New Treatments for Rhabdomyosarcoma: The Importance of Target Practice

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To address the poor outcomes in rhabdomyosarcoma, particularly the alveolar subtype, new therapies are needed. Potential cancer-specific alterations that may be molecular targets include gene fusions or copy number changes. Following the latter strategy, an attractive antigene approach was developed to inhibit MYCN oncogene expression in rhabdomyosarcoma. Clin Cancer Res; 18(3); 1–3. ©2011 AACR.

In this issue of Clinical Cancer Research, Tonelli and colleagues describe a molecular therapeutic approach directed against the MYCN gene in the pediatric soft tissue cancer rhabdomyosarcoma (RMS; ref. 1).

A major strategy for developing new therapeutics is to target molecular features that are only present in cancer cells. For such a strategy, RMS, and particularly the alveolar subtype (ARMS), can be considered a paradigm for the issues inherent in developing novel molecular-based therapeutics for a rare cancer. Genetic investigations revealed the existence of 2;13 and 1;13 chromosomal translocations in the far majority of ARMS cases but not in any other cancer category (2). These tumor-specific translocations generate novel chimeric transcription factors. On the basis of the presence of these tumor-specific proteins, interest has been growing in developing drugs that target these fusion proteins. However, though tumor-specific fusion proteins may seem to be ideal targets because of their specificity, the full development and testing of agents targeted against these proteins may be complicated by the small number of patients with fusion-positive disease.

Instead of focusing on a change specific to 1 rare cancer category, an alternative strategy for development of a targeted drug may be to find more generalizable genetic alterations that occur in larger patient numbers. Such an approach has stimulated interest in identifying molecular changes found in subsets of ARMS that also are found in other cancer categories, particularly common adult cancers, because the potential and motivation for targeted drug development increases in such situations. For example, frequent amplification events have been found in ARMS involving the 2p24, 12q13-14, and 13q31 chromosomal regions; the target genes of these 3 amplicons include MYCN, CDK4, and MIR17HG, respectively (3, 4). Each of these amplicons has been described in multiple cancer categories, thus providing a reasonably large number of potential patients for testing and ultimately treatment. It should also be noted that, for a change such as amplification, which results in overexpression, other mechanisms may also result in this molecular outcome, thereby further increasing the applicable number of cases.

On the basis of these considerations, Tonelli and colleagues focused on the MYCN locus in the 2p24 chromosomal region (1). These authors report a 25% MYCN amplification frequency in ARMS, with an additional 51% frequency of lower level MYCN copy number gains. Previous studies of 2p24 amplification in ARMS confirmed this amplification frequency, and localized MYCN to the minimum common region of 2p24 amplification in ARMS (3). In addition to ARMS, there is also 6% MYCN amplification in ERMS, as well as a 48% frequency of lower level MYCN copy number gains (1). Further impetus for developing MYCN as a therapeutic target is prompted by its involvement as an amplified oncogene in multiple other cancer categories including neuroblastoma, small cell lung carcinoma, medulloblastoma, and retinoblastoma (5).

Expression studies have provided additional information to assess the potential of MYCN as a therapeutic target. First, the confinement of normal MYCN expression to embryonic tissues provides encouragement that interventions on MYCN expression in these cancers will not affect normal tissue function (6). Previous studies showed that the highest mRNA levels in ARMS correspond to amplified cases with a clear subset of nonamplified cases with lower but readily detectable expression (3, 7). It should be noted that there are rare MYCN-amplified ARMS cases with no apparent mRNA overexpression. Several large surveys of RMS cases have also shown higher MYCN mRNA levels in fusion-positive ARMS cases compared with fusion-negative ERMS cases (8). Explanations for this expression difference include the higher frequency of MYCN amplification in ARMS and the upregulation of MYCN by the PAX3-FOXO1 (and probably PAX7-FOXO1) fusion protein. Using immunohistochemistry, Tonelli and associates provided MYCN...
protein data that are concordant with these previous MYCN RNA studies (1). In particular, high and moderate MYCN protein expression levels were found in 20% and 34% of ARMS cases, respectively, and 8% and 17% of ERMS cases, respectively. Therefore, as a result of amplification and other mechanisms, a majority of ARMS cases and a significant subset of ERMS cases express high and/or intermediate MYCN protein levels.

To take advantage of these MYCN expression patterns, Tonelli and associates tested an antigene construct targeting the MYCN gene in ARMS and ERMS cell lines (1). This construct, which is directed against an antisense sequence in MYCN exon 2 and is linked to a nuclear localization signal peptide at the amino terminus, was previously developed as part of studies inhibiting MYCN expression in neuroblastoma (9). Similar to the results in neuroblastoma, this construct downregulates MYCN RNA and protein levels in ARMS and ERMS lines and inhibits cell growth (1). The antigene construct, which was administered every 3 days in cell culture experiments, was more effective than a transduced short hairpin RNA lentiviral construct or daily-administered pools of siRNA constructs. In comparisons of ARMS and ERMS lines, the level of growth inhibition by the antigene construct was higher in ARMS lines, which expressed higher basal MYCN levels. In addition, focused studies on 1 ARMS line show induction of apoptosis in culture and tumor regression in vivo.

Though antigene targeting of MYCN resulted in phenotypic effects in both RMS subtypes, the most dramatic and surprising effects were in ARMS cell lines. Within the fusion-positive ARMS environment, previous published findings in combination with results from Tonelli and associates indicate that there is a dynamic interaction between MYCN and PAX3-FOXO1 at both the expression and functional levels (Fig. 1). (Similar effects may also involve MYCN and PAX7-FOXO1, but the data are not as complete.) As 1 component of this dynamic interaction, the PAX3-FOXO1 gene seems to be a downstream transcriptional target of the MYCN protein (1), and, thus, decreases in MYCN expression will result in decreases in fusion protein expression. Second, to further compound this effect, the MYCN gene is a direct downstream transcriptional target of the PAX3-FOXO1 fusion protein (8, 10), and thus as fusion expression decreases, there will be further decreases in MYCN expression. Finally, studies using constitutive MYCN and PAX3-FOXO1 constructs in mouse fibroblasts or human myoblasts showed significant functional collaboration between the 2 oncoproteins in cell culture assays of transformation as well as in vivo assays of tumorigenesis (8, 11, 12). These latter findings indicate that MYCN expression changes will also lead to inhibition of the oncogenic action of PAX3-FOXO1, in addition to the diminished growth and other effects mediated by MYCN. As a consequence of these feedback and collaborative effects, MYCN antigen therapy in ARMS will serendipitously lead to inhibition of PAX3-FOXO1, as well as MYCN at both the expression and functional levels. However, this feedback inhibition of PAX3-FOXO1 will only occur if MYCN is initially expressed at sufficient levels to be inhibited by the antigene construct.

In summary, impressive initial steps have been made toward establishing a molecularly targeted therapy against the MYCN gene in RMS, with most striking results in fusion-positive ARMS. Instead of targeting the tumor specific-fusion proteins in ARMS, Tonelli and associates have focused on the MYCN oncogene that is amplified and/or overexpressed in ARMS, as well as in multiple other cancer categories, thus providing large numbers of applicable patients for testing and ultimately treatment with MYCN-directed therapy. For ARMS tumors, the unexpected finding is that MYCN expression and function are so closely intertwined with that of PAX3-FOXO1 that this therapy may in fact be a surrogate method for inhibiting fusion protein expression and function.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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