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Mutation signature of adenoid cystic carcinoma: evidence for transcriptional and epigenetic reprogramming

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Adenoid cystic carcinoma (ACC), a relatively rare malignancy usually of salivary gland origin, has a signature v-myb avian myeloblastosis viral oncogene homolog–nuclear factor I/B (MYB-NFIB) gene fusion that activates MYB transcriptional regulatory activity. A new study in this issue by Stephens et al. is a comprehensive genomic mutation profiling analysis of this neoplasm and documents a common theme of alteration in chromatin regulatory genes. Also, mutations in SPEN (split ends, homolog of *Drosophila*), which encodes an RNA-binding coregulatory protein, suggest that other changes in transcriptional regulation may involve the NOTCH, FGFR, or other signaling pathways in which SPEN participates. Since there is a low prevalence of mutations in common oncogenes and tumor-suppressor genes, it is likely that alterations primarily in specific transcriptional regulatory genes, augmented by changes in chromatin structure, drive the neoplastic process in ACC.

In this issue of *JCI*, Stephens et al. (1) report the results of exome sequencing of 24 cases of adenoid cystic carcinoma (ACC), a relatively rare tumor, but one that is among the most common malignancies arising in salivary glands. ACC has distinctive clinical and pathologic features, including an often lengthy clinical course before the majority of patients succumb to their disease (2), a proclivity for tumor cells to

invade nerves, which may lead to incomplete surgical resection and recurrence, and distinct myoepithelial/luminal epithelial cellular differentiation. The elucidation of the specific molecular events that underlie ACC may lead to targeted therapies for patients who have distant metastases for whom there currently are no effective chemotherapeutic agents.

MYB-NFIB is the signature molecular alteration

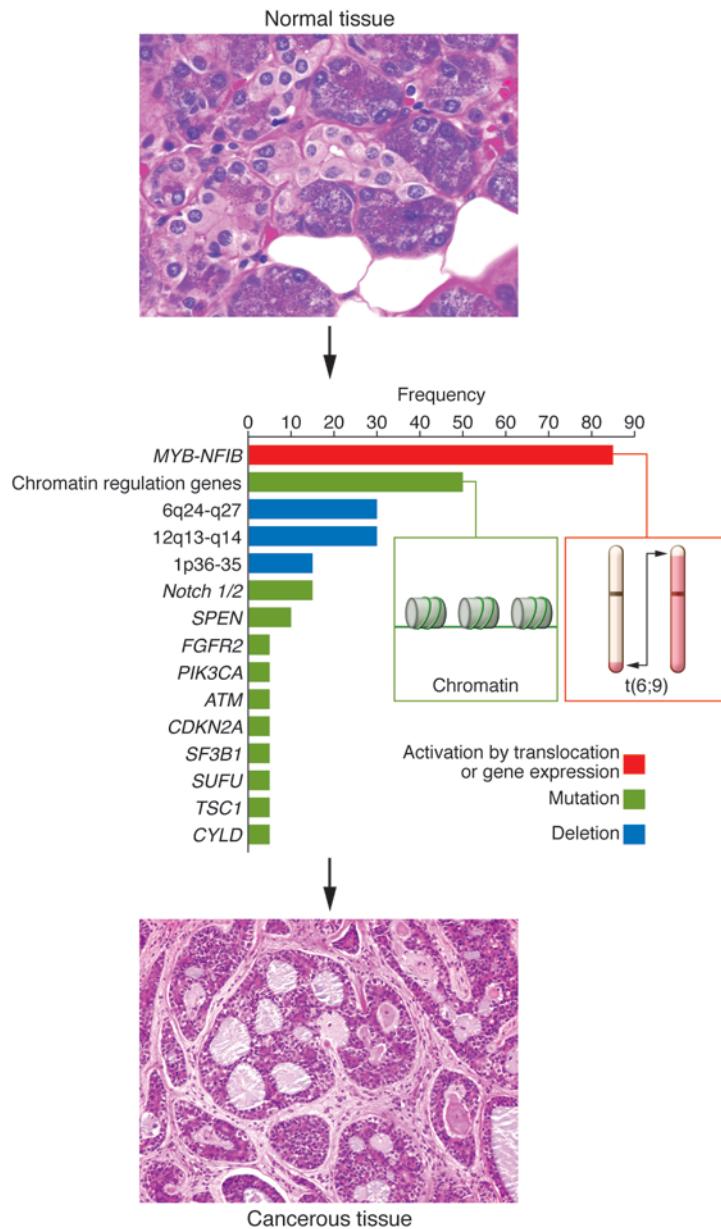
The study by Stephens et al. (1) confirms the presence of activation of v-myb avian myeloblastosis viral oncogene homolog (MYB)

(on chromosome 6) in the majority of ACC (19/24, 79%); this occurs chiefly by chromosomal translocation and fusion to nuclear factor I/B (NFIB) (on chromosome 9). This key oncogenic event, first discovered in 2009 by Persson et al. (3) in Goran Stenman's laboratory, appears to result in increased concentration and activity of the MYB transcriptional regulatory protein domains. The overexpression of MYB, which may be dysregulated by other mechanisms in ACC that lack MYB-NFIB fusion (4), leads to altered expression of its putative target genes involved in cell-cycle control, apoptosis, cell growth, angiogenesis, and cell adhesion (3). Which of these genes is the most critical for the growth and maintenance of ACC remains to be proven experimentally.

Aside from MYB alterations, Stephens et al. (1) report a mean of 13 mutations per exome in ACC, a mutation rate lower than that reported in comprehensive sequencing analyses of the most common types of carcinoma. The relative stability of the ACC genome at the nucleotide level is in keeping with comparative genomic hybridization (CGH) and array CGH studies that have revealed relatively few copy number alterations per genome

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**Figure 1**

The neoplastic transformation of normal salivary gland (upper histologic figure) to adenoid cystic carcinoma (lower histologic figure) is the culmination of genetic alterations including translocation, deletions, and mutations, catalogued from comprehensive array CHG (4) and whole exome sequencing (1) analyses. Key targets include the activation of MYB, most commonly by a t(6;9) chromosomal translocation (karyotype shown) and mutations in genes that regulate chromatin structure (shown graphically). Original magnification, $\times 400$ (upper panel); $\times 200$ (lower panel).

demethylase (12). While it is not known whether or how the chromatin modifications brought about by these mutations interact with the altered transcriptional activity caused by MYB activation (as well as perhaps other transcriptional regulatory pathways), it is interesting to speculate that changes in histone structure may either permit or augment reprogramming of transcriptional regulatory networks that drive the neoplastic cellular phenotype. Indeed, investigations in model systems suggest that joint action by a series of chromatin remodeling and transcriptional activation complexes is required for a more robust alteration in transcriptional regulation (13).

Of note, the single highest mutation frequency after MYB-NFIB occurred in SPEN (split ends, homolog of *Drosophila*) (7/66, 11%), whose gene product is an RNA-binding protein that acts as a coregulator with other transcriptional regulatory proteins. SPEN (also known as SHARP and MINT), a downstream effector of NOTCH signaling, generally represses the transcription of specific genes in the absence of NOTCH signaling (14); it also participates in the transcriptional response in other signaling pathways, including those of the FGFR family (15). Mutations in NOTCH1/2 and FGFR2 were also identified, further suggesting that these pathways play a role in ACC tumorigenesis and may have some overlap in regulating the effects of SPEN in this particular cellular phenotype.

Secondary ACC mutations target other transcriptional and chromatin regulators

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Secondary ACC mutations target other transcriptional and chromatin regulators

While no other single gene has been found to be mutated at a frequency approaching that of the MYB-NFIB fusion, a combination of genes whose protein products are involved in chromatin regulation were mutated in approximately 50% of ACC. These include ARID1A, a member of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex (9), CREBBP, a histone acetylase and transcriptional coactivator (10), EP300, a histone acetylase (11) and KDM6A, a histone lysine

(4–8), including the absence of high copy number amplifications. These data suggest that MYB activation is the primary driver event in ACC and that genomic instability appears to be a less important mechanism of tumorigenesis. It is also noteworthy that some of the most commonly altered oncogenes and tumor suppressor genes in cancer were underrepresented in this study; mutations in PIK3CA and CDKN2A occurred in only one ACC, and mutations in *TP53*, *RB1*, *ERBB2*, *BRAF*, *EGFR*, *KRAS*, *PTEN*, and *KIT* were absent, again indicating the unique and limited mutation signature of this neoplasm (Figure 1).

Model systems and potential downstream therapeutic targets

For patients with ACC who develop distant metastases, new modes of therapy are essential, as both conventional single and combination chemotherapeutic agents have largely been ineffective (16). The investigation of



biochemical mechanisms in cell signaling in ACC and preclinical studies of potential therapies would be aided by the analysis of model systems, but, to our knowledge, no validated and available ACC cell lines currently exist (17). However, xenografts derived from primary and metastatic ACC and serially passaged in mice have been found to be histopathologically identical and have gene expression profiles similar to those of their corresponding original tumors (18). Indeed, we have found these models to be extremely useful in screening compounds for their activity in ACC (unpublished observations).

Although *MYB* is clearly the driver oncogene in most ACC, targeting this transcription factor is not currently possible therapeutically. A reasonable approach would be to identify a pathway often activated in ACC for which kinase inhibitors are in current clinical usage. Indeed, our group has found that *FGFR1* is overexpressed and activated in many ACC compared with normal salivary glands (unpublished observations). *FGFR1* has *MYB* consensus binding sites just upstream of its promoter (19), and the major ligand of *FGFR1*, *FGF2*, is regulated by *MYB* in melanoma cells (20). These findings have led to the testing of various chemotherapeutic agents in ACC xenografts. The multi-tyrosine kinase inhibitor Dovitinib shows significant activity in growth suppression (unpublished observations), prompting a clinical trial of Dovitinib for patients with progressive, metastatic ACC (21). The identification of likely activating mutations in *FGFR2* in a few cases of ACC (1) supports the disruption of *FGFR* signaling as a rational therapeutic approach and suggests that mutational analysis of this gene in any type of neoplasm responsive to Dovitinib should be considered.

Lessons from a rare disorder

With a fairly stable genome, ACC provides a remarkably clear genetic fingerprint that points to specific molecular alterations likely responsible for neoplastic transformation. The identification of the signature *MYB-NFIB* fusion (3) together with the comprehensive mutation profiling by Stephens et al. (1) are key events in the unraveling of the molecular landscape of ACC. As *MYB-NFIB* fusion is the obvious driver for this cancer type, the transcriptional reprogramming that it causes will be a key focus of future

research. Modeling *MYB* activation in cell culture and transgenic animal models, especially in combination with disruption of chromatin remodeling complexes, will help us more fully understand the secondary changes that are required for full neoplastic transformation. The mutational signature in ACC suggests that activated transcriptional regulators require the participation of altered chromatin structure to fully effect durable changes in cell phenotype, and it appears that signal transduction events that culminate in complexes with the *SPEN* coregulatory factor are likely targets. It is intriguing, however, that ACC has so few mutations in the “upstream” portion of signal transduction pathways, which are so dominant in other cancers. Deciphering the specific transcriptional control elements in ACC may help highlight the mechanistic differences that underlie the diversity of cellular differentiation and clinical behavior of human neoplasms.

Note added in proof. Ho and colleagues recently reported the exome or whole-genome sequences for 60 ACC and found a low exonic somatic mutation rate, *MYB* translocations in 57%, and a 35% frequency of mutations targeting chromatin-remodeling genes (22).

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