line, SiHa, E6/E7 mRNA is induced by EGF treatment, in the presence of AP-1 transcription factor[71]. Controversially, EGF treatment reduces E6/E7 mRNA levels in HPV-16 immortalized human keratinocyte cell line, PHK160b[72]. Furthermore, both E6 and E7 oncoproteins from HPV16 markedly increase EGFR expression in transformed keratinocytes[73,74]. The E5 oncoprotein also enhances EGFR activity and upregulates ERK1/2 levels in both LR and HR-HPV types[75–77] (Figure 1B). In addition, 16E6 oncoprotein expressed in primary human foreskin keratinocytes (HFKs) can prolong EGFR internalization, leading to activation of the MAPK pathway[78]. The partners of the MAPK signaling pathway, including PTPN3, PTPN4, and PTPN13, which are tumor suppressors and PDZ domain-containing proteins, can also be targeted by E6 to promote tumor aggressiveness. E6 associates with and degrades these PDZ domain-containing proteins via E6-PBM in a proteasome-dependent manner [79–81]. Furthermore, hyperactivation of mutated H-Ras leads to the loss of PTPN13 function and aberrant activation of the MAPK signaling, thereby promoting invasive growth *in vivo*[82].

HPV variants may have a greater oncogenic potential. For instance, the HPV16 E6 L83V mutant, which carries a nucleotide substitution of T to G at residue 350 of the E6 oncogene, was found to increase MAPK signaling. This variant was shown to be notably associated with high-grade intraepithelial lesions (HSIL)[83]. Another study suggested that papillomaviruses manipulate the MEK/ERK pathway to control their own replication cycles. Moreover, there is a direct link between neoplastic progression and levels of ERK and HPV E6/E7 oncogenes. During normal cellular differentiation, the activity of the EGFR/MEK/ERK signal decreases. This suppresses E6 and E7 oncogene expression and promotes epithelial differentiation, essential for late viral gene expression. However, when cervical intraepithelial neoplasia (CIN) progresses from low- (LSIL) to HSIL, the EGFR level increases concomitantly. Overexpressed EGFR leads to increased phospho-ERK levels and subsequently increases HR-HPV E6 and E7 mRNA levels[70]. Seemingly, there is a direct linkage between the increase of the MAPK pathway and HPV-mediated tumor progression.

MAPK inhibitors in therapy

The MAPK signaling pathway plays a crucial role in cancer development, making pharmaceutical interventions targeting this pathway attractive treatment options. Over 27 MAPK-based drug compounds have been patented, and preclinical studies have yielded encouraging results[84]. For example, a study evaluated the combination effects of a p38 inhibitor, BIRB796, and an Aurora kinase inhibitor, VX680, on CC. The combination treatment synergistically inhibits CC cell growth *in vitro* and *in vivo*[85]. Another study reported that an ERK5 inhibitor, MHJ-627, had anti-cancer and anti-metastatic effects in CC cells[86]. Trametinib was the first MEK inhibitor approved by the FDA as a monotherapy or in combination with dabrafenib, specifically for BRAF-mutated melanoma and NSCLC[87]. A phase II study (NCT01958112) evaluated the effect of the combination of Tremetinib and GSK2141795, a pan-AKT inhibitor in persistent or recurrent CC. Unfortunately, the results did not reveal a significant clinical benefit from the treatment. The trial was halted due to the discontinuation of the clinical development of GSK2141795[88]. Many other MAPK inhibitors also fail in HNC clinical trials, such as cobimetinib (NCT00467779), selumetinib (NCT00085787), and AZD8330(NCT00454090)[89–92]. Conversely, Tipifarnib, a farnesyltransferase (FT) inhibitor that disrupts HRAS function, demonstrated a promising clinical result. A Phase 2 study (NCT03719690) revealed that tipifarnib treatment resulted in a 55% overall response rate in HRAS-mutated HNC patients. Less than 10% of patients experienced grade 3 hematological-related adverse events[93,94]. To conclude, past MAPK inhibitors showed low efficacy and potency. The promising clinical outcomes of tipifarnib indicate precision drugging to target specific MAPK mutations could be a good anti-cancer approach.

PI3K/AKT/mTOR signaling

The PI3K/Akt/mTOR cascade plays a pivotal role in the regulation of numerous cellular processes linked to energy metabolism, cell survival and growth, proliferation, and migration[95,96]. External stimuli, such as growth factors or cytokines, are required for the stimulation of AKT signaling. Under those conditions, phosphoinositide 3-kinases (PI3Ks), which belong to lipid kinases, are under the control of G protein-coupled receptors (GPCRs), GTPases or by receptor tyrosine kinases (RTKs), and activated by phosphorylation. Firstly, after being activated, PI3K induces phosphorylation of the membrane lipid phosphatidylinositol-4,5-bisphosphate ($PI4,5P_2$), so that phosphatidylinositol-3,4,5-trisphosphate (PIP3) can be produced and additionally converted to phosphatidylinositol-3,4-bisphosphate ($PI3,4P_2$)[97,98] (Figure 1C). As a consequence of this, AKT accumulates on the cell membrane where it undergoes phosphorylation by the mammalian target of rapamycin complex 2 (mTORC2), which results in its active form. Once activated, AKT phosphorylates several substrates which regulate various processes, such as cell proliferation, cell cycle control, angiogenesis, cell survival, and anti-apoptotic signaling[96,99]. One of these substrates is tuberous sclerosis complex 2 (TSC2), which gets inhibited by

AKT, consequently resulting in rapamycin complex 1 (mTORC1) activation. This activity is important for ensuring substantial amounts of oxygen, growth factors, amino acids, and other nutrients important for activating downstream cellular processes[100]. PI3K-induced activation of AKT is negatively regulated by the phosphoinositide phosphatase PTEN (phosphatase and tensin homolog) that dephosphorylates PIP3, or by PP2A and PHLPP1/PHLPP2 that dephosphorylate AKT. Furthermore, AKT can also be regulated by feedback inhibition which is mediated by a subset of its downstream substrates, such as mTORC1[101] (Figure 1C).

HPV and PI3K/AKT/mTOR

The PI3K/AKT/mTOR signaling pathway is very complex and involves various components which form a network, that is subject to a plethora of stimuli. In this way, this pathway affects numerous cellular key processes, which when unfunctional, might lead to malignant development[96]. In fact, the PI3K/AKT/mTOR cascade is often deregulated in various tumors, which drives malignant progression and stimulates resistance to therapy[98,102]. CC is one of the tumors where PKI3CA mutations are most commonly detected[103]. In addition to this, there are other modulations, such as the loss of PTEN or genomic amplifications in PIK3CA, detected in this type of cancer[96,104]. Furthermore, modulations in the PI3K/AKT/mTOR axis have also been shown to be linked with CC patient prognosis. In particular, a worse patient prognosis and shorter survival period after clinical treatments were demonstrated to be associated with PIK3CA mutations, elevated levels of phosphorylated AKT, and induced mTOR[96,105–107]. As previously mentioned, HPVs are also causative agents of several head and neck squamous cell carcinomas (HNSCCs), in particular oropharyngeal squamous cell carcinomas (OPSCCs). As in CC, the PI3K/AKT/mTOR signaling cascade is one of the most commonly modified signaling pathways in HNCs[108]. Getting a better understanding of alterations in this pathway could provide important information about HPV-mediated pathologies and help in designing potential novel therapies.

The importance of this cascade in HPV-mediated pathogenesis was further supported by the fact that HPV oncoproteins were shown to be involved in the PI3K/AKT/mTOR signaling pathway activation, while modulations in this signaling axis were demonstrated to be critical for cellular transformation[109,110]. HPV E5 was shown to induce expression of VEGF via activation of EGFR and phosphorylation of AKT and ERK1/2. This has a crucial role in stimulating angiogenesis during the early stages of cervical tumorigenesis[111,112]. In addition, a study has demonstrated that E5 expression significantly impacts the expression of numerous genes including the increase of PI3K and PKCδ and the decrease of lamin A/C protein. This appears to be important for blocking apoptosis and the establishment of persistent infection in epithelial cells[112,113]. HPV E6 and its spliced product E6*I also interfere with various components of the PI3K/AKT/mTOR signaling pathway. In particular, it was demonstrated for 18 E6 that mediated degradation of a PDZ domain-containing protein hDlg, the

homolog of disc-large tumor suppressor, reflected in increased levels of activated PTEN and Akt. Furthermore, 18 E6 also upregulated p-PI3K, which greatly corresponds with activated MAPKs and cell proliferation[114]. HPV18 E6*I was shown to be able to downregulate the expression levels of hDlg and AKT independently of full-length E6[115]. As already mentioned, HPV16 E6 also targets a PDZ domain-containing protein NHERF1. E6 binds NHERF1 via its C-terminus PDZ-binding motif (PBM) and, together in complex with E6AP, targets NHERF-1 for proteasomal degradation[39,116]. NHERF-1 is associated with the activation of the PI3K/AKT signaling pathway[39]. Interestingly, E6 can also directly activate AKT, or interact with TSC2, which drives its degradation and results in activation of mTORC1[98,117]. HPV E7 was also shown to impact the PI3K/AKT/mTOR pathway. By inactivating pRb and the other pocket proteins p107 and p130, HPV E7 upregulates AKT activity in differentiating keratinocytes[118]. E7 can also directly activate AKT by inducing its phosphorylation at threonine 308 and serine 473, leading to the phosphorylation of BAD, which is a downstream substrate of AKT[109]. Furthermore, to keep AKT active, E7 binds to protein phosphatase 2A (PP2A) subunits, blocking their interaction with p-AKT[109] (Figure 1C).

PI3K/AKT/mTOR inhibitors in therapy

Since the fact that the PI3K/AKT/mTOR signaling pathway is altered in many different cancer types, there are possibilities of examining this pathway for potential therapeutic approaches. Unfortunately, so far there is only a limited number of inhibitors available for targeting this pathway since the majority of them exhibit a limited impact and bear a certain level of toxicity for patients[96]. Therefore, there is a great urge to discover new and more reliable inhibitors, especially for cancers that still lack directed therapies, as is the case with HPV-driven malignancies[14,119]. There are ongoing clinical trials for CC patients with PI3K/AKT/mTOR pathway inhibitors, such as NCT01958112, NCT01217177, NCT01026792, or NCT01226316[120]. Importantly, it has been shown that mutations in PIK3CA were associated with better response to the treatment with PI3K/AKT/mTOR cascade inhibitors. This opens new avenues for developing specific approaches for treating patients with this genetic phenotype, which could overall improve their quality of life and survival rates[121]. Interestingly, mutations in PIK3CA and PTEN are more frequently detected in HPV+ than in HPV- HNCs, which could also be of benefit for applying available inhibitors[96,122]. Currently, there are clinical trials on HNC patients that involve the application of PI3K/AKT/mTOR inhibitors and they most commonly include rapamycin analogs, which target mTORC1, and some of them have shown potentially promising results[96,123]. In addition, a recent study demonstrated that AKT inhibitors inhibit the cell proliferation of HPV+ cells regardless of unaffected E6/E7 expression levels[96]. Finally, PI3K inhibitors have been shown to likely be a promising approach for acting upon the PI3K/AKT/mTOR signaling pathway. In particular, these inhibitors seem to prevent resistance to the immune checkpoint barrier in transgenic mice, and currently, clinical trials

are being undertaken in which PI3K inhibitors are used as combined therapy with immune checkpoint inhibitors[124–126].

Notch signaling

The Notch signaling pathway is an evolutionarily conserved pathway important for the development and homeostasis of many tissues. Its molecular details vary in different cell types and contexts, but the basic mechanism is the same[127]. Signaling is initiated by the direct interaction between Notch receptors (NOTCH1-4) located on one cell and Notch ligands (JAGGED1, JAGGED2, DLL1, DLL3, and DLL4) present on the surface of an adjacent cell (Figure 2A). The ligand-receptor interaction induces two successive proteolytic cleavages of the Notch receptor. The first cleavage occurs by a metalloprotease, such as ADAM10 or ADAM17, and results in the release of the extracellular domain of the Notch receptor from the plasma membrane. The remaining membrane-bound fragment of the Notch receptor called the transmembrane fragment or Notch intracellular domain (NICD), then gets engulfed inside of an endocytic vesicle where it undergoes proteolytic cleavage by the γ-secretase complex. This cleavage releases NICD from the membrane and allows its translocation to the nucleus. In the nucleus, NICD associates with the DNA-binding protein RBPJκ, Mastermind-like protein (MAML1), and other co-activators to form a Notch transcriptional factor complex (Figure 2A). The complex binds to Notch-responsive elements within the promoters of target genes, which leads to the recruitment of additional co-activators, chromatin remodeling complexes, and the initiation of gene transcription[128].

Within keratinocytes, Notch signaling plays a crucial role in spinous differentiation - a process known as keratinocyte stratification or epidermal maturation. Firstly, Notch expression helps in maintaining the undifferentiated state of basal keratinocytes, and ensures the compactness of the proliferative compartment of the skin. Localized expression of the Delta ligand on basal cells can induce a transit-amplifying state in the neighboring cells, ultimately leading to cell differentiation. For this reason, DLL1 is expressed on stem cells bordering the proliferative compartment[129]. Furthermore, activated Notch signaling also induces the expression of early differentiation markers in differentiating keratinocytes[130], as well as stimulates $p21^{WAF1/CIP1}$ and caspase 3 expression, through which it causes growth suppression and abrogates proliferative signals in differentiating cells[131]. Therefore, the activation of canonical Notch signaling in the suprabasal cell layers of the epidermis is considered to be a commitment to the epidermal lineage. Interestingly, skin keratinocytes show a layer-specific expression of Notch receptors and ligands[132]. This suggests there are differences in signaling pathway activation based on the receptors and ligands involved and that these differences have biological consequences. Additionally, there is evidence that asymmetric cell division is at least partially responsible for epithelial cell differentiation and that Notch signaling is involved in this process[133]. Activated Notch signaling leads

to target gene expression, the best described of which are the HES and HEY family of genes. One of the proteins that is most important for keratinocyte cell faith, and also a prominent Notch1 antagonist, is p63. Elevated p63 expression counteracts Notch1 growth suppression, while activated Notch signaling leads to p63 downregulation, suggesting that this balance is important for epidermal maintenance and control[134].

Overall, Notch signaling in the spinous differentiation of basal keratinocytes acts as a crucial regulator, which maintains the undifferentiated state of basal keratinocytes, in this way suppressing premature differentiation, while also committing cells to the epidermal lineage. The proper balance of Notch signaling ensures the progression of basal keratinocytes through the epidermal layers, leading to the formation of a fully stratified and differentiated epidermis.

HPV and Notch

With a somewhat delineated role in skin development, the impact of Notch signaling on the cellular transformation of keratinocytes would be expected to be well-defined, but this is not the case. While it is clear that HPV modulates Notch signaling, the details of this process are still being explored. The interference of HPV oncoproteins with the pathway was initially demonstrated for cutaneous β-HPV types, whose E6 oncoprotein binds the MAML1 coactivator and impedes its interaction with NICD, resulting in the abrogation of Notch signaling[135–137]. The inhibition of Notch signaling delays differentiation and keeps the immature cells proliferating in the basal layer. Interestingly, it was recently demonstrated that a number of α-HPV E6 oncoproteins also bind MAML1 and that this interaction has a positive impact on E6 protein stability and subsequently impacts cell proliferation and migration[119], but it is currently unknown whether this interaction affects Notch signaling. Nevertheless, it has long been known that HPV oncoproteins induce elevated Notch signaling through transcriptional and post-transcriptional mechanisms which has a positive impact on cell proliferation and tumorigenicity[138]. Some studies have indicated that HR-HPV E6 regulates NOTCH1 expression through a mechanism dependent on p53 or on p63[139–141]. HPV16 E6 was found to interact with the protein NFX1-123, together with which it upregulates NOTCH1 expression. NFX-123 was found to be highly expressed in primary cervical cancer cells[142]. Surprisingly, increased NOTCH1 activity could downregulate E6 expression, while overexpression of active NOTCH1 leads to cell growth arrest of HPV18-positive HeLa CC cells[143–145]. Conversely, HR-HPV E7 has been found to induce the expression of p63 – the aforementioned NOTCH1 antagonist – in order to improve DNA damage repair efficiency[146,147] (Figure 2A).

In patient samples, Notch was found to be dysregulated in both HNC and CC. Notch is frequently mutated in HNC with 40% of mutations being gene-inactivating[148–150]. As HPV- HNCs carry a larger

mutational burden, NOTCH1 was also more likely to be inactivated in HPV- HNC[151], but gene-activating mutations were also found in some premalignant lesions in oral cavities[152]. The higher activation of Notch was linked to elevated expression of FGF1 and an increased invasion in oral squamous cell carcinomas (OSCCs)[153]. This indicates the dual nature of Notch in HNC – it can act both as a tumor suppressor and as an oncogene. Nevertheless, early research of Notch abrogation via gamma-secretase inhibition in HNC cell lines shows anti-proliferative, anti-migratory, anti-clonogenic, and pro-apoptotic effects[154,155], so it would be interesting to see how this research could be expanded further.

The role of Notch in CC is even more confounding, as both activated and suppressed signaling was found to be an important factor in CC progression[156–159]. Its pathway components were found to be upregulated with cervical lesions progression to cancer[157,160], and the increase in nuclear NOTCH1 expression was associated with a poorer patient prognosis[158]. As it is usually the case with Notch, the exact opposite has also been found[161–163]. Stable overexpression of Notch intracellular domains in HeLa cells suppressed their growth and induced cell cycle arrest through the upregulation of nuclear receptor NR4A2 and somatostatin, and a decreased NF-κB p50 activation[144,145,164]. Even though there is some doubt about Notch expression in CC, a significant amount of evidence points out the oncogenic role of Notch. Notch abrogation can lead to a partial annulation of the epithelial-mesenchymal transition phenotype, as well as to overcome chemotherapy resistance in paclitaxel-resistant HeLa cell line[165]. Similarly, inhibition or downregulation of Notch, as well as upregulation of negative Notch modulators, in the HPV16-positive CaSki cell line had anti-proliferative and apoptotic effects[166–168].

As is the case with HNC, Notch signaling in CC could also be contextual and depend on the specific combination of receptors and ligands. High expression of DLL4, as opposed to DLL1, was found to mark a proliferative phenotype even before HPV infection and metaplasia. This proliferative phenotype seems to continue through cell transformation and cancer progression, as high DLL4 was associated with pelvic lymph node metastasis, cancer-related death, and an overall poorer prognosis for CC patients[169,170]. Likewise, NOTCH3 expression was significantly higher in cervical squamous cell carcinomas compared with adenocarcinomas, normal tissues, and CIN, and the expression of nuclear NOTCH3 in squamous cell carcinomas was an indicator of an overall shorter survival[171] (Figure 2A).

Determining the significance of a particular pathway based on its expression in cancer tissue samples is always a useful tool and a great starting point, but, in the case of Notch at least, it seems to be highly dependent on the patient cohort and tissue in question. In regards to the impact of Notch on the development and maintenance of HPV-related cancers, there seem to be underlying mechanisms and additional factors that have not been uncovered

Notch inhibitors in therapy

Because Notch signaling is important for the development of several different cancers, great efforts have been expended on utilizing this fact for cancer therapy. There are two main strategies for targeting Notch signaling currently being tested. One of them includes monoclonal antibodies which target various ligands or receptors participating in Notch signaling, and the other is based on small molecule inhibitors that target either γ-secretase, preventing the release of NICD, or the formation of Notch transcription factor complex[172]. The downside of small molecular inhibitors is the non-selective systemic inhibition of Notch signaling which, while having promising anti-proliferative effects on cancers, also has significant side effects, primarily gut toxicity[173,174]. Nevertheless, malignancy-specific inhibitors are being created and tested which will, hopefully, yield better results[175]. In regards to this, the inhibitor of the Notch transcriptional complex CB-103 is currently being evaluated and, so far, has shown promising results and better tolerability for patients[176]. Monoclonal antibodies for a number of ligands and receptors are also being tested but these, unfortunately, also bear similar problems for patients as the γ-secretase inhibitors and also demonstrate very limited or inconsistent results[172,177] .

While the impactful and ubiquitous nature of Notch signaling, as well as its common involvement in malignancies, made it a compelling target for cancer therapy, Notch has again proved to be more complex than initially expected. It seems that more research and a deeper understanding of this pathway are necessary to successfully utilize it for our means.

JAK/STAT signaling

The JAK/STAT pathway is a critical signaling cascade involved in various cellular processes, including cell growth, differentiation, immune response, and inflammation. Janus kinases (JAK1-3 and TYK2) are a family of intracellular enzymes that play a crucial role in the signaling pathways of various cytokines and growth factors. These enzymes are associated with cell surface receptors, typically growth factor or cytokine receptors. Upon ligand binding, the receptors dimerize, leading to the activation of the associated JAK enzymes. Once activated, JAKs phosphorylate themselves and their associated receptors, creating docking sites for STAT proteins (STAT1-4, STAT5a, STAT5b, and STAT6). The STATs bind to the receptors and are themselves phosphorylated by JAKs. Phosphorylated STATs form dimers and translocate to the nucleus, regulating gene expression and mediating cellular responses to cytokines and growth factors[178] (Figure 2B).

In keratinocytes, JAK/STAT signaling is involved in the regulation of epidermal differentiation, immune responses, inflammation, and wound healing. JAK/STAT signaling contributes to the regulation of keratinocyte differentiation. Activation of JAK/STAT signaling promotes the expression of late differentiation markers, such as involucrin, filaggrin, and loricrin, crucial in the terminal differentiation

of keratinocytes and the development of the cornified envelope, with STAT3 being particularly important [179,180]. JAK/STAT signaling also plays a role in the wound-healing process in the skin. Various cytokines and growth factors released upon tissue injury activate JAK/STAT signaling, promoting cell proliferation and migration to facilitate wound closure[181]. Additionally, the release of pro-inflammatory cytokines activates JAK/STAT signaling in immune cells and keratinocytes, promoting the additional release of cytokines and chemokines and the recruitment of immune cells. Thus, JAK/STAT signaling helps regulate the inflammatory response, facilitating the clearance of pathogens and debris from the wound[182].

HPV and JAK/STAT

JAK/STAT activation through interferon signaling triggers an immune response to viral infections. This occurs through IFN-I, IFN-II, and IFN-III type receptors, with the downstream STAT molecules typically being STAT1 and STAT2, although STAT4 can also be involved in IFN-I responses[183]. IFN-I signaling is known to be downregulated through a variety of mechanisms in keratinocytes expressing HR-HPV oncogenes[184]. The E7 oncoprotein interacts with IRF9, an IFN regulatory factor that can cause IFN expression after PRR activation, and inhibits its binding to the STAT dimer[185]. The E6 oncoprotein directly impairs *STAT1* expression, thereby contributing to the downmodulation of anti-viral signaling[186]. In addition to STAT1, the other members of the ISGF3 complex - STAT2 and IRF9 - are also downregulated and, consequentially, so are several interferon-stimulated genes (ISGs), which happens as a consequence of the loss of IFNκ expression and attenuates the innate immune response[187]. IFNκ and other members of the IFN-I group are downregulated by the E6-mediated promoter methylation[188–190]. Finally, HR-HPV E6 inhibits STAT1/2 signaling by binding to TYK2, a non-receptor tyrosine kinase member of the JAK family. This binding inhibits the interaction of TYK2 with IFNAR1 and, through this, the activation of the ISGF3 complex and the expression of ISGs[191] (Figure 2B).

Interestingly, some members of the JAK/STAT signaling pathway were found to be crucial for HR-HPV genome amplification. STAT3 and STAT5 are associated with the development of several malignancies and are considered oncogenes. Importantly, STAT3 is also involved in the proliferation and inhibition of differentiation of keratinocytes[192–194]. E6 mediates STAT3 activation and activated STAT3, in turn, induces the expression of proliferative and anti-apoptotic genes. Active STAT3 was found to be essential for the life cycle of HPV18, as the loss of STAT3 impairs genome maintenance and amplification[195]. STAT5 is also thought to be involved in keratinocyte differentiation and, in accordance with this, is also activated during HR-HPV infection[196]. STAT5 phosphorylation and activation were found to be a consequence of E7 expression and found to amplify the viral genome in HR-HPV-infected cells[197,198] (Figure 2B).

Unfortunately, the roles of JAK/STAT protein components in HPV-related tumor samples are not as clear as they are in *in vitro* studies. STAT1 expression was found to be positively correlated with the viral load and lesion progression in cervical lesions[199]. Conversely, HeLa samples show decreased proliferation and increased apoptosis when STAT1 is expressed[200]. CC samples show increased radiosensitivity as a consequence of STAT1 expression[201]. There is research describing STAT1 expression in HNC but, to our knowledge, no connection has been established with HPV presence in these patient samples. On the other hand, the *in vitro* findings on the abrogation of IFNκ signaling have been confirmed in cervical lesion samples[202,203]. The importance of STAT3 for viral genome maintenance and amplification is demonstrated by the findings that the activation status of STAT3 correlated with viral load and integration in cervical precancer and cancer lesions[204]. Accordingly, STAT3 was confirmed to be activated in CC samples, with the level of activation correlating to the histopathological grade[204]. Surprisingly, HPV- HNC shows higher levels of STAT3 activation than HPV+ HNC[205,206]. This suggests that there might be mechanistic differences in the way that E6 impacts STAT3 based on the tissue in question. STAT5 was also found to be constitutively expressed in CC samples, but there is also research suggesting that STAT5 expression correlates with better survival in cervical cancer patients[192,207]. In this case, as in the case of HNC, STAT5b, not STAT5a, was found to be activated[192,208]. STAT5 in HPV-related cancers is activated by both JAK2 and JAK3 and inhibiting either of these kinases leads to a decrease in cell proliferation[194,209] (Figure 2B). Like with most signaling pathways, the expression and activation of JAK/STAT is more complex than could be extrapolated from *in vitro* findings.

JAK/STAT inhibitors in therapy

With over 50 upstream cytokines, roughly the same number of receptors, and 19 genes coding for intracellular JAK/STAT components, there is ample opportunity to find inhibitors of individual molecules or interacting partners involved in the progression of malignancies[210]. One approach is to block cytokine signaling through engineered or fusion cytokines. This was, for the most part, found to be insufficiently selective and toxic, but there are still ongoing and promising trials, such as the preclinical trial with recombinant Il-2[211]. Similar to this, various cytokine antibodies, such as those for IL-2, IL-12, IL-17, and TNF, are currently being tested and clinical trials are underway[178]. Several small molecular inhibitors have also been evaluated in pre-clinical and clinical trials. STAT3 inhibitors seemed like a logical course of action, but they have shown high toxicity, which stalled their development and testing[212]. Some JAK inhibitors, like ruxolitinib, are currently being tested for the treatment of a number of diseases, including malignancies[178,213]. Promising new drugs called proteolysis targeting chimeras, which induce targeted protein degradation, are currently being developed for several oncogenes, including STAT3[214]. Another approach to JAK/STAT inhibition is small decoy oligonucleotides which would sponge the activated STAT or directly bind to STAT mRNA[215,216]. It is worth noting that JAK/STAT

inhibitors have shown therapeutic benefits in various diseases, and they have tremendous potential for several others. While JAK/STAT inhibitors have shown potential in cancer treatment, their use in specific cancer types and settings is still being investigated, and clinical trials are ongoing to assess their safety and efficacy.

Conclusions

The influence of HPV oncoproteins on signaling pathways within the host cell is multifaceted and intricate, underpinning the pathogenesis of HPV-associated diseases. The critical roles played by the three HPV oncoproteins are central to cellular reprogramming. Each of the signaling pathways discussed in this review serves crucial cellular functions during tissue development and homeostasis. All of these pathways regulate differentiation and proliferation. Additionally, JAK/STAT is crucial for cell growth and immune response and, together with Notch and Wnt/β-catenin, for keratinocyte differentiation. For these reasons, they are particularly important for viral replication and are, consequentially, often found to be dysregulated in HPV-induced tumors. The knowledge gained from analyzing the signaling pathway modulation of HPV-infected cells illuminates the comprehensive strategies employed by HPV oncoproteins and opens new therapeutic directions for HPV-associated cancers. The implications of these molecular interactions are profound, in which the association between the virus-host results in the dysregulation of cellular signaling pathways, and in this way, provides a foundation for developing targeted therapeutic strategies to mitigate the deleterious effects of HPV infection. They underscore the importance of ongoing research efforts in elucidating the mechanisms underlying viral oncogenesis. To date, the treatment of HPV-induced malignancies remains a formidable challenge, and the emergence of specific inhibitors targeting signaling pathways offers a glimmer of hope. Most kinase inhibitors are small molecules that often display off-target properties, while the biological therapeutic agents might have low cell permeability. The use of antibody/peptide-conjugated drugs/nanoparticles as avant-garde therapeutics, which combine the benefits of small and biologic molecule inhibitors, in demonstrating high cell permeability and target specificity, could be an avenue in the invention of innovative and target-specific directed therapeutics. In addition, repurposing FDA-approved drugs, and incorporating machine learning and computer-aided tools could be cost-effective to fast-track the drug discovery path. These innovative strategies hold the potential to mitigate the oncogenic influence of HPV oncoproteins, offering new directions for precision medicine in the management of HPV-associated cancers. Continued research, clinical trials, and interdisciplinary collaborations are essential to further advance the field and improve the prognosis and quality of life for individuals affected by these challenging malignancies.

Author contributions

Conceptualization, J.S., H.Y.L., S.S.B. and V.T.; writing—original draft preparation, J.S. and H.Y.L., writing—review and editing, D.B., S.S.B. and V.T. All authors read and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no conflicts of interest.

Data availability statement

All relevant data are within the manuscript.

References

1. Wong MCS, Vlantis AC, Liang M, et al. Prevalence and epidemiologic profile of oral infection with alpha, beta, and gamma papillomaviruses in an asian Chinese population. *Journal of Infectious Diseases*. 2018;218(3):388-397. doi:10.1093/infdis/jiy160

2. Della Fera AN, Warburton A, Coursey TL, Khurana S, McBride AA. Persistent human papillomavirus infection. *Viruses*. 2021;13(2). doi:10.3390/v13020321

3. Skelin J, Sabol I, Tomaić V. Do or Die: HPV E5, E6 and E7 in Cell Death Evasion. *Pathogens*. 2022;11(9). doi:10.3390/pathogens11091027

4. Burk RD, Harari A, Chen Z. Human papillomavirus genome variants. *Virology*. 2013;445(1-2):232-243. doi:10.1016/j.virol.2013.07.018

5. Pešut E, Đukić A, Lulić L, et al. Human papillomaviruses-associated cancers: An update of current knowledge. *Viruses*. 2021;13(11). doi:10.3390/v13112234

6. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660

7. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nat Rev Dis Primers*. 2020;6(1):92. doi:10.1038/s41572-020-00224-3

8. Argiris A, Karamouzis M V., Raben D, Ferris RL. Head and neck cancer. *The Lancet*. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X

9. Perri F, Longo F, Della Vittoria Scarpati G, et al. Metastatic HPV-related oropharyngeal carcinoma cured with chemoradiotherapy: importance of pretherapy biomolecular assessment. *Clin Case Rep*. 2018;6(1):56-62. doi:10.1002/ccr3.1107

10. Tornesello ML, Perri F, Buonaguro L, Ionna F, Buonaguro FM, Caponigro F. HPV-related oropharyngeal cancers: From pathogenesis to new therapeutic approaches. *Cancer Lett*. 2014;351(2):198-205. doi:10.1016/j.canlet.2014.06.003

11. De Villiers EM, Fauquet C, Broker TR, Bernard HU, Zur Hausen H. Classification of papillomaviruses. *Virology*. 2004;324(1):17-27. doi:10.1016/j.virol.2004.03.033

12. Meiring TL, Mbulawa ZZA, Lesosky M, Coetzee D, Williamson AL. High diversity of alpha, beta and gamma human papillomaviruses in genital samples from HIV-negative and HIV-positive heterosexual South African men. *Papillomavirus Research*. 2017;3:160-167. doi:10.1016/j.pvr.2017.05.001

13. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. *New England Journal of Medicine*. 2003;348(6):518-527. doi:10.1056/nejmoa021641

14. Tomaić V. Functional roles of E6 and E7 oncoproteins in HPV-induced malignancies at diverse anatomical sites. *Cancers (Basel)*. 2016;8(10). doi:10.3390/cancers8100095

15. D'Souza G, Kreimer AR, Viscidi R, et al. Case–Control Study of Human Papillomavirus and Oropharyngeal Cancer. *New England Journal of Medicine*. 2007;356(19):1944-1956. doi:10.1056/nejmoa065497

16. Oliva C, Carrillo-Beltrán D, Boettiger P, Gallegos I, Aguayo F. Human Papillomavirus Detected in Oropharyngeal Cancers from Chilean Subjects. *Viruses*. 2022;14(6). doi:10.3390/v14061212

17. Mittal S, Banks L. Molecular mechanisms underlying human papillomavirus E6 and E7 oncoprotein-induced cell transformation. *Mutat Res Rev Mutat Res*. 2017;772:23-35. doi:10.1016/j.mrrev.2016.08.001

18. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell*. 1993;75(3):495-505. doi:10.1016/0092-8674(93)90384-3

19. Gammoh N, Isaacson E, Tomaić V, Jackson DJ, Doorbar J, Banks L. Inhibition of HPV-16 E7 oncogenic activity by HPV-16 E2. *Oncogene*. 2009;28(23):2299-2304. doi:10.1038/onc.2009.78

20. Huh K, Zhou X, Hayakawa H, et al. Human Papillomavirus Type 16 E7 Oncoprotein Associates with the Cullin 2 Ubiquitin Ligase Complex, Which Contributes to Degradation of the Retinoblastoma Tumor Suppressor. *J Virol*. 2007;81(18):9737-9747. doi:10.1128/jvi.00881-07

21. Bodily J, Laimins LA. Persistence of human papillomavirus infection: Keys to malignant progression. *Trends Microbiol*. 2011;19(1):33-39. doi:10.1016/j.tim.2010.10.002

22. Levine AJ. P53, the Cellular Gatekeeper for Growth and Division. *Cell*. 1997;88(3):323-331. doi:10.1016/S0092-8674(00)81871-1

23. Doorbar J. Model systems of human papillomavirus-associated disease. *Journal of Pathology*. 2016;238(2):166-179. doi:10.1002/path.4656

24. Kranjec C, Doorbar J. Human papillomavirus infection and induction of neoplasia: a matter of fitness. *Curr Opin Virol*. 2016;20:129-136. doi:10.1016/j.coviro.2016.08.011

25. Paolini Paoletti M. Five Sources of Contingency for Dispositionalism. *Biol Chem*. 2020;401(5):585-599. doi:10.1515/hsz-2019-0408

26. Akhatova A, Azizan A, Atageldiyeva K, et al. Prophylactic Human Papillomavirus Vaccination: From the Origin to the Current State. *Vaccines (Basel)*. 2022;10(11). doi:10.3390/vaccines10111912

27. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. *Cell*. 2012;149(6):1192-1205. doi:10.1016/j.cell.2012.05.012

28. Niehrs C. The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol*. 2012;13(12):767-779. doi:10.1038/nrm3470

29. Nakamura T, Hamada F, Ishidate T, et al. Axin, an inhibitor of the Wnt signalling pathway, interacts with β- catenin, GSK-3β and APC and reduces the β-catenin level. *Genes to Cells*. 1998;3(6):395-403. doi:10.1046/j.1365-2443.1998.00198.x

30. Krishnamurthy N, Kurzrock R. Targeting the Wnt/beta-catenin pathway in cancer: Update on effectors and inhibitors. *Cancer Treat Rev*. 2018;62:50-60. doi:10.1016/j.ctrv.2017.11.002

31. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. *Cell*. 2012;149(6):1192-1205. doi:10.1016/j.cell.2012.05.012

32. Imajo M, Miyatake K, Iimura A, Miyamoto A, Nishida E. A molecular mechanism that links Hippo signalling to the inhibition of Wnt/β-catenin signalling. *EMBO Journal*. 2012;31(5):1109-1122. doi:10.1038/emboj.2011.487

33. Rampias T, Boutati E, Pectasides E, et al. Activation of Wnt signaling pathway by human papillomavirus E6 and E7 oncogenes in HPV16-positive oropharyngeal squamous carcinoma cells. *Molecular Cancer Research*. 2010;8(3):433-443. doi:10.1158/1541-7786.MCR-09-0345

34. Üren A, Fallen S, Yuan H, et al. Activation of the canonical Wnt pathway during genital keratinocyte transformation: A model for cervical cancer progression. *Cancer Res*. 2005;65(14):6199-6206. doi:10.1158/0008-5472.CAN-05-0455

35. Pérez-Plasencia C, Vázquez-Ortiz G, López-Romero R, Piña-Sanchez P, Moreno J, Salcedo M. Genome wide expression analysis in HPV16 Cervical Cancer: Identification of altered metabolic pathways. *Infect Agent Cancer*. 2007;2(1):16. doi:10.1186/1750-9378-2-16

36. Chakraborty B, Mukhopadhyay D, Roychowdhury A, et al. Differential Wnt-β- catenin pathway activation in HPV positive and negative oral epithelium is transmitted during head and neck tumorigenesis: clinical implications. *Med Microbiol Immunol*. 2021;210(1):49-63. doi:10.1007/s00430-020-00697-9

37. Banister CE, Liu C, Pirisi L, Creek KE, Buckhaults PJ. Identification and characterization of HPV-independent cervical cancers. *Oncotarget*. 2017;8(8):13375-13386. doi:10.18632/oncotarget.14533

38. Lichtig H, Gilboa DA, Jackman A, et al. HPV16 E6 augments Wnt signaling in an E6AP-dependent manner. *Virology*. 2010;396(1):47-58. doi:10.1016/j.virol.2009.10.011

39. Accardi R, Rubino R, Scalise M, et al. E6 and E7 from Human Papillomavirus Type 16 Cooperate To Target the PDZ Protein Na/H Exchange Regulatory Factor 1. *J Virol*. 2011;85(16):8208-8216. doi:10.1128/jvi.00114-11

40. Bonilla-Delgado J, Bulut G, Liu X, et al. The E6 oncoprotein from HPV16 enhances the canonical Wnt/β-catenin pathway in skin epidermis in vivo. *Molecular Cancer Research*. 2012;10(2):250-258. doi:10.1158/1541-7786.MCR-11-0287

41. Yin W, Egawa N, Zheng K, et al. HPV E6 inhibits E6AP to regulate epithelial homeostasis by modulating keratinocyte differentiation commitment and YAP1 activation. *PLoS Pathog*. 2023;19(6):e1011464. doi:10.1371/journal.ppat.1011464

42. Michaloglou C, Lehmann W, Martin T, et al. The tyrosine phosphatase PTPN14 is a negative regulator of YAP activity. *PLoS One*. 2013;8(4):e61916. doi:10.1371/journal.pone.0061916

43. Wilson KE, Li YW, Yang N, Shen H, Orillion AR, Zhang J. PTPN14 forms a complex with Kibra and LATS1 proteins and negatively regulates the YAP oncogenic function. *J Biol Chem*. 2014;289(34):23693-23700. doi:10.1074/jbc.M113.534701

44. White EA, Münger K, Howley PM. High-Risk Human Papillomavirus E7 Proteins Target PTPN14 for Degradation. *mBio*. 2016;7(5). doi:10.1128/mBio.01530-16

45. Szalmás A, Tomaić V, Basukala O, et al. The PTPN14 Tumor Suppressor Is a Degradation Target of Human Papillomavirus E7. *J Virol*. 2017;91(7). doi:10.1128/jvi.00057-17

46. Yun HY, Kim MW, Lee HS, et al. Structural basis for recognition of the tumor suppressor protein PTPN14 by the oncoprotein E7 of human papillomavirus. *PLoS Biol*. 2019;17(7):e3000367. doi:10.1371/journal.pbio.3000367

47. Webb Strickland S, Brimer N, Lyons C, Vande Pol SB. Human Papillomavirus E6 interaction with cellular PDZ domain proteins modulates YAP nuclear localization. *Virology*. 2018;516:127-138. doi:10.1016/j.virol.2018.01.003

48. Dacus D, Cotton C, McCallister TX, Wallace NA. Beta Human Papillomavirus 8E6 Attenuates LATS Phosphorylation after Failed Cytokinesis. *J Virol*. 2020;94(12). doi:10.1128/jvi.02184-19

49. Wu Sharon C, Grace M, Munger K. The HPV8 E6 protein targets the Hippo and Wnt signaling pathways as part of its arsenal to restrain keratinocyte differentiation. *mBio*. 2023;14(5):e01556-23. doi:10.1128/mbio.01556-23

50. Sominsky S, Shterzer N, Jackman A, Shapiro B, Yaniv A, Sherman L. E6 proteins of α and β cutaneous HPV types differ in their ability to potentiate Wnt signaling. *Virology*. 2017;509:11-22. doi:10.1016/j.virol.2017.05.020

51. Deng F, Peng L, Li Z, et al. YAP triggers the Wnt/β-catenin signalling pathway and promotes enterocyte self-renewal, regeneration and tumorigenesis after DSS-induced injury. *Cell Death Dis*. 2018;9(2):153. doi:10.1038/s41419-017-0244-8

52. Muñoz-Bello JO, Olmedo-Nieva L, Castro-Muñoz LJ, et al. HPV-18 E6 oncoprotein and its spliced isoform E6*I regulate the Wnt/β-catenin cell signaling pathway through the TCF-4 transcriptional factor. *Int J Mol Sci*. 2018;19(10). doi:10.3390/ijms19103153

53. Brimer N, Vande Pol S. Human papillomavirus type 16 E6 induces cell competition. *PLoS Pathog*. 2022;18(3):e1010431. doi:10.1371/journal.ppat.1010431

54. Hu Z, Müller S, Qian G, et al. Human papillomavirus 16 oncoprotein regulates the translocation of β-catenin via the activation of epidermal growth factor receptor. *Cancer*. 2015;121(2):214-225. doi:10.1002/cncr.29039

55. Lee J, Yoon YS, Chung JH. Epigenetic silencing of the WNT antagonist DICKKOPF-1 in cervical cancer cell lines. *Gynecol Oncol*. 2008;109(2):270-274. doi:10.1016/j.ygyno.2008.01.034

56. Wang X, Moon J, Dodge ME, et al. The development of highly potent inhibitors for porcupine. *J Med Chem*. 2013;56(6):2700-2704. doi:10.1021/jm400159c

57. Liu J, Pan S, Hsieh MH, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proc Natl Acad Sci U S A*. 2013;110(50):20224-20229. doi:10.1073/pnas.1314239110

58. Rodon J, Argilés G, Connolly RM, et al. Phase 1 study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours. *Br J Cancer*. 2021;125(1):28-37. doi:10.1038/s41416-021-01389-8

59. Tabernero J, Van Cutsem E, Garralda E, et al. A Phase Ib/II Study of WNT974 + Encorafenib + Cetuximab in Patients With BRAF V600E-Mutant KRAS Wild-Type Metastatic Colorectal Cancer. *Oncologist*. 2023;28(3):230-238. doi:10.1093/oncolo/oyad007

60. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. *J Hematol Oncol*. 2020;13(1):165. doi:10.1186/s13045-020-00990-3

61. Neiheisel A, Kaur M, Ma N, Havard P, Shenoy AK. Wnt pathway modulators in cancer therapeutics: An update on completed and ongoing clinical trials. *Int J Cancer*. 2022;150(5):727-740. doi:10.1002/ijc.33811

62. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta Mol Basis Dis*. 2010;1802(4):396-405. doi:10.1016/j.bbadis.2009.12.009

63. Lavoie H, Gagnon J, Therrien M. ERK signalling: a master regulator of cell behaviour, life and fate. *Nat Rev Mol Cell Biol*. 2020;21(10):607-632. doi:10.1038/s41580-020-0255-7

64. Brown MD, Sacks DB. Protein scaffolds in MAP kinase signalling. *Cell Signal*. 2009;21(4):462-469. doi:10.1016/j.cellsig.2008.11.013

65. Witzel F, Maddison L, Blüthgen N. How scaffolds shape MAPK signaling: What we know and opportunities for systems approaches. *Front Physiol*. 2012;3 DEC:475. doi:10.3389/fphys.2012.00475

66. Cargnello M, Roux PP. Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. *Microbiology and Molecular Biology Reviews*. 2011;75(1):50-83. doi:10.1128/mmbr.00031-10

67. Guo Y, Pan W, Liu S, Shen Z, Xu Y, Hu L. ERK/MAPK signalling pathway and tumorigenesis (Review). *Exp Ther Med*. 2020;19(3):1997-2007. doi:10.3892/etm.2020.8454

68. Hou SW, Zhi HY, Pohl N, et al. PTPH1 dephosphorylates and cooperates with p38gamma MAPK to increase ras oncogenesis through PDZ-mediated interaction. *Cancer Res*. 2010;70(7):2901-2910. doi:10.1158/0008-5472.Can-09-3229

69. Chen KE, Lin SY, Wu MJ, et al. Reciprocal allosteric regulation of p38γ and PTPN3 involves a PDZ domain-modulated complex formation. *Sci Signal*. 2014;7(347):ra98. doi:10.1126/scisignal.2005722

70. Luna AJ, Sterk RT, Griego-Fisher AM, et al. MEK/ERK signaling is a critical regulator of high-risk human papillomavirus oncogene expression revealing therapeutic targets for HPV-induced tumors. *PLoS Pathog*. 2021;17(1):e1009216. doi:10.1371/journal.ppat.1009216

71. Peto M, Tolle-Ersu I, Kreysch HG, Klock G. Epidermal growth factor induction of human papillomavirus type 16 E6/E7 mRNA in tumour cells involves two AP-1 binding sites in the viral enhancer. *Journal of General Virology*. 1995;76(8):1945-1958. doi:10.1099/0022-1317-76-8-1945

72. Yasumoto S, Taniguchi A, Sohma K. Epidermal growth factor (EGF) elicits down-regulation of human papillomavirus type 16 (HPV-16) E6/E7 mRNA at the transcriptional level in an EGF-stimulated human keratinocyte cell line: functional role of EGF-responsive silencer in the HPV-16 long control. *J Virol*. 1991;65(4):2000-2009. doi:10.1128/jvi.65.4.2000-2009.1991

73. Zyzak LL, MacDonald LM, Batova A, Forand R, Creek KE, Pirisi L. Increased levels and constitutive tyrosine phosphorylation of the epidermal growth factor receptor contribute to autonomous growth of human papillomavirus type 16 immortalized human keratinocytes. *Cell Growth and Differentiation*. 1994;5(5):537-547.

74. Akerman GS, Tolleson WH, Brown KL, et al. Human papillomavirus type 16 E6 and E7 cooperate to increase epidermal growth factor receptor (EGFR) mRNA levels, overcoming mechanisms by which excessive EGFR signaling shortens the life span of normal human keratinocytes. *Cancer Res*. 2001;61(9):3837-3843.

75. Crusius K, Auvinen E, Steuer B, Gaissert H, Alonso A. The human papillomavirus type 16 E5-protein modulates ligand-dependent activation of the EGF receptor family in the human epithelial cell line HaCaT. *Exp Cell Res*. 1998;241(1):76-83. doi:10.1006/excr.1998.4024

76. Crusius K, Rodriguez I, Alonso A. The human papillomavirus type 16 E5 protein modulates ERK1/2 and p38 MAP kinase activation by an EGFR-independent process in stressed human keratinocytes. *Virus Genes*. 2000;20(1):65-69. doi:10.1023/A:1008112207824

77. Cartin W, Alonso A. The human papillomavirus HPV2a E5 protein localizes to the Golgi apparatus and modulates signal transduction. *Virology*. 2003;314(2):572-579. doi:10.1016/S0042-6822(03)00509-9

78. Spangle JM, Munger K. The HPV16 E6 Oncoprotein Causes Prolonged Receptor Protein Tyrosine Kinase Signaling and Enhances Internalization of Phosphorylated Receptor Species. *PLoS Pathog*. 2013;9(3):e1003237. doi:10.1371/journal.ppat.1003237

79. Jing M, Bohl J, Brimer N, Kinter M, Vande Pol SB. Degradation of tyrosine phosphatase PTPN3 (PTPH1) by association with oncogenic human papillomavirus E6 proteins. *J Virol*. 2007;81(5):2231-2239. doi:10.1128/jvi.01979-06

80. Li MY, Lai PL, Chou YT, et al. Protein tyrosine phosphatase PTPN3 inhibits lung cancer cell proliferation and migration by promoting EGFR endocytic degradation. *Oncogene*. 2015;34(29):3791-3803. doi:10.1038/onc.2014.312

81. Lee HS, Yun HY, Lee EW, Shin HC, Kim SJ, Ku B. Structural and biochemical analysis of the PTPN4 PDZ domain bound to the C-terminal tail of the human papillomavirus E6 oncoprotein. *Journal of Microbiology*. 2022;60(4):395-401. doi:10.1007/s12275-022-1606-1

82. Hoover AC, Strand GL, Nowicki PN, et al. Impaired PTPN13 phosphatase activity in spontaneous or HPV-induced squamous cell carcinomas potentiates oncogene signaling through the MAP kinase pathway. *Oncogene*. 2009;28(45):3960-3970. doi:10.1038/onc.2009.251

83. Chakrabarti O, Veeraraghavalu K, Tergaonkar V, et al. Human Papillomavirus Type 16 E6 Amino Acid 83 Variants Enhance E6-Mediated MAPK Signaling and Differentially Regulate Tumorigenesis by Notch Signaling and Oncogenic Ras. *J Virol*. 2004;78(11):5934-5945. doi:10.1128/jvi.78.11.5934-5945.2004

84. Wydra VR, Ditzinger RB, Seidler NJ, Hacker FW, Laufer SA. A patent review of MAPK inhibitors (2018–present). *Expert Opin Ther Pat*. 2023;33(6):421-444. doi:10.1080/13543776.2023.2242584

85. Jin X, Mo Q, Zhang Y, et al. The p38 MAPK inhibitor BIRB796 enhances the antitumor effects of VX680 in cervical cancer. *Cancer Biol Ther*. 2016;17(5):566-576. doi:10.1080/15384047.2016.1177676

86. Hwang J, Moon H, Kim H, Kim KY. Identification of a Novel ERK5 (MAPK7) Inhibitor, MHJ-627, and Verification of Its Potent Anticancer Efficacy in Cervical Cancer HeLa Cells. *Curr Issues Mol Biol*. 2023;45(7):6154-6169. doi:10.3390/cimb45070388

87. Hoffner, MSN, ANP-BC, AOCNP B, Benchich, MSN, NP-C, AOCNP K. Trametinib: A Targeted Therapy in Metastatic Melanoma. *J Adv Pract Oncol*. 2018;9(7):741-745. doi:10.6004/jadpro.2018.9.7.5

88. Liu JF, Gray KP, Wright AA, et al. Results from a single arm, single stage phase II trial of trametinib and GSK2141795 in persistent or recurrent cervical cancer. *Gynecol Oncol*. 2019;154(1):95-101. doi:10.1016/j.ygyno.2019.05.003

89. Rosen LS, LoRusso P, Ma WW, et al. A first-in-human phase I study to evaluate the MEK1/2 inhibitor, cobimetinib, administered daily in patients with advanced solid tumors. *Invest New Drugs*. 2016;34(5):604-613. doi:10.1007/s10637-016-0374-3

90. Adjei AA, Cohen RB, Franklin W, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. *Journal of Clinical Oncology*. 2008;26(13):2139-2146. doi:10.1200/JCO.2007.14.4956

91. Cohen RB, Aamdal S, Nyakas M, et al. A phase I dose-finding, safety and tolerability study of AZD8330 in patients with advanced malignancies. *Eur J Cancer*. 2013;49(7):1521-1529. doi:10.1016/j.ejca.2013.01.013

92. Ngan HL, Law CH, Choi YCY, Chan JYS, Lui VWY. Precision drugging of the MAPK pathway in head and neck cancer. *NPJ Genom Med*. 2022;7(1):20. doi:10.1038/s41525-022-00293-1

93. Haddad RI, Adkins D, Licitra LF, et al. The AIM-HN Study: A pivotal study evaluating the efficacy of tipifarnib in patients with recurrent or metastatic head and neck squamous cell carcinoma with HRAS mutations. . *Journal of Clinical Oncology*. 2021;39(15_suppl):TPS6087-TPS6087. doi:10.1200/jco.2021.39.15_suppl.tps6087

94. Ho AL, Brana I, Haddad R, et al. Tipifarnib in head and neck squamous cell carcinoma with HRAS mutations. *Journal of Clinical Oncology*. 2021;39(17):1856-1864. doi:10.1200/JCO.20.02903

95. Medda A, Duca D, Chiocca S. Human papillomavirus and cellular pathways: Hits and targets. *Pathogens*. 2021;10(3):1-23. doi:10.3390/pathogens10030262

96. Bossler F, Hoppe-Seyler K, Hoppe-Seyler F. PI3K/AKT/mTOR signaling regulates the virus/host cell crosstalk in HPV-positive cervical cancer cells. *Int J Mol Sci*. 2019;20(9). doi:10.3390/ijms20092188

97. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: Lessons learned from early clinical trials. *Nat Rev Clin Oncol*. 2013;10(3):143-153. doi:10.1038/nrclinonc.2013.10

98. Medda A, Duca D, Chiocca S. Human papillomavirus and cellular pathways: Hits and targets. *Pathogens*. 2021;10(3):1-23. doi:10.3390/pathogens10030262

99. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell*. 2017;169(3):381-405. doi:10.1016/j.cell.2017.04.001

100. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell*. 2017;168(6):960-976. doi:10.1016/j.cell.2017.02.004

101. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell*. 2017;169(3):381-405. doi:10.1016/j.cell.2017.04.001

102. Lim HJ, Crowe P, Yang JL. Current clinical regulation of PI3K/PTEN/Akt/mTOR signalling in treatment of human cancer. *J Cancer Res Clin Oncol*. 2015;141(4):671-689. doi:10.1007/s00432-014-1803-3

103. Wright AA, Howitt BE, Myers AP, et al. Oncogenic mutations in cervical cancer: Genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. *Cancer*. 2013;119(21):3776-3783. doi:10.1002/cncr.28288

104. Wright AA, Howitt BE, Myers AP, et al. Oncogenic mutations in cervical cancer: Genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. *Cancer*. 2013;119(21):3776-3783. doi:10.1002/cncr.28288

105. McIntyre JB, Wu JS, Craighead PS, et al. PIK3CA mutational status and overall survival in patients with cervical cancer treated with radical chemoradiotherapy. *Gynecol Oncol*. 2013;128(3):409-414. doi:10.1016/j.ygyno.2012.12.019

106. Schwarz JK, Payton JE, Rashmi R, et al. Pathway-specific analysis of gene expression data identifies the PI3K/Akt pathway as a novel therapeutic target in cervical cancer. *Clinical Cancer Research*. 2012;18(5):1464-1471. doi:10.1158/1078-0432.CCR-11-2485

107. Faried LS, Faried A, Kanuma T, et al. Expression of an activated mammalian target of rapamycin in adenocarcinoma of the cervix: A potential biomarker and molecular target therapy. *Mol Carcinog*. 2008;47(6):446-457. doi:10.1002/mc.20402

108. Marquard FE, Jücker M. PI3K/AKT/mTOR signaling as a molecular target in head and neck cancer. *Biochem Pharmacol*. 2020;172:113729. doi:10.1016/j.bcp.2019.113729

109. Pim D, Massimi P, Dilworth SM, Banks L. Activation of the protein kinase B pathway by the HPV-16 E7 oncoprotein occurs through a mechanism involving interaction with PP2A. *Oncogene*. 2005;24(53):7830-7838. doi:10.1038/sj.onc.1208935

110. Accardi R, Rubino R, Scalise M, et al. E6 and E7 from Human Papillomavirus Type 16 Cooperate To Target the PDZ Protein Na/H Exchange Regulatory Factor 1. *J Virol*. 2011;85(16):8208-8216. doi:10.1128/jvi.00114-11

111. Kim SH, Juhnn YS, Kang S, et al. Human papillomavirus 16 E5 up-regulates the expression of vascular endothelial growth factor through the activation of epidermal growth factor receptor, MEK/ERK1,2 and PI3K/Akt. *Cellular and Molecular Life Sciences*. 2006;63(7-8):930-938. doi:10.1007/s00018-005-5561-x

112. Zhang W, Chen H, Chen Y, et al. Cancerous inhibitor of protein phosphatase 2A contributes to human papillomavirus oncoprotein E7-induced cell proliferation via E2F1. *Oncotarget*. 2015;6(7):5253-5262. doi:10.18632/oncotarget.2867

113. Kivi N, Greco D, Auvinen P, Auvinen E. Genes involved in cell adhesion, cell motility and mitogenic signaling are altered due to HPV 16 E5 protein expression. *Oncogene*. 2008;27(18):2532-2541. doi:10.1038/sj.onc.1210916

114. Contreras-Paredes A, De la Cruz-Hernández E, Martínez-Ramírez I, Dueñas-González A, Lizano M. E6 variants of human papillomavirus 18 differentially modulate the protein kinase B/phosphatidylinositol 3-kinase (akt/PI3K) signaling pathway. *Virology*. 2009;383(1):78-85. doi:10.1016/j.virol.2008.09.040

115. Pim D, Tomaić V, Banks L. The Human Papillomavirus (HPV) E6* Proteins from High-Risk, Mucosal HPVs Can Direct Degradation of Cellular Proteins in the Absence of Full-Length E6 Protein. *J Virol*. 2009;83(19):9863-9874. doi:10.1128/jvi.00539-09

116. Drews CM, Case S, Pol SBV. E6 proteins from high-risk HPV, low-risk HPV, and animal papillomaviruses activate the Wnt/ß-catenin pathway through E6AP-dependent degradation of NHERF1. *PLoS Pathog*. 2019;15(4):e1007575. doi:10.1371/journal.ppat.1007575

117. Lu Z, Hu X, Li Y, et al. Human papillomavirus 16 E6 oncoprotein interferences with insulin signaling pathway by binding to tuberin. *Journal of Biological Chemistry*. 2004;279(34):35664-35670. doi:10.1074/jbc.M403385200

118. Menges CW, Baglia LA, Lapoint R, McCance DJ. Human papillomavirus type 16 E7 up-regulates AKT activity through the retinoblastoma protein. *Cancer Res*. 2006;66(11):5555-5559. doi:10.1158/0008-5472.CAN-06-0499

119. Skelin J, Đukić A, Filić V, et al. MAML1-induced HPV E6 oncoprotein stability is required for cellular proliferation and migration of cervical tumor-derived cells. *J Med Virol*. 2023;95(3):e28624. doi:10.1002/jmv.28624

120. Bahrami A, Hasanzadeh M, Hassanian SM, et al. The Potential Value of the PI3K/Akt/mTOR Signaling Pathway for Assessing Prognosis in Cervical Cancer and as a Target for Therapy. *J Cell Biochem*. 2017;118(12):4163-4169. doi:10.1002/jcb.26118

121. Janku F, Wheler JJ, Westin SN, et al. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. *Journal of Clinical Oncology*. 2012;30(8):777-782. doi:10.1200/JCO.2011.36.1196

122. Gillison ML, Akagi K, Xiao W, et al. Human papillomavirus and the landscape of secondary genetic alterations in oral cancers. *Genome Res*. 2019;29(1):1-17. doi:10.1101/gr.241141.118

123. Day TA, Shirai K, O'Brien PE, et al. Inhibition of mTOR signaling and clinical activity of rapamycin in head and neck cancer in a window of opportunity trial. *Clinical Cancer Research*. 2019;25(4):1156-1164. doi:10.1158/1078-0432.CCR-18-2024

124. De Henau O, Rausch M, Winkler D, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. *Nature*. 2016;539(7629):443-447. doi:10.1038/nature20554

125. Tawbi HAH, Peng W, Milton D, et al. Phase I/II study of the PI3Kβ inhibitor GSK2636771 in combination with pembrolizumab (P) in patients (pts) with PD-1 refractory metastatic melanoma (MM) and PTEN loss. *Journal of Clinical Oncology*. 2018;36(15_suppl):TPS9596-TPS9596. doi:10.1200/jco.2018.36.15_suppl.tps9596

126. Tolcher A, Hong D, Sullivan R, et al. Abstract CT089: IPI-549-01 - A Phase 1/1b, first-in-human study of IPI-549, a PI3K-γ inhibitor, as monotherapy and in combination with nivolumab in patients with advanced solid tumors. *Cancer Res*. 2017;77(13_Supplement):CT089-CT089. doi:10.1158/1538-7445.am2017-ct089

127. Zhou B, Lin W, Long Y, et al. Notch signaling pathway: architecture, disease, and therapeutics. *Signal Transduct Target Ther*. 2022;7(1):95. doi:10.1038/s41392-022-00934-y

128. Vázquez-Ulloa E, Lin KL, Lizano M, Sahlgren C. Reversible and bidirectional signaling of notch ligands. *Crit Rev Biochem Mol Biol*. 2022;57(4):377-398. doi:10.1080/10409238.2022.2113029

129. Lowell S, Jones P, Le Roux I, Dunne J, Watt FM. Stimulation of human epidermal differentiation by Delta-Notch signalling at the boundaries of stem-cell clusters. *Current Biology*. 2000;10(9):491-500. doi:10.1016/S0960-9822(00)00451-6

130. Rangarajan A, Talora C, Okuyama R, et al. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO Journal*. 2001;20(13):3427-3436. doi:10.1093/emboj/20.13.3427

131. Okuyama R, Nguyen BC, Talora C, et al. High commitment of embryonic keratinocytes to terminal differentiation through a Notch1-caspase 3 regulatory mechanism. *Dev Cell*. 2004;6(4):551-562. doi:10.1016/S1534-5807(04)00098-X

132. Nickoloff BJ, Qin JZ, Chaturvedi V, Denning MF, Bonish B, Miele L. Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-κB and PPARγ. *Cell Death Differ*. 2002;9(8):842-855. doi:10.1038/sj.cdd.4401036

133. Williams SE, Beronja S, Pasolli HA, Fuchs E. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. *Nature*. 2011;470(7334):353-358. doi:10.1038/nature09793

134. Nguyen BC, Lefort K, Mandinova A, et al. Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes Dev*. 2006;20(8):1028-1042. doi:10.1101/gad.1406006

135. Meyers JM, Spangle JM, Munger K. The Human Papillomavirus Type 8 E6 Protein Interferes with NOTCH Activation during Keratinocyte Differentiation. *J Virol*. 2013;87(8):4762-4767. doi:10.1128/jvi.02527-12

136. Brimer N, Lyons C, Wallberg AE, Vande Pol SB. Cutaneous papillomavirus E6 oncoproteins associate with MAML1 to repress transactivation and NOTCH signaling. *Oncogene*. 2012;31(43):4639-4646. doi:10.1038/onc.2011.589

137. Tan MJA, White EA, Sowa ME, Harper JW, Aster JC, Howley PM. Cutaneous β-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. *Proc Natl Acad Sci U S A*. 2012;109(23):1473-1480. doi:10.1073/pnas.1205991109

138. Weijzen S, Zlobin A, Braid M, Miele L, Kast WM. HPV16 E6 and E7 oncoproteins regulate Notch-1 expression and cooperate to induce transformation. *J Cell Physiol*. 2003;194(3):356-362. doi:10.1002/jcp.10217

139. Kranjec C, Holleywood C, Libert D, et al. Modulation of basal cell fate during productive and transforming HPV-16 infection is mediated by progressive E6-driven depletion of Notch. *Journal of Pathology*. 2017;242(4):448-462. doi:10.1002/path.4917

140. Khalifa Y, Teissier S, Tan MKM, et al. The human papillomavirus e6 oncogene represses a cell adhesion pathway and disrupts focal adhesion through degradation of tap63β upon transformation. *PLoS Pathog*. 2011;7(9). doi:10.1371/journal.ppat.1002256

141. Yugawa T, Handa K, Narisawa-Saito M, Ohno S ichi, Fujita M, Kiyono T. Regulation of Notch1 Gene Expression by p53 in Epithelial Cells. *Mol Cell Biol*. 2007;27(10):3732-3742. doi:10.1128/mcb.02119-06

142. Vliet-Gregg PA, Hamilton JR, Katzenellenbogen RA. NFX1-123 and Human Papillomavirus 16E6 Increase Notch Expression in Keratinocytes. *J Virol*. 2013;87(24):13741-13750. doi:10.1128/jvi.02582-13

143. Wang L, Qin H, Chen B, Xin X, Li J, Han H. Overexpressed active Notch1 induces cell growth arrest of HeLa cervical carcinoma cells. *International Journal of Gynecological Cancer*. 2007;17(6):1283-1292. doi:10.1111/j.1525-1438.2007.00927.x

144. Yao J, Duan L, Fan M, Yuan J, Wu X. Notch1 induces cell cycle arrest and apoptosis in human cervical cancer cells: Involvement of nuclear factor kappa B inhibition. *International Journal of Gynecological Cancer*. 2007;17(2):502-510. doi:10.1111/j.1525-1438.2007.00872.x

145. Sun L, Liu M, Sun GC, et al. Notch signaling activation in cervical cancer cells induces cell growth arrest with the involvement of the nuclear receptor NR4A2. *J Cancer*. 2016;7(11):1388-1395. doi:10.7150/jca.15274

146. Eldakhakhny S, Zhou Q, Crosbie EJ, Sayan BS. Human papillomavirus E7 induces p63 expression to modulate DNA damage response article. *Cell Death Dis*. 2018;9(2). doi:10.1038/s41419-017-0149-6

147. Citro S, Bellini A, Medda A, et al. Human Papilloma Virus Increases ΔNp63α Expression in Head and Neck Squamous Cell Carcinoma. *Front Cell Infect Microbiol*. 2020;10(April):1-6. doi:10.3389/fcimb.2020.00143

148. Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science (1979)*. 2011;333(6046):1154-1157. doi:10.1126/science.1206923

149. Pickering CR, Zhang J, Yoo SY, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov*. 2013;3(7):770-781. doi:10.1158/2159-8290.CD-12-0537

150. Das T, Zhong R, Spiotto MT. Notch signaling and human papillomavirus–associated oral tumorigenesis. *Adv Exp Med Biol*. 2021;1287:105-122. doi:10.1007/978-3-030-55031-8_8

151. Rettig EM, Chung CH, Bishop JA, et al. Cleaved NOTCH1 expression pattern in head and neck squamous cell carcinoma is associated with NOTCH1 mutation, HPV status, and high-risk features. *Cancer Prevention Research*. 2015;8(4):287-295. doi:10.1158/1940-6207.CAPR-14-0366

152. Izumchenko E, Sun K, Jones S, et al. Notch1 mutations are drivers of oral tumorigenesis. *Cancer Prevention Research*. 2015;8(4):277-286. doi:10.1158/1940-6207.CAPR-14-0257

153. Weaver AN, Benjamin Burch M, Cooper TS, et al. Notch signaling activation is associated with patient mortality and increased FGF1-mediated invasion in squamous cell carcinoma of the oral cavity. *Molecular Cancer Research*. 2016;14(9):883-891. doi:10.1158/1541-7786.MCR-16-0114

154. Pongjantarasatian S, Nowwarote N, Rotchanakitamnuai V, et al. A γ-Secretase Inhibitor Attenuates Cell Cycle Progression and Invasion in Human Oral Squamous Cell Carcinoma: An In Vitro Study. *Int J Mol Sci*. 2022;23(16). doi:10.3390/ijms23168869

155. Varatanovic S, Maier T, Al-Gboore S, et al. In vitro effects of gamma-secretase inhibition in HPV-positive and HPV-negative head and neck squamous cell carcinoma. *Invest New Drugs*. 2023;41(2):193-201. doi:10.1007/s10637-023-01334-x

156. Ramdass B, Maliekal TT, Lakshmi S, et al. Coexpression of Notch1 and NF-κB signaling pathway components in human cervical cancer progression. *Gynecol Oncol*. 2007;104(2):352-361. doi:10.1016/j.ygyno.2006.08.054

157. Rong C, Feng Y, Ye Z. Notch is a critical regulator in cervical cancer by regulating numb splicing. *Oncol Lett*. 2017;13(4):2465-2470. doi:10.3892/ol.2017.5683

158. Vázquez-Ulloa E, Ramos-Cruz AC, Prada D, et al. Loss of nuclear NOTCH1, but not its negative regulator NUMB, is an independent predictor of cervical malignancy. *Oncotarget*. 2018;9(27):18916-18928. doi:10.18632/oncotarget.24828

159. Tripathi R, Rath G, Hussain S, et al. Jagged-1 induced molecular alterations in HPV associated invasive squamous cell and adenocarcinoma of the human uterine cervix. *Sci Rep*. 2018;8(1):9359. doi:10.1038/s41598-018-27699-1

160. Tripathi R, Rath G, Sharma V, et al. HES1 protein modulates human papillomavirus–mediated carcinoma of the uterine cervix. *J Glob Oncol*. 2019;2019(5):1-10. doi:10.1200/JGO.18.00141

161. Talora C, Sgroi DC, Crum CP, Paolo Dotto G. Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. *Genes Dev*. 2002;16(17):2252-2263. doi:10.1101/gad.988902

162. Talora C, Cialfi S, Segatto O, et al. Constitutively active Notch1 induces growth arrest of HPV-positive cervical cancer cells via separate signaling pathways. *Exp Cell Res*. 2005;305(2):343-354. doi:10.1016/j.yexcr.2005.01.015

163. Kadian LK, Gulshan G, Ahuja P, et al. Aberrant promoter methylation of NOTCH1 and NOTCH3 and its association with cervical cancer risk factors in North Indian population. *Am J Transl Res*. 2020;12(6):2814-2826.

164. Franko-Tobin LG, Mackey LV, Huang W, et al. Notch1-Mediated Tumor Suppression in Cervical Cancer with the Involvement of SST Signaling and Its Application in Enhanced SSTR-Targeted Therapeutics. *Oncologist*. 2012;17(2):220-232. doi:10.1634/theoncologist.2011-0269

165. Sun T, Zhang D, Wang Z, et al. Inhibition of the notch signaling pathway overcomes resistance of cervical cancer cells to paclitaxel through retardation of the epithelial–mesenchymal transition process. *Environ Toxicol*. 2021;36(9):1758-1764. doi:10.1002/tox.23296

166. Yue Y, Zhou K, Li J, Jiang S, Li C, Men H. Msx1 induces g0/g1 arrest and apoptosis by suppressing notch signaling and is frequently methylated in cervical cancer. *Onco Targets Ther*. 2018;11:4769-4780. doi:10.2147/OTT.S165144

167. D.S P, Chaturvedi PK, Shimokawa T, Kim KH, Park WY. Silencing of Fused Toes Homolog (FTS) Increases Radiosensitivity to Carbon-Ion Through Downregulation of Notch Signaling in Cervical Cancer Cells. *Front Oncol*. 2021;11:730607. doi:10.3389/fonc.2021.730607

168. Khan F, Pandey P, Jha NK, Khalid M, Ojha S. Rutin mediated apoptotic cell death in Caski cervical cancer cells via notch-1 and hes-1 downregulation. *Life*. 2021;11(8). doi:10.3390/life11080761

169. Yang S, Liu YD, Xia BR, et al. DLL4 as a predictor of pelvic lymph node metastasis and a novel prognostic biomarker in patients with early-stage cervical cancer. *Tumor Biology*. 2016;37(4):5063-5074. doi:10.1007/s13277-015-4312-3

170. Khelil M, Griffin H, Bleeker MCG, et al. Delta-like ligand-notch1 signaling is selectively modulated by HPV16 e6 to promote squamous cell proliferation and correlates with cervical cancer prognosis. *Cancer Res*. 2021;81(7):1909-1921. doi:10.1158/0008-5472.CAN-20-1996

171. Yeasmin S, Nakayama K, Rahman MT, et al. Expression of nuclear Notch3 in cervical squamous cell carcinomas and its association with adverse clinical outcomes. *Gynecol Oncol*. 2010;117(3):409-416. doi:10.1016/j.ygyno.2010.03.004

172. Allen F, Maillard I. Therapeutic Targeting of Notch Signaling: From Cancer to Inflammatory Disorders. *Front Cell Dev Biol*. 2021;9(May):1-17. doi:10.3389/fcell.2021.649205

173. El-Khoueiry AB, Desai J, Iyer SP, et al. A phase I study of AL101, a pan-NOTCH inhibitor, in patients (pts) with locally advanced or metastatic solid tumors. *Journal of Clinical Oncology*. 2018;36(15_suppl):2515-2515. doi:10.1200/jco.2018.36.15_suppl.2515

174. Massard C, Azaro A, Soria JC, et al. First-in-human study of LY3039478, an oral Notch signaling inhibitor in advanced or metastatic cancer. *Annals of Oncology*. 2018;29(9):1911-1917. doi:10.1093/annonc/mdy244

175. Habets RA, De Bock CE, Serneels L, et al. Safe targeting of T cell acute lymphoblastic leukemia by pathology-specific NOTCH inhibition. *Sci Transl Med*. 2019;11(494). doi:10.1126/scitranslmed.aau6246

176. Lehal R, Zaric J, Vigolo M, et al. Pharmacological disruption of the Notch transcription factor complex. *Proc Natl Acad Sci U S A*. 2020;117(28):16292-16301. doi:10.1073/pnas.1922606117

177. Ferrarotto R, Eckhardt G, Patnaik A, et al. A phase I dose-escalation and dose-expansion study of brontictuzumab in subjects with selected solid tumors. *Annals of Oncology*. 2018;29(7):1561-1568. doi:10.1093/annonc/mdy171

178. Hu X, li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther*. 2021;6(1):402. doi:10.1038/s41392-021-00791-1

179. Kim Y, Lim KM. Skin barrier dysfunction and filaggrin. *Arch Pharm Res*. 2021;44(1):36-48. doi:10.1007/s12272-021-01305-x

180. Furue M. Regulation of filaggrin, loricrin, and involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic implications in atopic dermatitis. *Int J Mol Sci*. 2020;21(15):1-25. doi:10.3390/ijms21155382

181. Jere SW, Abrahamse H, Houreld NN. The JAK/STAT signaling pathway and photobiomodulation in chronic wound healing. *Cytokine Growth Factor Rev*. 2017;38:73-79. doi:10.1016/j.cytogfr.2017.10.001

182. Villarino A V., Kanno Y, O'Shea JJ. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat Immunol*. 2017;18(4):374-384. doi:10.1038/ni.3691

183. Ezeonwumelu IJ, Garcia-Vidal E, Ballana E. Jak-stat pathway: A novel target to tackle viral infections. *Viruses*. 2021;13(12). doi:10.3390/v13122379

184. Nees M, Geoghegan JM, Hyman T, Frank S, Miller L, Woodworth CD. Papillomavirus Type 16 Oncogenes Downregulate Expression of Interferon-Responsive Genes and Upregulate Proliferation-Associated and NF-κB-Responsive Genes in Cervical Keratinocytes. *J Virol*. 2001;75(9):4283-4296. doi:10.1128/jvi.75.9.4283-4296.2001

185. Barnard P, McMillan NAJ. The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-α. *Virology*. 1999;259(2):305-313. doi:10.1006/viro.1999.9771

186. Hong S, Mehta KP, Laimins LA. Suppression of STAT-1 Expression by Human Papillomaviruses Is Necessary for Differentiation-Dependent Genome Amplification and Plasmid Maintenance. *J Virol*. 2011;85(18):9486-9494. doi:10.1128/jvi.05007-11

187. James CD, Fontan CT, Otoa R, et al. Human Papillomavirus 16 E6 and E7 Synergistically Repress Innate Immune Gene Transcription. *mSphere*. 2020;5(1). doi:10.1128/msphere.00828-19

188. LaFleur DW, Nardelli B, Tsareva T, et al. Interferon-κ, a Novel Type I Interferon Expressed in Human Keratinocytes. *Journal of Biological Chemistry*. 2001;276(43):39765-39771. doi:10.1074/jbc.M102502200

189. Reiser J, Hurst J, Voges M, et al. High-Risk Human Papillomaviruses Repress Constitutive Kappa Interferon Transcription via E6 To Prevent Pathogen Recognition Receptor and Antiviral-Gene Expression. *J Virol*. 2011;85(21):11372-11380. doi:10.1128/jvi.05279-11

190. Habiger C, Jäger G, Walter M, Iftner T, Stubenrauch F. Interferon Kappa Inhibits Human Papillomavirus 31 Transcription by Inducing Sp100 Proteins. *J Virol*. 2016;90(2):694-704. doi:10.1128/jvi.02137-15

191. Li S, Labrecque S, Gauzzi MC, et al. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-α. *Oncogene*. 1999;18(42):5727-5737. doi:10.1038/sj.onc.1202960

192. Sobti RC, Singh N, Hussain S, Suri V, Bharadwaj M, Das BC. Deregulation of STAT-5 isoforms in the development of HPV-mediated cervical carcinogenesis. *Journal of Receptors and Signal Transduction*. 2010;30(3):178-188. doi:10.3109/10799891003786218

193. Orecchia V, Regis G, Tassone B, et al. Constitutive STAT3 activation in epidermal keratinocytes enhances cell clonogenicity and favours spontaneous immortalization by opposing differentiation and senescence checkpoints. *Exp Dermatol*. 2015;24(1):29-34. doi:10.1111/exd.12585

194. Morgan EL, Macdonald A. Autocrine STAT3 activation in hpv positive cervical cancer through a virus-driven Rac1— NFκB—IL-6 signalling axis. *PLoS Pathog*. 2019;15(6):e1007835. doi:10.1371/journal.ppat.1007835

195. Morgan EL, Wasson CW, Hanson L, et al. *STAT3 Activation by E6 Is Essential for the Differentiation-Dependent HPV18 Life Cycle*. Vol 14. Public Library of Science; 2018. doi:10.1371/journal.ppat.1006975

196. Dai X, Sayama K, Shirakata Y, et al. STAT5a/PPARγ pathway regulates involucrin expression in keratinocyte differentiation. *Journal of Investigative Dermatology*. 2007;127(7):1728-1735. doi:10.1038/sj.jid.5700758

197. Hong S, Laimins LA. The JAK-STAT Transcriptional Regulator, STAT-5, Activates the ATM DNA Damage Pathway to Induce HPV 31 Genome Amplification upon Epithelial Differentiation. *PLoS Pathog*. 2013;9(4):e1003295. doi:10.1371/journal.ppat.1003295

198. Hong S, Cheng S, Iovane A, Laimins LA. STAT-5 Regulates transcription of the topoisomerase IIβ-Binding protein 1 (TopBP1) gene to activate the ATR pathway and promote human papillomavirus Replication. *mBio*. 2015;6(6):e02006-15. doi:10.1128/mBio.02006-15

199. Wu S, Wu Y, Lu Y, et al. Stat1 expression and HPV16 viral load predict cervical lesion progression. *Oncol Lett*. 2020;20(4):28. doi:10.3892/ol.2020.11889

200. Zhang M, Liang L, He J, et al. Fra-1 Inhibits Cell Growth and the Warburg Effect in Cervical Cancer Cells via STAT1 Regulation of the p53 Signaling Pathway. *Front Cell Dev Biol*. 2020;8:579629. doi:10.3389/fcell.2020.579629

201. Raspaglio G, Buttarelli M, Filippetti F, et al. Stat1 confers sensitivity to radiation in cervical cancer cells by controlling Parp1 levels: a new perspective for Parp1 inhibition. *Cell Death Dis*. 2021;12(10):933. doi:10.1038/s41419-021-04229-y

202. Decarlo CA, Rosa B, Jackson R, Niccoli S, Escott NG, Zehbe I. Toll-like receptor transcriptome in the HPV-positive cervical cancer microenvironment. *Clin Dev Immunol*. 2012;2012:785825. doi:10.1155/2012/785825

203. Rincon-Orozco B, Halec G, Rosenberger S, et al. Epigenetic silencing of interferon-κ in human papillomavirus type 16-positive cells. *Cancer Res*. 2009;69(22):8718-8725. doi:10.1158/0008-5472.CAN-09-0550

204. Shukla S, Shishodia G, Mahata S, et al. Aberrant expression and constitutive activation of STAT3 in cervical carcinogenesis: Implications in high-risk human papillomavirus infection. *Mol Cancer*. 2010;9:282. doi:10.1186/1476-4598-9-282

205. Sriuranpong V, Park JI, Amornphimoltham P, Patel V, Nelkin BD, Gutkind JS. Epidermal growth factor receptor-independent constitutive activation of STAT3 in head and neck squamous cell carcinoma is mediated by the autocrine/paracrine stimulation of the interleukin 6/gp130 cytokine system. *Cancer Res*. 2003;63(11):2948-2956.

206. Gaykalova DA, Manola JB, Ozawa H, et al. NF-κB and stat3 transcription factor signatures differentiate HPV-positive and HPV-negative head and neck squamous cell carcinoma. *Int J Cancer*. 2015;137(8):1879-1889. doi:10.1002/ijc.29558

207. Chen HHW, Chou CY, Wu YH, et al. Constitutive STAT5 activation correlates with better survival in cervical cancer patients treated with radiation therapy. *Int J Radiat Oncol Biol Phys*. 2012;82(2):658-666. doi:10.1016/j.ijrobp.2010.11.043

208. Leong PL, Xi S, Drenning SD, et al. Differential function of Stat5 isoforms in head and neck cancer growth control. *Oncogene*. 2002;21(18):2846-2853. doi:10.1038/sj.onc.1205385

209. Rocha-Zavaleta L, Huitron C, Cacéres-Cortés JR, et al. Interleukin-2 (IL-2) receptor-βγ signalling is activated by c-Kit in the absence of IL-2, or by exogenous IL-2 via JAK3/STAT5 in human papillomavirus-associated cervical cancer. *Cell Signal*. 2004;16(11):1239-1247. doi:10.1016/j.cellsig.2004.03.011

210. Morris R, Kershaw NJ, Babon JJ. The molecular details of cytokine signaling via the JAK/STAT pathway. *Protein Science*. 2018;27(12):1984-2009. doi:10.1002/pro.3519

211. Lopes JE, Fisher JL, Flick HL, et al. ALKS 4230: A novel engineered IL-2 fusion protein with an improved cellular selectivity profile for cancer immunotherapy. *J Immunother Cancer*. 2020;8(1):1-13. doi:10.1136/jitc-2020-000673

212. Wong ALA, Hirpara JL, Pervaiz S, Eu JQ, Sethi G, Goh BC. Do STAT3 inhibitors have potential in the future for cancer therapy? *Expert Opin Investig Drugs*. 2017;26(8):883-887. doi:10.1080/13543784.2017.1351941

213. Qureshy Z, Johnson DE, Grandis JR. Targeting the JAK/STAT pathway in solid tumors. *J Cancer Metastasis Treat*. 2020;6. doi:10.20517/2394-4722.2020.58

214. Bai L, Zhou H, Xu R, et al. A Potent and Selective Small-Molecule Degrader of STAT3 Achieves Complete Tumor Regression In Vivo. *Cancer Cell*. 2019;36(5):498-511.e17. doi:10.1016/j.ccell.2019.10.002

215. Reilley MJ, McCoon P, Cook C, et al. STAT3 antisense oligonucleotide AZD9150 in a subset of patients with heavily pretreated lymphoma: Results of a phase 1b trial. *J Immunother Cancer*. 2018;6(1):119. doi:10.1186/s40425-018-0436-5

216. Sen M, Grandis JR. Nucleic acid-based approaches to STAT inhibition. *JAKSTAT*. 2012;1(4):285-291. doi:10.4161/jkst.22312

Figure legends

Fig 1. **Cellular signaling pathways and their perturbation by HPV oncoproteins.** A) Wnt pathway - The activity of this pathway is increased by HPV oncoproteins, but it is still not completely clear whether the upregulation of its components is due to E6/E7 (yellow and light blue) overexpression. Nevertheless, Wnt is activated by the binding of E6 (yellow) to β-catenin, which leads to its activation and the stabilization of TCF-4. B) MAPK pathway -The pseudooncoprotein E5 (pink) increases the expression and activation of EGFR, one of the most important receptors of the pathway, while E6 (yellow) oncoprotein prolongs the receptor's activity. Additionally, E5 (pink) upregulates the expression ERK1/2, an executive kinase of the pathway. C) PI3K/AKT/mTOR pathway - HPV oncoproteins increase the activity of this pathway by either upregulating the expression of pathway members such as PI3K and PKCγ, or by directly activating other components such as AKT, ERK1/2, or mTOR. PTEN, a negative regulator of the pathway, was also found to be activated in the presence of HPV. Figure was created using Servier Medical Art and MS Office Power Point.

Fig 2. **The modulations of cellular signaling pathways by HPV oncoproteins.** A) Notch pathway - HR α-HPV E6 (yellow) oncoproteins form a complex with MAML1, a Notch co-activator, resulting in E6 (yellow) protein stability. The expression of NOTCH1 was found to be upregulated as a consequence of NFX1-123 and E6 (yellow) interaction. Interestingly, the expression of p63, a NOTCH1 antagonist, was also found to be increased. In addition, the upregulation of other receptors and ligands was detected in HPV-associated cancers, but the mechanistic details of this have not yet been resolved. B) JAK/STAT pathway - The oncoproteins inhibit IFN-I signaling and, through it, the immune response by interacting with IRF9 and inhibiting its co-factor activity. Additionally, E6 (yellow) downregulates STAT1, STAT2, and STAT9, as well as IFNκ expression, also attenuating innate immunity. E6 (yellow) also binds TYK2, a member of JAK, thereby inhibiting the signaling. Conversely, signaling through STAT3 and STAT5 is essential for HPV genome amplification, which is why they are activated as a consequence of HPV infection. Figure was created using Servier Medical Art and MS Office Power Point.