Harnessing the Power of Antisense Technology for Combination Chemotherapy

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The Bcr-abl fusion protein, which results when the abl (also known as ABL) proto-oncogene translocates from chromosome 9 to chromosome 22 (i.e., to the site of the bcr [also known as BCR] gene), is found in patients with hematologic cancers such as chronic myelogenous leukemia (CML). The concept of multistage carcinogenesis is well illustrated in CML, whose clinical course consists of two well-defined stages: a long-lasting, indolent stage and a terminal, more aggressive blast crisis stage (1). During the indolent stage, the 9 to 22 translocation of abl (creating the “Philadelphia” chromosome) and the expression of the Bcr-abl fusion protein can be readily detected. Bcr-abl expression confers a growth advantage to the CML cells, at least in part by protecting them from apoptosis (2,3). The later, blast crisis stage of CML involves the development of additional genetic aberrations, such as the inactivation of the p53 tumor suppressor gene (also known as TP53) and further chromosomal translocations and abnormalities (4). Because Bcr-abl is expressed only in the leukemia cells, the encoding gene offers a highly selective molecular target for therapeutic intervention. The antisense technique, by virtue of its predicted specificity, holds the potential to inhibit gene expression with a functional consequence [see (5-8) for review]. Several reports (9-15) have documented the use of antisense techniques to target the bcr-abl fusion product in both in vitro and in vivo models.

Effective treatment of cancer, a complex disease with a multitude of causes involving aberrations in many different genes, requires appropriate combinations of drugs that target multiple gene products to eradicate all tumor cells successfully. The combination therapy approach has become the method of choice in order to overcome the development of drug resistance to single agents. The preclinical experience with antisense oligodeoxynucleotides (ODNs) in diverse disease models rightfully raises the possibility of using antisense oligomers in combination therapy for refractory cancers [see (7,8)]. Skorski et al. (12) previously demonstrated that the combination of a low dose of mafosfamide (an in vitro active metabolite of the alkylating agent cyclophosphamide) with an antisense phosphorothioate ODN designed to target the bcr-abl fusion junction was highly effective in eliminating Philadelphia chromosome-positive leukemia cells from a mixture of leukemia cells and normal bone marrow cells in vitro.

In this issue of the Journal, Skorski et al. (16) have extended their earlier observations (12) to an in vivo model. Moreover, they have attempted to provide a molecular explanation for their findings, which can be summarized as follows: (a) In severe combined immunodeficient mice bearing CML-blast crisis cells, progression of leukemia was delayed by combination therapy involving a single suboptimal dose of cyclophosphamide and multiple doses of the bcr-abl antisense ODN; (b) 50% of the treated mice were cured of leukemia; (c) increased apoptosis and alterations in the expression profile of apoptosis-related genes were seen in treated leukemia cells; and (d) enhanced uptake of the bcr-abl ODN was seen in vitro in response to treatment with mafosfamide. These findings define a new role for ODNs in a combination therapy approach.

Notwithstanding the substantial advances of the past decade in identifying molecular targets, including oncogenes and tumor suppressor genes, cancer therapy still relies heavily on cytotoxic agents. One of the major obstacles to successful treatment and eradication of cancer is the frequent development of drug resistance. While drug resistance can often be overcome by low-dose combination chemotherapy, toxicity remains a major concern, and too many patients still succumb to refractory disease despite the best efforts of clinicians. In this context, combination chemotherapy with the addition of ODNs would offer two advantages: 1) the possibility of avoiding the early development of drug resistance and 2) the potential of using the compounds at low doses, minimizing toxicity. While at this point several phosphorothioate ODNs are in clinical trials for diverse diseases, including acquired immunodeficiency syndrome (AIDS) and cancer [see (8) for review], the efficacy of these ODNs as single agents is still to be demonstrated. The results currently presented by Skorski et al. (16) open a fresh and exciting approach to harnessing the power of ODNs by demonstrating their efficacious use in combination chemotherapy.

Apoptosis is becoming a major paradigm in our understanding of the mechanism of action of many cancer therapeutic agents (17). While an increasing number of pro- and apoptotic genes are being discovered and no single gene can be the sole causative agent of so complex a process as apoptosis, the induction of apoptosis in tumors is enormously attractive as a therapeutic approach. The current findings of Skorski et al. (16) suggest that it is feasible to accelerate the induction of apoptosis by combining ODNs with mafosfamide (or cyclophos-
phamide). In parallel, this study also implicates the apoptosis-related genes: A suppression of bcl-2 (an anti-apoptotic gene) expression and an increase in p53 and bax (pro-apoptotic genes) expression occur in response to the combination therapy. While it may be difficult to ascertain whether the enhanced apoptosis is solely a consequence of antisense effects in suppressing Bcr-abl protein expression, the results certainly lend credibility to the starting premise of combining ODNs with existing chemotherapeutic agents. Further experiments are needed to discover what effects this combination therapy might exert on normal cells at the level of apoptosis.

The uptake of ODNs by cells is complex and involves both active and passive processes [see (5, 8) for review]. Regardless of the mechanism, the enhanced uptake of ODNs seen by Skorski et al. in response to mafosfamide treatment can certainly be advantageous in ODN combination therapy. These results also open new avenues for ODN uptake by testing other compounds that may be functionally similar to mafosfamide.

The theory that led to the development of antisense technology is straightforward, but the mechanisms underlying the action of these powerful pharmacologic agents have proven to be exceedingly complex. It is almost certain that not all of the biologic effects observed with ODNs are due to antisense effects; some effects arise from sequence-specific, non-antisense activity (5, 7). The bcr-abl antisense ODNs are no exception to these complications—in fact, the contradictions between several recent reports (18-21) attest to the complexity of bcr-abl antisense research. Despite the compelling efficacy seen with the bcr-abl antisense ODNs, it is difficult to make an argument for antisense as the sole mechanism of action. This is no reason to discourage the exploration of CML therapy with bcr-abl ODNs. The efficacy seen with antisense ODNs in preclinical models cannot be simply dismissed, despite concerns about nonspecificity; it is unrealistic to expect a high level of specificity with any antitumor drug, whether an ODN or a chemical compound. The history of anticancer drug discovery teaches us that only after discovery of a given drug are the underlying mechanisms elucidated. New mechanisms are continually being discovered for compounds with a long history of clinical use, mechanisms that often have little to do with those postulated when the compounds were developed. For example, most of the cytotoxic agents were originally intended to target essential cellular processes (for both cancer cells and normal cells), but recent investigations are beginning to implicate apoptosis in their antitumor activities. In this regard, the study by Skorski et al. presented in this issue of the Journal provides a sound framework for expansion of the use of ODNs in combination approaches for the treatment of cancer.

References

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