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Dysregulated Molecular Networks in Head and Neck Carcinogenesis

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Abstract

Multiple genetic and epigenetic events, including the aberrant expression and function of molecules regulating cell signaling, growth, survival, motility, angiogenesis, and cell cycle control, underlie the progressive acquisition of a malignant phenotype in squamous carcinomas of the head and neck (HNSCC). In this regard, there has been a recent explosion in our understanding on how extracellular components, cell surface molecules, and a myriad of intracellular proteins and second messenger systems interact with each other, and are organized in pathways and networks to control cellular and tissue functions and cell fate decisions. This emerging ability to understand the basic mechanism controlling inter- and intra-cellular communication has provided an unprecedented opportunity to understand how their dysregulation contributes to the growth and dissemination of human cancers. Here, we will discuss the emerging information on how the use of modern technologies, including gene array and proteomic studies, combined with the molecular dissection of aberrant signaling networks, including the EGFR, *ras*, NF κ B, Stat, Wnt/ β -catenin, TGF- β , and PI3K-AKT-mTOR signaling pathways, can help elucidate the molecular mechanisms underlying HNSCC progression. Ultimately, we can envision that this knowledge may provide tremendous opportunities for the diagnosis of premalignant squamous lesions, and for the development of novel molecular-targeted strategies for the prevention and treatment of HNSCC.

Introduction

With approximately 500,000 new cases annually, squamous cell carcinomas of the head and neck (HNSCC), represent one of the six most common cancers in the world (1). This disease, which includes malignant lesions arising in the oral cavity, larynx and pharynx, results in nearly ~11,000 deaths each year in the United States alone (2). In spite of the many advances in our understanding in prevention and treatment of other types of cancers, the five-year survival rate after diagnosis for HNSCC remains low, approximately 50%, which is considerably lower than that for other cancers, such as those of colorectal, cervix and breast origin (2). The limited survival of HNSCC patients is likely due to a high proportion of patients presenting with advanced disease stages, lack of suitable markers for early detection, and failure to respond to available chemotherapy (3,4). Their poor prognosis may be also a reflection of the fact that while many of the most common risk factors involved in HNSCC development, such as alcohol

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and tobacco use, betel nut chewing, and infection with the human papillomavirus (HPV) are well recognized (3-5), we still have an incomplete knowledge of the mechanisms underlying the malignant progression of this cancer type.

Recent discoveries have dramatically increased our understanding of the most basic mechanisms controlling normal cell growth, and have also greatly enhanced our ability to investigate the nature of the biological processes that lead to cancer. We now know that the majority of cancer cells are derived from the clonal expansion and aberrant growth of a single stem cell or from few tumor-initiating cells that have re-acquired self-renewal capacity (6). Normal cells proliferate only when needed, as a result of a delicate balance between growth promoting and growth inhibiting factors under the influence of biochemical cues provided by neighboring cells and circulating factors. Cancer cells override these controlling mechanisms and follow their own internal program for timing their reproduction. These cells usually grow in an unrestricted manner, and over time, cancer cells can escape cell senescence and death programs thereby becoming immortal, enhance their supply of oxygen and nutrients by promoting the formation of new blood vessels, and acquire the ability to migrate from their original site, invade nearby tissues, and metastasize to distant anatomical sites. These progressive changes in cellular behavior, from slightly deregulated proliferation to full malignancy, are a result of the accumulation of genetic and epigenetic changes in a limited set of genes. Among them, two classes of genes, oncogenes and tumor suppressor genes, play major roles in triggering and promoting cancerous growth (7). Whereas activated oncogenes promote cell proliferation, tumor suppressor genes inhibit cell growth and contribute to the carcinogenic process when inactivated by mutations or by genetic and/or epigenetic events (7,8).

An emerging concept is that several activating and inactivating events must occur in oncogenes and tumor suppressor genes for the initiation and progression of many types of cancer. In HNSCC, these genetic changes occur in a multistep process (9). Thus, if molecular markers representing early and late events could be pinpointed, it would be possible to identify persons at high risk of HNSCC, namely, those whose lesions are progressing through the premalignant state. Furthermore, the availability of biochemical markers heralding malignancy would be key for monitoring cancer recurrence, as well as for the evaluation of the efficacy of novel chemoprevention agents. Clearly, the ability to gain a mechanistic insight into the complex molecular events leading to the development of HNSCC will have important implications for the early diagnosis, therapy, and prognosis of HNSCC patients.

Genetic and epigenetic alterations in HNSCC

Cancer arises in a multistep process resulting from the sequential accumulation of genetic and epigenetic defects and the clonal expansion of selected cell populations (7). As described in detail in the papers by Sidransky (*in this issue*) and Califano (*in this issue*), in the case of HNSCC, tumor progression involves genetic alterations leading to dysplasia (9p21, 3p21, 17p13), carcinoma *in situ* (11q13, 13q21, 14q31) and invasive tumors (4q26-28, 6p, 8p, 8q) (10). These and several recent studies suggest the contribution of several known tumor suppressor genes in HNSCC, such as *p16* and *p14^{ARF}* (9p21), which are responsible for G1 cell cycle regulation and MDM2 mediated degradation of p53, respectively, *APC* (5q21-22) and *P53* (17p13), as well as the existence of many putative tumor suppressor genes affected in HNSCC, including *FHIT* (3p14), and *RASSF1* (3p21) (11). Among them, loss of chromosomal region 9p21 is found in 70-80% of dysplastic lesions of the oral mucosa, and together with the inactivation of the remaining alleles of *p16* and *14^{ARF}* by promoter hypermethylation, represent one of the earliest and most frequent events in HNSCC progression (4,10).

Gain of cell immortality in HNSCC. Do these tumors arise from oral epithelial stem cells?

The ability to proliferate continuously, without undergoing senescence, is one of the hallmarks of cancer (7). In HNSCC, limitless replicative potential is most likely acquired through the genetic and epigenetic inactivation of *p16*, together with mutations in *P53* and enhanced activity of the telomerase (12). The lack of a functional p16 enables cells to bypass replicative stress-induced senescence (13), while the enhanced telomerase activity prevents the shortening of the telomeres and the consequent generation of signals from uncapped telomeres that impinge on p53 and other molecules involved in DNA-damage response (13). While HNSCC cells can replicate indefinitely, our newly gained knowledge of the intervening process may help us identify new approaches to re-established their mortality potential and promote their demise.

The basal layer of the oral epithelium contains cells with self-renewing capacity. This population of stem cells contributes to the physiological renewal of the epithelium lining the oral cavity and tongue, and contributes to its rapid regeneration upon damage (14). As these stem cells are the only keratinocytes that would reside long enough to accumulate the number of mutations observed in oral cancer, it is highly likely that HNSCC may arise from the malignant transformation of cells within the stem cell compartment, or from more differentiated cells that have regained self-renewing capacity (14). On the other hand, recent studies from both hematologic malignancies and solid tumors have suggested that there are only minor populations of cells in each malignancy, designated tumor stem cells, which are capable of tumor initiation (15). These tumor-initiating cells divide infrequently, in an asymmetric fashion, and self renew. Their potential survival following chemotherapy and radiation may represent a frequent cause of treatment failure, even after killing most, or all, of the rapidly proliferating cells that constitute the bulk of the tumor (16). In HNSCC, these tumor-initiating stem cells can be distinguished by the expression of E-cadherin and CD44 and the lack of lymphoid and monomyeloid markers (17). Although these cells represent only a fraction of the total tumor mass, they can give rise to tumors in xenografted immuno-compromised mice, though further characterization may be required to define this cell population more precisely.

Loss of tumor suppressor function in HNSCC

As outlined above, most HNSCCs lose the ability to restrain aberrant growth primarily due to the inactivation of p16, whose normal function is to block cyclin-bound cyclin-dependent kinases (CDKs) CDK4 and CDK6 (18). The latter orchestrate cell cycle progression and repress the growth inhibitory activity of the retinoblastoma (*RBI*) gene product (18). When hypophosphorylated, pRb forms a complex with the transcription factor E2F, thereby inhibiting E2F-mediated transcription of growth promoting genes (18). Mitogen stimulation leads to the phosphorylation and inactivation of pRb by CDKs, particularly CDK2/Cyclin E, or CDK4/CyclinD and CDK6/CyclinD complexes, thus enabling cells to initiate the synthesis of DNA (18).

RBI mutations are rare in HNSCC, but loss of Rb in premalignant and advanced oral cancer lesions have been reported with variable rate (19-21), reflecting perhaps that in the presence of p16 inactivation, further mutations or alterations in the p16-Rb tumor suppressor pathway would have limited growth advantage. Instead, nearly 50% of the HNSCC cases harbor mutations in the *P53* tumor suppressor gene (22,23), which halts cell-cycle progression upon DNA-damage, and can trigger apoptotic cell death if the cellular DNA is not repaired. *P53* is one of the most frequently mutated tumor suppressor gene in human malignancies (24). In HNSCC, the presence of mutations that render p53 functionally inactive are associated with

tumor progression and decreased overall survival (22). Indeed, loss of heterozygosity of p53 and the presence of tobacco carcinogen-induced inactivating mutations in the coding sequence of *P53*, or the accelerated destruction of its protein product, p53, by viral oncoproteins, such as by HPV E6, represent common molecular alterations in HNSCC (3,4). In the absence of *P53* mutations, p53 can also be inactivated by its ubiquitin-dependent degradation, which is caused either by binding the E6 protein from oncogenic human papillomavirus types, such as HPV16 and HPV18, or the cellular MDM2 protein, a protein that is functionally inactivated by the p14^{ARF} tumor suppressor protein (24). The former is of particular importance for the growing number of HPV-related HNSCC cases, the vast majority of which occur in the oropharynx, including base of tongue and tonsils (25,26). Thus, either infection with oncogenic papillomaviruses, such as HPV16 and HPV18, overexpression of MDM2, or inactivation of p14^{ARF}, may result in the reduced function of p53, which in turn may enable the further accumulation of unchecked genetic alterations due to the absence of an appropriate cellular response to DNA-damage.

Aberrant gene and protein expression in HNSCC

While a comprehensive review of all possible molecular mechanisms involved in cancerous growth is beyond our current scope, we will focus instead on key biochemical routes participating in inter- and intracellular communication whose deregulation contributes to the acquisition of the malignant phenotype in human HNSCC. In this regard, a major scientific challenge in HNSCC is to unravel the nature of the molecular events that drive tumor progression *in vivo*. As an approach, several research teams have focused on the analysis of gene expression patterns in normal oral mucosa and in HNSCC. Indeed, these efforts have already helped identify many altered gene products, which might contribute to the conversion of normal epithelium to a malignant phenotype. This emerging wealth of information is expected to yield novel biomarkers of tumor development and progression, as well as candidate drug targets for pharmacological intervention.

Initial gene discovery efforts in this area involved the generation of cDNA libraries from HNSCC cell lines and microdissected or bulk HNSCC tissues (27). However, with the completion of the Human Genome Project, the identification of most transcribed genes, and the development of robust techniques for gene expression analysis using array technologies, the use of gene arrays has become the method of choice for the study of gene expression patterns in HNSCC. While a complete description of the variety of platforms utilized for gene array analysis of HNSCC is beyond the scope of this review, in general these studies involve either small sets of normal and HNSCC tissue samples in which normal and tumoral epithelial cells are first isolated by laser capture microdissection (LCM) and their RNA amplified prior to labeling, or larger collections of clinical HNSCC samples in which gene array analysis is performed using total RNA isolated from bulk tissue specimens (28,29).

These approaches have yielded considerable information regarding genes contributing to HNSCC development, such as the observation that members of the Wnt and Notch family of signaling molecules may contribute to HNSCC progression (30). It also provided the first glimpse of the altered expression of genes associated with cell signaling, gene transcription, cell cycle regulation, oncogenesis, tumor suppression, differentiation, motility and invasion in HNSCC (30,31). Similar approaches were utilized to study gene expression in nasopharyngeal carcinoma (NPC), a major public health problem in Southeast Asia (32). More recently, LCM and gene array analysis have been used to examine gene expression in oral cavity cancer exhibiting distinct cervical lymph node metastasis status, leading to the identification of a predictive gene expression signature distinguishing patient samples with and without metastasis (33).

The ability to identify distinct groups of HNSCC lesions by investigating their gene expression pattern was nicely reflected by performing bioinformatic analysis of gene array data using RNA a large collection of bulk HNSCC tissues (34). This analysis also enabled the identification of a set of genes discriminating primary bulk tumors for the presence of lymph node metastases at the time of diagnosis (34). Some of the genes present in this sub-group included STK6, MAD2, ECT2, and CENPA. A 102-predictor gene set for lymph node metastases was identified soon after (35). Some of the genes in this list include those encoding extracellular matrix components, genes involved in cell adhesion, cell growth, and proteases involved in extracellular matrix degradation and tissue remodeling. A more targeted effort aimed at exploring relevant biomarkers of prognostic value in primary tongue tumors identified a signature gene-set for classifying tongue tumors and normal groups (36), and genes that characterize tumors that had metastasized to regional lymph nodes, including cases of extracapsular spread of metastatic nodes (37). In general, some of the frequently upregulated genes in HNSCC include matrix metalloprotease (MMP) family members, such as MMP-1, MMP-3, MMP-10, and MMP-12, pro-angiogenic chemokines, including IL-8 (CXCL8) and Gro- α (CXCL1), and those often downregulated genes include KRT4, MAL, SPINK5, and TGM3 (36,38).

Ultimately, the aberrant expression and activity of molecules present in HNSCC cells are responsible for their malignant behavior. Consequently, several groups have explored the use of a variety of proteomics techniques to investigate the nature of the proteins expressed in HNSCC. Initially, these studies were based on the separation of proteins in two-dimensional gel-based systems followed by mass spectroscopy proteomic analysis of individual isolated protein spots. For example, this approach enabled the identification of several proteins such as heat shock protein (HSP)60, HSP27, calgranulin B, myosin, tropomyosin and galectin 1 in tongue carcinoma tissues, when compared with their paired normal mucosa (39). In a related study in HNSCC of the oral cavity, alpha B-crystallin was detected at reduced levels, while several glycolytic enzymes, heat-shock proteins, tumor antigens, cytoskeleton proteins, enzymes involved in detoxification and anti-oxidation systems, and proteins involved in mitochondrial and intracellular signaling pathways were deemed to be overexpressed in HNSCC. (40). Other studies have used a fluorescent two-dimensional in-gel electrophoresis system combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to perform proteomic analysis of HNSCC cell lines and normal oral keratinocytes, thereby identifying numerous differentially expressed proteins, some of which were validated in HNSCC tissue samples (41).

Rather than relying on an initial bi-dimensional separation, recently available techniques enable the global proteolysis of whole cell and tissue lysates, followed by the separation of complex peptide mixtures by reverse phase liquid chromatography (LC) and analysis by mass spectrometry (MS) followed by tandem MS sequencing of selected peptides. While this strategy has been used to investigate protein expression in HNSCC cell lines (42), it has been recently adapted to the study proteins expressed in bulk HNSCC tissues. For example, from a large sample collection it was possible detect approximately 48 proteins that were differentially expressed between healthy oral mucosa and HNSCC including calgizarin (S100A11), the cysteine proteinase inhibitor cystatin A, stratifin (14-3-3 sigma), histone H4, and the alpha-defensins 1-3 (43). By using a quantitative proteomics technique, it was also possible to extend these studies and identified approximately 800 proteins differentially expressed in HNSCC (44). Stratifin, several calcium binding proteins (S100A2, S100A7), glutathione S transferase-Pi and APC-binding protein EB1 were among the molecules detected.

Recently, the development of new highly sensitive proteomic strategies enabled their application to laser-captured normal oral epithelial and HNSCC cells, thus opening the possibility of revealing the oral cancer cell proteome as it exist in vivo. For example, in an

initial study, 94-105 proteins were identified in the laser assisted isolated cells from each tissue sample, among which Wnt6 and Wnt14 were represented in normal and tumoral oral epithelial cells, respectively, and placental growth factor (PIGF) in tumor samples (45). The ability to combine laser-capture microdissection with novel liquid protein extraction techniques and mass spectrometry enabled the identification of proteins expressed in normal oral squamous epithelium and HNSCCs displaying distinct differentiation patterns. Indeed, approximately 20,000 cells procured from formalin fixed paraffin embedded tissue sections of clinically defined cases of well, moderately, and poorly differentiated squamous cell carcinoma and normal epithelial cells, were sufficient to identify up to 900 unique proteins within each individual sample. Proteins identified include a significant number of cytokeratins and other intermediary filament proteins, as well as differentiation markers, signal transduction and cell cycle regulatory molecules, growth and angiogenic factors, and matrix degrading proteases. Examples from this study include EGFR, STAT1, cathepsin D, HuR, the potential oncogenic molecules SET and AF1q, the pro-metastatic integrin β 4, and the stem cell protein PIWIL3, among many others (46). These recently developed techniques for the proteomic analysis of formalin-fixed paraffin embedded tissues may enable retrospective biomarker investigations of the vast archive of pathologically characterized HNSCC samples that exist worldwide. This may help expedite the identification of markers of clinical value, and proteins whose expression and activity contribute to HNSCC progression.

On the other hand, saliva is a biofluid in close contact with HNSCC lesions, and thus it may offer an excellent potential for clinical diagnostics, and specifically for the detection of biomarkers in HNSCC patients. In this regard, several studies conducted to search for markers of interest in the saliva from healthy and HNSCC patients have identified over 300 proteins which include several cytokeratins, defensin alpha-1 precursor, CXCR2 (interleukin-8 receptor B), kallikrein 1, notch 1, vav-3 protein and numerous salivary gland associated molecules (47,48). Over 1100 proteins were recently detected in submandibular-sublingual gland biofluids collected as ductal secretions, which included cystatin, histatin, proline-rich proteins, and mucins, and a large number of proteins of potential diagnostic value (49). Similarly, several studies have focused on the cells shed into the saliva, leading to the identification of over 1000 human proteins, which may play a role in oral squamous cell carcinogenesis (50). Overall, these efforts are expected to facilitate the development of novel markers of disease progression, which may facilitate the point-of-care diagnosis of HNSCC.

Dysregulated signaling networks in HNSCC

There has been a recent explosion in our knowledge on how the flow of information through intercellular signaling networks regulates cell fate decisions, cell differentiation, survival, metabolism, motility, and normal and aberrant cell growth. Indeed, the emerging understanding of the basic mechanism controlling intercellular and cell-to-cell communication is providing an unprecedented opportunity to understand physiological processes at the molecular, cellular, and organismal levels, thereby identifying novel targets for pharmacological intervention in a myriad of diseases. In this regard, we will discuss the emerging information on the nature of the dysregulated signaling mechanisms in HNSCC, their potential contribution to disease progression, and how this knowledge could provide novel molecular targeted approaches to prevent and treat HNSCC patients.

Overexpression of Epidermal Growth Factor Receptors (EGFR) in HNSCC

Since the first connection between a viral oncogene, a constitutively-active truncated mutant of *ERBB1*, and human cancer was made in 1984, it has been well known that aberrant signaling by growth factor receptors is critically involved in human neoplasias (51). Indeed, many human cancers express high levels of growth factors and corresponding receptors, and many malignant

cells exhibit highly active receptor tyrosine kinases due to their activation by an autocrine or paracrine mechanism, or by activating mutations in their coding sequence. Among the best-studied group of these receptors is the EGF receptor (EGFR) family (also known as type I receptor tyrosine kinases or ErbB tyrosine kinase receptors), which is essential for numerous normal cellular processes. The aberrant activity of this receptor family has also been linked to the development and growth of numerous tumor types including 80-90% of all HNSCCs (52). Indeed, EGFR overexpression may represent an independent prognostic marker correlating with increased tumor size, decreased radiation sensitivity, and increased risk of recurrence (53-55). The predominant mechanism leading to EGFR overexpression is *EGFR* gene amplification, with more than 12 copies per cell reported in HNSCC (56). In general, elevated levels of EGFR expression leads to the activation of their kinase activity by spontaneous dimerization. Constitutive EGFR activation in HNSCC is also caused by its autocrine stimulation through the co-expression of EGFR with one of its ligands, TGF α , which is frequently observed in HNSCC and correlates with a poor prognosis (57). The presence of truncated mutant forms of EGFR, EGFR variant III (EGFRvIII), which causes its constitutive activation, has also been detected in a fraction of HNSCC cases (58). Of particular interest to the current efforts targeting EGFR for HNSCC therapy, EGFRvIII may be resistant to EGFR-blocking antibodies and cisplatin (58). Interestingly, G protein-coupled receptor (GPCR)-induced cleavage of EGF-like growth factors leads to EGFR transactivation and EGFR-related signaling in cancer cells, suggesting that GPCR-EGFR cross-communication may play a role in the development and progression of HNSCC and other human cancers (59,60).

Once activated, EGFR stimulates a number of downstream signaling events, whose contribution to normal and aberrant cell growth has been the center of intense experimental scrutiny over the past decades. Among them, EGFR activates the Ras/Raf/mitogen activated protein kinase (MAPK) signaling route, the transcription factor signal transducer and activator transcription (STAT), and the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, which in turn contribute to the malignant growth and metastatic potential of HNSCC. Each of these biochemical routes will be discussed in further detail, as they are often persistently active in HNSCC dependently or independent of EGFR overactivity, and thus may represent potential targets for pharmacological intervention in HNSCC.

Ras

Members of the *ras* family (*H-ras*, *K-ras*, *N-ras*) are some of the most frequently mutated oncogenes in human cancer (61). A high incidence of *ras* mutation has been found in oral cancer, mainly in Asian populations, where it has been associated with areca nut chewing (62). However, *H-ras* mutations are found much less often (less than 5%) in HNSCC cases in the West, and the other *ras* genes are also infrequently mutated in HNSCC (3,63,64). This shows nicely how different etiological factors and risk habits can result in distinct genetic alterations, which may have a remarkable impact in disease progression and clinical response to the available treatment options and emerging targeted therapies. It also suggests that the repertoire of signaling molecules contributing to HNSCC progression may differ depending on the associated risk factors and patient population. In particular, while in oral cancer related to areca quid chewing the Ras/RAF/MAPK pathway may be constitutively activated due to gain of function mutations in *ras* genes, in those cancers associated with prolonged exposure to tobacco carcinogens this pathway may be activated downstream from the persistent autocrine or paracrine stimulation of EGFR and other growth factor receptors.

Aberrant activity of the transcription factor NF κ B in HNSCC

The transcription factor NF κ B was initially described based on its central role in controlling the expression of genetic programs involved in innate and adaptive immune responses, and it

is now known to act as an essential component of intracellular regulatory circuitries regulating cell proliferation and survival (65). Growing evidence supports that the dysregulated function of NFκB can contribute to many pathological processes, including cancer (65).

The NFκB transcription factors are assembled by the dimerization of 5 family members: p50 (*NFKB1*), p52 (*NFKB2*), p65, also known as RelA (*RELA*), c-Rel (*REL*), and RelB (*RELB*) (66) which, upon activation, translocate to the nucleus where they participate in the expression of genes involved in inflammatory and immune responses, as well as in cell proliferation and survival (67). In particular, the basal activity of the p65 NFκB is repressed by its association with IκB, an ankyrin repeat-containing protein that binds to NFκB and masks its nuclear localization signal, thus retaining NFκB in its inactive state in the cytosol (67). The activation of NFκB by its classical mechanism involves the phosphorylation of IκB on two serine residues, Ser 32 and Ser 36, which triggers the rapid ubiquitination and degradation of phosphorylated IκB in the proteasome, and the consequent nuclear translocation and activation of NFκB (67). The IκB kinase (IKK) includes a regulatory subunit, NEMO (IKKγ), and two catalytic kinase subunits, IKKα (IKK1) and IKKβ (IKK2) (65), all of which are readily detected and activated by pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α in HNSCC cells (68).

The role of NFκB in tumor development is nicely exemplified by the early discovery that v-Rel, an oncoprotein encoded by the turkey retrovirus REV-T, is a homolog of the mammalian p65 NFκB DNA binding subunit (69). The constitutive activation of NFκB is a frequent event in a variety of human neoplasias, such as melanoma, breast and prostate carcinoma, T cell leukemia, Hodgkin's and B cell lymphomas, multiple myeloma, and HNSCC (65,70). Dysregulation of NFκB promotes tumor angiogenesis and metastasis, and suppresses the apoptotic potential of chemotherapeutic agents and radiation, thus leading to tumor treatment resistance (65,71).

In HNSCC, the expression and activity of NFκB is often upregulated, and its protein levels increases gradually from premalignant lesions to invasive cancer (70,72-74), which suggests that NFκB signaling may play an important role at the early stages of HNSCC carcinogenesis. In fact, NFκB promotes the expression of the anti-apoptotic protein Bcl-2 in HNSCC (75). Interfering with NFκB function in HNSCC leads to a remarkable reduction in cell survival and tumor growth (76), and downregulation of IL-6 gene and protein expression, concomitant with a decreased release of a number of cytokines and chemokines, including IL-2, IL-5, IL-8, IL-10, IL-12, IL-13, IL-17, GM-CSF and G-CSF (68), many of which are highly elevated in the serum of HNSCC patients (77). In addition, in a surprising twist, aberrant function of NFκB leads to the stimulation of STAT3 by an autocrine/paracrine mechanism that is independent from EGFR, which is initiated by the release of IL-6, thereby establishing a crosstalk between NFκB and STAT3 pathways in HNSCC (68). These findings support the emerging notion that the aberrant activity of a network of interrelated signaling pathways, rather than a single deregulated biochemical route, contribute to squamous carcinogenesis.

What leads to the persistent activation of NFκB in HNSCC is still unclear. Recent studies associate the elevated function of NFκB with the activation of the TNF-α initiated pathway and the overexpression of casein-kinase 2 (CK2) (78,79), which may lead to the overactivity of IKKα and IKKβ, with IKKβ playing a more prominent role (68). Nonetheless, probably multiple mechanisms may contribute to NFκB activation in HNSCC patients, whose elucidation may warrant further investigation. On the other hand, a systems level analysis of gene and protein expression is now helping define NFκB regulons that may contribute to the classifications and stratification of HNSCC, which in turn may help identify those patients that may benefit the most from the treatment with therapies targeting NFκB in HNSCC (80,81).

Activation of Signal Transducer and Activator of Transcription (STAT) proteins in HNSCC

A network of cytokines controls stem cell function, lineage commitment, and organ development during embryogenesis (82), many of which rely on the activation of signal-transducer-and-activator-of-transcription (STAT) family proteins to regulate gene expression, thereby orchestrating these intricate processes (82). To date, seven STAT family members have been identified, STATs 1, 2, 3, 4, 5a, 5b and 6, which participate in the transcription of genes involved in immune responses, growth, and cell fate decisions (83). Whereas STAT activity is essential for normal cellular functions, deregulation of the STAT pathway can contribute to a number of human diseases. Indeed, gain of function of STATs is often associated with cellular transformation and oncogenic potential (84).

Cytokines and growth promoter factors stimulate STAT proteins by acting on their cognate receptors, which leads to the recruitment and phosphorylation of Janus kinase 1 and 2 (JAK-1 and JAK-2) that in turn phosphorylate STAT proteins at specific tyrosine residues, thus promoting their homo- and heterodimerization (82,85). Among STAT family members, STAT1 and STAT3 are often phosphorylated in serine residues, which further activate STATs (82). STAT dimers translocate to the nucleus where they bind to consensus DNA sequences and activate the expression of growth promoting genes, such as *c-myc* and *cyclin D* (85).

Whereas constitutive activation of STAT3 has been demonstrated in many cancers, including breast cancer, leukemia, lymphoma, lung and thyroid cancers (86,87), early studies indicated that HNSCC and their derived cell lines exhibit remarkably elevated levels of the phosphorylated active forms of STAT3 (88). Moreover, quenching STAT3 activity leads to growth inhibition of HNSCC (88-90), thus supporting the importance of signaling through STAT3 in HNSCC oncogenesis. In HNSCC, these elevated STAT3 levels alter cell cycle progression, and promote the proliferation and survival of tumor cell (91). In fact, STAT3 activation may represent an early event in oral carcinogenesis, as both tumor and the adjacent normal epithelia of HNSCC patients show higher levels of STAT3 expression and phosphorylation (89). Activated STAT3 also correlates with lymph node metastasis and poor prognosis (88,92). Although STAT3 is the most prominent STAT molecule in HNSCC, some lesions also present constitutively active STAT5, with STAT5A and STAT5B being overexpressed and phosphorylated (93,94). STAT5A has been associated to upregulation of cyclin D1 and inhibition of STAT5B results in growth arrest in HNSCC tumors (94).

Many mechanisms may converge to promote the persistent activation of STATs in HNSCC. For example, whereas the direct activation of STAT3 by EGFR has been clearly shown in HNSCC cells (88,91), STAT3 can also be activated by an EGFR-independent mechanism (95). The latter often involves the autocrine activation of the gp130 cytokine receptor in HNSCC cells by tumor-released cytokines, such as IL-6, which activates STAT3 independently from the activation status of EGFR (68,95). Furthermore, interfering with this cytokine-initiated pathway of STAT3 activation can result in the reduced growth and apoptotic death of HNSCC cells (95). Other cytokines, such as erythropoietin, can also stimulate STATs in HNSCC (96), suggesting that the paracrine and autocrine activation of STATs may ultimately represent a general mechanism by which these transcription factors can be activated in HNSCC in the tumor microenvironment. In addition, two members of the suppressors of cytokine signaling (SOCS) family of STAT-inhibitory proteins, SOCS-1 and SOCS-3, have been recently shown to be downregulated by promoter hypermethylation in a large fraction of HNSCC tissue samples and cell lines (97,98) suggesting that this protein family may represent signal transduction tumor suppressor genes in HNSCC, whose epigenetic inactivation may contribute to the multistep process of HNSCC development.

Wnt and oral cancer

The Wnt protein family consists of 19 secreted cysteine-rich glycoproteins that act on cells by interacting with the N-terminal extracellular cysteine-rich domain of the seven-span transmembrane receptors of the Frizzled family, and to LRP5 or LRP6, two members of the low-density-lipoprotein receptor-related (LDL-R) protein family (99). Wnt can initiate the activation of several major signaling pathways, often referred to as the canonical Wnt/ β -catenin pathway, where β -catenin is stabilized and translocated to the nucleus, and the non-canonical Wnt pathways, which include the PCP (Planar Cell Polarity), c-Jun amino-terminal kinase (JNK), Rho, and calcium signaling pathways (reviewed in (99,100)).

Whereas activation of the Wnt- β -catenin pathway is a frequent event in colon, kidney, prostate, thyroid cancer and melanoma, among others (101), there is limited knowledge on the contribution of this signaling mechanism in HNSCC. However, we now know that several components of the Wnt pathway are also altered in oral cancers. For example, several Wnt receptors, Frizzleds, and their downstream target, Dishevelled, are highly expressed in HNSCC when compared to matching normal tissues as judged by gene array analysis (30), and high levels of Wnt14 were detected by mass spectrometric analysis of microdissected HNSCC cells (45). On the other hand, reduced expression of natural Wnt antagonists is a frequent epigenetic event in HNSCC. For example, promoter methylation in the gene for soluble frizzled receptor proteins (SFRP) SFRP1, SFRP2, SFRP4 and SFRP5, which sequester Wnt proteins and prevent their function, have been observed in ~30-40% of HNSCC cases, some of which are associated with drinking, smoking and HPV infection (102). Deregulated function of the APC tumor suppressor protein, which is required for the integrity and function of the β -catenin destruction complex, is often compromised in HNSCC by loss of heterozygosity (LOH) and hypermethylation of the *APC* gene and its consequent reduced expression level in 25% to 39% of patient samples (103,104). This suggests the existence of a subpopulation of HNSCC in which the Wnt pathway may contribute to carcinogenesis. The expression of β -catenin is altered in HNSCC (105,106), but no activating mutations in this molecule have yet been identified (107), which suggests that the deregulation of the Wnt pathway and consequent overactivity of normal β -catenin protein, rather than β -catenin mutations, may contribute to HNSCC progression.

On the other hand, the inhibition of the secreted Wnt-1 protein by the use of anti-Wnt-1 antibodies inhibits the proliferation and induces the apoptosis of HNSCC cancer cell lines, which correlates with the reduction of the activity of the transcription factor LEF/TCF activity, and the consequent reduction in cyclin D1 protein expression (108). This suggests that Wnt may represent a potential target for immunotherapy strategies. Indeed, given the broad activity of Wnt signaling in organ development, maintenance of adult stem/progenitor cell, and tumor development, it is likely that the therapeutic inhibition of Wnt and its signaling pathway may emerge as an effective approach to halt HNSCC progression.

TGF- β

The transforming growth factor- β (TGF- β) superfamily of growth factors consists of more than 35 secreted polypeptides including members of the TGF- β and bone morphogenic proteins (BMPs), among others. TGF- β was initially described based on its ability to induce anchorage-independent growth of fibroblasts, it behaves as a potent tumor suppressor and inhibitor of cell proliferation in many epithelial cells (reviewed in (109)). The most studied members of this family are TGF- β 1, TGF- β 2 and TGF- β 3, which are secreted as inactive precursors called latent TGF- β s (L-TGF- β). Under physiological conditions, TGF- β s are activated by acidic environment and proteolysis, such as by matrix metalloproteinases and plasmin. Cleavage or conformational changes of the precursor protein latency-associated peptide from the L-TGF-

β results in the formation of biologically active TGF- β . Upon activation, TGF- β binds to its receptors (TGF β RI, TGF β RII, and TGF β RIII) and initiates intracellular signaling via Smad and mitogen-activated protein kinase (MAPK) pathways. TGF β RI and TGF β RII are single pass transmembrane proteins containing a glycosylated extracellular domain, a short transmembrane domain, and an intracellular serine/threonine kinase domain. TGF β RIII are also called accessory receptors and include betaglycan and endoglin (CD105), although members of the glycosylphosphatidylinositol-anchored protein DRAGON family have also been identified as co-receptors for BMPs (109). Upon TGF- β binding, TGF β RII interacts with TGF β RI forming an heteromeric complex, leading to the phosphorylation of TGF β RI by TGF β RII receptor kinase, resulting in activation of type I receptor kinase domain and phosphorylation of the Smad signaling mediators (110). The mammalian Smad family consists of 3 functional classes: receptor-regulated Smads (R-Smads; Smad1, 2, 3, 5 and Smad8), co-mediator Smads (Co-Smad; Smad4) and inhibitory Smads (I-Smads; Smad6 and Smad7) and they are ubiquitously expressed in most adult tissue, stressing the importance of TGF- β signaling in tissue development and homeostasis (111).

The role TGF- β in epithelial malignancy is complex, and still not completely understood. Available evidence supports a dual role; TGF- β acts as a potent tumor suppressor during the early stages of carcinogenesis while promoting tumor progression at later stages (112). The pro-oncogenic functions of TGF- β may be associated with loss of response to the ligand, defects of components of the TGF- β signal transduction pathway, over-expression and/or activation of the latent complex, epithelial-mesenchymal transition, and engagement of other signaling mechanisms which act in concert with TGF- β to facilitate the metastatic phenotype (112). In HNSCC, loss of TGF β RII has been identified in human HNSCC (113). Furthermore, activation of either K-*ras* or H-*ras* in combination with TGF β RII deletion from mouse oral epithelia could induce metastatic HNSCC with complete penetrance. Conditional deletion of TGF- β RI in mice can lead to acantholytic SCCs in periorbital areas, a histological type frequently seen in lips squamous cell carcinomas in humans (114). Similar acantholytic tumors appear to arise from oral cavity cancers in mice lacking TGF β RII and expressing *ras* oncogenes, suggesting an association between the TGF β R and pathways affecting cell-to-cell adhesion (113). In humans, decreased immunoreactivity for TGF β RII is associated with decreased p-Smad2, and increased disease aggressiveness, likely resulting from the loss of cell cycle-inhibitory mechanisms that mediate the growth suppressive effect of TGF- β 1 on HNSCC cells (115).

Aberrant function of the phosphatidylinositol 3-kinase (PI3Ks), PTEN, AKT and mTOR signaling network is a frequent event in HNSCC

The PI3K pathway has emerged as one of the most frequently targeted pathways in all sporadic human cancer, as suggested by the fact that mutation in one or another PI3K component accounts for up to 30% of all human cancer (116). Genomic aberrations include mutation, amplification, and rearrangements in a variety of components of the PI3K signaling route, resulting in the dysregulation of cellular growth control and survival, which contributes to a competitive growth advantage, metastatic potential, and resistance to therapy (117). PI3Ks are grouped into three classes (I-III) according to their substrate preference and sequence homology (118). The class I PI3Ks are activated by growth factor tyrosine kinase receptors (class IA), such as EGFR, or by GPCRs (class IB). Class IA PI3Ks are heterodimers of a p85 regulatory subunit and a p110 catalytic subunit. p85 binds and integrates signals from various cellular proteins, including growth factor tyrosine kinase-linked receptors, and oncogenic proteins, such as mutated Ras and Src, providing an integration point for activation of PI3K and its downstream molecules.

The direct product of PI3K activity, the lipid second messenger PtdIns(3,4,5)P₃ (PIP₃), is a constituent of the inner leaflet of the plasma membrane and serves as docking sites for proteins

that contain PH domains, including AKT proteins and phosphoinositide-dependent kinase 1 (PDK1) (118), which phosphorylates AKT proteins within their catalytic domains in the so-called T-loop (Thr308 in AKT1) resulting in its activation. The second activation-specific AKT phosphorylation site lies within a hydrophobic motif proximal to the C-terminus (Ser473 in AKT1) and is targeted by a distinct protein kinase(s), most likely the mammalian target of rapamycin (mTOR)-Rictor complex (119). The AKT family of kinases consists of three members, AKT1, AKT2 and AKT3, which are derived from distinct genes (117). All three AKT isoforms possess these conserved phosphorylatable threonine and serine residues (T308/S473 in AKT1) that together with the PH domain are critical for AKT function. A wealth of genetic, biochemical, and biological evidence supports a key role for AKT in the transmission of the proliferative and transforming pathways initiated by growth factors and oncogenes that stimulate PI3K (120). Thus, the identification of AKT substrates has been the focus of numerous studies to understand the mechanisms by which this kinase impacts on insulin signaling, cell growth, apoptosis, and cancer (120). Among them, AKT prevents cell death by inactivating proapoptotic factors including BAD, procaspase-9 and Forkhead transcription factor family proteins (FOXOs), activates transcription factors that upregulate antiapoptotic genes, including NF- κ B, inactivates p53 through Mdm2, and phosphorylates the CDK inhibitors p21^{CIP1/WAF1} and p27^{KIP1}, resulting in their exclusion from the nucleus and subsequent cytoplasmic sequestration/degradation and thus in increased cell proliferation (117). AKT also phosphorylates and inhibits glycogen synthase kinase-3 (GSK3), thus enhancing β -catenin and cyclin D1 stabilization (121).

The fact that many frequently occurring oncogenic mutations (e.g., in the small GTPase Ras, PI3K, and receptor and non-receptor tyrosine kinases) result in the constitutive activation of AKT, and that many tumor-suppressor proteins (e.g., PTEN, TSC1, TSC2, and LKB1) act by inhibiting the activity of AKT and its downstream targets, underscores the critical role of the dysregulation of the AKT-pathway in cancer (122,123). In this regard, emerging work suggests that AKT is persistently activated in the vast majority of HNSCC cases. Indeed, the presence of phosphorylated, active forms of AKT can be readily detected in both experimental and human HNSCCs and in HNSCC-derived cell lines (124), and blockade of PDK1, which acts upstream of AKT, potently inhibits tumor cell growth (124,125). Moreover, AKT activation is an early event in HNSCC progression, as it is detected in nearly 50% of tongue preneoplastic lesions (126), and its activation represents an independent prognostic marker of poor clinical outcome in tongue and oropharyngeal HNSCC (126,127).

Multiple genetic and epigenetic events may converge to promote the activation of the PI3K-AKT pathway in HNSCC (Figure 1). These include EGFR overexpression and alterations in its coding sequence and the expression of oncogenic *ras* mutants (see above). Of interest, copy number gain and amplification at 3q26, where the *PI3K α* gene is located, represents a frequent (~40%) and early genomic aberration in HNSCC (128), which contributes with still unclear epigenetic events to *PI3K α* overexpression and AKT activation (129,130). Furthermore, activating mutations in the *PI3K α* gene, referred to as the *PI3KCA* oncogene, can be observed in a small fraction (<10%) of HNSCC tumors (131,132). In addition, PIP₃ is rapidly metabolized by PTEN, a lipid phosphatase that is mutated or epigenetically inactivated in a large fraction of human tumors, rivaling only p53 as one of the most important tumor suppressor proteins (133). In HNSCC, genetic alterations in *PTEN*, located at 10q23.3, occur in 5-10% of HNSCC lesions but, remarkably, loss of PTEN expression can be observed in ~30% of HNSCCs, and this lack of PTEN expression may be an independent prognostic indicator of poor clinical outcome (134,135). Overall, AKT can be activated in HNSCC due to the overactivity of EGFR, *ras* mutations, *PI3K α* gene amplification, overexpression or activating mutations, together with defective PTEN activity due to genetic alterations or decreased expression. Indeed, the presence of multiple convergent pathways resulting in enhanced AKT

function may explain why activation of the AKT pathway represents one of the most frequent events in HNSCC (136).

Despite accumulating evidence supporting an important role for the AKT pathway in the development of HNSCC, the nature of the biologically relevant pathway(s) through which AKT acts in this tumor type is still not fully understood. Of interest, recent findings suggest that the ability of AKT to coordinate mitogenic signaling with nutrient-sensing pathways controlling protein synthesis may represent an essential mechanism whereby AKT ultimately regulates cell growth (137,138). This pathway is initiated by AKT phosphorylation and inactivation of a tumor-suppressor protein, tuberous sclerosis complex protein 2 (TSC2), which is also known as tuberin (139). TSC2 associates with a second tumor-suppressor protein, tuberous sclerosis complex protein 1 (TSC1), and act together as a GTPase activating protein (GAP) for the small GTPase Rheb1 (139,140). Thus, inactivation of TSC2 by AKT leads to the accumulation of the GTP-bound (active) form of Rheb1, which in turn promotes the phosphorylation and activation of an atypical serine/threonine kinase known as the mammalian target of rapamycin (mTOR) (141). mTOR then phosphorylates key eukaryotic translation regulators, including p70-S6 kinase (p70S6K) and the eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) (142). The latter event prevents the repressing activity of 4E-BP1 on the eukaryotic initiation factor 4E (eIF4E), ultimately resulting in enhanced translation from a subset of genes that are required for cell growth (142). Of direct relevance to HNSCC, eIF4E gene amplification and protein overexpression is often associated with malignant progression of this cancer type (143), and eIF4E-positive surgical margins have a more than 6-fold risk of developing local recurrences (144,145). Furthermore, by monitoring the accumulation of the phosphorylated form of the ribosomal S6 protein, pS6, the most downstream target of the mTOR pathway, we have recently documented that activation of mTOR is an early and one of the most frequent events in HNSCC (136,146). In addition, the inhibition of mTOR with its specific inhibitor, rapamycin, provokes the rapid death of HNSCC xenografts, resulting in tumor regression (146). These findings provide a strong rationale for ongoing studies evaluating the clinical efficacy of rapamycin and its related rapalogs in HNSCC treatment.

Of interest, whereas in some HNSCC cell lines EGFR inhibition does affect the activity of the mTOR pathway, in others a reduction in the status of phosphorylation of S6 after EGFR blockade was observed, albeit often requiring high concentrations of EGFR inhibitors (146). These findings may have important clinical implications, as they may provide a mechanistic framework for using molecules interfering with mTOR function alone or in combination with chemotherapeutic agents or EGFR inhibitors, depending on the interplay between the mTOR pathway and EGFR activity in individual HNSCC patients. On the other hand, the use of a reverse-pharmacology approach, which involved the expression of a rapamycin-insensitive form of mTOR in HNSCC cells, revealed that cancer cells are the primary targets of rapamycin *in vivo*, and that mTOR controls the expression of HIF-1 α , a key transcription factor that orchestrates the cellular response to hypoxic stress, including the regulation of the expression of angiogenic factors (147), thus providing a likely mechanism by which rapamycin exerts its tumor suppressive and antiangiogenic effects.

Conclusion. A case for pathway dependence in HNSCC?

Multiple genetic and epigenetic events, including the aberrant expression and function of molecules regulating cell signaling, growth, survival, angiogenesis, cell cycle control, and cell motility underlie the progressive acquisition of a malignant phenotype in HNSCC progression. While the ability to restore the function of defective genes may hold great therapeutic potential, we have also learned recently that, as they progress, most HNSCC cancers may become addicted to, and therefore dependent on, the aberrant activation of multiple signaling pathways,

including NF κ B, Stat, and AKT-mTOR. While their relative contribution to HNSCC progression may be highly dependent on individual factors, ultimately we can envision that the ability to examine their activation status using readily available approaches and the development of novel molecular targeted therapies may soon enable exploiting this pathway dependence for the prevention and treatment of HNSCC.

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Bibliography

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55(2): 74–108. [PubMed: 15761078]
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58(2):71–96. [PubMed: 18287387]
3. Mao L, Hong WK, Papadimitrakopoulou VA. Focus on head and neck cancer. *Cancer Cell* 2004;5(4): 311–6. [PubMed: 15093538]
4. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med* 2001;345(26): 1890–900. [PubMed: 11756581]
5. Bagan JV, Scully C. Recent advances in Oral Oncology 2007: epidemiology, aetiopathogenesis, diagnosis and prognostication. *Oral Oncol* 2008;44(2):103–8. [PubMed: 18252251]
6. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 2007;23:675–99. [PubMed: 17645413]
7. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100(1):57–70. [PubMed: 10647931]
8. Esteller M. Epigenetics in cancer. *N Engl J Med* 2008;358(11):1148–59. [PubMed: 18337604]
9. Partridge M, Emilion G, Pateromichelakis S, Phillips E, Langdon J. Field cancerisation of the oral cavity: comparison of the spectrum of molecular alterations in cases presenting with both dysplastic and malignant lesions. *Oral Oncol* 1997;33(5):332–7. [PubMed: 9415332]
10. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996;56(11):2488–92. [PubMed: 8653682]
11. Hunter KD, Parkinson EK, Harrison PR. Profiling early head and neck cancer. *Nat Rev Cancer* 2005;5(2):127–35. [PubMed: 15685196]
12. Todd R, Hinds PW, Munger K, Rustgi AK, Opitz OG, Suliman Y, et al. Cell cycle dysregulation in oral cancer. *Crit Rev Oral Biol Med* 2002;13(1):51–61. [PubMed: 12097237]
13. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. *Cell* 2007;130(2):223–33. [PubMed: 17662938]
14. Costea DE, Tsinkalovsky O, Vintermyr OK, Johannessen AC, Mackenzie IC. Cancer stem cells - new and potentially important targets for the therapy of oral squamous cell carcinoma. *Oral Dis* 2006;12(5):443–54. [PubMed: 16910914]
15. Hill RP, Perris R. “Destemming” cancer stem cells. *J Natl Cancer Inst* 2007;99(19):1435–40. [PubMed: 17895479]
16. Knoblich JA. Mechanisms of asymmetric stem cell division. *Cell* 2008;132(4):583–97. [PubMed: 18295577]
17. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A* 2007;104(3):973–8. [PubMed: 17210912]
18. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995;81(3):323–30. [PubMed: 7736585]
19. Pande P, Mathur M, Shukla NK, Ralhan R. pRb and p16 protein alterations in human oral tumorigenesis. *Oral Oncol* 1998;34(5):396–403. [PubMed: 9861348]

20. Pavelic ZP, Lasmar M, Pavelic L, Sorensen C, Stambrook PJ, Zimmermann N, et al. Absence of retinoblastoma gene product in human primary oral cavity carcinomas. *Eur J Cancer B Oral Oncol* 1996;32B(5):347–51. [PubMed: 8944840]
21. Xu J, Gimenez-Conti IB, Cunningham JE, Collet AM, Luna MA, Lanfranchi HE, et al. Alterations of p53, cyclin D1, Rb, and H-ras in human oral carcinomas related to tobacco use. *Cancer* 1998;83(2):204–12. [PubMed: 9669801]
22. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 2007;357(25):2552–61. [PubMed: 18094376]
23. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, et al. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993;53(19):4477–80. [PubMed: 8402617]
24. Vousden KH, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol* 2007;8(4):275–83. [PubMed: 17380161]
25. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol* 2008;26(4):612–9. [PubMed: 18235120]
26. Gillison ML. Current topics in the epidemiology of oral cavity and oropharyngeal cancers. *Head Neck* 2007;29(8):779–92. [PubMed: 17230556]
27. Leethanakul C, Knezevic V, Patel V, Amornphimoltham P, Gillespie J, Shillitoe EJ, et al. Gene discovery in oral squamous cell carcinoma through the Head and Neck Cancer Genome Anatomy Project: confirmation by microarray analysis. *Oral Oncol* 2003;39(3):248–58. [PubMed: 12618197]
28. Glanzer JG, Eberwine JH. Expression profiling of small cellular samples in cancer: less is more. *Br J Cancer* 2004;90(6):1111–4. [PubMed: 15026786]
29. Petersen D, Chandramouli GV, Geoghegan J, Hilburn J, Paarlberg J, Kim CH, et al. Three microarray platforms: an analysis of their concordance in profiling gene expression. *BMC Genomics* 2005;6(1):63. [PubMed: 15876355]
30. Leethanakul C, Patel V, Gillespie J, Pallente M, Ensley JF, Koontongkaew S, et al. Distinct pattern of expression of differentiation and growth-related genes in squamous cell carcinomas of the head and neck revealed by the use of laser capture microdissection and cDNA arrays. *Oncogene* 2000;19(28):3220–4. [PubMed: 10918578]
31. Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Kohno Y, Posner M, et al. Oral cancer in vivo gene expression profiling assisted by laser capture microdissection and microarray analysis. *Oncogene* 2001;20(43):6196–204. [PubMed: 11593428]
32. Sriuranpong V, Mutirangura A, Gillespie JW, Patel V, Amornphimoltham P, Molinolo AA, et al. Global gene expression profile of nasopharyngeal carcinoma by laser capture microdissection and complementary DNA microarrays. *ClinCancer Res* 2004;10:4944–58.
33. Nguyen ST, Hasegawa S, Tsuda H, Tomioka H, Ushijima M, Noda M, et al. Identification of a predictive gene expression signature of cervical lymph node metastasis in oral squamous cell carcinoma. *Cancer Sci* 2007;98(5):740–6. [PubMed: 17391312]
34. Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell* 2004;5(5):489–500. [PubMed: 15144956]
35. Roepman P, Wessels LF, Kettelarij N, Kemmeren P, Miles AJ, Lijnzaad P, et al. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nat Genet* 2005;37(2):182–6. [PubMed: 15640797]
36. Ye H, Yu T, Temam S, Ziober BL, Wang J, Schwartz JL, et al. Transcriptomic dissection of tongue squamous cell carcinoma. *BMC Genomics* 2008;9:69. [PubMed: 18254958]
37. Zhou X, Temam S, Oh M, Pungpravat N, Huang BL, Mao L, et al. Global expression-based classification of lymph node metastasis and extracapsular spread of oral tongue squamous cell carcinoma. *Neoplasia* 2006;8(11):925–32. [PubMed: 17132224]
38. Ziober AF, Patel KR, Alawi F, Gimotty P, Weber RS, Feldman MM, et al. Identification of a gene signature for rapid screening of oral squamous cell carcinoma. *Clin Cancer Res* 2006;12(20 Pt 1):5960. [PubMed: 17062667]

39. He QY, Chen J, Kung HF, Yuen AP, Chiu JF. Identification of tumor-associated proteins in oral tongue squamous cell carcinoma by proteomics. *Proteomics* 2004;4(1):271–8. [PubMed: 14730689]
40. Chen J, He QY, Yuen AP, Chiu JF. Proteomics of buccal squamous cell carcinoma: the involvement of multiple pathways in tumorigenesis. *Proteomics* 2004;4(8):2465–75. [PubMed: 15274141]
41. Onda T, Uzawa K, Nakashima D, Saito K, Iwadate Y, Seki N, et al. Lin-7C/VELI3/MALS-3: an essential component in metastasis of human squamous cell carcinoma. *Cancer Res* 2007;67(20):9643–8. [PubMed: 17942893]
42. Sudha R, Kawachi N, Du P, Nieves E, Belbin TJ, Negassa A, et al. Global proteomic analysis distinguishes biologic differences in head and neck squamous carcinoma. *Lab Invest* 2007;87(8):755–66. [PubMed: 17558418]
43. Roesch-Ely M, Nees M, Karsai S, Ruess A, Bogumil R, Warnken U, et al. Proteomic analysis reveals successive aberrations in protein expression from healthy mucosa to invasive head and neck cancer. *Oncogene* 2007;26(1):54–64. [PubMed: 16819514]
44. Ralhan R, Desouza LV, Matta A, Chandra Tripathi S, Ghanny S, Datta Gupta S, et al. Discovery and verification of head-and-neck cancer biomarkers by differential protein expression analysis using iTRAQ-labeling and multidimensional liquid chromatography and tandem mass spectrometry. *Mol Cell Proteomics*. 2008
45. Baker H, Patel V, Molinolo AA, Shillitoe EJ, Ensley JF, Yoo GH, et al. Proteome-wide analysis of head and neck squamous cell carcinomas using laser-capture microdissection and tandem mass spectrometry. *Oral Oncol* 2005;41:183–99. [PubMed: 15695121]
46. Patel V, Hood BL, Molinolo AA, Lee NH, Conrads TP, Braisted JC, et al. Proteomic analysis of laser-captured paraffin-embedded tissues: a molecular portrait of head and neck cancer progression. *Clin Cancer Res* 2008;14(4):1002–14. [PubMed: 18281532]
47. Hu S, Xie Y, Ramachandran P, Ogorzalek Loo RR, Li Y, Loo JA, et al. Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. *Proteomics* 2005;5(6):1714–28. [PubMed: 15800970]
48. Hu S, Loo JA, Wong DT. Human saliva proteome analysis and disease biomarker discovery. *Expert Rev Proteomics* 2007;4(4):531–8. [PubMed: 17705710]
49. Denny P, Hagen F, Hardt M, Liao L, Yan W, Arellanno M, et al. The Proteomes of Human Parotid and Submandibular/Sublingual Gland Salivas Collected as the Ductal Secretions. *J Proteome Res* 2008;7(5):1994–2006. [PubMed: 18361515]
50. Xie H, Onsongo G, Popko J, de Jong EP, Cao J, Carlis JV, et al. Proteomics analysis of cells in whole saliva from oral cancer patients via value-added three-dimensional peptide fractionation and tandem mass spectrometry. *Mol Cell Proteomics* 2008;7(3):486–98. [PubMed: 18045803]
51. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, et al. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 1984;307(5951):521–7. [PubMed: 6320011]
52. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. *Cancer Res* 1993;53(15):3579–84. [PubMed: 8339264]
53. Grandis JR, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, et al. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Inst* 1998;90(11):824–32. [PubMed: 9625170]
54. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res* 2002;62(24):7350–6. [PubMed: 12499279]
55. Gupta AK, McKenna WG, Weber CN, Feldman MD, Goldsmith JD, Mick R, et al. Local recurrence in head and neck cancer: relationship to radiation resistance and signal transduction. *Clin Cancer Res* 2002;8(3):885–92. [PubMed: 11895923]
56. Temam S, Kawaguchi H, El-Naggar AK, Jelinek J, Tang H, Liu DD, et al. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J Clin Oncol* 2007;25(16):2164–70. [PubMed: 17538160]

57. Quon H, Liu FF, Cummings BJ. Potential molecular prognostic markers in head and neck squamous cell carcinomas. *Head Neck* 2001;23(2):147–59. [PubMed: 11303632]
58. Sok JC, Coppelli FM, Thomas SM, Lango MN, Xi S, Hunt JL, et al. Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. *Clin Cancer Res* 2006;12(17):5064–73. [PubMed: 16951222]
59. Dorsam RT, Gutkind JS. G-protein-coupled receptors and cancer. *Nat Rev Cancer* 2007;7(2):79–94. [PubMed: 17251915]
60. Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A. Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene* 2001;20(13):1594–600. [PubMed: 11313906]
61. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res* 1989;49(17):4682–9. [PubMed: 2547513]
62. Saranath D, Chang SE, Bhoite LT, Panchal RG, Kerr IB, Mehta AR, et al. High frequency mutation in codons 12 and 61 of H-ras oncogene in chewing tobacco-related human oral carcinoma in India 1991;63(4):573–8.
63. Das N, Majumder J, DasGupta UB. ras gene mutations in oral cancer in eastern India. *Oral Oncol* 2000;36(1):76–80. [PubMed: 10889924]
64. Clark LJ, Edington K, Swan IR, McLay KA, Newlands WJ, Wills LC, et al. The absence of Harvey ras mutations during development and progression of squamous cell carcinomas of the head and neck 1993;68(3):617–20.
65. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005;5(10):749–59. [PubMed: 16175180]
66. Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature* 2006;441(7092):431–6. [PubMed: 16724054]
67. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008;132(3):344–62. [PubMed: 18267068]
68. Squarize CH, Castilho RM, Sriuranpong V, Pinto DS Jr. Gutkind JS. Molecular crosstalk between the NFkappaB and STAT3 signaling pathways in head and neck squamous cell carcinoma. *Neoplasia* 2006;8(9):733–46. [PubMed: 16984731]
69. Wilhelmson KC, Eggleton K, Temin HM. Nucleic acid sequences of the oncogene v-rel in reticuloendotheliosis virus strain T and its cellular homolog, the proto-oncogene c-rel. *J Virol* 1984;52(1):172–82. [PubMed: 6090694]
70. Ondrey FG, Dong G, Sunwoo J, Chen Z, Wolf JS, Crowl-Bancroft CV, et al. Constitutive activation of transcription factors NF-(kappa)B, AP-1, and NF-IL6 in human head and neck squamous cell carcinoma cell lines that express pro-inflammatory and proangiogenic cytokines. *Mol Carcinog* 1999;26(2):119–29. [PubMed: 10506755]
71. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer* 2005;5(4):297–309. [PubMed: 15803156]
72. Mishra A, Bharti AC, Varghese P, Saluja D, Das BC. Differential expression and activation of NF-kappaB family proteins during oral carcinogenesis: Role of high risk human papillomavirus infection. *Int J Cancer* 2006;119(12):2840–50. [PubMed: 16998793]
73. Sawhney M, Rohatgi N, Kaur J, Shishodia S, Sethi G, Gupta SD, et al. Expression of NF-kappaB parallels COX-2 expression in oral precancer and cancer: association with smokeless tobacco. *Int J Cancer* 2007;120(12):2545–56. [PubMed: 17354234]
74. Bindhu OS, Ramadas K, Sebastian P, Pillai MR. High expression levels of nuclear factor kappa B and gelatinases in the tumorigenesis of oral squamous cell carcinoma. *Head Neck* 2006;28(10):916–25. [PubMed: 16823875]
75. Jordan RC, Catzavelos GC, Barrett AW, Speight PM. Differential expression of bcl-2 and bax in squamous cell carcinomas of the oral cavity. *Eur J Cancer B Oral Oncol* 1996;32B(6):394–400. [PubMed: 9039223]
76. Chen Z, Malhotra PS, Thomas GR, Ondrey FG, Duffey DC, Smith CW, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res* 1999;5(6):1369–79. [PubMed: 10389921]

77. Allen C, Duffy S, Teknos T, Islam M, Chen Z, Albert PS, et al. Nuclear factor-kappaB-related serum factors as longitudinal biomarkers of response and survival in advanced oropharyngeal carcinoma. *Clin Cancer Res* 2007;13(11):3182–90. [PubMed: 17545521]
78. Jackson-Bernitsas DG, Ichikawa H, Takada Y, Myers JN, Lin XL, Darnay BG, et al. Evidence that TNF-TNFR1-TRADD-TRAF2-RIP-TAK1-IKK pathway mediates constitutive NF-kappaB activation and proliferation in human head and neck squamous cell carcinoma. *Oncogene* 2007;26(10):1385–97. [PubMed: 16953224]
79. Yu M, Yeh J, Van-Waes C. Protein kinase casein kinase 2 mediates inhibitor-kappaB kinase and aberrant nuclear factor-kappaB activation by serum factor(s) in head and neck squamous carcinoma cells. *Cancer Res* 2006;66(13):6722–31. [PubMed: 16818647]
80. Yan B, Chen G, Saigal K, Yang X, Jensen ST, Van Waes C, et al. Systems biology-defined NF-kappaB regulons, interacting signal pathways and networks are implicated in the malignant phenotype of head and neck cancers differing in p53 status. *Genome Biol* 2008;9(3):R53. [PubMed: 18334025]
81. Yan B, Yang X, Lee TL, Friedman J, Tang J, Van Waes C, et al. Genome-wide identification of novel expression signatures reveal distinct patterns and prevalence of binding motifs for p53, nuclear factor-kappaB and other signal transcription factors in head and neck squamous cell carcinoma. *Genome Biol* 2007;8(5):R78. [PubMed: 17498291]
82. O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 2002;109(Suppl):S121–31. [PubMed: 11983158]
83. Darnell JE Jr. Transcription factors as targets for cancer therapy. *Nat Rev Cancer* 2002;2(10):740–9. [PubMed: 12360277]
84. Bromberg JF, Darnell JE Jr. Potential roles of Stat1 and Stat3 in cellular transformation. *Cold Spring Harb Symp Quant Biol* 1999;64:425–8. [PubMed: 11232317]
85. Reich NC, Liu L. Tracking STAT nuclear traffic. *Nat Rev Immunol* 2006;6(8):602–12. [PubMed: 16868551]
86. Darnell JE. Validating Stat3 in cancer therapy. *Nat Med* 2005;11(6):595–6. [PubMed: 15937466]
87. Bromberg J. Stat proteins and oncogenesis. *J Clin Invest* 2002;109(9):1139–42. [PubMed: 11994401]
88. Grandis JR, Drenning SD, Chakraborty A, Zhou MY, Zeng Q, Pitt AS, et al. Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth *In vitro*. *J Clin Invest* 1998;102(7):1385–92. [PubMed: 9769331]
89. Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. *Proc Natl Acad Sci U S A* 2000;97(8):4227–32. [PubMed: 10760290]
90. Rubin Grandis J, Zeng Q, Drenning SD. Epidermal growth factor receptor--mediated stat3 signaling blocks apoptosis in head and neck cancer. *Laryngoscope* 2000;110(5 Pt 1):868. [PubMed: 10807365]
91. Leeman RJ, Lui VW, Grandis JR. STAT3 as a therapeutic target in head and neck cancer. *Expert Opin Biol Ther* 2006;6(3):231–41. [PubMed: 16503733]
92. Masuda M, Suzui M, Yasumatu R, Nakashima T, Kuratomi Y, Azuma K, et al. Constitutive activation of signal transducers and activators of transcription 3 correlates with cyclin D1 overexpression and may provide a novel prognostic marker in head and neck squamous cell carcinoma. *Cancer Res* 2002;62(12):3351–5. [PubMed: 12067972]
93. Kar P, Supakar PC. Expression of Stat5A in tobacco chewing-mediated oral squamous cell carcinoma. *Cancer Lett* 2006;240(2):306–11. [PubMed: 16303247]
94. Xi S, Zhang Q, Gooding WE, Smithgall TE, Grandis JR. Constitutive activation of Stat5b contributes to carcinogenesis *in vivo*. *Cancer Res* 2003;63(20):6763–71. [PubMed: 14583472]
95. Sriuranpong V, Park JJ, Amornphimoltham P, Patel V, Nelkin BD, Gutkind JS. Epidermal growth factor receptor-independent constitutive activation of STAT3 in head and neck squamous cell carcinoma is mediated by the autocrine/paracrine stimulation of the interleukin 6/gp130 cytokine system. *Cancer Res* 2003;63(11):2948–56. [PubMed: 12782602]
96. Lai SY, Childs EE, Xi S, Coppelli FM, Gooding WE, Wells A, et al. Erythropoietin-mediated activation of JAK-STAT signaling contributes to cellular invasion in head and neck squamous cell carcinoma. *Oncogene* 2005;24(27):4442–9. [PubMed: 15856028]

97. Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, et al. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. *Oncogene* 2005;24(44):6699–708. [PubMed: 16007169]
98. Lee TL, Yeh J, Van Waes C, Chen Z. Epigenetic modification of SOCS-1 differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas. *Mol Cancer Ther* 2006;5(1):8–19. [PubMed: 16432158]
99. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004;5(9):691–701. [PubMed: 15372092]
100. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;434(7035):843–50. [PubMed: 15829953]
101. Polakis P. Wnt signaling and cancer. *Genes Dev* 2000;14(15):1837–51. [PubMed: 10921899]
102. Marsit CJ, McClean MD, Furniss CS, Kelsey KT. Epigenetic inactivation of the SFRP genes is associated with drinking, smoking and HPV in head and neck squamous cell carcinoma. *Int J Cancer* 2006;119(8):1761–6. [PubMed: 16708382]
103. Worsham MJ, Chen KM, Meduri V, Nygren AO, Errami A, Schouten JP, et al. Epigenetic events of disease progression in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 2006;132(6):668–77. [PubMed: 16785414]
104. Chang KW, Lin SC, Mangold KA, Jean MS, Yuan TC, Lin SN, et al. Alterations of adenomatous polyposis Coli (APC) gene in oral squamous cell carcinoma. *Int J Oral Maxillofac Surg* 2000;29(3):223–6. [PubMed: 10970088]
105. Ueda G, Sunakawa H, Nakamori K, Shinya T, Tsuchiko W, Tamura Y, et al. Aberrant expression of beta- and gamma-catenin is an independent prognostic marker in oral squamous cell carcinoma. *Int J Oral Maxillofac Surg* 2006;35(4):356–61. [PubMed: 16288849]
106. Mahomed F, Altini M, Meer S. Altered E-cadherin/beta-catenin expression in oral squamous carcinoma with and without nodal metastasis. *Oral Dis* 2007;13(4):386–92. [PubMed: 17577324]
107. Lo Muzio L, Goteri G, Capretti R, Rubini C, Vinella A, Fumarulo R, et al. Beta-catenin gene analysis in oral squamous cell carcinoma. *Int J Immunopathol Pharmacol* 2005;18(3 Suppl):33–8. [PubMed: 16848985]
108. Rhee CS, Sen M, Lu D, Wu C, Leoni L, Rubin J, et al. Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. *Oncogene* 2002;21(43):6598–605. [PubMed: 12242657]
109. Siegel PM, Massague J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 2003;3(11):807–21. [PubMed: 14557817]
110. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003;113(6):685–700. [PubMed: 12809600]
111. Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev* 2005;19(23):2783–810. [PubMed: 16322555]
112. Prime SS, Davies M, Pring M, Paterson IC. The role of TGF-beta in epithelial malignancy and its relevance to the pathogenesis of oral cancer (part II). *Crit Rev Oral Biol Med* 2004;15(6):337–47. [PubMed: 15574678]
113. Lu SL, Herrington H, Reh D, Weber S, Bornstein S, Wang D, et al. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. *Genes Dev* 2006;20(10):1331–42. [PubMed: 16702406]
114. Honjo Y, Bian Y, Kawakami K, Molinolo A, Longenecker G, Boppana R, et al. TGF-beta receptor I conditional knockout mice develop spontaneous squamous cell carcinoma. *Cell Cycle* 2007;6(11):1360–6. [PubMed: 17534148]
115. Peng H, Shintani S, Kim Y, Wong DT. Loss of p12CDK2-AP1 expression in human oral squamous cell carcinoma with disrupted transforming growth factor-beta-Smad signaling pathway. *Neoplasia* 2006;8(12):1028–36. [PubMed: 17217620]
116. Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006;6(3):184–92. [PubMed: 16453012]
117. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* 2005;4(12):988–1004. [PubMed: 16341064]

118. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;296(5573):1655–7. [PubMed: 12040186]
119. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005;307(5712):1098–101. [PubMed: 15718470]
120. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer Cell* 2003;4(4):257–62. [PubMed: 14585353]
121. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *NatRevCancer* 2002;2(7):489–501.
122. Brazil DP, Yang ZZ, Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 2004;29(5):233–42. [PubMed: 15130559]
123. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet* 2005;37(1):19–24. [PubMed: 15624019]
124. Amornphimoltham P, Sriuranpong V, Patel V, Benavides F, Conti CJ, Sauk J, et al. Persistent activation of the Akt pathway in head and neck squamous cell carcinoma: a potential target for UCN-01. *Clin Cancer Res* 2004;10(12 Pt 1):4029. [PubMed: 15217935]
125. Patel V, Lahusen T, Leethanakul C, Igishi T, Kremer M, Quintanilla-Martinez L, et al. Antitumor activity of UCN-01 in carcinomas of the head and neck is associated with altered expression of cyclin D3 and p27(KIP1). *Clin Cancer Res* 2002;8(11):3549–60. [PubMed: 12429646]
126. Massarelli E, Liu DD, Lee JJ, El-Naggar AK, Lo Muzio L, Staibano S, et al. Akt activation correlates with adverse outcome in tongue cancer. *Cancer* 2005;104(11):2430–6. [PubMed: 16245318]
127. Yu Z, Weinberger PM, Sasaki C, Egleston BL, Speier WF, Haffty B, et al. Phosphorylation of Akt (Ser473) predicts poor clinical outcome in oropharyngeal squamous cell cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16(3):553–8. [PubMed: 17372251]
128. Woenckhaus J, Steger K, Werner E, Fenic I, Gamberdinger U, Dreyer T, et al. Genomic gain of PIK3CA and increased expression of p110alpha are associated with progression of dysplasia into invasive squamous cell carcinoma. *J Pathol* 2002;198(3):335–42. [PubMed: 12375266]
129. Fenic I, Steger K, Gruber C, Arens C, Woenckhaus J. Analysis of PIK3CA and Akt/protein kinase B in head and neck squamous cell carcinoma. *Oncol Rep* 2007;18(1):253–9. [PubMed: 17549376]
130. Pedrero JM, Carracedo DG, Pinto CM, Zapatero AH, Rodrigo JP, Nieto CS, et al. Frequent genetic and biochemical alterations of the PI 3-K/AKT/PTEN pathway in head and neck squamous cell carcinoma. *Int J Cancer* 2005;114(2):242–8. [PubMed: 15543611]
131. Murugan AK, Hong NT, Fukui Y, Munirajan AK, Tsuchida N. Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. *IntJOncol* 2008;32(1):101–11.
132. Kozaki K, Imoto I, Pimkhaokham A, Hasegawa S, Tsuda H, Omura K, et al. PIK3CA mutation is an oncogenic aberration at advanced stages of oral squamous cell carcinoma. *Cancer Sci* 2006;97(12):1351–8. [PubMed: 17052259]
133. Sulis ML, Parsons R. PTEN: from pathology to biology. *Trends Cell Biol* 2003;13(9):478–83. [PubMed: 12946627]
134. Lee JJ, Soria JC, Hassan KA, El-Naggar AK, Tang X, Liu DD, et al. Loss of PTEN expression as a prognostic marker for tongue cancer. *Arch Otolaryngol Head Neck Surg* 2001;127(12):1441–5. [PubMed: 11735811]
135. Squarize CH, Castilho RM, Santos Pinto D Jr. Immunohistochemical evidence of PTEN in oral squamous cell carcinoma and its correlation with the histological malignancy grading system. *J Oral Pathol Med* 2002;31(7):379–84. [PubMed: 12224530]
136. Molinolo AA, Hewitt SM, Amornphimoltham P, Keelawat S, Rangdaeng S, Meneses Garcia A, et al. Dissecting the Akt/mammalian target of rapamycin signaling network: emerging results from the head and neck cancer tissue array initiative. *Clin Cancer Res* 2007;13(17):4964–73. [PubMed: 17785546]
137. Shamji AF, Nghiem P, Schreiber SL. Integration of growth factor and nutrient signaling: implications for cancer biology. *Mol Cell* 2003;12(2):271–80. [PubMed: 14536067]
138. Sekulic A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, et al. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res* 2000;60(13):3504–13. [PubMed: 10910062]

139. Inoki K, Ouyang H, Li Y, Guan KL. Signaling by target of rapamycin proteins in cell growth control. *Microbiol Mol Biol Rev* 2005;69(1):79–100. [PubMed: 15755954]
140. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 2003;17(15):1829–34. [PubMed: 12869586]
141. Manning BD, Cantley LC. Rheb fills a GAP between TSC and TOR. *Trends Biochem Sci* 2003;28(11):573–6. [PubMed: 14607085]
142. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;18(16):1926–45. [PubMed: 15314020]
143. Sorrells DL Jr, Ghali GE, De Benedetti A, Nathan CA, Li BD. Progressive amplification and overexpression of the eukaryotic initiation factor 4E gene in different zones of head and neck cancers. *J Oral Maxillofac Surg* 1999;57(3):294–9. [PubMed: 10077200]
144. Nathan CA, Amirghahri N, Rice C, Abreo FW, Shi R, Stucker FJ. Molecular analysis of surgical margins in head and neck squamous cell carcinoma patients. *Laryngoscope* 2002;112(12):2129–40. [PubMed: 12461330]
145. Nathan CA, Amirghahari N, Abreo F, Rong X, Caldito G, Jones ML, et al. Overexpressed eIF4E is functionally active in surgical margins of head and neck cancer patients via activation of the Akt/mammalian target of rapamycin pathway. *ClinCancer Res* 2004;10(17):5820–7.
146. Amornphimoltham P, Patel V, Sodhi A, Nikitakis NG, Sauk JJ, Sausville EA, et al. Mammalian Target of Rapamycin, a Molecular Target in Squamous Cell Carcinomas of the Head and Neck. *Cancer Res* 2005;65(21):9953–61. [PubMed: 16267020]
147. Abraham RT. mTOR as a positive regulator of tumor cell responses to hypoxia. *Curr Top Microbiol Immunol* 2004;279:299–319. [PubMed: 14560965]

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