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REVIEW ARTICLE

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The role of Hippo-YAP signaling in squamous cell carcinomas

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Abstract

The Hippo-YAP pathway regulates organ size, tissue homeostasis, and tumorigenesis in mammals. In response to cell density, external mechanical pressure, and/or other stimuli, the Hippo core complex controls the translocation of YAP1/TAZ proteins to the nucleus and thereby regulates cell growth. Abnormal upregulation or nuclear localization of YAP1/TAZ occurs in many human malignancies and promotes their formation, progression, and metastasis. A key example is squamous cell carcinoma (SCC) genesis. Many risk factors and crucial signals associated with SCC development in various tissues accelerate YAP1/TAZ accumulation, and mice possessing constitutively activated YAP1/TAZ show immediate carcinoma in situ (CIS) formation in these tissues. Because CIS onset is so rapid in these mutants, we propose that many SCCs initiate and progress when YAP1 activity is sustained and exceeds a certain oncogenic threshold. In this review, we summarize the latest findings on the roles of YAP1/TAZ in several types of SCCs. We also discuss whether targeting aberrant YAP1/TAZ activation might be a promising strategy for SCC treatment.

KEYWORDS

cervical cancer, esophageal cancer, head-and-neck cancer, hippo-YAP pathway, lung cancer, squamous cell carcinomas

1 | INTRODUCTION

Stratified squamous epithelium (SSE) consists of layers of squamous epithelial cells arranged over top of a basement membrane. The outermost layers of the nasal-oral cavity, esophagus, trachea, bronchi, cervix, vagina, and skin are all SSE. SSE constantly interacts with various stimuli, including harmful stresses. In so doing, SSE becomes the origin of several human malignancies, including esophageal squamous cell carcinoma (ESCC), head-and-neck SCC (HNSCC), cutaneous (skin) SCC (CuSCC), lung SCC (LSCC), and cervical SCC (CvSCC), which are among the most common forms of human cancers. This transformation is often associated with dysregulation of the Hippo-YAP intracellular signaling pathway. In this review, we provide an overview of how Hippo-YAP signaling drives SCC onset and development and discuss how this knowledge might lead to new therapeutic avenues for these insidious cancers.

2 | GENETIC ALTERATIONS IN SCCS

The most common genomic alterations in human SCCs are summarized in Table 1.[1-12] The frequencies of these mutations differ among different SCC types. CvSCCs have the lowest mutation rate

TABLE 1 DNA alteration frequencies in various human SCCs

Gene name	Alteration[a] frequency (%)					
	HPV$^+$ HNSCC[1-3]	HPV$^-$ HNSCC[1-3]	CvSCC[4,5,12]	CuSCC[6,7,12]	ESCC[8,9,12]	LSCC[10-12]
Cell cycle control						
TP53	3-4	81-84	5	65-95	83-93	73-84
RB1	6	3-4	4-6	18-27	2-9	7-16
MYC	3	9-14	5	5-10	2-9	10
SRC			2	5-21		7
CDKN2A	0	21-58	2	45-46	5-20	44
CDKN2B	0	43	1	3	6	28
CCND1	31	3	3-8	33-46	14	
FBXW7	4-22	6	16	10-23	4-8	4
E2F1	19	2	5	8-18	14	5
CDK6	0	8	2	3-8		5
RTK						
EGFR	0-6	12-15	5	14-31	1-25	2-9
FGFR1	0-1	2-10	2	10-31	1	7-19
FGFR2	4	0-2	4	7-15	1-4	2-4
FGFR3	11-12	1-2	2	13-31	33	2-5
DDR2	2-6	3-5	5	18-51		7
ERBB2	3	3-4	0-6	18-44		2-6
IGF1R	0	4	4	13-33		4
EPHA2	2-3	4-5	5	15-21	1	2
MET	0	2	8	21-23	1	4
MAPK-PI3K pathway						
HRAS	0-4	3-5	1	20-26		3
KRAS	6-11	0-1	4	7-10	4	2-5
NRAS	0	1	1	5-8		1
BRAF		3	1	18-26	1-2	4-5
MAPK1			8	3		4
NF1	0-6	3	8	15-56	2-4	11-14
PIK3CA	22-56	13-34	14-20	10-23	5-17	49
PIK3R1	0-3	1	3-4	5-15	1	4
PIK3CG	0	7	2	33-44	3	11
PIK3CB	13	13	10	13-31		17
PTEN	2-6	4-12	6	7-10	2-5	20
AKT1	5	7	3	8-10	1-16	4
AKT2			5	3		4-8
AKT3			3	8	1	3-16
RICTOR	11	0	7	10-18	1	16
Cell death						
BIRC2	3	7	13	5	1	2
FADD	6	32	4	3		15
TRAF3	22	1	5	13-18		4
CASP8	3	11	5-12	23-36		4
BCL2L1	11	0	5	3-5	15	7

(Continues)

TABLE 1 (Continued)

Gene name	Alteration[a] frequency (%)					
	HPV$^+$ HNSCC[1-3]	HPV$^-$ HNSCC[1-3]	CvSCC[4,5,12]	CuSCC[6,7,12]	ESCC[8,9,12]	LSCC[10-12]
Cell differentiation						
TP63	28	19	4-23	15-17		16-41
SOX2	16	6	20	10		21-48
NOTCH1	8-17	16-26	7	40-64	9-14	8
NOTCH2	0	9	5-20	50-62	4-5	4-10
NOTCH3			4-5	36-54	2-6	6-10
NOTCH4			4-8	26-41		5
MAML1			3	10-23		3
JAG1		11	3	8-26		4
JAG2		9	3	13-33		5
Transcriptional regulation						
EZH2			1	8-10	1	3
EP300			13-16	34-38	8-10	5
KMT2D (MLL2)	18	14	69-79	12-19	20-24	
KMT2C (MLL3)	6	19	38-62	4-8	17	
NSD1	2	10	6	18-21	2-4	8
MED1			4-5	26-44	1	4
DDX3X			4-6	5-13		3
Oxidative stress						
NFE2L2	0	14	4-7	0-7	5-10	15-19
KEAP1	0	5	3	8	3-4	12-16
CUL3	0-3	6-10	3	3-23	1-2	7-8
Hippo-YAP related pathway						
FAT1	3	32	11	44-62	11-15	20
FAT2			6	49-85	6-9	11
FAT3			7	21-72	7-11	18
FAT4			6	26-74	7	16
LATS1			4	13-18		4
LATS2			2	13-26		2
YAP1			10-16	5	6	2
WWTR1 (TAZ)		13	3		27	
AJUBA	0	7	2	18-26	2-7	2
NF2			3-5	10-17	1	2
SCRIB			2-6	23-44		9

[a]DNA alterations include somatic mutations and copy number alterations.

(4.2 mutations/megabase),[5] with CuSCC having the highest (50-60 mutations/megabase).[6,7]

TP53 is the gene most frequently altered across all SCCs, except SCCs caused by human papillomavirus (HPV) infection [HPV$^+$ SCCs] (Table 1). In general, HPV$^+$ SCCs display fewer mutations compared with HPV$^-$ SCCs, occur in younger people, and have a better prognosis.[1,4] HPV$^-$ SCCs tend to occur in older individuals with a history of tobacco and/or alcohol abuse, and almost always show *TP53* alterations. In addition, HPV$^-$ SCCs often bear mutations of *CDKN2A* and *CCND1* (Table 1). HPV$^-$ CuSCCs exhibit mutations of *KMT2C/D*, *RAS/RAF/AJUBA* or *NOTCH* more frequently than other HPV$^-$ SCCs. All SCCs commonly show some mutation/amplification of *PIK3CA/PTEN* or *TP63* (Table 1).

Although *TP53* shows the highest alteration rate in human SCCs, mice with loss of *TP53* alone never develop spontaneous SCCs.[13] Even when TP53 inactivation is coupled with K-RAS activation in the skin[14] or with Akt activation in the oral cavity,[15] SCCs take 6-7 mo to appear. Similarly, mice lacking TP53 or RB/p107/p130 develop carcinoma in situ (CIS) or invasive CvSCC only after estrogen exposure for 5-6 mo.[16,17] Thus, other essential gene mutations and/or epigenetic alteration(s)

appear to be required for SCC onset. The fact that clones with multiple mutations of the above-mentioned genes are present not only in human tumors but also in normal SSE bolsters this hypothesis.[18,19]

3 | OVERVIEW OF HIPPO-YAP SIGNALING

3.1 | Pathway elements

Hippo-YAP signaling (Figure 1) is triggered by changes to extracellular matrix (ECM) rigidity, external mechanical forces, high cell density, adherens junction or integrin-SRC signaling, or G-protein-coupled receptor signaling induced by certain growth factors or hormones.[20] The mammalian Hippo pathway core consists of the large tumor suppressor homolog (LATS) kinases and their adaptor proteins mps 1 binder kinase activator-1 (MOB1A/B), and the mammalian STE20-like protein (MST) kinases with their adaptor protein salvador homolog-1 (SAV1).[20] MOB1A/B binding to LATS kinases greatly enhances their kinase activities. The Hippo pathway terminates with the transcriptional co-factors Yes-associated protein-1 (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) [WW domain containing transcriptional regulator 1 (WWTR1)]. The function of YAP1 and TAZ is to bind to components of the tight and adherens junctions, including Angiomotin (AMOT),[21] α-catenin,[22] Protein Tyrosine Phosphatase, Non-Receptor Type 14 (PTPN14),[23] and Scribble.[24] This binding induces LATS to phosphorylate YAP1/TAZ, restricting them to the cytoplasm where E3-ubiquitin ligase $SCF^{\beta TRCP}$ ubiquitinates them and facilitates their proteasome-mediated degradation. Unphosphorylated YAP1/TAZ interact principally with nuclear TEA domain transcription factors (TEADs) that activate expression of target genes controlling cell growth.[20] Therefore, YAP1/TAZ

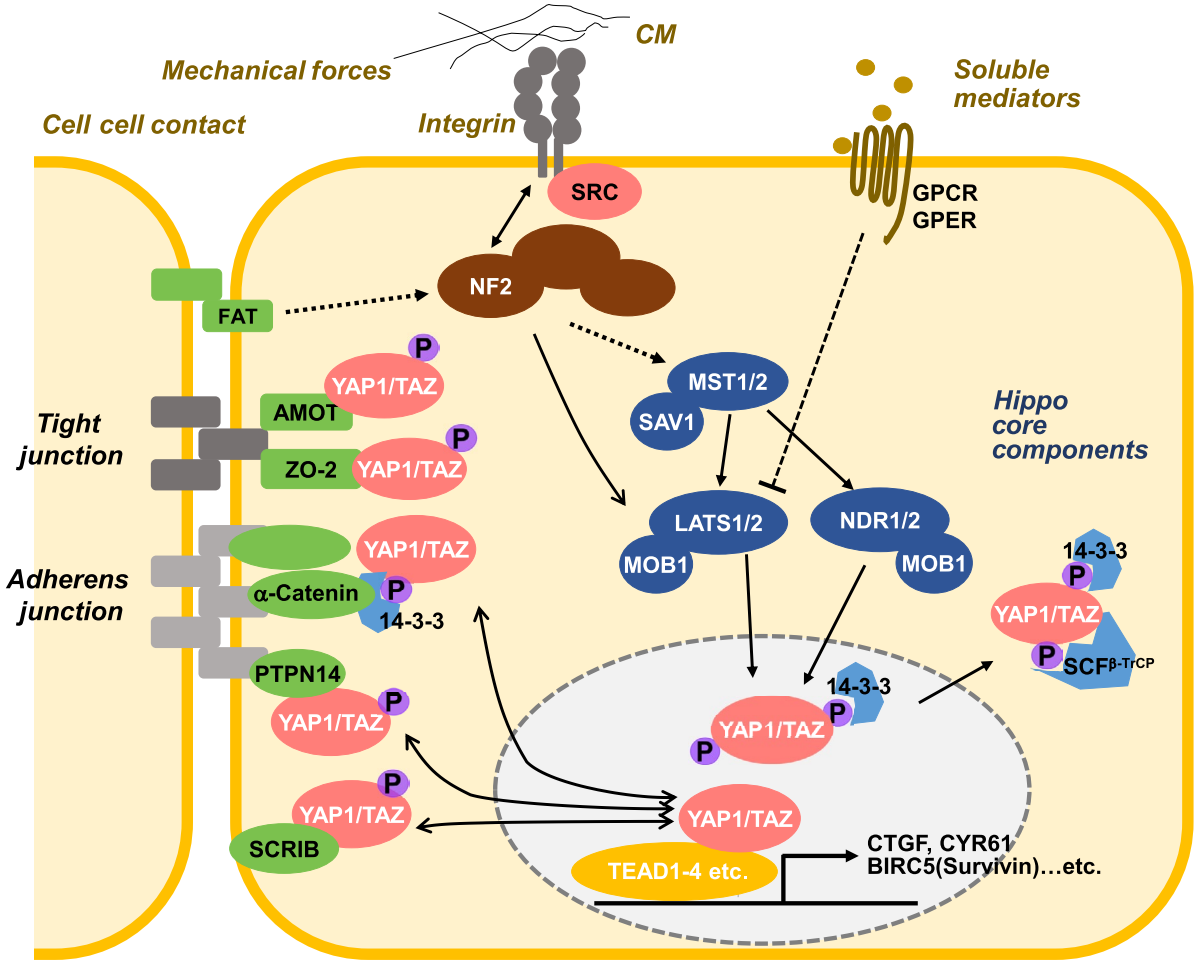

FIGURE 1 Mammalian Hippo-YAP signaling network. Hippo-YAP signaling is controlled by high cell density, external mechanical forces, rigidity of the ECM, integrin-SRC signaling, and the binding of certain growth factors or hormones to G-protein-coupled receptors. The core components of the mammalian canonical Hippo pathway are MST kinases, LATS/NDR kinases, the SAV1 adaptor and the MOB1 adaptor. When LATS/NDR phosphorylates YAP1/TAZ, these effectors bind to 14-3-3 proteins and are retained in the cytoplasm. Phosphorylated YAP1/TAZ also bind to $SCF^{\beta TRCP}$, promoting their degradation and preventing the activation of TEAD transcription factors driving the expression of cell survival/anti-apoptosis genes (such as *CTGF*, *CYR61*, *AXL*, *BIRC5*). Components of the tight and adherens junctions (such as AMOT, α-Catenin, PTPN14, Scribble) also bind to phospho-YAP1/TAZ to control their cytoplasmic localization and activity

generally promote cell proliferation in a manner negatively regulated by Hippo signaling.

3.2 | Interaction with cancer-related genes

Dysregulation of the Hippo-YAP pathway has been implicated in numerous cancer types.[25] The YAP1/TAZ-TEADs complex directly targets genes encoding growth factors and cytokines, including connective tissue growth factor (*CTGF*), cysteine-rich angiogenic inducer-61 (*CYR61*), and tyrosine-protein kinase receptor gene (*AXL*). Other targets are involved in the epithelial-mesenchymal transition, anti-apoptosis (BIRC5, DDIT4, Trail, NuRD), stem cell maintenance, and cell proliferation, invasion, and/or metastasis.[26] YAP1/TAZ also bind to other transcription factors driving morphogenesis, differentiation, proliferation and apoptosis, including PAX3, SMADs, P73, T-box transcription factor-5 (TBX5), RUNT-related transcription factors (RUNX1/2), erythroblastic oncogene-B4 (ERBB4) and NK2 Homeobox-1 (NKX2-1).[27] Reducing YAP1/TAZ activation has thus been contemplated as a treatment for YAP1/TAZ-driven tumors. Interestingly, however, even though the activated YAP1 protein itself frequently accumulates in tumors, actual mutation of the *YAP1* and *WWTR1* (*TAZ*) genes is uncommon in human cancers,[28] including in SCCs (Table 1).

3.3 | Crosstalk with SCC-related pathways

Hippo signaling participates in crosstalk with the WNT/β-catenin, NOTCH, SHH, SRC, TGFβ, PI3K/Akt and other pathways crucial for SSE morphogenesis and homeostasis.[29] Several lines of evidence link this interaction with general SCC carcinogenesis:

(1) Activated nuclear YAP1 that is recruited to TCF/LEF binding sites along with β-catenin enhances WNT signaling. Phosphorylated cytoplasmic YAP1 inhibits Disheveled (DVL), supporting β-catenin degradation that suppresses WNT signaling. Conversely, YAP1 mRNA is upregulated by WNT signaling.

(2) SHH pathway activation upregulates YAP1 mRNA, stabilizes YAP1 protein, and promotes nuclear YAP accumulation. SHH also induces nuclear translocation of the YAP/TEAD/IRS1 complex, which regulates GLI1/2.

(3) YAP1 upregulates transcription of the NOTCH ligand Jagged-1. Conversely, Jagged-1 appears to activate YAP, and γ-secretase inhibitors that abrogate NOTCH signaling also suppress YAP-induced intestinal dysplasia in mice.[30]

(4) Phosphorylated R-SMADs bind to YAP/TAZ, linking Hippo signaling to the bone morphogenetic protein receptor (BMPR) and TGF-β pathways. Generally speaking, nuclear YAP/TAZ enhances nuclear SMAD activity, whereas cytoplasmic YAP/TAZ limits nuclear SMAD accumulation and regulates sensitivity to TGF-β ligands.

(5) YAP1 is directly and indirectly activated by SRC signaling.[31,32] Dasatinib-mediated suppression of SRC prevents YAP1 activation.[33]

(6) AKT blocks MST1/2 activity, resulting in YAP activation.[29]

(7) Finally, a recent comprehensive study of genetic alterations across a range of SCCs also supports the importance of Hippo/YAP signaling in SCC carcinogenesis.[34]

4 | LINKS BETWEEN HIPPO-YAP SIGNALING AND SCC ONSET

In this section, we discuss recent findings linking Hippo-YAP signaling aberrations to specific SCC subtypes.

4.1 | Head-and-neck SCCs

The mammalian head-and-neck region comprises the oral cavity, pharynx, and larynx, all of which are covered with SSE. HNSCC is the 7th most common human cancer worldwide, with tongue cancer being the most frequent subtype. About 15% of HNSCC patients are infected with HPV, making this virus a key cause of HNSCC (although the underlying molecular mechanism is not clear).[35] HPV that integrates into the host cell genome generates the viral E6 and E7 proteins known to target p53 and RB, respectively, compromising tumor suppression.[36]

HPV^+ HNSCC is found principally in the oropharynx.[35] These patients tend to have better prognoses and some are even cured.[36] The remaining 85% of HNSCC patients that are HPV^- have poor prognoses because they do not benefit from either intensified chemo/radio therapy or current molecular targeting drugs.[36] As noted above, *TP53* is mutated in 84% of HPV^- HNSCC cases, with mutations of *EGFR*, *CDKN2A/B*, *FADD*, *FAT Atypical Cadherin1* (*FAT1*), *NFE2L2*, and *CCND1* also frequently observed. These mutations, including that of TP53 (3%), are rare in HPV^+ HNSCC. In contrast, mutations of *NOTCH*, *TP63*, and *PI3K/PTEN* are common in both HPV^- and HPV^+ HNSCC. Because the E6 oncoprotein of HPV efficiently inactivates TP53, TP53 inhibition is likely to be a critical tumorigenic event in HNSCC genesis. However, as noted above, mice lacking *TP53* alone never develop spontaneous HNSCC.[13,37]

Several lines of evidence tie YAP1 activation to HNSCC genesis:

(1) We generated mice with tongue-specific loss of *Mob1a/b* that led to constitutive activation of endogenous YAP1 in this tissue.[33] These mutants developed tongue CIS with surprising rapidity and exhibited invasive HNSCC within 4 wk of *Mob1a/b* deletion.

(2) YAP1 activation can be detected in human precancerous dysplasia in the tongue and is linked to decreased patient survival.[33]

(3) Lastly, 8.6% of human HNSCC show amplification of the 11q22 chromosomal region containing the *YAP1* locus.[38] Excess YAP1 activity is a feature of such malignant SCCs and leads to a poor prognosis.[38]

4.2 | Cervical SCCs

Cervical SCCs (CvSCCs) are the 4th most frequent cancer in women worldwide. CvSCC most often arises due to infection with high-risk HPV, but the complete molecular mechanism has yet to be elucidated.

Numerous studies implicate YAP1 in human CvSCC genesis:

(1) YAP1 activation or LATS1 inactivation is linked to heightened proliferation and invasiveness of CvSCC cell lines,[39] as well as with unfavorable histologic grading, early recurrence, and lymph node metastasis in patients.[40]

(2) Nuclear YAP1 protein is upregulated both in invasive CvSCCs and in precancerous high-grade squamous intraepithelial lesions (HSIL).[41]

(3) LATS1 protein is downregulated in 45% of CvSCC cases.[39]

(4) Human chromosomal region 11q22 is amplified in 16% of CvSCCs.[4]

In mice, uterus-specific loss of MOB1A/B leads to cervical CIS within 1 wk in a manner dependent on YAP1.[42] Additional p53 deficiency in these mutants produces invasive tumors within 3 wk. In contrast, transgenic (Tg) mice showing modest activation of YAP (S127A), a constitutively active mutant form of YAP1, exhibit invasive CvSCC only after 6-8 mo.[41] Tg mice that both lack YAP1 and express HPV16 under the control of the K14 promoter do not show the cervical phenotype of *HPV16* Tg mice alone.[42] The HPV E6 protein prevents the YAP1 protein from undergoing proteasome-mediated degradation.[41] HPV E7 protein binds to and targets PTPN14 for proteasome-mediated degradation, which likely relieves the cytoplasmic retention of YAP by PTPN14.[43,44] This combination of HPV E6/E7-induced accumulation of YAP protein plus its release to the nucleus leads to vigorous YAP activation that may promote human cervical epithelial cell proliferation and eventually CIS onset.[23,42] HPV E6 also inhibits p53, spurring the progression of CIS to invasive CvSCC.

4.3 | Cutaneous SCCs

About 20%-50% of human skin cancers are cutaneous squamous cell carcinomas (CuSCCs) arising from malignant transformation of epidermal keratinocytes in the cutaneous SSE. CuSCCs exhibit the highest mutation rate among SCCs,[6,7] with frequent alterations to TP53, NOTCH1, NOTCH2, KMT2D, FAT1-4 and HRAS/BRAF/AJUBA (Table 1). Mutations of CDKN2A (INK4A/ARF) and PI3KCA/PTEN are also common, as is amplification of tyrosine kinase receptor genes (Table 1).

Normal epidermal cell development and skin homeostasis are thought to be maintained by tight and adherens junctions and catenin-based cell adhesion structures that sense skin status via Hippo-YAP signaling.[22] YAP1 and TAZ act redundantly to regulate the proliferation of basal layer stem/progenitor cells and balance epidermal growth.[22,45] When the skin suffers a wound, keratinocytes boost both their proliferation and YAP protein.[45]

Little information is known about Hippo/YAP signaling in CuSCCs, but accumulating evidence suggests that YAP1 activation is involved in CuSCC genesis:

(1) LATS1 and LATS2 are mutated in 18% and 26% of CuSCCs, respectively[12].

(2) YAP protein levels are elevated in human CuSCCs and correlate with disease progression.[46]

(3) YAP promotes human CuSCC cell proliferation in vitro.[47]

(4) Tg mice expressing constitutively active YAP have thicker epithelium compared with controls and their immature keratinocytes display increased mitogenic activity.[22]

CIS forms in the skin of these mutants starting on day 9 after YAP activation and invasive CuSCC appears in 2-4 wk. Also in these Tg mice, epithelial scratch wounding leads to synergism between the wound healing response and YAP1 activation that promotes spindle cell SCC development.[48]

(5) YAP-deficient mice show delayed wound healing and epidermal shrinkage.[45] YAP deficiency also prevents the formation of AP-1-dependent CuSCCs in mice.[49]

4.4 | Esophageal SCCs

Esophageal cancer is the 6th leading cause of cancer-related deaths worldwide. Most patients are diagnosed only after the malignancy has become advanced because it is difficult to detect early and grows aggressively. Although esophageal adenocarcinoma (EADC) predominates in Western countries, esophageal SCC (ESCC) is the most common form in East Asia. Alcohol and smoking are key risk factors for ESCC, as are genetic polymorphisms that inactivate alcohol-metabolizing enzymes.[50] Like other HPV$^-$ SCCs, ESCCs usually exhibit mutations of genes involved in the cell cycle, apoptosis regulation, and histone modification, including *TP53* (93%), *FGFR3*, *CCND1*, *NOTCH1-3*, *CDKN2A*, and *KMT2D* (Table 1).

The risk of ESCC in Eastern China has been associated with certain polymorphisms of the *FAT4* gene, which acts upstream of the Hippo core. YAP1 protein is often overexpressed in ESCC[51] and correlates positively with ESCC histological grade, stage, and diameter, as well as with overall and disease-free patient survival.[51] YAP1 knockdown promotes the apoptosis of ESCC cells or suppresses their proliferation and invasion.[52] Fewer ESCC cells show properties of cancer stem cells in the absence of YAP1 activation.[53] Among human ESCCs, 65% show LATS downregulation and 62% display TAZ upregulation.[54] This profile correlates positively with histological grade, clinical stage, and lymph node metastasis of ESCCs as well as with decreased overall and progression-free patient survival. High TAZ expression is also associated with low LATS2 in human ESCCs. To date, whether YAP and TAZ differ in their roles in ESCC onset and development is unknown. In mice, loss of YAP1 specifically in the esophagus decreases esophageal progenitor cell proliferation and stratification. Conversely, Tg mice with constitutive *Yap1* expression show enhanced cell proliferation that induces abnormal stratification of esophageal layers at E18.5.[55] It is unclear if they would go on to develop esophageal CIS or invasive ESCC, but it is quite likely that these animals would die during the perinatal period before any malignancy could progress.

4.5 | Lung SCCs

Lung cancer is the 2nd most common cancer in humans and the most lethal. A lung cancer is classified as either a small-cell lung cancer (SCLC) or a non–small-cell lung cancer (NSCLC). NSCLCs

occur more frequently compared with SCLCs and have a worse prognosis. About 30% of NSCLCs are lung SCCs (LSCCs). LSCC is essentially incurable due to a current lack of effective treatments. Although lung adenocarcinomas (LADCs) often exhibit EGFR mutations or ALK fusions, LSCCs are not driven by these oncogenic changes and so rarely respond to agents targeting them.[56] Almost all LSCCs feature *TP53* alterations as well as mutations of oxidative stress response genes (*KEAP1*, *NFE2L2*), PI3K/PTEN pathway genes, squamous differentiation genes (*TP63*, *SOX2*), and cell cycle-related genes (*CDKN2A/B*) (Table 1).

In a mouse model, the trans-differentiation of LADCs into LSCCs was associated with LKB1 inactivation and led to malignant progression as well as drug resistance.[57] YAP activation levels differ between mouse LADCs and LSCCs, with the former demonstrating YAP1 hyperactivation. Indeed, forced YAP activation largely abolishes the LADC-to-LSCC transition,[58] suggesting that YAP functions may differ between these NSCLC subtypes. Work in mouse models and human LADC cell lines has confirmed that YAP hyperactivation promotes malignant progression of LADC.[59] In striking contrast, YAP activation in LSCCs downregulates GPX2 in a ΔNp63-dependent manner.[60] As a result, excessive intracellular reactive oxygen species (ROS) accumulate and kill the LSCC cells. Analyses of human cancer patient-derived xenograft (PDX) models have shown that higher YAP activation correlates positively with suppression of LSCC growth and negatively with lymph node metastasis.[61] These observations were unexpected, and why YAP activation would promote LADC growth but suppress LSCCs is still unknown. Importantly, these findings indicate that caution should be exercised in administering YAP inhibitors to LSCC patients.

5 | PUTATIVE MECHANISM BY WHICH HIPPO/YAP SIGNALING DRIVES SCC ONSET AND DEVELOPMENT

The above body of work establishes that MOB1 deletion or YAP1 activation induces CIS formation within 5-9 d in the context of HNSCCs, CvSCCs, and CuSCCs. This timeline is unusually rapid, given that most cancers initiate over a long period of multi-step carcinogenesis in which several mutated genes act additively or synergistically. However, it should be noted that: (a) a CIS diagnosis in these SCCs is obtained only after the dysplastic cells in the basal layer rise to the top layer of the epidermis; and (b) it takes about 7 d for MOB1 protein to be completely lost in conditional mutants.[33] Thus, dysplastic SCC cells may be created immediately after YAP1 activation. These factors have led us to propose that CIS in almost any SSE tissue is initiated when YAP1 activity is sustained and exceeds a certain oncogenic threshold (Figure 2). The resulting dysplastic cells are able to expand uncontrollably without any other genetic alterations, setting the stage for eventual SCC progression.

Studies of SCCs in the aforementioned tissues have pinpointed ongoing exposure to carcinogens such as smoking, HPV infection, and excessive epidermal growth factor (EGF) or estrogen, as well as abrasion of the mucosa due to mechanical irritation, as major causes of SCCs.[35] Each of these factors has been shown to activate YAP1 directly in 1 or more SSE cell types.[62-64] In addition, as detailed above, loss-of-function or gain-of-function mutations of genes that result in YAP activation are linked to SCCs.[62,65-67] When these gene mutations combine with carcinogens, YAP1 activity rises even higher.[33,42] In short, there is substantial evidence supporting our hypothesis that, for many SCCs, the original CIS

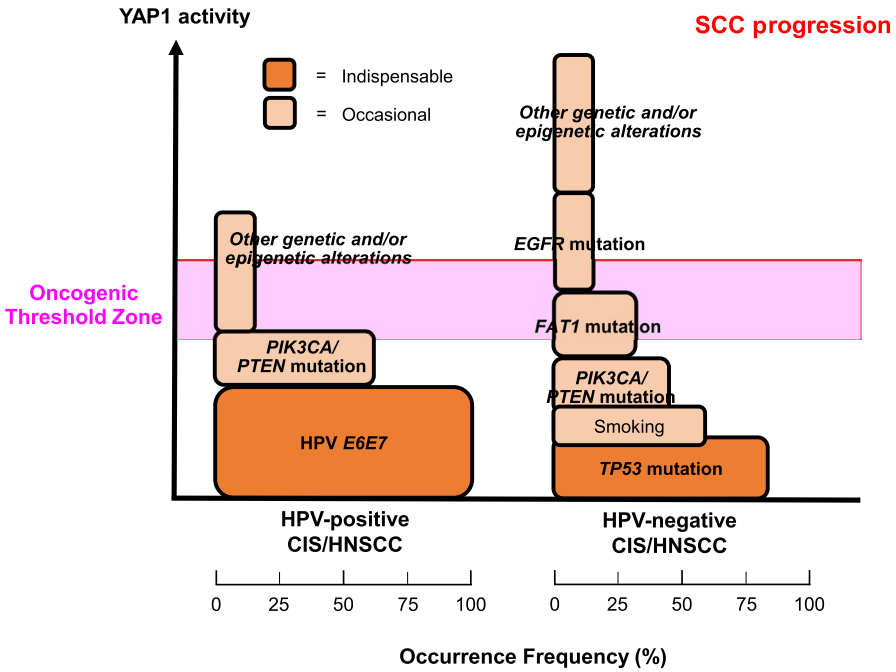

FIGURE 2 Proposed mechanism for SCC genesis using HNSCC as an example. Both HPV$^+$ HNSCCs and HPV$^-$ HNSCCs may be caused by an accumulation of YAP1 activity that is driven by either mutation of the indicated genes, HPV infection, or risk factors such as smoking. When the oncogenic threshold of YAP1 activity is surpassed, transformation ensues. Each rounded rectangle represents a factor promoting HNSCC genesis. The height of each rectangle represents relative YAP1 activation intensity,[33] and the width represents the frequency at which this gene or factor contributes to HNSCC malignancies

is caused by a simple sustaining of YAP1 activity over a particular threshold. When additional genetic and epigenetic alterations occur, the already elevated level of YAP activation accelerates SCC development. Depending on the degree of YAP1 activation induced by 1 alteration, the oncogenic threshold may be overcome with relatively few additional mutations. This scenario may explain why patients with HPV$^+$ HNSCC exhibit favorable therapeutic responses and have better prognoses. In the case of HNSCC, the HPV E6/E7 proteins are strong activators of YAP1, meaning that the threshold is exceeded by few other deleterious mutations to trigger the transition to SCC.[35] Considering that, in mice, YAP1 inhibition not only prevents SCC onset but also delays its progression, YAP1 may be an appealing target for molecular therapy of these devastating malignancies.

One possible reason for the frequent and early onset of SCCs in mice bearing mutated elements of the Hippo pathway is the activation of ΔNp63, the master regulator of proliferation, differentiation, and adhesion of SSE cells. By binding directly to ΔNp63 protein, activated YAP1 stabilizes this regulator.[68] Thus, YAP1 hyperactivation leading to abundant stabilized ΔNp63 may drive SSE cell differentiation and proliferation, possibly promoting 1st CIS and then SCC development. A 2nd reason for early SCC onset in Hippo-mutant mice may be elevated production of BMP4, a soluble growth factor that also regulates ΔNp63.[69] Indeed, we detected increased BMP4 in *Mob1*-deficient tongue and cervical epithelial cells compared to controls.[33,42] A 3rd reason for our findings may be potential positive feedback between YAP1 and EGF signaling. YAP1 promotes the transcription of EGF receptors such as EGFR and ERBB3 as well as that of various EGF-like ligands (NRG1, NRG2, HBEGF, AREG, TGFα).[62] Conversely, all EGF-like ligands can apparently activate YAP1,[62] so that an autocrine loop related to YAP1-EGF/EGFR signaling may stimulate SCC genesis and progression. A 4th contributor to our results may be an interaction between YAP1 and cell adhesion genes. Excessive YAP activation reduces the expression of integrin receptor genes and epithelial cell adhesion genes such as CDH1, removing critical negative regulators of SCC growth.[70,71] The proliferation of SSE cells, which are tightly connected to one another, is greatly influenced by cell adhesion, ΔNp63 signaling, and EGF-like ligands. Thus, all 4 of the above mechanisms may in fact contribute to the genesis of most SCCs (except LSCC), perhaps explaining why the phenotype is so strong in SSE cells showing YAP1/TAZ activation.

6 | CONCLUSION

An oncogenic driver gene for CIS formation in SCCs has been difficult to identify. We propose that, because YAP hyperactivation induces immediate CIS onset in mouse models, YAP must be the key molecule driving CIS with no need for any other gene alteration. We therefore advance a new concept positing that human CIS can simply initiate when sustained YAP1 activity exceeds an oncogenic threshold. SCCs are generally refractory to conventional treatments, and so remain largely incurable. Thus, targeting the Hippo-YAP pathway may be a promising strategy for suppressing the growth of most SCCs (except LSCCs, for the reasons elaborated above). In addition, our mouse models featuring mutations in the Hippo-YAP pathway currently constitute the world's fastest spontaneous cancer onset models. Cancer progression is synchronized in these mutant animals, and the tumors are easily visualized on the mouse exterior (especially in the HNSCC and CuSCC models). These characteristics are ideal for cancer research and the development of novel anti-cancer drugs, and we humbly take pride in sharing these new tools with the international scientific community to devise fresh approaches to treating human SCCs.

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DISCLOSURE

The authors have no conflicts of interest.

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