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REVIEW

Head and neck squamous cell carcinoma: a potential therapeutic target for the Wnt signaling pathway

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Abstract

Squamous cell carcinoma (SCC) of the head and neck region accounts for 3% of all tumors worldwide. The incidence is higher in men, with most carcinomas found in the oral cavity. At the point of initial diagnosis, distant metastases are rare. The Wnt signaling pathway is critically involved in cell development and stemness and has been associated with SCC. Understanding precisely how Wnt signaling regulates SCC progression and how it can, therefore, be modulated for the therapeutic benefit has enormous potential in the treatment of head and neck SCC. In this review, we will describe the underlying mechanisms of Wnt signaling and outline how Wnt signaling controls cellular processes both in homeostasis and in the development and progression of SCC.

Level of evidence: Not gradable.

Keywords Stem cells · Carcinoma · Wnt signaling

Introduction

Over 85% of cancers in the head and neck area are squamous cell carcinomas (HNSCCs), and the oral cavity is the most common location. [1] More than 500,000 new HNSCCs are diagnosed every year, placing them amongst the top ten cancers worldwide. [2] Etiologically, HNSCCs result from mutations in the DNA of keratinocytes. [3] Mutated keratinocytes are at risk of becoming pre-malignant or malignant cells with the potential for unregulated growth. [4] Once growth is autologous, HNSCCs develop. Growth of cancerous cells beyond the basement membrane leads to metastases in the lymph nodes, bones, brain, liver, and other organs. [5]

Mutations to keratinocytes can occur spontaneously, but often follow exposure to mutagenic substances. Mutagens may be chemicals (e.g., tobacco and alcohol), physical, or microbiological (e.g., viruses like human papillomavirus (HPV) or Epstein-Barr virus (EBV)). [6, 7] Radiation exposure, immunosuppression, and poor oral hygiene are all risk factors for HNSCC in the mouth and throat. [8] Worldwide, 25% of oral SCC is associated with tobacco consumption, and 7–19% is associated with alcohol consumption. [9] Combined consumption of alcohol and tobacco increases the risk of developing mouth or throat SCC sevenfold compared

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to the sole exposition to one of the noxious agents. [10] This synergistic effect may relate to how alcohol facilitates penetration of carcinogens into the oral mucosa as well as how chronic alcohol consumption extends the carcinogen-mucosal surface contact time due to reduced salivary gland function. [11]

Recent advances in sequencing technology and characterization of cancer genomes highlighted the involvement of the Wnt signaling pathway in human cancers including SCCs. [12] The Wnt signaling pathway is a complex and ancient signal transduction pathway critical in regulating cellular stemness and development throughout embryology and adult life. Consequently, Wnt signaling is ubiquitous to all cells, tissues, and organ systems and has been highly conserved across evolution, dating back to anaerobic metazoans. [13] A considerable amount of cancer research has centered on Wnt signaling; many of the critical protein regulators are defined, and recent genetic and biochemical studies have further identified novel Wnt pathway components and their functions [14, 15] including Wnt secretory machinery, Wnt co-receptors, components of the β-catenin destruction complex, and nuclear co-factors. [16] This review aims to discuss the involvement of Wnt signaling in the maintenance of stemness in cancer stem cells (CSC) with particular reference to HNSCC. We will then discuss the different therapeutic and diagnostic options for HNSCCs and shed light on the most promising therapeutic agent for the HNSCC treatment to date.

Wnt signaling pathways

Wnt-mediated signal transduction processes regulate basic processes that determine the functionality or dysfunction of numerous cell types. [17] Specifically, Wnt signaling is a critical regulator of embryonic development and the creation of embryonic cell patterns, as well as the differentiation, proliferation, invasion, polarity, and apoptosis of cells in adult organisms. [18, 19] Disorders of the Wnt signaling can result in various diseases, including cancer and osteoporosis. [20, 21]

To date, at least three different intracellular Wnt signaling pathways have been described; (1) the canonical Wnt/β-catenin signaling pathway, (2) the non-canonical pathways Wnt/PCP (planar cell polarity), and (3) the Wnt/Ca^{2+} signaling pathway. [22] Common to all three pathways are the Wnt proteins, which function as ligands to specific cell surface receptors (Frizzled receptors or Fzds), which trigger intracellular signaling cascades (Fig. 1). The term "canonical Wnts" came from the observation that strongly transforming Wnts transmit signals via β-catenin. [23] At first, it was assumed

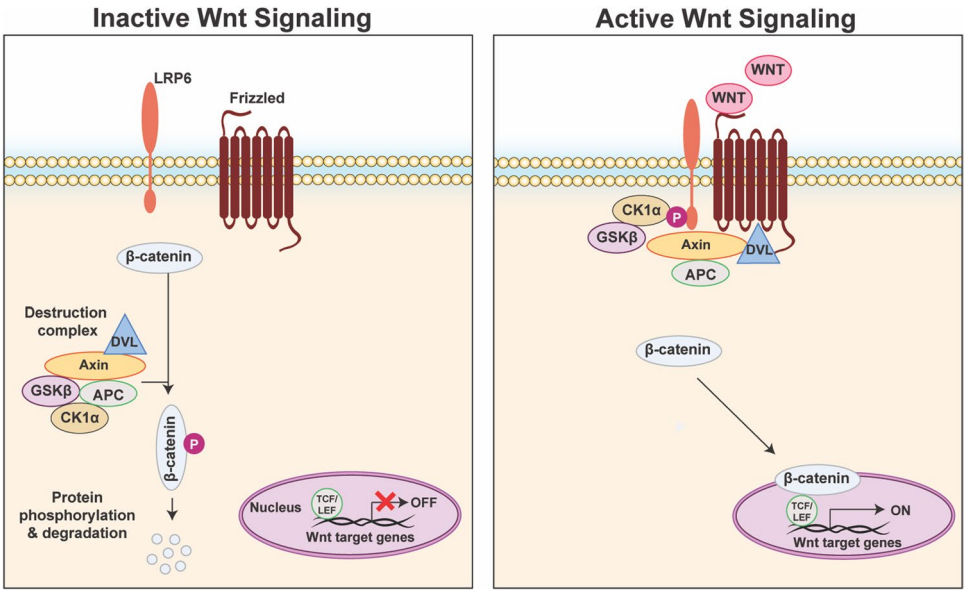

Fig. 1 Canonical Wnt signaling. *Left*: In the inactive state, Wnt ligands are absent, and intracellular β-catenin is phosphorylated by the destruction complex (a complex of Axin, APC, GSK3β, casein kinase (CK1α)). Phosphorylated β-catenin is then ubiquitinated and targeted for proteasomal degradation. The absence of intranuclear β-catenin allows the TCF (lymphoid enhancer factor)/LEF (lymphoid enhancer factor) proteins to promote histone deacetylases which leads to repression of target genes. *Right*: In the active state, secreted Wnt ligands bind Frizzled (Fzd) receptors and lipoprotein receptor-related protein (LRP) co-receptors leading to LRP phosphorylation by CK1α and GSK3β, and recruitment of disheveled (Dvl) proteins. The accumulation of Dvl proteins leads to their polymerization and activation which in turn inactivates the destruction complex. The cytoplasmic β-catenin is free to accumulate and translocate to the nucleus where it associates with LEF and TCF and recruits histone-modifying co-activators to promote gene expression and activation of numerous cellular processes

that all other Wnts signaled via β-catenin-independent signaling pathways and hence were termed "non-canonical Wnts." [24] However, the *canonical* Wnt3a can also signal independent of β-catenin by activating Rho and Rho-kinase. [25] In addition, under certain conditions, the non-canonical Wnt5a activates both a β-catenin-dependent and a β-catenin-independent signaling pathway. [26] Consequently, the classic distinction between canonical and non-canonical Wnts can no longer be strictly maintained.

The canonical Wnt/β-catenin signaling pathway primarily regulates the developmental trajectory of cells and the invasiveness and proliferation potential of CSCs. Binding of Wnts to Fzd receptors destabilizes a degradation complex that initiates the breakdown of β-catenin typically. Intracellular β-catenin consequently accumulates and translocates to the cell nucleus and acts as a transcription factor for various Wnt/β-catenin target genes. [16]

The two non-canonical signal pathways cannot be completely separated from one another. Although both pathways have been extensively studied in Drosophila and Xenopus, little is known about the function of these pathways in mammals [27]. It is known that the Wnt/PCP pathway participates in the control of cell polarity and cell movement during gastrulation. [28] In the Wnt/PCP signaling pathway, Wnt-Fzd binding activates disheveled (Dvl). Activated Dvl, in turn, can transmit the signal in two independent ways. [22] This is how Daam1 enables the complex formation of Dvl and Rho, a G protein, followed by the activation of Rho kinase (RhoK) via Rac, another G protein, Dvl leads in parallel to the activation of JNK (JUN N-terminal kinase). [29]

In the Wnt/Ca^{2+} signaling pathway, Wnt-Fzd binding leads to an increased intracellular Ca^{2+} concentration and activates heterotrimeric G proteins, which in turn activate phospholipase C and phosphodiesterase. [30] The high intracellular Ca^{2+} and activated G proteins also activate the calcium/calmodulin-dependent protein kinase II (CaMKII) and the protein kinase C. [31] It is suspected that the Wnt/Ca^{2+} signaling pathway regulates cell proliferation and migration and also functions to inhibit the Wnt/β-catenin pathway. CamKII and TAK1- activate NLK mitogen-activated protein kinase (NLK-MAPK), which then phosphorylates the T-cell factor (TCF) and thereby prevents the binding of the β-catenin/TCF complex to the DNA and thus prevents transcription. [32]

The molecular genetics of tumor development

The genetic changes predispose to tumor initiation and progression can occur as relatively small sequence changes to the DNA or chromosomal deviations. [33] The sequence changes affect two classes of genes: the proto-oncogenes and the tumor suppressor genes. [34]

Proto-oncogenes code for proteins that stimulate cell proliferation or progression through the cell cycle. They are converted into oncogenes by amplification of the gene locus, chromosomal translocation, or point mutations, i.e., to activated forms (gain of function), which results in uncontrolled growth of the cells. [35] The mutations in the proto-oncogenes are dominant, which means that the mutation in one of the two alleles is sufficient for the activation of the proto-oncogene. [36] Today, more than 100 known proto-oncoproteins have been described, including growth factors (e.g., PDGF, Wnt-1), their receptors (e.g., EGF receptors), proteins of signal transduction (e.g., Ras proteins, Src kinase), and transcription factors (e.g., c-Jun, c-Fos, c-Myc). [37]

Tumor suppressor genes, in contrast, have an inhibitory effect on cell growth, [38] and thus mutations that impair tumor suppressor function predispose to unregulated cell growth (i.e., cancer). Tumor suppressor gene function may be altered following faulty chromosome or chromatid division during cell division, mitotic recombination, or due to point mutations or deletions. [39] However, the functioning of both tumor suppressor gene alleles must be lost before tumorigenesis occurs; [40] this is referred to as a "loss of heterozygosity" (LOH). Some individuals inherited a mutated allele through the germline, and are thus at an increased risk for LOH and developing cancer. For example, individuals with familial adenomatous polyposis (FAP) syndrome are born with a mutated APC gene and show increased risk of colon cancer. [41] An alternative mechanism of tumor suppressor gene inactivation is epigenetic modification. Various studies have shown that the promoters of different tumor suppressor genes are often methylated in tumors but are free of methylation in healthy tissues. [42] The methylation of the DNA affects 5'-CG-3' dinucleotides (CpG), which are located in the promoter regions of the genes, [43] and methylation is thus involved in the regulation of gene expression and chromosome condensation. [44] Today, about 30 tumor suppressor genes with different functions are known, including APC, PTEN, SASH1, and p21CIP1/WAF1. [45] One of the most extensively studied tumor suppressor genes is p53, which is reported to be mutated in 50% of all cancer cells. p53 functions to either block the cell cycle or induce apoptosis, and is a transcription factor with an important role in maintaining genomic integrity, hence its name the "guardian of the genome." [46]

In addition to the classic oncogenes and tumor suppressor genes, there is a large number of additional "tumor genes" that act as modulators of tumor development or that play an essential role in clinical practice as diagnostic and prognostic tumor markers. [47] The chromosomal aberrations lead to aneuploidies — deviations from the normal diploid chromosome set. The changes can affect both the number of chromosomes and the structure of the chromosomes and lead

to chromosomal instability (CIN). [48] Genetic instability within the cell can also arise at the nucleotide sequence level (MIN, microsatellite instability). In this case, the tumor cell chromosome set is usually diploid. [49] Furthermore, gene function can also be modified at an epigenetic level, which recent research has increasingly highlighted. [50]

The importance of the Wnt signaling during the Embryonic development as well as in regeneration and tumor development

Cancer is not a single disease, but a term for many different types of disease. It is a genetic disease that is based on the uncontrolled growth of specific cells. Cancer is now the second leading cause of death after cardiovascular disease in western countries and the third leading cause of adult deaths in developing countries. [51] Tumor formation is a multi-stage process in which the physiological control of cell proliferation, cell differentiation, and cell–cell interactions are gradually lost. [52] Apart from the inherited forms, most tumors are caused by somatic mutations. [53] The majority of tumors are presumably monoclonal, meaning they arise from a *single cell* through the accumulation of several genetic and epigenetic changes through proliferation and clonal selection. [54] Tumors start as benign growths of cells maintained in differentiated states exhibition organization to their tissue architecture. As tumorigenesis progresses, additional genetic changes accumulate and lead to the formation of a malignant tumor comprised of poorly differentiated cells, which acquired the ability to penetrate the neighboring tissues and metastasize to distant organs. [55] Six changes in the physiology of the cell define the malignant phenotype: (1) independence from growth-promoting signals, (2) insensitivity to growth-inhibiting signals, (3) resistance to apoptosis, (4) unlimited multiplication potential, (5) persistent angiogenesis, and (6) ability to invade tissue and metastasize. [56]

The Wnt/β-catenin signaling regulates the development and function of many tissues and organs throughout embryonic development and in adult life. [57, 58] The Wnt pathways also interact with many other important signaling pathways, such as the transforming growth factor-β(TGF-β)/Bmp-, Fgf-, Notch-, and hedgehog signaling pathways, which influence each other either in series or in parallel. [19, 59] Even at a very early stage of embryonic development, before gastrulation, Wnt β-catenin signaling is critical for the induction of the mesoderm and the direction the correct formation of the embryonic body axis, [19] as highlighted by experiments where injection of Wnt agonists (Lithium Chloride or Wnt1 mRNA) induced a second body axis in Xenopus embryos. [60] The Wnt/β-catenin signaling pathway is also essential for the development of the primitive endoderm, formation of the head region, and development of many organs, as highlighted by various mouse models. [61] Activation of the Wnt/β-catenin signaling pathway is also necessary to maintain the pluripotency of embryonic stem (ES) cells and for the process of reprogramming differentiated fibroblasts into pluripotent stem cells (iPS). [62]

In certain organs, such as the pituitary gland, dorsal spinal cord, and bone, the Wnt/β-catenin pathway controls the formation, maintenance, and/or specification of progenitor cells. [63] For example, β-catenin/LEF/TCF induces the expression of Pitx2 in the pituitary gland, which is responsible for the formation of Pit1-expressing progenitor cells. [64] During bone development, β-catenin controls the differentiation of osteo-chondrogenic progenitor cells into osteoblasts. [65] The importance of the maintenance of progenitor cells is also observed in the development of the dorsal spinal cord, [63] where Wnt/β-catenin signaling activation promotes the proliferation of Olig3-expressing progenitor cells and to differentiate excess Foxd3 and Isl1/2-expressing interneurons (dI2 and 3 regions). [66] In contrast, the 0 mutation of β-catenin leads to the absence of progenitor cells expressing Olig3, and the loss of Olig3 prevents the formation of dI2- and dI3 neurons. [67] The combination of Olig3 null mutation and Stabilization of β-catenin causes the loss of the dI2 and dI3 neurons and the increase in Lbx1-expressing neurons (dI4-dI6) while maintaining the proliferative effect on the progenitor cells. [68] These results indicate that Wnt/β-catenin in the dorsal spinal cord is important for the formation and maintenance of Olig3-expressing progenitor cells, but not for differentiation into dI2 and dI3 neurons. [68]

In continuously renewing tissues and organs, the Wnt/β-catenin signaling pathway is responsible for the self-renewal of multipotent tissue stem cells. [69, 70] The intestinal stem cells of the intestinal crypts which continually renew the intestinal epithelium require active Wnt/β-catenin signaling for their proliferative function. [71] Newly formed intestinal cells lose the ability to divide, and the Wnt/β-catenin signaling pathway is switched off as they differentiate into terminal cells. [16] However, mutations that result in permanent activation of the Wnt pathway (e.g., involving APC or β-catenin genes) lead to the development of intestinal polyps, characteristic of early stages of colon cancer. [72] On the other hand, in the case of degenerative diseases of the intestinal mucosa, such as Crohn's disease, there is assumed to be insufficient Wnt/β-catenin activity. [73]

The skin is another organ that continuously renews throughout life. In embryology, Wnt signaling directs the embryonic ectoderm to differentiate into the epithelium by promoting production of keratin and blocking fibroblast growth factor (FGR). [74] Wnt signaling also inducts formation of skin appendages, especially hair follicles, by orchestrating dynamic singaling between the epidermis and dermis. [75] In adult life, Wnt/β-catenin signaling continues to

control hair follicle cycling by coordinating the differentiation of follicular stem cells into hair cells in the bulges of the hair follicle. [76] Within hair follicle stem cells, β-catenin is found within the nucleus during anagen (growth phase), but in the cell membrane during telogen (rest phase), and is thus activation of the Wnt pathway is thought to induce the onset of anagen from telogen. [77] Wnt signaling is then further thought to control cell fate within hair follicles. [78] In addition, Wnt/β-catenin is inhibited by the Bmp signaling pathway during the hair cycle — an important criterion for the oscillating renewal and differentiation of the hair follicle stem cells. [79] The signal pathway can induce the development of hair follicle tumors. It could also be shown that cancer stem cells from ras-induced epidermal tumors require active β-catenin for their self-maintenance, which illustrates the great importance of Wnt/β-catenin for the cancer stem cells of the skin. [80] β-catenin is also involved in epidermal proliferation and maintenance of the epidermal stem cell population, as shown in lineage-tracing work in mice. [81, 82] However, interestingly, loss of β-catenin leads to hair loss but has little impact on the integrity of the epidermis. [83] Wnt/β-catenin signaling also helps to orchestrate cutaneous wound healing. *In vitro* work has shown that human keloid keratinocytes stimulate fibroblasts to secrete R-Spondin2, a Wnt agonist which promotes epidermal proliferation and is thought to lead to the thickened epidermis characteristic of keloid scars. [84] Further work has also shown that TGF-β, a potent regulator of fibrosis, induces activation of β-catenin within dermal fibroblasts, and β-catenin staining is increased in hypertrophic scars and keloid, compared to normal skin. [85] Regarding skin cancer, and specifically SSCs, chromosomes containing WNT and FZD genes are amplified in SSCs in genomic hybridization studies. [86] Messenger RNA levels of Wnt ligands and receptors are increased, and levels of Wnt inhibitors are decreased, further implicating role for Wnt activation in SSC development. [87–89]

Together, these findings highlight the critical roles of the canonical Wnt signaling pathway in cell regulation in normal functioning and in the development of cancer through altering the regulation of cell expansion and survival of mature cells and stem cells by their direct and/or indirect target genes (Table 1).

Wnt/β-catenin signaling as a therapeutic target for HNSCC

Head and neck tumors are treated with surgery, radiotherapy, and chemotherapy. [90] Early-stage cancers are often resected entirely with adjuvant radiotherapy and/or without chemotherapy. Cancer too large to be removed can be debulked and are typically treated with a combination of radiotherapy and chemotherapy. Progress in all three therapeutic areas has been small and the average 5-year survival for patients with head and neck cancer remains at 61%. [91]

Although numerous Wnt receptors and Wnt signaling components are highly expressed in head and neck cancers, [13] there has been hesitation to target β-catenin therapeutically due to its structural role in apical junctions (AJs) and its functional role in CSC self-renewal. [16, 70] However, numerous Wnt inhibitors have been investigated for their antitumor effects in both preclinical and clinical trials. [92] The most promising candidates are inhibitors of porcupine, an acyl-transferase and critical protein in the Wnt/β-catenin cascade. For example, the porcupine inhibitor LGK974 can disrupt HNSCC growth and reduce distant metastases. [93, 94] Recent insight into the specific function of β-catenin within the cell nucleus, however, has supported its potential as a druggable target; [95] antibodies which target Wnt-1

Table 1 Elevated expression of upstream and downstream of Wnt signaling pathway genes and their associated diseases are reported

Gene transduction level	Gene	Disease (cell type)	Associated biological gene function
Upstream	WNT	Head and neck squamous cell carcinoma (cancer stem cell)	Self-renewal
	FZD		
Downstream	C-MYC	Colon cancer (cancer stem cell)	
	AXIN2	Breast cancer (cancer stem cell)	
	LEF1		
	OCT14	Non-disease (embryonic stem cell) Head and neck squamous cell carcinoma (cancer stem cell)	
	NANOG SOX2	Non-disease (embryonic stem cell)	
	MMP7	Oral squamous cell carcinoma (cancer cell	Metastasis
	TWIST	Breast cancer (immortalized human breast epithelial cell) Head and neck squamous cell carcinoma (cancer cell)	
	ABCB1	Colon cancer, neuroblastoma (cancer cell)	Drug resistance

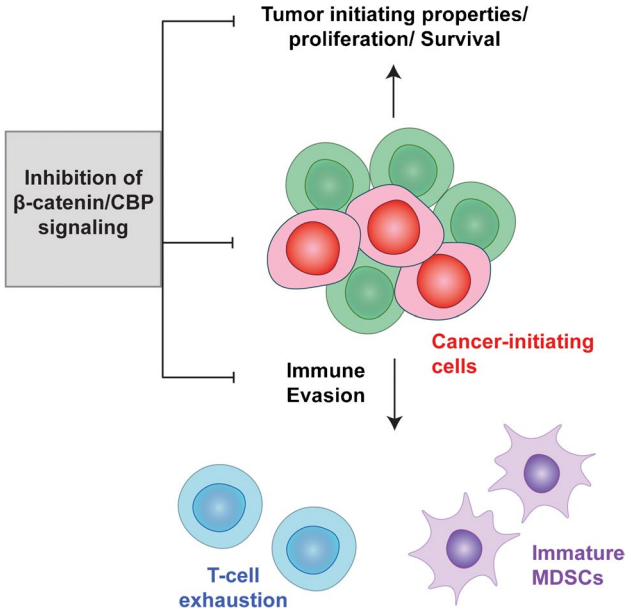

Fig. 2 Therapeutic targeting the β-catenin/cAMP-responsive element-binding protein (CBP) axis for head and neck squamous cell carcinoma (HNSCC). It is believed that inhibition of the association between β-catenin and CBP can deplete tumor cancer stem cells (CSCs), stimulate epithelial cell differentiation, deplete the exhausted T cells, immature heterogeneous cells of the myeloid lineage, and/or myeloid-derived suppressor cells (MDSCs) involved in immune evasion

and inhibit cell proliferation can decrease tumor burden, [12, 96] and β-catenin/CBP axis inhibitors can intercept oncogenic activities in the tumor niche. Axitinib is a small-molecular inhibitor of nuclear β-catenin which works by stabilizing ubiquitin ligase SHPRH (SNF2, histone linker, PHD, and RNIG finger domaincontaining helicase), and thus decreasing the availability of nuclear β-catenin. [97] The association of β-catenin with fibrosis and immune cell depletion suggests that targeting the β-catenin-CBP interaction will have intratumoral inhibitory effects (Fig. 2). It is likely that targeting oncogenic pathways in combination with Wnt/β-catenin signaling may be more effective than monotherapies and standard radiation therapy/chemotherapy for the treatment of HNSCC. For example, combining β-catenin/CBP axis inhibitors with immune checkpoint blockades may effectively deplete aggressive CSCs and promote anticancer immune cell function.[98]

Conclusions and future directions

Preclinical and early-phase clinical trials have highlighted the clinical utility of targeting the canonical Wnt signaling pathway to disrupt the malignant potential of CSCs. Cancer is a complex disease and Wnt signaling is ubiquitous, thus therapeutic manipulation a challenge. Specifically, since Wnt signaling regulates many essential activities within every cell during homeostasis, it is critical to develop inhibitors specific to cancer cells to avoid unfavorable side effects. The major hurdles facing the development of Wnt pathway antagonists include limited efficacy, non-specific binding, and undeteremined therapeutic windows. Many current molecules suffer low bioavailability as single-agent treatments and may benefit from combination treatments which facilitate drug delivery. Ongoing work is focused on exploiting advances in computational and system biology methodologies to map signaling networks and cellular metabolism in different tumor cell populations. Combining this knowledge with emerging epigenome editing technologies can further elucidate the causal relationships between cellular epigenetic landscapes and regulatory processes in order to better understand how β-catenin pathways are deregulated in HNSCC.

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Declarations

Ethics approval and consent to participate Ethical approval was not required for this study.

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