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Exploiting the head and neck cancer oncogenome: Widespread PI3K-mTOR pathway alterations and novel molecular targets

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Summary

Two studies published in this issue of Cancer Discovery describe the emerging mutational landscape of head and neck squamous cell carcinomas (HNSCC) and their genomic and epigenetic alterations, thus identifying novel actionable cancer drivers and predictive biomarkers for targeted therapies. Most genomic alterations in HNSCC converge in a handful of molecular pathways resulting in cell cycle deregulation, genomic instability, cell differentiation defects, and persistent mitogenic signaling, the latter involving aberrant PI3K/mTOR pathway activation thereby rendering HNSCC responsive to PI3K/mTOR inhibitors.

The recent development of deep sequencing approaches to study human cancer genomes in individual tumor lesions is already revolutionizing medical oncology and translational medicine (1). These unbiased approaches provide an unprecedented knowledge of the multiplicity of somatic mutations and genetic and epigenetic alterations underlying each human cancer type. This large and growing body of information is now contributing to the elucidation of aberrant molecular mechanisms and signaling circuitries driving tumor progression, hence revealing novel druggable targets for therapeutic intervention to prevent and treat human malignancies. Two studies published in this issue of Cancer Discovery join these efforts (2, 3), exploiting the emerging genomic landscape of head and neck squamous cell carcinoma (HNSCC) to identify actionable cancer drivers and biomarkers predicting favorable therapeutic responses to targeted anticancer agents.

HNSCC, which includes malignant squamous lesions arising in the oral cavity, larynx and pharynx, is the sixth most common cancer in the world, with approximately 500,000 new cases annually, and resulting in nearly 11,000 deaths each year in the United States alone (4). The use of tobacco and the excess consumption of alcohol have been long recognized as risk factors for HNSCC development, with added risk caused by betel quid chewing, primarily in Southeast Asia, and the rising incidence of HNSCC associated with high risk human papilloma virus (HPV) infection, now accounting for 10–20% of all cases (5). The striking evidence emerging from recent reports (6, 7) and these new HNSCC genomic studies (2, 3) is the remarkable multiplicity and diversity of genetic alterations in HNSCC. This makes the search for cancer-driving molecular events daunting, especially regarding

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the ability to distinguish them from passenger mutations that may have minimal impact on tumor progression and/or clinical response. Nonetheless, the emerging picture from the in depth analysis of the HNSCC oncogenome is that while the specific molecules altered in each individual tumor may be distinct, they all participate in a handful of dysregulated molecular pathways that are likely shared among most HNSCC lesions.

Building on this concept, Pickering et al. performed a detailed integrated multi-platform analysis of the genomic alterations in HNSCC (2), including genome-wide copy number alterations (CNA), tumor ploidy, gene expression, methylation and point mutations. This approach revealed many more somatic events than previously reported. While 32% of the HNSCC cases were triploid, 37% were tetraploid or had higher ploidy, and 11 regions of focal chromosomal gain and 33 regions of focal loss were identified (2). Overall, 74% of the tumors exhibited at least 20 CNAs, reflecting the high genomic instability of HNSCC. These include gains in 8q (63%), 3q (58%), and focal gains in regions containing *CCND1* (22%), *EGFR* (16%), *MYC* (9%), and *TP63* (26%), which represent candidate cancer drivers (2). Also identified were losses of 3p (76%), 18q (58%), and 8p (53%), which harbor multiple tumor suppressor genes, together with focal losses in 9p (32%) that includes the *CDKN2A* locus (2). Gene CNA alterations often correlated with changes in mRNA levels of the encoded genes, but microRNAs were much less affected. Changes in DNA methylation were also observed, particularly in HNSCC lesions from smokers.

Remarkably, hundreds of genetic alterations were also identified, which extend recent published reports (6, 7). However, most of these alterations fell within four major driver biological processes (Figure 1): 1) mitogenic signaling (63%), with particular emphasis on aberrant activation of the PI3K/mTOR pathway (including 11% mutations on *PIK3CA*, encoding the catalytic subunit of PI3K-α); 2) defective cell differentiation (including 9% *NOTCH1* gene mutations and 66% predicted NOTCH signaling pathway alterations); 3) a nearly universal (94%) cell cycle deregulation due to inactivation of the *CDKN2A* ($p16^{INK4A}$) tumor suppressor gene by copy number loss or promoter methylation, together with *CCND1* (CYCLIN D1) amplification; and 4) genomic stability controlled by *TP53* and other candidate genes, such as those involved in DNA-damage recognition and repair. This study also identified two additional key genes likely affecting cell-cell communication and cell death: *FAT1* (30% mutations) and *CASP8* (10% mutations), respectively. The latter appears to be associated with a cohort of HNSCC harboring activating *HRAS* mutations, suggesting that these tumors may survive apoptotic stimuli arising from *HRAS* gene mutations in the tumor microenvironment. These data revealed that together with a widespread loss of function in tumor suppressor genes, the majority (80%) of HNSCC patients harbor aberrant activity of at least one oncogenic molecular pathway, which could be targeted for pharmacological intervention as part of novel genomically-driven therapeutic strategies (2).

In a pathway-specific effort, Lui *et al.* (3) studied targetable mitogenic signaling routes genomically altered in HNSCC, including the MAPK, JAK/STAT, and the PI3K pathways. Among these, the PI3K pathway harbored the highest percentage of mutations (30.5%), while the MAPK and JAK/STAT pathways were mutated in less than 10% of the cases, further emphasizing that PI3K is the most altered mitogenic signaling pathway in head and

neck cancer. *PIK3CA* was the most mutated gene in the pathway (12.6%), and mutations in *PIK3* genes were the only identifiable oncogenes in 20% of the HPV positive tumors, suggesting that that PI3K fuels the growth of these HPV-associated HNSCC. However, the emerging picture is that *PIK3CA* mutations are not the only genetic alterations resulting in the persistent activation of PI3K and its downstream targets, including AKT and mTOR, in HNSCC. Indeed the PI3K/AKT/mTOR pathway may represent the most frequently activated signaling route in both HPV− and HPV+ HNSCCs (>80% of HNSCC cases, (8–10)), suggesting that multiple genetic and epigenetic changes may act in concert with *PIK3CA* mutations to sustain pathway activation in these malignancies (Figure 1).

In this regard, copy number gain and mRNA overexpression in the *PIK3CA* gene (within 3q) are frequent events in HNSCC at 20% and 52%, respectively (Figure 1). Furthermore, 4% of HNSCCs display mutations in *PIK3CG* (PI3Kγ), a member of a distinct class of G protein-linked PI3K catalytic subunit. Mutations were also identified in four of the PI3K regulatory subunits (each ~2%), and low frequency of mutations (<2%) were also observed in genes for AKT2, mTOR, its associated subunits, RICTOR and RAPTOR, and the tumor suppressor genes *TSC1* and *TSC2* ((3) and Figure 1). Interestingly, mutations and gene copy number loss were identified (4% and 8.16%, respectively) in the tumor suppressor *PTEN*, one of the most effective negative regulators of the PI3K pathway (3). Reduced PTEN protein expression has been also observed in approximately 30% of HNSCCs (11), supporting PTEN functional inactivation in a subset of HNSCCs.

All together, these findings confirm that despite the remarkable complexity of genomic alterations found in HNSCC, most of them fall within a few major driver-signaling pathways (Figure 1), with the majority of the HNSCC lesions harboring genetic and epigenetic alterations that converge on the persistent activation of the PI3K-AKT-mTOR pathway. Surprisingly, in some advanced stage HNSCC cases, tumors can even harbor concomitant genomic alterations in more than one component of this pathway (3). While representing a major HNSCC driver, this likely overreliance on PI3K-mTOR signaling for tumor growth can in turn expose a cancer vulnerability, which can be exploited for therapeutic purposes. Indeed, the high sensitivity of HNSCC to mTOR inhibition has been documented in multiple experimental models and encouraging recent clinical studies (8–10, 12). The presence of genomic alterations in the PI3K pathway may therefore represent a suitable biomarker predicting a clinical response to its pharmacological inhibitors (3). HNSCC cells or patient tumorgrafts with genomic alterations in PI3K were highly sensitive to a PI3K/mTOR inhibitor, while a patient tumorgraft that did not exhibit PI3K pathway mutations was not (3). Thus, the future clinical evaluation of new PI3Kα inhibitors and PI3K/mTOR inhibitors could be enriched for patients harboring activating PIK3CA mutations or other PI3K pathway genetic alterations as predictive biomarkers (3).

Nonetheless, it may be premature to exclude from these future clinical trials patients without the described PI3K pathway genetic changes, given the multiple additional alterations that may result in the activation of downstream targets of PI3K, such as mTOR. For example, while only over 30% of tumors have genomic alterations in the PI3K pathway, more than 80–90% of HNSCC lesions present activation of the PI3K/AKT/mTOR axis, including those cases associated with HPV infection (10). This suggests that while genomic alterations in

the PI3K pathway might be excellent predictors of a response to its inhibitors, this genomic analysis alone may miss a substantial number of patients that have PI3K/mTOR pathway activation arising from other factors and hence could benefit from the same pharmacological intervention. For example, STK11 (also known as LKB1), REDD1, SESTRIN1, and SESTRIN2, all converge to inhibit the mTOR pathway downstream of PI3K. STK11 links mTOR inhibition to cell metabolic and energy sensing, and is mutated in 1% and downregulated in >10% of the HNSCC cases (Figure 1). Of specific relevance to HNSCC, REDD1, SESTRIN1, and SESTRIN2 are all downstream targets of TP53, and hence their mTOR inhibiting activity is disabled in HNSCC lesions harboring TP53 mutations or expressing high risk HPV oncogenes, thereby resulting in mTOR activation in the absence of obvious PI3K pathway genomic alterations (Figure 1).

Clearly, a comprehensive genetic and biochemical approach to evaluate the status of activation of PI3K/mTOR network will likely yield valuable information predicting a clinical response to PI3K/mTOR pathway inhibitors. Newly developed PI3K/mTOR inhibitors are also excellent candidates for combination therapies with currently available treatment options for HNSCC, such as chemotherapy and chemoradiation, or by concomitantly targeting EGFR, which acts upstream of PI3K/mTOR, with biological or small molecule inhibitors. One can envision that building on similar integrated studies, it will soon be possible to harness the power of modern genomics and functional proteomics analytical strategies to study cancer-associated signaling circuitries, and to identify molecular pathways that each specific cancer and its tumor initiating cells are addicted to. This will help identify the patients that may benefit the most from a growing repertoire of signal transduction-based anticancer therapies, either as single agents or as part of rational combinations that may bypass intrinsic and acquired resistant mechanisms.

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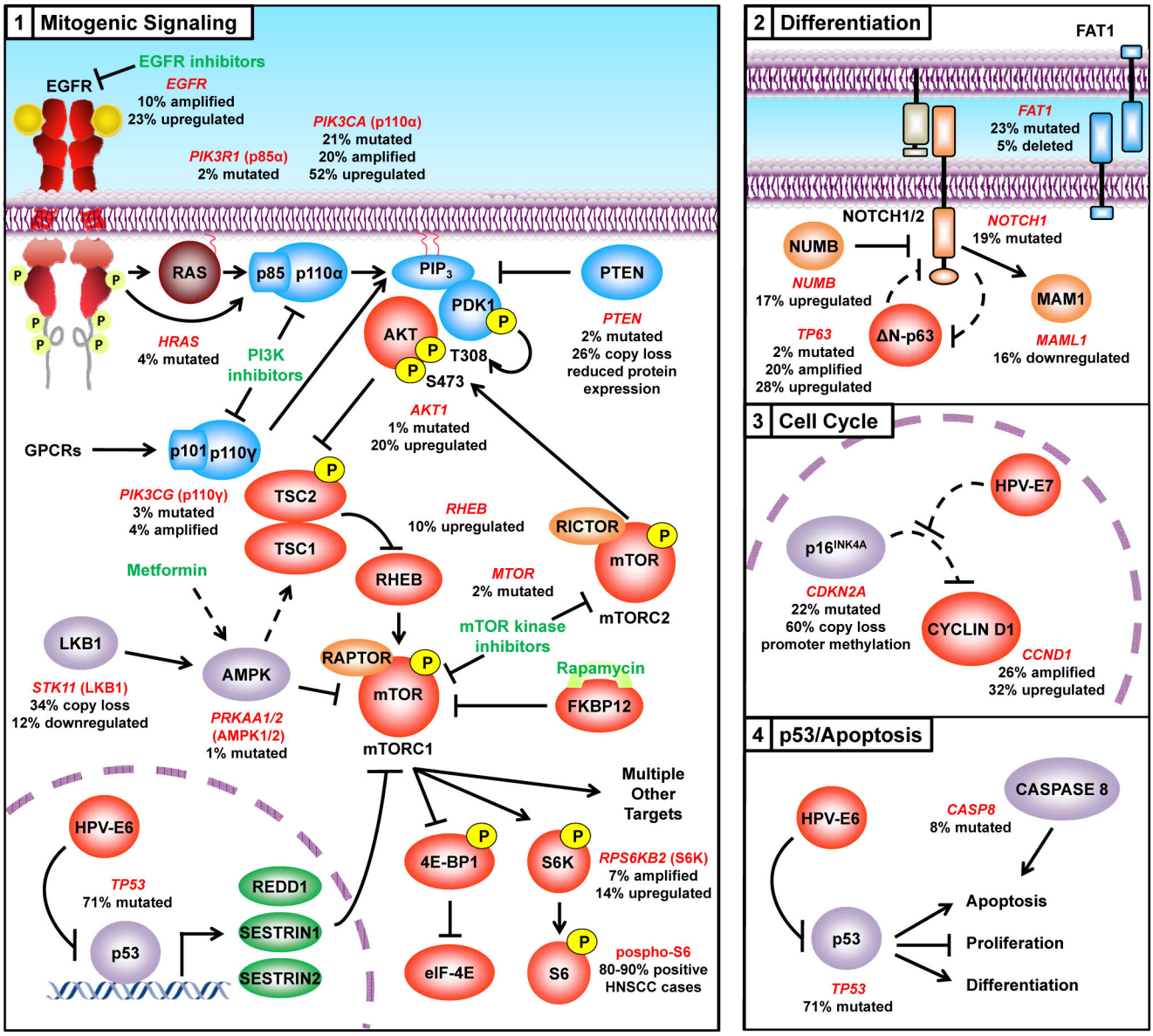

Figure 1. The head and neck cancer oncogenome

Despite the remarkable complexity of genomic alterations found in HNSCC, most of them fall within few major driver-signaling pathways. Alterations found in each key gene are shown. Copy loss refers to homozygous and heterozygous gene deletion. Data were extracted from the publicly available TCGA (The Cancer Genome Atlas) consortium (http://cancergenome.nih.gov/), HNSCC provisional dataset containing CNA, mutational and gene expression data from 295 HNSCC samples.