

Review

Somatic Host Cell Alterations in HPV Carcinogenesis

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Abstract: High-risk human papilloma virus (HPV) infections cause cancers in different organ sites, most commonly cervical and head and neck cancers. While carcinogenesis is initiated by two viral oncoproteins, E6 and E7, increasing evidence shows the importance of specific somatic events in host cells for malignant transformation. HPV-driven cancers share characteristic somatic changes, including apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC)-driven mutations and genomic instability leading to copy number variations and large chromosomal rearrangements. HPV-associated cancers have recurrent somatic mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) and phosphatase and tensin homolog (*PTEN*), human leukocyte antigen A and B (*HLA-A* and *HLA-B*)-A/B, and the transforming growth factor beta (TGF β) pathway, and rarely have mutations in the tumor protein p53 (*TP53*) and RB transcriptional corepressor 1 (*RB1*) tumor suppressor genes. There are some variations by tumor site, such as *NOTCH1* mutations which are primarily found in head and neck cancers. Understanding the somatic events following HPV infection and persistence can aid the development of early detection biomarkers, particularly when mutations in precancers are characterized. Somatic mutations may also influence prognosis and treatment decisions.

Keywords: HPV; somatic mutation; cervical cancer; APOBEC; significantly mutated gene; copy number variation; chromosomal instability; head and neck cancer; integration

1. Introduction

High-risk human papilloma virus (HPV) infections cause cancers at many sites. It is estimated that almost all cervical cancers [1], 20–70% of oropharyngeal cancers and 5–30% of other head and neck cancers [2–4], 88% of anal cancers [5], 48% of penile cancers [6,7], 19% of vulvar cancers [5], and 71% of vaginal cancers [5] are caused by HPV, with some geographic variation observed for the non-cervical cancers. Together, these cancers resulted in approximately 610,000, or 5%, of all cancer diagnoses worldwide in 2008 [8,9].

HPV infection alone is an insufficient cause of carcinogenesis. Most HPV infections become undetectable after a few months and never result in malignancies, with 91% becoming undetectable after two years, although it has been proposed that there may be some level of persistent latent infection that is undetectable by PCR [10,11]. High-risk HPV types persist longer on average than low-risk types [12]. A failure to clear the virus results in viral persistence, but many persistent infections never develop into precancerous lesions [13]. Finally, even advanced precancerous cervical intraepithelial neoplasias grade 3 (CIN3) only progress to invasive cancer in 30% of cases over 30 years [14]. When

infections persist over time, somatic mutations may accumulate and contribute to the development of precancerous lesions, and then finally to malignant cancers. Understanding the complete carcinogenic pathways is important for developing new strategies to prevent HPV-associated cancer mortality, both through early detection and through targeted therapies [15,16].

HPV-derived cancers share many carcinogenic features across cancer sites, suggesting that the viral oncoproteins E6 and E7 work similarly at different sites. A previous review on this topic [17] predates recent publications of large genomic data from HPV-driven cervical and head and neck cancers in The Cancer Genome Atlas (TCGA) [2,18]. Here, we review common somatic mutations, copy number alterations, and related pathways identified by TCGA and other recent efforts. While the focus of this review is on somatic changes, genome-wide association (GWAS) studies of cervical and HPV-related head and neck cancers have shown that there is also a heritable component. At both cancer sites, human leukocyte antigen (HLA) variants are among the few consistent, independently replicated findings from GWAS studies [19–21].

2. Mechanisms of HPV-Mediated Mutagenesis

There is a great diversity of HPV genotypes, but only a small subset is carcinogenic; among these, HPV16 alone accounts for 50–90% of HPV-driven cancers depending on the site, with some regional variations [22,23]. Most cancers evaluated in studies included in this review are caused by HPV16, and there may be variations in somatic mutation load and type by HPV genotype that are currently not adequately captured. Two of the eight proteins encoded by the HPV genome, E6 and E7, account for most carcinogenic effects of high-risk HPV types [15]. They promote carcinogenesis in several ways, including creating genomic instability and inhibiting tumor suppressor genes. E6 and E7 directly promote genomic instability, which can result in large chromosomal rearrangements and copy number variations, by interfering with centromere duplication during mitosis [24,25]. Both oncoproteins interfere with important cellular tumor suppressor pathways: E6 inhibits the p53 tumor suppressor by promoting its proteasomal degradation [26,27], while E7 disrupts the retinoblastoma (Rb) pathway resulting in uncontrolled activation of the cell cycle and induction of p16^{INK4A}, a cyclin-dependent kinase inhibitor, through a disrupted feedback loop (Figure 1) [28–30]. Theoretically, since HPV oncoproteins are important carcinogenic drivers interfering with several cellular pathways, it could be expected that fewer somatic alterations are required for malignant transformation in HPV-associated compared to non-HPV associated cancers. There is some evidence of lower mutation load in HPV-positive compared to HPV-negative penile cancers [31]. However, the evidence is inconclusive for head and neck cancers, with one study showing evidence of a reduced somatic mutation load in HPV-positive compared to HPV-negative cancers [3] while the TCGA head and neck study did not find evidence of a difference [2].

In addition to direct viral effects, specific mutation signatures may be overrepresented in HPV-positive cancers due to host-viral interactions. The apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) mutation signature in particular is very common in HPV-positive cancers, likely triggered by the host response to HPV infection [32].

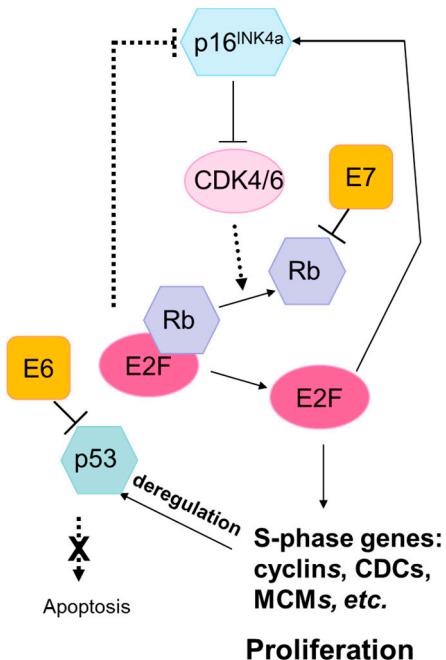


Figure 1. The Rb and p53 pathways are disrupted by the human papilloma virus (HPV) oncoproteins E7 and E6, respectively. The HPV E7 protein binds to Rb with high affinity, disrupting its interaction with the transcription factor E2F. This results in the release and activation of E2F, driving expression of S-phase genes and cell cycle progression. P16^{INK4A} is a cyclin-dependent kinase inhibitor that regulates the cell cycle by inactivating cyclin-dependent kinases involved in Rb phosphorylation. Upregulation of p16^{INK4A} is induced by HPV-mediated disruption of E7, leading to the accumulation of p16^{INK4A} in HPV-transformed cells. The HPV E6 protein inhibits apoptosis by targeting the tumor suppressor protein, p53, for degradation. HPV E6 inhibition of p53 promotes cell proliferation and can lead to genomic instability and the accumulation of somatic mutations. Abbreviations: Rb, retinoblastoma protein; p16^{INK4A}, cyclin-dependent kinase inhibitor 2A; CDK, cyclin-dependent kinases; E2F, E2F transcription factor; CDC, cell-division-cycle genes; MCM, minichromosome maintenance family.

2.1. Genomic Instability

Rates of copy number alterations vary across cancer sites. Cervical cancers average 88 copy number alterations in the TCGA dataset, including 26 amplifications and 37 losses [18]. Focal amplifications of loci containing genes discussed elsewhere in this review in order of frequency include 3q28 (tumor protein p63 (*TP63*), altered in 77% of samples), 3q24.1 (transforming growth factor beta receptor 2 (*TGFBR2*), 36%), 10q23.31 (phosphatase and tensin homolog (*PTEN*), 31%), 18q21.2 (SMAD family member 4 (*SMAD4*), 28%), and 7p11.2 (epidermal growth factor receptor (*EGFR*), 17%) [18]. Greater numbers of copy number variations were reported in cervical squamous cell carcinomas than in cervical adenocarcinomas [18]. A review of cervical squamous cell carcinomas from other datasets as well as limited information on HPV-positive vulvar squamous cell carcinomas also showed gains at 3q (55%), losses at 3p (36%), and losses at 11q (33%) [33]. A study of CIN3 lesions and invasive cancers reported an average of 36.3 copy number alterations in cancers, with the most frequent amplification at 3q (50% of cancers and 25% of CIN3) [34]. Notably, this region contains the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene, which is the most commonly mutated gene in HPV-driven cancers across sites (see below). Losses were most common in 3p (40% of cancers and 10% of CIN3) [34]. A summary of copy number alterations reported in HPV-driven cancers can be found in Table 1. Figure 2 shows the frequency of chromosomal amplifications and deletions across the whole genome in cervical cancers from TCGA [18].

Table 1. Common copy number alterations in HPV-driven cancers.

Arm or Location ¹	Gene	Pathway	Alteration	Cancer Site ²	Fraction Altered in HPV+ Cancers (%)	References
1p	—	—	Gain	Cervix squamous	33	[35]
1q	—	—	Gain	Cervix squamous	29–36	[33,35]
	—	—	Gain	Cervix adeno	22–35	[33,35]
2q	—	—	Loss	Cervix squamous	22	[33]
3p	—	—	Loss	Cervix squamous	36–51	[33–35]
	—	—	Loss	Vulva	45	[33]
3p24.1	TGFBR2	TGFβ	Gain	Cervix	36	[18]
3p14.1	FOXP1	Transcription	Loss	Cervix squamous	42	[34]
3q	—	—	Gain	Cervix	66	[18,33,35]
	—	—	Gain	Cervix squamous	44–62	[33–35]
	—	—	Gain	Cervix adeno	29–39	[33,35]
	—	—	Gain	Vulva	58	[33]
3q25.32	MLF1	Phenotypic determination	Gain	Cervix squamous	60	[34]
3q26.32	PIK3CA	PI3K/AKT	Gain	Head and neck	30–56	[2,36]
3q26.33	SOX2	Transcription-Sox2	Gain	Head and neck	11–28	[2,36]
3q27.1	KLHL6	Immune signaling	Gain	Head and neck	1–25	[2,36]
3q27.3	BCL6	RTK-JAK-STAT	Gain	Head and neck	1–25	[2,36]
3q28	TP63	p53	Gain	Cervix	77	[18]
3q28	LPP	Cell-cell adhesion	Gain	Cervix squamous	60	[34]
4p	—	—	Loss	Cervix squamous	24–47	[33,35]
	—	—	Loss	Cervix adeno	10–46	[33,35]
	—	—	Loss	Vulva	27	[33]
4q	—	—	Loss	Cervix squamous	21–34	[33,35]
	—	—	Loss	Cervix adeno	17–42	[33,35]
4q31.3	FBXW7	Notch	Loss	Head and neck	3–12	[2,36]
5p	—	—	Gain	Cervix squamous	27–28	[33,35]
	—	—	Gain	Vulva	15	[33]
5p13.1	RICTOR	PI3K/AKT	Gain	Head and neck	4–6	[2,36]
6p	—	—	Loss	Cervix squamous	24	[35]
6q	—	—	Loss	Cervix squamous	20–29	[33,35]
7p	EGFR	RAS/EGFR/ERK	Gain	Cervix	17	[18]
8p	—	—	Loss	Cervix squamous	27	[35]
8q	—	—	Gain	Cervix squamous	25	[34,35]
8q24.21	MYC	TGFβ	Gain	Head and neck	3–6	[2,36]
9p	CD274	Immune response	Gain	Cervix	8	[18]
10q23.31	PTEN	PI3K/AKT	Loss	Cervix	31	[2,18,36]
			Loss	Head and neck	3–15	[2,36]
11p	—	—	Loss	Cervix squamous	32	[35]
	—	—	Loss	Cervix adeno	35	[35]
11q	—	—	Loss	Cervix squamous	32–33	[33,35]
	—	—	Loss	Cervix adeno	9–35	[33,35]
	—	—	Loss	Vulva	30	[33]
11q13.3	FGF19	RAS/EGFR/ERK	Gain	Head and neck	4–6	[2,36]
11q13.3	FGF3	RAS/EGFR/ERK	Gain	Head and neck	4–6	[2,36]
11q13.3	FGF4	RAS/EGFR/ERK	Gain	Head and neck	4–6	[2,36]
11q22.1	YAP1	Hippo	Gain	Cervix	16	[18]
13q	—	—	Loss	Cervix squamous	24–41	[33,35]
	—	—	Loss	Cervix adeno	21	[33]
	—	—	Loss	Vulva	12	[33]
13q14.2	RB1	Rb	Loss	Head and neck	6–24	[2,36]
14q	—	—	Gain	Cervix squamous	26	[35]
14q32.32	TRAF3	NF-κB	Loss	Head and neck	14	[2]
14q32.33	AKT1	PI3K/AKT	Gain	Head and neck	5	[2]
16p	—	—	Loss	Cervix adeno	33	[35]
16p13.13	BCAR4	Hedgehog	Gain	Cervix	7	[18]
16q	—	—	Loss	Cervix adeno	45	[35]
17p	—	—	Loss	Cervix squamous	34	[35]
18q	—	—	Loss	Cervix adeno	54	[35]
18q21.2	SMAD4	TGFβ	Gain	Cervix	28	[18]

Table 1. Cont.

Arm or Location ¹	Gene	Pathway	Alteration	Cancer Site ²	Fraction Altered in HPV+ Cancers (%)	References
19p	—	—	Loss	Cervix adeno	30	[35]
19q	—	—	Gain	Cervix squamous	23	[35]
	—	—	Gain	Cervix adeno	32	[35]
20p	—	—	Gain	Cervix squamous	33	[35]
	—	—	Gain	Cervix adeno	26	[35]
20q	—	—	Gain	Cervix squamous	31	[35]
Xp11.3	KDM6A	Chromatin organization	Loss	Head and neck	3–7	[2,36]

¹ Ordered by chromosome arm or location. ² Cervix is listed when references did not differentiate between squamous cell carcinoma and adenocarcinoma. Sites other than cervix always refer to squamous cell carcinoma. Abbreviations: Adeno, adenocarcinoma; TGFBR2, transforming growth factor beta receptor 2; TGF β , transforming growth factor beta; FOXP1, forkhead box P1; MLF1, myeloid leukemia factor 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; SOX2, SRY-box 2; KLHL6, kelch like family member 6; BCL6, B-cell CLL/lymphoma 6; RTK-JAK-STAT, RTK, receptor tyrosine kinase-Janus kinase-signal transducer and activator of transcription; TP63, tumor protein p63; LPP, LIM domain containing preferred translocation partner in lipoma; FBXW7, F-box and WD repeat domain containing 7; RICTOR, RPTOR independent companion of MTOR complex 2; EGFR, epidermal growth factor receptor; RAS, retrovirus-associated DNA sequences; ERK, extracellular signal-regulated kinases; MYC, MYC proto-oncogene, bHLH transcription factor; PTEN, phosphatase and tensin homolog; FGF19, fibroblast growth factor 19; FGF3, fibroblast growth factor 3; FGF4, fibroblast growth factor 4; YAP1, Yes associated protein 1; RB1, RB transcriptional corepressor 1; Rb, retinoblastoma; TRAF3, TNF receptor associated factor 3; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; AKT1, AKT serine/threonine kinase 1; BCAR4, breast cancer anti-estrogen resistance 4; SMAD4, SMAD family member 4; KDM6A, lysine demethylase 6A.

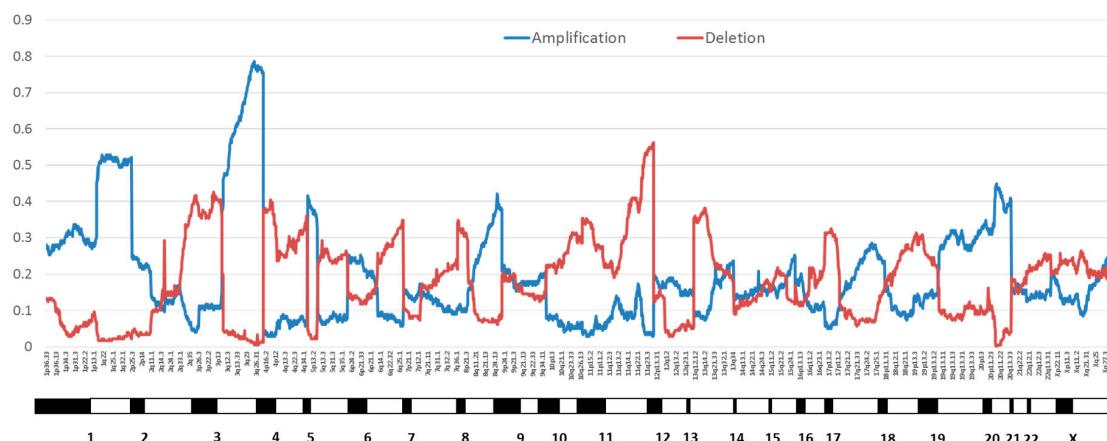


Figure 2. Proportion of cervical cancers with copy number variation by chromosome position from The Cancer Genome Atlas (TCGA) cervical cancer data [18]. Amplifications are in blue and deletions are in red.

In HPV-positive head and neck cancers, significant copy number losses have been reported in 22 genes and gains in 65 genes, including RB transcriptional corepressor 1 (*RB1*) and *PIK3CA* [37]. The 3q26-28 region is amplified in both HPV-positive and HPV-negative cancers, while 3p deletions are primarily found in HPV-negative head and neck cancers [37].

In penile cancers, greater copy number gains in 15 regions and losses in four regions are seen in HPV-positive compared to HPV-negative cancers [38]. Autosomal copy number variations are most frequently observed on chromosomes 3 and 8, including losses in 3p and gains in 3q, and are also associated with worse prognosis [38]. A small study of HPV-positive anal cancers reported recurrent gains in 17q, 3q, 19p, and 19q [39].

In HPV-driven cancers of the cervix and head and neck, copy number variations often co-localize with sites of viral integration [2,18], a phenomenon that occurs in many HPV-associated cancers, and has been shown to vary by HPV type [40–42]. Though the mechanisms by which HPV integrates

into the host cell genome are not well understood, these events tend to occur at regions of genomic instability [34,42–45]. It has been proposed that copy number alterations commonly occur in regions of genomic instability, which in turn may promote viral integration in those locations, explaining why viral integration is more common at sites with copy number alterations than expected by chance [34]. Viral integration has also been observed in short regions of HPV and host genome sequence homology (i.e., “micro-homologies”), suggesting a potential role for DNA repair processes to integrate HPV and host cell genomes based on nucleotide sequence similarities [45,46].

Recurrent large chromosomal rearrangements have been reported in 23 locations in cervical cancers in TCGA [18]. One notable recurrent rearrangement is the 16p13 zinc finger CCCH-type containing 7Abreast cancer anti-estrogen resistance 4 (*ZC3H7A-BCAR4*) fusion, which together with copy number gain of the locus containing *BCAR4* (16p13.13, found in 20% of tumors) and duplication detected by whole genome sequencing suggest a potential role of this gene in cervical carcinogenesis [18].

HPV-driven cancers of the cervix, head and neck, and penis share copy number alteration sites, most notably copy number gains in 3q, which in addition to *PIK3CA* contains the telomerase RNA component (*TERC*), *MDS1* and *EVI1* complex locus (*MECOM*), SRY-box 2 (*SOX2*), and *TP63* genes [18,34,37,38]. It is worth noting that both HPV-positive and HPV-negative cancers display recurrent focal amplifications of this region [2]. Together with the extremely high somatic mutation rate of *PIK3CA* (see Section 3.2), this supports an important role for *PIK3CA* in HPV-mediated carcinogenesis.

2.2. Mutational Signatures

2.2.1. APOBEC

The APOBEC family of cytosine deaminases causes cytosine to thymine or guanine mutations [47–49]. APOBEC3B, a subclass of these proteins, causes characteristic mutations that are enriched in many cervical and head and neck cancers [18,35,50–52]. During DNA repair, APOBEC-mediated cytosine deamination can result in characteristic mutational signatures that occur at motifs involving a thymine immediately 5' to the target cytosine, collectively referred to as “TCW” mutations, where W corresponds to an A or T [52]. APOBEC-mediated mutagenesis is also enriched in HPV-positive subsets of many head and neck cancers [53] as well as in penile cancers [54] suggesting the activation of APOBEC enzymes in HPV-driven cancers across sites.

APOBEC-associated mutations are responsible for many mutations of genes in the HPV-associated carcinogenesis pathways discussed below, including common *PIK3CA* point mutations [53]. APOBEC signature enrichment was reported in 150 of 192 exomes in TCGA cervical cancer data, with the fraction of ABOPEC signature mutations by gene reproduced in Figure 3 [18].

The APOBEC pathway drives mutations in many cancer sites including cervix, head and neck, bladder, lung, and breast [51,52]. However, APOBEC mutations are likely enriched in HPV-positive cancers due to its role in the host response to the viral infection. The APOBEC3A protein may inhibit HPV infectivity, so upregulation assists in viral clearance and reduces persistence [32], although it has also been suggested that APOBEC3B is likely to be the primary APOBEC involved in HPV-related carcinogenesis because unlike APOBEC3A it is expressed in the nucleus [51]. The APOBEC mutagenesis pathway has also been reported to be upregulated by the HPV oncogene E6 [55]. Upregulation of APOBEC proteins in response to viral infection can cause “collateral damage” to the host DNA [56]. However, the exact mechanism of induction of the APOBEC pathway and its contribution to carcinogenesis once activated remain unclear, since it is also found in many cancer types not associated with infectious agents, including breast cancer and ovarian serous carcinoma [57–59]. Due to insufficient data from cancer precursors, it is currently not clear at what stage in the carcinogenic process APOBEC mutations start to accumulate and whether APOBEC mutations occur before non-APOBEC mutations.

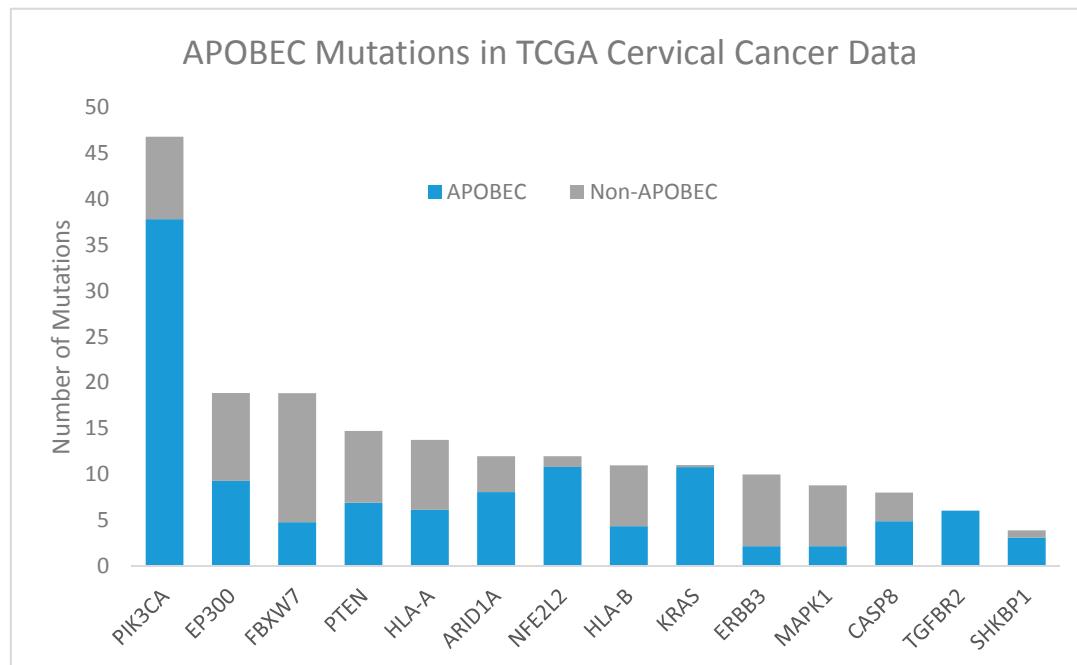


Figure 3. Apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC, blue) and non-APOBEC (gray) mutations in significantly mutated genes in TCGA cervical cancer data [18]. Abbreviations: PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; EP300, E1A binding protein p300; FBXW7, F-box and WD repeat domain containing 7; PTEN, phosphatase and tensin homolog; HLA-A, human leukocyte antigen A; NFE2L2, nuclear factor, erythroid 2 like 2; ARID1A, AT-rich interaction domain 1A; HLA-B, human leukocyte antigen B; KRAS, KRAS proto-oncogene, GTPase; ERBB3, erb-b2 receptor tyrosine kinase 2; MAPK1, mitogen-activated protein kinase 1; CASP8, caspase 8; TGFBR2, transforming growth factor beta receptor 2; SHKBP1, SH3KBP1 binding protein 1.

2.2.2. Other Mutational Signatures

Cervical cancer, which has an attributable risk for HPV of close to 100% [1], has two primary mutational signatures, classified as signature 1B and signature 2 by Alexandrov et al. [50]. Signature 2 is the above-discussed APOBEC signature. Signature 1B is a common pattern across many cancer sites that is characterized by cytosine to thymine mutations at methylated cytosine-guanine (CpG) sites along the DNA and is associated with age [50]. Other cancers associated with signature 1B include head and neck, the only other HPV-associated cancer characterized by this study, as well as ovarian and endometrial, the other major gynecological cancers [50].

3. Genes and Pathways

Many somatic mutations overlap across HPV-associated cancer sites. Frequently somatically mutated genes are summarized in Table 2. In the following sections, common mutations are discussed in the context of their respective pathways.

Table 2. Common Somatic Mutations in HPV-Driven Cancers.

Gene ¹	Pathway	Mutation	Cancer Site ²	Fraction Mutated in HPV+ Cancers (%)	References
<i>PIK3CA</i> ³	PI3K/AKT	Activating	Cervix squamous	6–42	[18,35,60–69]
			Cervix adeno	10–42	[18,35,60–69]
			Head and neck	22–56	[2,36,37]
<i>EGFR</i>	RAS/EGFR/ERK	Activating	Cervix squamous	3–33	[18,62,70,71]
			Cervix adeno	6	[18,62,70]
<i>SMAD4</i>	TGFβ	Inactivating	Cervix	28	[18]
<i>ERBB2</i>	PI3K/AKT	Activating	Cervix squamous	4	[18]
			Cervix adeno	26	[18]
<i>TP53</i>	DNA repair	Inactivating	Cervix squamous	5	[35]
			Head and neck	0–25	[2,3,36,37,72]
			Vulvar	8–16	[73,74]
<i>RB1</i>	Rb	Inactivating	Head and neck	6–24	[2,36,37]
<i>FGFR2 & FGFR3</i>	RAS/EGFR/ERK	Activating	Head and neck	1–24	[2,36,37]
<i>KRAS</i>	RAS/EGFR/ERK	Activating	Cervix squamous	4	[18,35,62]
			Cervix adeno	8–23	[18,35,62,75]
			Head and neck	6	[37]
<i>MLL2</i>	Chromatin organization	Activating	Head and neck	10–20	[2,36]
<i>ASXL1</i>		Inactivating	Head and neck	5–19	[36]
<i>NOTCH1</i>	Notch	Activating, inactivating	Head and neck	6–18	[2,36,37,76,77]
<i>EP300</i>	TGFβ	Inactivating	Cervix squamous	13–16	[18]
			Cervix adeno	10	[18]
			Head and neck	10–14	[36]
<i>ERBB3</i>	PI3K/AKT	Activating	Cervix squamous	4	[18]
			Cervix adeno	16	[18]
<i>ATM</i>	DNA repair	Inactivating	Head and neck	1–16	[36]
<i>FBXW7</i>	Notch	Inactivating	Cervix	11–15	[18,35]
			Head and neck	3–12	[36]
<i>PTEN</i>	PI3K/AKT	Inactivating	Cervix squamous	6–13	[18,35]
			Cervix adeno	13	[18,35]
			Head and neck	3–15	[2,36]
<i>BRCA1</i>	DNA repair	Inactivating	Head and neck	2–14	[36]
<i>NF1</i>	RAS/EGFR/ERK	Inactivating	Head and neck	0–14	[2,36]
<i>ELF3</i>	PI3K/AKT	Inactivating	Cervix adeno	13	[35]
<i>FLG</i>			Head and neck	12	[2]
<i>BRCA2</i>	DNA repair	Inactivating	Head and neck	3–12	[36]
<i>LRP1B</i>	RTK		Head and neck	2–12	[36]
<i>HRAS</i>	RAS/EGFR/ERK	Activating	Head and neck	1–12	[36]
<i>HLA-A/B</i>	MHC	Inactivating	Cervix	6–9	[18,35,78]
			Head and neck	11	[2,37]
<i>MLL3</i>	Chromatin organization	Activating	Head and neck	10	[2]
<i>TGFBR2</i>	TGFβ	Inactivating	Cervix squamous	8	[18]
<i>CREBBP</i>	TGFβ	Inactivating	Cervix squamous	8	[18]
			Cervix adeno	6	[18]
<i>TRAF3</i>	NF-κB	Truncating mutations	Head and neck	8	[2]
<i>MAPK1</i>	MEK/ERK	Activating	Cervix squamous	8	[18,35]
<i>CBFB</i>	RUNX1/RUNX2	Inactivating	Cervix adeno	8	[35]
<i>DDX3X</i>		Inactivating	Head and neck	8	[2]
<i>ARID1A</i>	Chromatin organization	Inactivating	Cervix	7	[18]
<i>NFE2L2</i>		Inactivating	Cervix squamous	4–7	[18,35]
<i>TPRX1</i>			Head and neck	6	[2]
<i>CYLD</i>	NF-κB	Inactivating	Head and neck	6	[2]
<i>RIPK4</i>	NF-κB, Notch		Head and neck	6	[2]
<i>UBR5</i>	Proteolysis		Head and neck	6	[2]
<i>CASP8</i>	Fas apoptosis	Inactivating	Cervix	4	[18]
<i>STK11</i>	PI3K/AKT	Inactivating	Cervix squamous	4	[35]
<i>SHKBP1</i>	RAS/EGFR/ERK		Cervix	2	[18]

¹ Ordered by higher reported fraction mutated. ² Cervix is listed when references did not differentiate between squamous cell carcinoma and adenocarcinoma. Sites other than cervix always refer to squamous cell carcinoma.

³ Significantly mutated genes in TCGA cervical cancer data are bolded [18]. Abbreviations: ERBB2, erb-b2 receptor tyrosine kinase 2; KRAS, KRAS proto-oncogene, GTPase; MLL2, lysine methyltransferase 2D; ASXL1, additional sex combs like 1, transcriptional regulator; EP300, E1A binding protein p300; ERBB3, erb-b2 receptor tyrosine kinase 3; ATM, ATM serine/threonine kinase; BRCA1, BRCA1, DNA repair associated; NF1, neurofibromin 1; ELF3, E74 like ETS transcription factor 3; FLG, filaggrin; BRCA2, BRCA2, DNA repair associated; LRP1B, LDL receptor related protein 1B; HRAS, HRas proto-oncogene, GTPase; HLA-A/B, human leukocyte antigen A/B; MHC, major histocompatibility complex; MLL3, lysine methyltransferase 2C; CREBBP, CREB binding protein; MAPK1, mitogen-activated protein kinase 1; CBFB, core-binding factor beta subunit; RUNX1/RUNX2, runt related transcription factor 1/2; DDX3X, DEAD-box helicase 3, X-linked; ARID1A, AT-rich interaction domain 1A; NFE2L2, nuclear factor, erythroid 2 like 2; TPRX1, tetrapeptide repeat homeobox 1; CYLD, CYLD lysine 63 deubiquitinase; RIPK4, receptor interacting serine/threonine kinase 4; UBR5, ubiquitin protein ligase E3 component n-recognition 5; CASP8, caspase 8; STK11, serine/threonine kinase 11; SHKBP1, SH3KBP1 binding protein 1.

3.1. Lack of Mutations in TP53 and RB1

The HPV oncogenic proteins E6 and E7 target the tumor suppressor proteins p53 and pRB, respectively, for degradation [79]. They therefore obviate the need for somatic deactivation of the *TP53* and *RB1* genes during the carcinogenesis process, and mutations in these genes infrequently occur in HPV-positive cancers compared to corresponding HPV-negative cancers at the same sites (Figure 1).

In cervical squamous cell carcinoma, *TP53* mutations have been reported with a frequency of 5% [35]. Although fewer than 1% of cervical squamous cell carcinomas are HPV-negative, one study reported a difference in *TP53* mutation status by classifying tumors in the TCGA-CESC data set as “HPV active” (expressing HPV transcripts; 4% *TP53* mutation rate) versus “HPV inactive” (not expressing HPV transcripts; 47% *TP53* mutation rate and 8% of the total number of HPV-positive samples) [80]. This is consistent with the idea that *TP53* inactivation is exceedingly common, and that the *TP53* mutation rates are negatively correlated with HPV activity. Vulvar squamous cell carcinoma has an 8–16% *TP53* mutation prevalence in HPV-positive tumors versus 30–76% prevalence in HPV-negative tumors, and vulvar intraepithelial neoplasia (VIN) precancerous lesions have a 3% *TP53* mutation prevalence in HPV-positive and 21% prevalence in HPV-negative lesions [73,74]. Likewise, *TP53* mutations appear to be more prevalent in HPV-negative than in HPV-positive penile squamous cell carcinomas [31].

Numerous studies have reported significantly higher *TP53* mutation rates in HPV-negative (52–86%) compared to HPV-positive (0–25%) head and neck tumors [2,3,36,37,72]. A complete absence of *TP53* mutations in tumors with high-risk HPV types present has also been found in laryngeal [81] and esophageal [82] cancers. It has been suggested that *TP53* inactivation, either through HPV infection or somatic mutation, is nearly ubiquitous in head and neck squamous cell carcinomas, even those that are HPV-negative and therefore must achieve this inactivation via other pathways [3].

Head and neck cancers with wild-type *TP53* have a better prognosis than those with *TP53* mutations [2,83]. HPV positivity and p16^{INK4A} expression, which are both related to retention of wild type *TP53*, are also positively correlated with overall 3-year survival in anal cancers [84]. Evidence in penile cancers is mixed [85–88].

The Rb pathway controls the cell cycle and regulates growth and proliferation [89]. *RB1* mutations are very rare in cervical cancers because HPV E7 activity inactivates Rb tumor suppression activity by disrupting its interaction with the transcription factor E2F, making mutations in this gene unnecessary in HPV-positive cancers [90,91]. *RB1* is mutated in 6–24% of HPV-positive head and neck cancers, a similar fraction to HPV-negative head and neck cancers (4%) [2,36,37]. Cyclin dependent kinase inhibitor 2A (*CDKN2A*) encodes p16^{INK4A}, an Rb pathway gene which as described above is nearly ubiquitously expressed in HPV-positive cancers due to activation of a negative feedback loop triggered by E2F release [92,93]. Overexpression of p16^{INK4A} is also common in HPV-related precancers, which has led to development of p16^{INK4A}-based biomarkers for cervical cancer screening and triage [94,95]. *CDKN2A* is rarely altered in HPV-positive (0%) compared with HPV-negative head and neck cancers (25% mutation rate, frequent alterations in 9p21.3 chromosomal region containing the *CDKN2A* gene) [2]. An absence of *CDKN2A* alterations in HPV-positive penile squamous cell carcinomas has also been reported, compared with 16% mutation prevalence and 24% copy number reduction in HPV-negative tumors [31].

3.2. PI3K/AKT Pathway

PIK3CA is a part of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, a very commonly disrupted pathway observed across several cancer sites that is involved in the regulation of cell growth, proliferation, differentiation, glucose metabolism, protein synthesis, and apoptosis [96–99] (Figure 4).

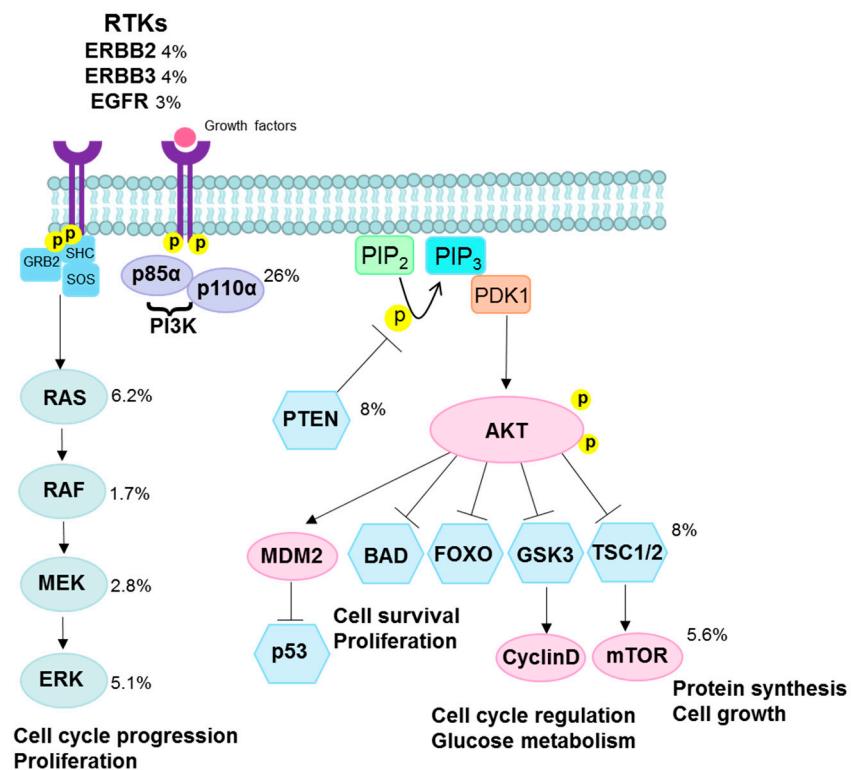


Figure 4. PI3K/AKT and RAS/EGFR/ERK pathways. Class IA PI3K are heterodimers consisting of a p85 α regulatory subunit and a p110 α catalytic subunit (encoded by PIK3CA). The p85 α regulatory subunit normally stabilizes p110 α and inhibits its catalytic activity. Activation of the PI3K pathway via ligand binding to transmembrane RTKs such as EGFR, ERBB2, and ERBB3, results in phosphorylation of p85 α and activation of the p110 α catalytic subunit. Once activated, PI3K phosphorylates PIP₂ at the plasma membrane to produce the lipid second messenger, PIP₃. This step is inhibited by PTEN, which dephosphorylates PIP₃ to PIP₂. PIP₃ binds to PDK1 which phosphorylates and activates AKT. Activated AKT phosphorylates TSC1/2, leading to mTOR activation and increased protein synthesis and cell growth. AKT increases cell proliferation by phosphorylating GSK3 which normally regulates the degradation of cyclin D. In addition, activation of AKT promotes cell survival by inhibiting proapoptotic factors such as BAD and FOXO transcription factors, and by phosphorylating MDM2 which antagonizes p53-mediated apoptosis. Other PI3K activation pathways depend on adaptor proteins such as GRB2, which binds to and activates SOS, stimulating RAS and independent activation of p110 α . A Ras-binding domain in p110 α also mediates activation by RAS. RAS-mediated recruitment to the plasma membrane activates RAF, which in turn activates MEK and ERK, respectively. ERK phosphorylates several proteins that control cell proliferation and cell cycle progression. Somatic mutation frequencies in cervical squamous cell carcinomas are shown next to each gene [18]. Abbreviations: RTKs, receptor tyrosine kinases; ERBB2, erb-b2 receptor tyrosine kinase 2; ERBB3, erb-b2 receptor tyrosine kinase 3; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3-kinases; AKT, protein kinase B; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; PIK3CA, phosphatidylinositol 3-kinase catalytic subunit alpha; PDK1, phosphoinositide-dependent kinase; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; TSC1/2, T-cell leukemia 1 and 2; GSK, glycogen synthase kinase; BAD, Bcl-2-associated death promoter; FOXO, forkhead box, O subclass; MDM2, mouse double minute 2 homolog; SHC, Src homology 2 domain-containing; GRB2, growth factor receptor-bound protein 2; SOS, son of sevenless; RAS, retrovirus-associated DNA sequences; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinases.

PIK3CA encodes p110 α , the catalytic subunit of PI3K, and is considered an oncogene; mutations and copy number variations of *PIK3CA* and other related genes in this pathway can contribute to unchecked growth, invasion, and metastasis [100]. *PIK3CA* is the most frequently mutated gene in HPV-positive cancers, with frequencies ranging from 6 to 42% in cervical squamous cell carcinoma, 10–42% in cervical adenocarcinoma, and 22–56% in HPV-positive head and neck cancers [2,18,35–37,60–69].

The most common *PIK3CA* mutations occur in “hotspots” E542K and E545K in the helical domain (exon 9) of p110 α . Mutations in these sites have been shown to increase phosphatidylinositol 3,4,5-trisphosphate (PIP₃) levels, activate downstream effectors such as phosphoinositide-dependent kinase (PDK1) and AKT, and promote cellular transformation. Although the mechanisms by which these mutations activate PI3K signaling are not fully understood, current data suggests these mutations block the inhibitory effect of the p85 α regulatory subunit on p110 α activity [101]. In HPV-positive head and neck and cervical squamous cancers, mutations in *PIK3CA* are almost exclusively found in E542K (c.1624G > A) and E545K (1633G > A) corresponding to a C to T single base change at a TCW motif, indicative of APOBEC-induced mutagenesis [35,53,102–105]. In contrast, these mutations are less common in HPV-negative head and neck cancers, suggesting that APOBEC activity is the major source of *PIK3CA* mutations in HPV-driven carcinogenesis. Evidence from a limited number of studies suggests that these mutations may represent a late event in cervical carcinogenesis [63,67,105]; however, a comprehensive deep-sequencing study of cervical precancers has not been conducted.

PTEN is a cell cycle regulator that inhibits rapid cell growth and functions as a tumor suppressor [106]. Signaling of the PI3K pathway is regulated by *PTEN* through dephosphorylation of PIP₃ (Figure 4) [107]. *PTEN* mutations are less frequent than *PIK3CA* mutations but are found in 6–13% of cervical carcinomas and 6–10% of HPV-positive head and neck cancers [2,18,35,36]. High rates of concurrent *PIK3CA* mutations with *PTEN* loss have been documented in HPV-positive tumors, ranging from 24 to 56% in head and neck cancers to over 80% in anal cancers [99,108]. In the context of *PTEN* loss or deficiency, helical mutations in *PIK3CA* have been shown to induce tumorigenesis through AKT-dependent signaling; whereas in tumors with intact *PTEN*, helical mutations in *PIK3CA* have been shown to promote cell growth and transformation through AKT-independent pathways involving PDK1 and its substrate serine/threonine protein kinase family member 3 (SGK3) [109].

Overall, more than 50% of cancers of the cervix and anus have at least one mutation in the PI3K/AKT pathway [110]. Similarly, mutations in this pathway have been reported in 61% of HPV-positive head and neck cancers (and a similar number of HPV-negative head and neck cancers) [2]. The average across all solid tumors was 38%, suggesting that compared with the known driver mutations in other cancers, PI3K pathway alterations are uniquely high in HPV-driven cancers [110]. It is interesting to note that *PIK3CA* is also commonly mutated in endometrial and some ovarian cancers [111,112], which could make it a hallmark of gynecological cancers as well as of HPV-driven cancers.

3.3. Human Leukocyte Antigen

Human leukocyte antigen (HLA) alleles are important components of host cell-mediated immune responses to viral infections and are essential to the major histocompatibility complex (MHC) immune response pathway. *HLA-A* and *HLA-B* are MHC class I molecules that present viral antigens on the cell surface to alert the immune system to infection [113] (Figure 5). Germline HLA variants have been associated with cervical cancer and with HPV-positive oropharyngeal cancer susceptibility [19–21]. Somatic mutations are found in *HLA-A* in 8% and *HLA-B* in 6–9% of cervical squamous cell carcinomas [18,35]. In a small study evaluating cervical cancer cell suspensions, 90% of tumors showed some *HLA* gene alterations including gene mutations, loss of heterozygosity, and other genetic changes [78]. *HLA* alterations are found frequently in cervical precancers as well, suggesting that it is an early event in cervical carcinogenesis [114]. Rates of *HLA-A/B* mutations are somewhat more common in HPV-positive (11%) than HPV-negative (7%) head and neck cancers [2,37]. Loss of *HLA-A*

or HLA-B could lead to loss of presentation of tumor antigens and immune cell recognition. One small study reported frequent mutations in the HLA pathway-associated transporter associated with antigen processing (TAP) gene (52%) in cervical carcinomas [115]. However, another candidate gene study failed to replicate this finding [116] and the large cervical cancer studies did not identify recurrent mutations in this gene [18,35]. Given the observed associations of both germline and somatic changes with the antigen presentation pathway, it is clear that it plays an important role in the host response to viral invasion that can alter the probability of persistence and potentially subsequent steps in the carcinogenesis process.

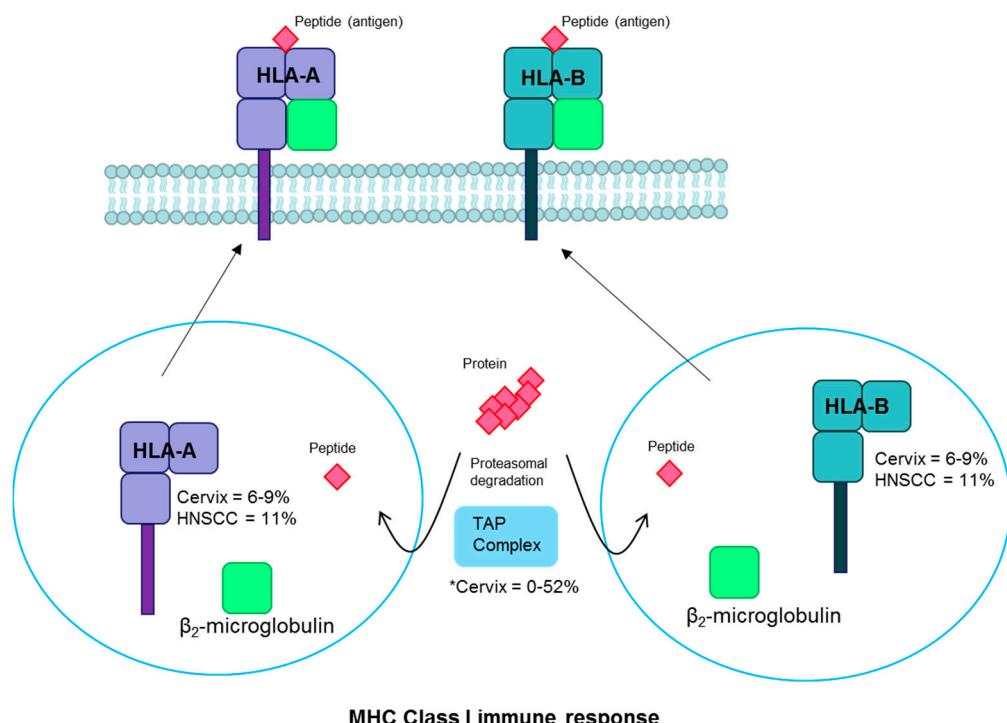


Figure 5. HLA pathway. Proteins undergo proteasomal degradation and the resulting peptides are transported to the endoplasmic reticulum by the TAP complex. There they are bound with MHC Class I into HLA-A or HLA-B and bound to β_2 -microglobulin. The complex is transported to the plasma membrane, where the peptide antigen is displayed for cytotoxic T-cell recognition. Fraction of cervical and head and neck cancers with each gene mutated are noted [2,18,115,116]. * There are conflicting reports of TAP mutation prevalence [115,116]. Abbreviations: MHC, major histocompatibility complex; HLA, human leukocyte antigen; TAP, transporter associated with antigen processing; HNSCC, head and neck squamous cell carcinoma.

3.4. Transforming Growth Factor Beta Pathway

The transforming growth factor beta (TGF β) pathway inhibits DNA synthesis and plays a tumor suppressor role, although it can also promote cancer progression once carcinogenesis has been initiated [117–119]. Inhibition of this pathway by the HPV oncoprotein E7 contributes to early tumor development in HPV-positive cervical and head and neck cancers [120–123] (Figure 6). Commonly mutated genes in the TGF β pathway include *TGFB2* (a receptor), CREB binding protein (*CREBBP*) and E1A binding protein p300 (*EP300*) (activators), and *SMAD4* (a transcription factor and tumor suppressor), and mutations in at least one of these genes have been reported in 30% of cervical squamous cell carcinomas [18]. In contrast, among TGF β genes, only *EP300* was in the top 30 mutated genes in head and neck squamous cell carcinomas [36]. This implies that somatic alterations in *TGFB2*, *CREBBP*, and *SMAD4* may be cervical squamous cell carcinoma-specific, although E7-driven expression effects in the TGF β pathway may still play a role in carcinogenesis in other HPV-positive

cancers. SMAD4 downregulation is also associated with HPV-negative head and neck cancers [124], and SMAD signaling pathway alterations have been found in both HPV-positive and HPV-negative tumors [37].

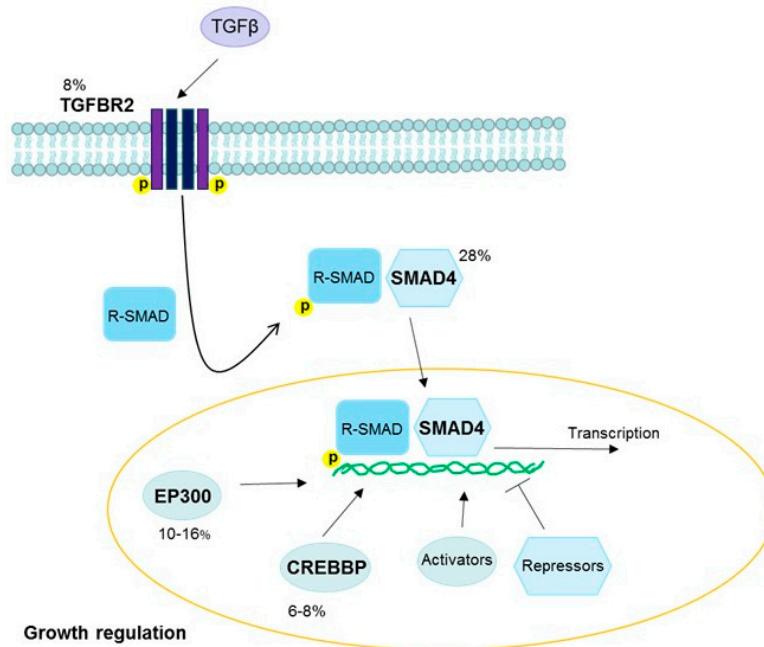


Figure 6. TGF β pathway. TGF β binds to TGFBR2 and other receptors to form a complex which becomes phosphorylated. This triggers the phosphorylation of R-SMADs. The phosphorylated R-SMADs form a complex with SMAD4 and are transported into the nucleus, where they promote transcription by binding to promotor regions of the DNA. EP300 and CREBBP are two activators commonly mutated in HPV-driven cancers, and many other activators and repressors also act to regulate this pathway. Fractions of cervical cancers with each gene mutated are noted [18]. Abbreviations: TGF β , transforming growth factor beta; TGFBR2, TGF β receptor 2; R-SMAD, receptor-regulated SMAD; EP300, E1A binding protein p300; CREBBP, CREB binding protein.

3.5. Notch Pathway

The Notch signaling pathway is responsible for cellular differentiation. Mutations in the *NOTCH1* receptor are found in both HPV-negative (12–26%) and in HPV-positive (6–17%) head and neck cancers, albeit somewhat more frequently in HPV-negative tumors, and are not commonly reported in cervix or other HPV-driven cancer sites [2,36,37,76,77]. This mutation may, therefore, be specific to head and neck carcinogenesis rather than to HPV infection, and *NOTCH1* has indeed been reported as a driver gene in oral tumorigenesis independent of HPV status [125]. F-box and WD repeat domain containing 7 (*FBXW7*) is involved in angiogenesis through regulation of the Notch pathway [126] and is mutated at higher rates in cervix (11–15%) and HPV-positive head and neck (12%) squamous cell carcinomas than in combined head and neck squamous cell carcinomas (HPV status not specified) (5%) [18,35,36].

3.6. RAS/EGFR/ERK Pathway

The RAS/EGFR/ERK (retrovirus-associated DNA sequences/ epidermal growth factor receptor/extracellular signal-regulated kinases) pathway is involved in cellular proliferation and survival (Figure 3). It consists of a signaling cascade that regulates transcription of genes affecting many functions including differentiation, growth, and senescence, which can contribute to carcinogenesis [127]. KRAS proto-oncogene, GTPase (*KRAS*) is an oncogene in which mutations are found in 8–23% in cervical adenocarcinomas but rarely in cervical squamous cell carcinomas [18,35,62,75]. The mutation rate of *KRAS* in head and neck cancers is 6% [37]. In contrast, *EGFR* is a tumor suppressor in the same

pathway in which mutations are found in 3–33% of cervical squamous cell carcinomas but rarely in cervical adenocarcinomas [18,62,70,71]. Other genes in this pathway are mutated in fewer than 10% of HPV-positive tumors except for *FGFR2* and *FGFR3*, which have combined mutation rates of 10–17% in HPV-positive head and neck cancers [2,18,35–37]. This is notable because, as kinases, the *FGFR* genes may potentially be therapeutic targets [37].

3.7. Other Genes

The tumor necrosis factor (TNF) receptor associated factor 3 (*TRAF3*) is involved in viral immune responses [128] and was recently reported to have truncating mutations (8%) or deletions (14%) in HPV-positive head and neck cancers [2]. It is not commonly mutated in cervical cancers [18], and it remains to be investigated whether this gene is mutated in HPV-positive cancers at other sites. Other genes differentially mutated in HPV-positive versus HPV-negative head and neck squamous cell carcinomas include *E2F1*, a cell cycle related gene more commonly mutated in HPV-positive cancers (19% versus 2%), and *FAT* atypical cadherin 1 (*FAT1*) and ajuba LIM protein (*AJUBA*), two genes involved in differentiation that are more commonly mutated in HPV-negative cancers (32% versus 3% and 7% versus 0%, respectively) [2].

4. Discussion

While HPV infection is a necessary cause of many cancers, the interplay between the virus and the host cell is what ultimately causes cancers to develop. There are many similarities across sites in the mechanisms and mutations found in HPV-driven cancers, suggesting that mechanisms are likely to be similar in rarer cancers such as penile and vaginal carcinomas in which it is difficult to complete large genomic studies. For example, one recent candidate gene study found no statistically significant differences in gene mutations in any of 48 candidate genes including *PIK3CA*, *EGFR*, *NOTCH1*, and *KRAS* or copy number alterations in any of six candidate genes across HPV-positive squamous cell carcinomas at four anatomical sites [99]. While HPV-positive cancers share many characteristic mutagenesis mechanisms and somatic mutations, there are also site-specific aspects. The other major gynecological cancers, endometrial and ovarian cancer, share with cervical cancer high rates of *PIK3CA* mutations and APOBEC and signature 1B mutational signatures. HPV-positive and HPV-negative tumors arising in the head and neck also share properties such as recurrent focal amplifications of the 3q26–28 chromosomal region. Recent data have shown that HPV genetic variation is very common and that HPV variant sublineages influence the risk of different histologic types of cervical precancer and cancer. It will be important to study the interplay between viral genetics and host genomic changes to better understand HPV-driven carcinogenesis [129,130].

Characterizing somatic mutations in HPV-related carcinogenesis could be highly relevant for early detection, prognosis, and treatment. To date, very few studies have attempted to characterize the somatic landscape of precancerous lesions, none comprehensively [63,74,131]. Several important steps are required to develop early detection assays based on somatic mutations. First, the sequence of somatic mutation events in the transition from precancers to cancers needs to be established. Next, a promising panel of mutations needs to be selected and evaluated in cervical cytology samples. Similar efforts have been evaluated for other gynecological cancers [132].

In addition to early detection, somatic characterization can be important for prognosis and targeted treatment strategies. For example, *PIK3CA*-mutated cervical cancers have worse prognosis than cancer with wild-type *PIK3CA* [61]. Site-specific mutations in *PIK3CA* have been shown to have varying responses to treatment, with evidence suggesting a greater response to PI3K/AKT/mTOR pathway inhibitors for tumors with mutations in the H1047R kinase domain (which are not commonly found in cervical cancers) compared with mutations at other sites [133]. Another prospective therapeutic target is *BCAR4*, in which amplifications and gene fusions have been found in cervical cancer and which is targeted by lapatinib [18,134]. *CD274* and *PDCD1LG2* are immunotherapy targets with amplifications reported in cervical cancer [18]. Erb-b2 receptor tyrosine kinase 2 (*ERBB2*;

HER2) and erb-b2 receptor tyrosine kinase 3 (*ERBB3*; *HER3*) are mutated in a subset of cervical adenocarcinomas and these tumors may be susceptible to targeted therapies, and *PTEN* and AT-rich interaction domain 1A (*ARID1A*) alterations are also potential targets [18]. The PI3K/AKT and TGF β signaling pathways, at least one of which is altered in over 70% of cervical cancers, are very promising in that targeted therapies may be broadly applicable due to their high prevalence [18]. The development of somatic marker panels for HPV-driven cancers will enable oncologists to more precisely tailor treatments.

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