IN THE SPOTLIGHT

Adenoid Cystic Carcinoma Can Be Driven by *MYB* or *MYBL1* Rearrangements: New Insights into MYB and Tumor Biology

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Summary: A majority of adenoid cystic carcinomas (AdCC)—rare tumors of the salivary gland and some other organs—have recently been found to be driven by chromosomal translocations resulting in *MYB–NFIB* fusions. Brayer and colleagues and Mitani and colleagues have now reported that AdCCs can alternatively be driven by similar rearrangements involving a second *MYB* family gene, *MYBL1*, and that these two drivers act in remarkably similar ways. *Cancer Discov; 6(2); 125–7. ©2016 AACR.*

See related article by Brayer et al., p. 176 (3).

Recent reports of gene fusions between *MYB* (a.k.a. *c-MYB*) and *NFIB* in a rare tumor of the salivary gland or breast called adenoid cystic carcinoma (AdCC) have provided new impetus to understand the protumorigenic actions of the transcription factor MYB. Yet, as in many areas of research endeavor, it can be the explorations of cases that do not fit into the general picture that are equally revealing. *MYB–NFIB* gene fusions generated by recurrent t(6;9) translocations account for the majority of AdCC cases (1, 2), and because the genomes of these tumors are by most standards minimally scrambled, the field has cautiously accepted that these tumors are *MYB* driven. This in turn has broadened the acceptance that *MYB* is a *bona fide* human oncogene.

However, such fusions are not universal in AdCC even if MYB protein expression remains robust, raising the prospect that other mechanisms and/or *MYB* fusion partners might be involved. To make matters more complicated, high MYB protein expression in AdCC is not universal either. Two new studies, one from Brayer and colleagues (3) and another from Mitani and colleagues (4), shine a light on why this might be the case.

By using the different technological approaches of RNA-seq from paraffin-embedded AdCC blocks (3) or whole genomic sequencing of fresh-frozen tumor samples (4), these two groups' findings converged on the discovery that *MYBL1* gene fusions, also frequently involving *NFIB*, are a recurrent feature of this cancer in cases where *MYB* is clearly not involved (Fig. 1, top). *MYBL1* (a.k.a. *A-MYB*) has restricted expression in normal tissues, most notably T cells, testis, and, interestingly, from the perspective of AdCC of the breast, the developing mammary gland. The third *MYB* family member, *MYBL2* (a.k.a. *B-MYB*), is arguably the most ubiquitously expressed and is primarily involved in cell-cycle progression. To date, *MYBL2* fusions have not been reported in AdCC or other tumors.

Of the three family members, aberrant *MYB* stands apart as being, until recently, the only one with clear oncogenic activity. This has been extensively reviewed elsewhere but can be traced back to *MYB*'s origins as a retroviral oncogene, a locus rearranged in various hematopoietic malignancies of animals and humans, and a critical contributor to common human epithelial cancers of the colon and breast (5). However, recent studies of pediatric low-grade gliomas (e.g., ref. 6) provided the first glimpse of *MYBL1*'s oncogenic potential. Each member (*MYB, MYBL1*, and *MYBL2*) shares a highly homologous DNA binding cassette which in evolutionary terms has been used extensively in other eukaryotes, most predominantly in plants. Nevertheless, the three mammalian family members seem to regulate genuinely different expression programs. The polypeptide regions that reside C-terminal to the highly conserved DNA binding domains dictate these differences, presumably via divergent transcriptional regulatory mechanisms and partnerships. This view, supported by the Ness group's previous domain-swapping studies (7), is also convergent with the evolutionary relationships among the *MYB* family. Evolutionary and conservation analysis has revealed that *MYB* and *MYBL1* are more closely related to each other than either is to *MYBL2*; the latter appears to have arisen earlier in evolution, as it is most similar to the single MYB family gene present in invertebrates such as *Drosophila* (8).

Such considerations may have prompted Brayer and colleagues (3) to look closely at the transcriptomes of *MYB* fusion– and *MYBL1* fusion–associated AdCCs. Here, they found two very interesting things. One was that expression of the nonrearranged homolog was low; that is, in AdCC with *MYBL1* fusions, *MYB* was low, and vice versa. A second observation, also made by Mitani and colleagues (4), was that the gene expression signatures and targets of both fusions were very similar, but clearly different from normal salivary gland or AdCCs not apparently involving *MYB* or *MYBL1* rearrangements. Therefore, it seems that the transforming/transcriptional functions of *MYB* and *MYBL1* appear to be, at least in the instance of AdCC, interchangeable.

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Another area of coming together of general principles that link MYB and cancer is that of patient outcome. High MYB expression in colon cancer and high MYB(L1) in AdCC are predictors of poor outcome. In contrast, high *MYB* in estrogen receptor–positive breast cancer tracks with better survival, in part perhaps because this cancer responds optimally to effective targeted therapies—i.e., those blocking estrogen action or production. However, unlike colon and breast cancers, where aberrant expression of full-length MYB protein is a general finding, AdCC uses a range of fusions that lead to removal, to different extents, of the C-terminal regulatory regions of the two MYB family proteins (Fig. 1, top). In some cases, the MYB truncations closely resemble highly activated forms of MYB found in other tumors or identified experimentally, whereas in other cases the fusions result in minimal structural alteration to the MYB protein. Nevertheless, it now appears that the common activating mechanism in AdCC is the juxtaposition of *NFIB* enhancer elements with *MYB* (and now presumably *MYBL1*) that consequently drives high-level *MYB* expression (9).

Intriguingly, the relative transcriptional potencies of the various MYB/MYBL1 fusion proteins in *in vitro* reporter assays are also broadly different, such that those with the most C-terminally truncated forms of MYB largely track with the highest activity (3). Moreover, analysis from Mitani and colleagues suggested that transcriptional profiles, albeit of a limited number of samples, more closely correlated with longer versus truncated MYB family proteins rather than whether the tumor is driven by *MYB* or *MYBL1*. This is in agreement with the functional equivalence discussed above. Intriguingly, in some cases the fusion protein is no more transcriptionally active than the wild-type protein. Perhaps part of the explanation that underpins these differences will come from understanding the balance of activation and repression by the various forms and the interplay with other transcriptional regulators, as well as how other pathways, most notably those involving NOTCH1, might shape AdCC transformation (10). On this last point, a precedent is that *MYB* and *NOTCH1* seem to work in concert in the transformation of around 25% of T-cell acute lymphoblastic leukemias.

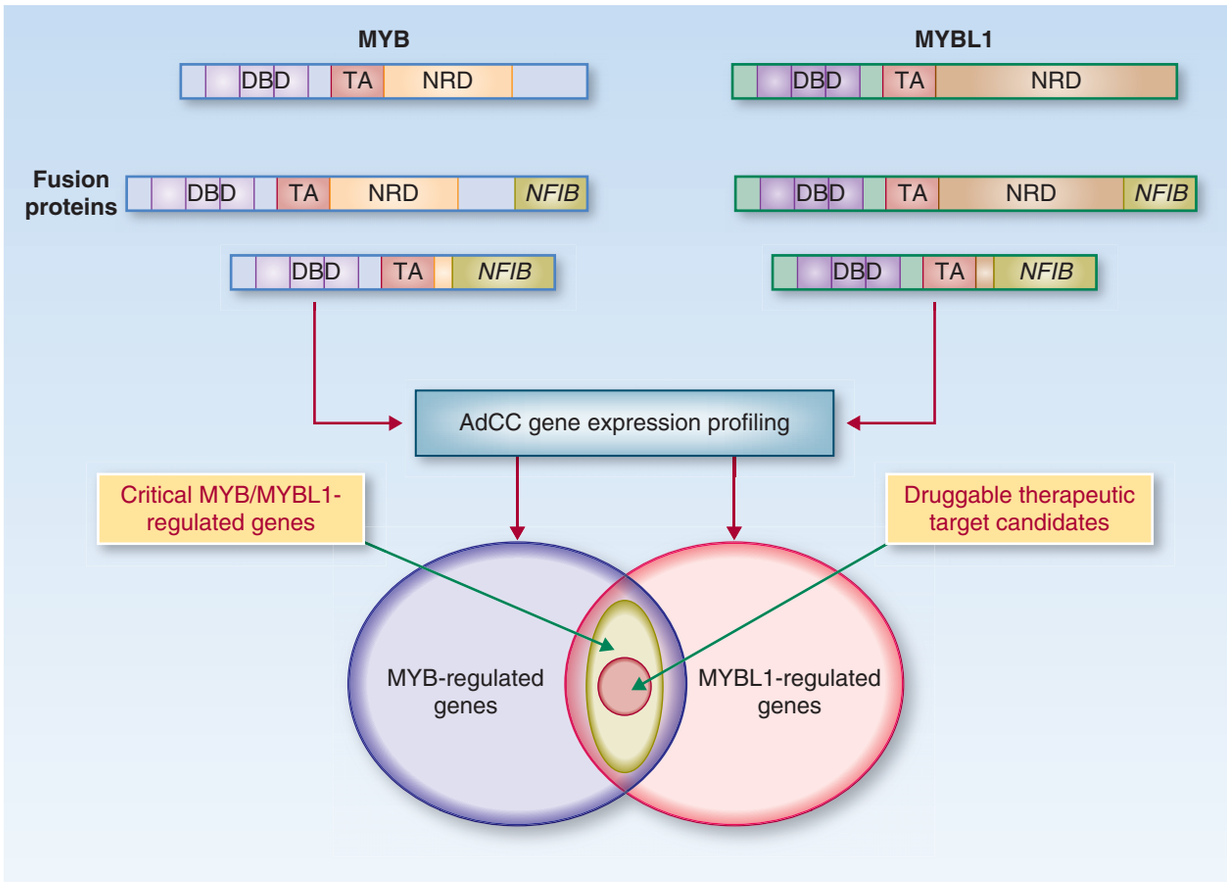

Figure 1. Top, simplified schematic showing the two major types of MYB and MYBL1 fusion proteins seen in AdCC, each of which are generated by translocations involving the *NFIB* gene. The approximate locations of the three main functional domains of each protein are indicated in the complete MYB/MYBL1 proteins and in the fusion products: DBD, DNA-binding domain; TA, transactivation domain; NRD, negative regulatory domain. Note that for both *MYB* and *MYBL1*, different translocations and/or splicing events generate some fusions containing all/most of the NRD as well as some where the NRD has been extensively disrupted. Bottom, a possible approach based on expression profiling data [e.g., as described by Brayer et al. (3) and Mitani et al. (4)] for identifying candidate therapeutic targets in AdCC, based on the notion that these will be common to *MYB*- and *MYBL1*-rearranaged tumors.

Beyond the original reports of *NFIB* being a common *MYB*, and now *MYBL1* fusion partner, both groups recognized new fusion partnerships in AdCC. Brayer and colleagues identified *RAD51B*, whereas Mitani and colleagues found *YTHDF3* as *MYB* and *MYBL1* fusion partners. Although the importance of NFIB (which is also a transcription factor) in transformation remains puzzling, at the genomic level *NFIB* seems to form reciprocal fusions (to *MYB* and *MYBL1*) with other genes that include *XRCC4*, *PTPRD*, *ANIN2*, and *AIG1*. An uneasy explanation would be that these arise as part of the excess of double-stranded breaks that are generated by happenstance and are of no consequence. It would be prudent at this time to hold this view in reserve.

Having so nicely discovered the predominance of *MYB* and now *MYBL1* rearrangements in AdCC, the next key question is how to exploit this knowledge in order to treat patients with this slow-developing but ultimately fatal cancer. This question has been given recent consideration more generally by those charged with managing patients with AdCC. The recognition that MYB and MYBL1 fusions generate common transcriptional signatures suggests that the MYB component must be engaging common or shared transcriptional machinery. Thus, this exceptionally high amino acid sequence homology between the DNA binding domains offers at least two therapeutic avenues. One might involve blocking protein–protein interactions mediated by this domain, and the other is to use immunologic approaches that recognize aberrant expression of cellular proteins processed and presented in the context of the MHC. Perhaps novel vaccination approaches directed against this domain might be efficacious against AdCC expressing MYB or MYBL1 fusions (11). Finally, this commonality of transcriptional profiles might be exploited to identify critical downstream target genes, because we would assume that these would be regulated by both MYB and MYBL1 fusions in AdCC. Integrated ChIP-seq and RNA-seq studies in each case would identify recurrent and direct targets, and this subset could then be interrogated to highlight candidate druggable gene products for subsequent functional validation (Fig. 1, bottom). The lack of AdCC cell lines and engineered animal models will remain a problem here, but one that can be partially bypassed using transplantable patient-derived tumor xenografts (12).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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