IN THE SPOTLIGHT

MYC, MAX, and Small Cell Lung Cancer

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Summary: In this issue of *Cancer Discovery*, Romero and colleagues identify somatic mutations and deletions of *MAX*, and also define what seem to be mutually exclusive alterations in MYC family members and other MYC-associated factors in small cell lung cancer. Taken together, these data highlight the importance of MYC signaling in small cell lung cancer and suggest possible avenues for therapeutic intervention. *Cancer Discov; 4(3); 273–4. ©2014 AACR.*

See related article by Romero et al., p. 292 (7).

Small cell lung cancer (SCLC) is an aggressive malignancy with a strong predilection for early metastasis. Approximately two thirds of patients have metastatic disease at the time of diagnosis. Metastatic disease is associated with a median survival of approximately 9 months from diagnosis, and a 5-year survival rate of less than 1%. Even limited-stage disease is associated with a median survival from the time of diagnosis of only 18 months. New therapeutic targets and new approaches are needed for management of this disease.

Recent genomic analyses of SCLC confirmed that concomitant inactivation of two key tumor suppressors, *TP53* and *RB1*, is exceptionally common, if not universal, and represents a primary driver of small cell lung cancer tumorigenesis (1, 2). Disappointingly, these studies, primarily based on exome sequencing, did not reveal consistently mutated and readily druggable oncogenes, such as the mutant tyrosine kinases found in subsets of lung adenocarcinoma. However, a number of known or suspected oncogenes were found to be focally amplified and overexpressed in SCLCs, notably including all members of the *MYC* family: *MYC* itself as well as homologs *MYCN* and *MYCL*. Intriguingly, a recurrent in-frame gene fusion of *MYCL* and a gene called *RLF* was also observed in a primary SCLC and four SCLC lines (1).

Aberrant MYC signaling represents a more general hallmark of malignancy, found in an estimated 70% of all human cancers (3). *MYC* was among the earliest identified and validated oncogenes, first cloned in an oncogenic viral form, *v-MYC*, from which the endogenous gene was subsequently identified by homology (4). The roles of MYC in promoting cancer growth are both numerous and complex: ongoing research is continuing to define mechanisms of MYC activity, and is also adding additional layers to our understanding of MYC action in both normal cell physiology and cancer, 35 years after its initial description.

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One striking example, in the current issue of *Cancer Discovery*, comes from the laboratory of Dr. Sanchez-Cespedes. MYC has been reported to have both transcriptional and nontranscriptional activities, but its function as a DNA-binding transcriptional activator has been most convincingly and broadly implicated in driving oncogenic growth (3). MYC binds a DNA motif, the E-box (CACGTG, with some variability), as a heterodimer with a primary partner, MAX. E-boxes are among the most commonly found motifs in gene promoters. MYC:MAX dimers bound to DNA serve as a hub for the binding of multiple additional proteins. Distinct MYC-containing DNA-bound protein complexes can act as either transcriptional activators or transcriptional repressors, depending on both site and context.

It was originally hypothesized that the transcriptional regulatory functions of MYC were entirely or largely dependent on heterodimeric partnering with MAX. However, inactivating mutations in *MAX* were found initially in a rat pheochromocytoma (5) and, subsequently, in human pheochromocytomas, including germline mutations associated with a hereditary form of this disease (6). Pheochromocytoma is a neuroendocrine cancer of the adrenal medulla. Cancer-specific inactivation of *MAX* was surprising, as the hypothesized requirement for MYC:MAX partnering for MYC oncogenic activity would be inconsistent with MAX having a tumor-suppressor function.

Romero and colleagues (7) now extend the observation of tumor-specific *MAX* inactivation to another neuroendocrine tumor type: SCLC. On the basis of the prior observations in pheochromocytoma, the investigators conducted a focused analysis of *MAX* in a set of 98 SCLC tumors and cell lines, identifying homozygous somatic alterations of *MAX* in six (6%). *MAX* inactivation was found to be mutually exclusive of gene amplification of *MYC*, *MYCL*, or *MYCN*, and was also mutually exclusive of mutations in *BRG1*, encoding an ATPase of the switch/sucrose nonfermentable (SWI/SNF) complex known previously to be a regulator of MAX, and of *MGA*, encoding a protein involved in MAX dimerization.

The authors further extend our understanding of the dynamic interactions between these interacting factors, in some surprising directions. They find that BRG1 binds to the *MAX* promoter, and that BRG1 depletion by short hairpin RNA (shRNA) leads to marked downregulation of

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MAX expression. Surprisingly, however, they find that BRG1 depletion specifically inhibits the growth of *MAX*-deficient cells, that is, cells in which BRG1 regulation of *MAX* is not operant. The authors offer a hypothetical model to account for this seemingly counterintuitive synthetic lethality, but clearly more work will be required to define the mechanisms involved.

This work begs several additional questions to be addressed. Is cancer-specific somatic *MAX* inactivation unique to neuroendocrine cancers? If so, why? If MYC is regulating transcription in a MAX-independent manner, is it doing so via MYC:MYC homodimers (as hypothesized by the authors) or is it preferentially heterodimerizing with another basic helix–loop–helix leucine zipper DNA-binding protein, of which there are many? Assessing the changes in MYC protein:protein interactions in the absence of MAX will be of interest. How does the loss of MAX in neuroendocrine tumors generally, and SCLC specifically, alter the spectrum of genes bound and regulated by MYC? ChIP-seq and transcriptional profiling could begin to address these questions in *MAX*-mutant versus wild-type tumors.

Most importantly, what are the therapeutic implications of various forms of aberrant MYC pathway activation in SCLC? It is certainly intriguing that *MYC* amplification, *MYCL* amplification, *MYCN* amplification, and mutations of genes encoding key MYC-interacting factors, including MAX, BRG1, and MGA, seem to be almost entirely mutually exclusive events in lung cancer, suggesting that each of these mechanisms may represent an alternative route to achieving aberrant MYC signaling in oncogenesis.

Attempts to therapeutically target MYC in cancer have been largely disappointing. Several groups have taken the strategy of attempting to disrupt MYC:MAX heterodimerization (e.g., ref. 8). This strategy is called into question if relevant oncogenic pathways in (at least some) aggressive tumors are regulated by MYC in a MAX-independent manner. MYC protein has a notably short intracellular half-life of approximately 15 minutes, and *MYC* mRNA is also short lived, prompting some groups to look at inhibiting key regulators of *MYC* transcription. *MYC* transcription in multiple tumor types seems to be dependent on activity of the histone modifier BRD4, a member of the BET family of bromodomain-containing chromatin modifiers (9). Bromodomain inhibitors with high affinity for BRD4 have been developed and have entered early-phase clinical testing in SCLC and other tumor types. It will be of particular interest in these trials to see whether activity correlates with specific alterations in *MYC* family members or genes encoding MYC-interacting factors. It may well be that different therapeutic approaches are optimal in the context of different mutations leading to aberrant MYC activity. BRG1 itself contains two bromodomains that could potentially be targeted by a novel small-molecule inhibitor to disrupt the SWI/SNF complex in the context of *MAX* alteration.

SCLC continues to have one of the highest case fatality rates of all human malignancies. The past 2 years, however, have been notable for several genomic, proteomic, high-throughput drug screening, and pathway-specific investigations, yielding new insights and new potential therapeutic targets for this aggressive disease (1, 2, 10, 11). Further definition of the biologic roles of possible oncogenic drivers, and prioritization of these targets for clinical translation in relevant preclinical models, may finally yield progress for patients diagnosed with SCLC.

Disclosure of Potential Conflicts of Interest

C.M. Rudin is a consultant/advisory board member of Celgene, Merck, and Aveo. No potential conflicts of interest were disclosed by the other author.

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