NF-κB Gene Signatures and p53 Mutations in Head and Neck Squamous Cell Carcinoma

□□ *Commentary on Lee et al., p. 5680*

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Head and neck squamous cell carcinoma (HNSCC) has traditionally been attributed to carcinogen exposure, through heavy tobacco and ethanol consumption. Disruption of tumor suppressor pathways, notably regulated through p53 and Rb, has served as a paradigm for studying HNSCC carcinogenesis and the development of therapeutic resistance. However, the inconsistencies with this simplistic model for a heterogeneous disease have become apparent. Not all HNSCC harbor mutated p53 and the importance of tumor/stromal and immune cell interactions in the microenvironment is increasingly appreciated, although its importance remains to be clarified. In particular, inflammatory gene expression, induced by transcription factors downstream of cytokine and growth factor receptors, such as signal transducers and activators of transcription 3 and nuclear factor-κB (NF-κB), has been implicated in the development of HNSCC from premalignancy and progression to invasion, metastasis, and treatment resistance (1, 2). The potential for differential contribution from one or the other of these crucial oncogenic transcriptional regulators in the context of tumor suppressor pathways has not been carefully explored, until recently, as described by Lee et al. (2), in this issue of *Clinical Cancer Research*.

Elucidation of the oncogenic mediators downstream of dysregulated signaling pathways that contribute to the development and progression of HNSCC is of translational importance. In general, these mediators have focused on cell cycle regulatory proteins, such as cyclinD1, p53, and $p16^{arf}$. It is becoming increasing clear that NF-κB–mediated inflammatory signals are overexpressed in HNSCC and transmit prosurvival and antiapoptotic effects through a set of genes under the transcriptional control of NF-κB. In this issue, a group led by Drs. Zhong Chen and Carter Van Waes at the NIH describes the role of two potentially disparate pathways, NF-κB and p53. Using expression profiling arrays and gene knockdown/overexpression strategies in HNSCC cell lines, two clusters of gene sets were identified, which roughly segregated based on NF-κB or p53 status. Based on these results, the authors suggest that HNSCC may arise from two divergent carcinogenic pathways. One set of tumors showed consistent activation of a cluster of NF-κB–mediated gene expression, whereas genes regulated primarily by p53 segregated the second cohort. These findings may explain, in part, the conflicting reports on the prognostic role of p53 alterations in this disease (3–5) and lend credence to the relationship of serum inflammatory mediators to clinical disease status (6, 7).

NF-κB is a transcription factor that modulates the expression of inflammatory genes in HNSCC. Constitutive NF-κB activation seems to be a common event induced by carcinogen exposure or infection with oncogenic viruses. This event causes up-regulation of genes important for tumor cell proliferation, survival, migration, and angiogenesis. Newer agents targeting the proteasome, inhibitor-κB kinase, and other upstream kinases involved in NF-κB activation have shown anticancer activity in clinical or preclinical studies. However, a clear understanding of the role and hierarchy of oncogenic signaling regulated by different transcription factors, such as NF-κB or p53, has been lacking. A large body of research and epidemiologic evidence indicates that activation of NF-κB via various stimuli plays direct or indirect roles in cellular promotion, transformation, and progression of HNSCC. EBV, an etiologic agent in nasopharyngeal carcinoma, encodes the proteins LMP-1 and human T-lymphocyte virus gene Tax, which can activate NF-κB, thereby promoting cell proliferation, survival, and inflammation (8). Human papillomavirus, now recognized as a major etiologic agent in oropharyngeal carcinomas, encodes the E6 and E7 proteins that inactivate p53 and Rb tumor suppressor gene products (9). E6 and E7 have also been implicated in NF-κB activation and are associated with premalignant and malignant lesions of the cervix and oropharynx, the most common sites of human papillomavirus–associated tumors.

There is now considerable evidence that basal and induced activation of NF-κB is prevalent in tumor cell lines and tissue specimens and contributes to carcinogenesis and tumor relapse in many cancer types, such as lymphomas and carcinomas of the breast, prostate, lung, colon, pancreas, head and neck, esophagus, and cervix (10). Autocrine or paracrine activation of NF-κB resulting from overexpression of epidermal growth factor, HER2, interleukin-1, hepatocyte growth factor, chemokine receptor (CCR7), and integrin family ligands and receptors has been reported. Epidermal growth factor receptor and cytokine receptor signaling pathways involving phosphatidylinositol 3-kinase, IκB kinase, Akt, and associated genes have been shown to mediate activation in HNSCC (11–13). Loss of the inhibitory effects of certain tumor suppressor genes may also contribute to the role of NF-κB as a prosurvival factor. Activation of functional ARF, ATR, and Chk2 may alternatively phosphorylate RelA (p65), and p53 can bind to CBP/p300, resulting in repression of NF-κB prosurvival genes when the ARF-ATR-p53 pathway and

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proapoptotic target genes are activated (14). Inactivation of this proapoptotic pathway through inhibition of p14ARF/p16INK4A, or human papillomavirus inactivation of p53 is among the most common alterations in human cancers. Thus, the findings by Lee et al. implicating mutually distinct gene clusters is not entirely consistent with data linking these pathways in tumor cells (15).

Several key genes in cancer are targets of and transcriptionally regulated by NF-κB, including the oncogenes, cyclin D, Bcl-XL, and IAP. Expression of key angiogenesis factors and adhesion molecules, such as interleukin-8 and vascular endothelial growth factor, is directly or indirectly enhanced by NF-κB activation. Together, these genes contribute to increased tumor proliferation and survival. In this way, the current paradigm for signaling by classic or alternative IκB kinase/NF-κB pathways has emerged. Interactions of NF-κB components with positive and negative regulators, such as phosphatidylinositol 3-kinase/Akt or the p14ARF/p53 pathway, respectively, may regulate whether DNA-bound NF-κB results in gene activation or repression. As shown in previous work by Loercher et al. (16), NF-κB has direct carcinogenic effects in HNSCC cells and may enable these cells to disable or evade innate or adaptive immune control in the microenvironment.

These findings have implications for several therapeutic agents directed at reducing tumor burden or enhancing the efficacy of existing chemotherapies or radiotherapies. Inhibition of NF-κB has been found to be an important mechanism of action of steroids, nonsteroidal anti-inflammatory drugs, and natural and synthetic compounds that show therapeutic and preventive activity in several cancers with acceptable safety profiles. Newer agents targeting the proteasome, IκB kinase, and other upstream kinases involved in NF-κB activation have shown anticancer activity in clinical or preclinical studies. Bortezomib (PS-341) is a proteasome inhibitor that has recently shown single-agent efficacy and may improve radiosensitivity in recurrent/metastatic HNSCC (17, 18). Accepted and emerging therapeutic and prevention approaches may benefit from a greater understanding of the pathways and mediators that are important to target. For instance, *cis*-platinum, one of the standard cytotoxic chemotherapeutic and radiation-sensitizing agents through inflicting DNA damage, is used frequently in HNSCC and has also been shown to inhibit NF-κB activation and expression of antiapoptotic genes (19). Thus, improvement in current cancer therapy may be achieved by approaches to specifically and selectively target the inflammatory NF-κB pathway when overexpressed in certain patients' tumors.

In summary, alterations in multiple signal pathways shown to positively and negatively regulate NF-κB activation are consistent with its frequent activation and important functional role in cancer. The model presented by Lee et al. raises the possibility that, in some tumors, mutated p53 leads to activation of a cluster of related gene pathways, whereas, in other tumors with wild-type p53, constitutive and inducible NF-κB activation results in a distinct cluster of target gene expression. Other studies by this group support the prognostic importance of these two transcriptional regulators (20). Inflammatory gene expression alterations may represent crucial host and stromal interactions in tumorigenesis, leading to reduced immune/inflammatory cell rejection of tumor cells. Activation of inflammatory genes reflects, at least in part, the contribution of the immune system to tumor development and progression. The model presented by Lee et al. may help explain the heterogeneity in response to therapy in HNSCC. Future studies can test various aspects of this model and its translational implications. Identification of a gene cluster that identifies therapeutic response in individual tumors would bring us another step closer to the realization of personalized cancer medicine.

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