Blocking oncogenes in malignant cells by RNA interference—New hope for a highly specific cancer treatment?

A little more than one year after the first demonstration that silencing of endogenous human genes is possible in cell culture, the new tool of RNA interference (RNAi) enters the field of tumor therapy.

Tumor gene therapy with the aim of specifically attacking the malignant cells takes direct advantage of our current understanding of carcinogenesis at the molecular level. This modern treatment option is largely based on the tremendous efforts that have been made in identification, cloning, sequencing and functional analysis of oncogenes during the last 20 years. In 1982, the development of the NIH 3T3 cell transfection assay made it possible, for the first time, to identify activated oncogenes in human tumors. With it, the ras genes were identified and subsequent work showed that ras genes encode a family of 21 kDa intracellular guanosine triphosphate (GTP) binding proteins. They are integral to cellular signal transduction and ultimately regulate differentiation, proliferation, survival and migration in a wide diversity of cell types. Ras cycles between its activated GTP-bound form and its inactive guanosine diphosphate (GDP)-bound form. A long-standing puzzle surrounding p21 ras proteins (there are 3 functional proteins, H-ras, K-ras, and N-ras) stems from the amazingly complex signaling network in which ras acts as a central player and is itself regulated through the control of the GTP/GDP cycling rate by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) (Bar-Sagi and Hall, 2000; Campbell et al., 1998).

In view of the central role of ras in cell signaling, it is perhaps not surprising that ras is one of the most commonly mutated oncogenes in human malignancies, accounting for about 30%–50% of human cancer. These ras mutations, often found in codons 12, 13, 60, and 61, result in its constitutive activation due to inability of the ras-bound GTP to be hydrolyzed to GDP (Bos, 1989). For many years there has been some hope that appropriate molecular therapy can be designed to attenuate ras activity. Accordingly, ras genes were among the first targets for which antisense strategies were applied. Despite encouraging results in preventing tumor growth in animals, the clinical phase I/II studies with antisense DNA oligonucleotides against ras did not ultimately enter into larger clinical treatment protocols. One major problem in designing a sequence-specific anti-ras therapy simply lies in the nature of its activation: a single point mutation suffices to turn the protein on and convert it to a dominant oncoprotein (Figure 1). Thus, wild-type ras and its oncogenic form differ only very slightly with regard to their DNA sequence but enormously in their biochemical consequences.

In the current issue of *Cancer Cell*, Brummelkamp et al. now open a new chapter in anti-ras-mediated tumor therapy (Brummelkamp et al., 2002b). Moreover, their approach may generally serve as a new prototype for blocking oncogene expression in human cancer—the use of RNA interference (RNAi). It was only one year ago that the Tuschl group published their pioneering paper in which they showed how small double-stranded RNA molecules (small interfering RNA, siRNA) silence gene expression in mammalian cell culture (Elbashir et al., 2001). Since then, siRNAs, either chemically synthesized or intracellularly expressed via a polymerase III-based transcription system, have been widely used for targeting genes in cell culture (Paddison et al., 2002; Brummelkamp et al., 2002a). Employing a method similar to that in a recent report about the application of a retroviral expression cassette for induction of RNAi (Paddison and Hannon, 2002), Brummelkamp et al. used a retroviral version of their plasmid vector system "pSUPER" (*sup*pression of *e*ndogenous *R*NA). With it, they strongly inhibited the expression of mutated K-ras^{V12} while leaving other ras isoforms unaffected. This extraordinary sequence specificity of RNAi, which clearly exceeds that of DNA antisense approaches, makes it a very attractive tool for cancer therapy. Moreover, in a set of elegantly performed experiments, Brummelkamp et al. demonstrated the power of RNAi-mediated gene therapy not only in cell culture but, encouragingly, in an animal model as well. The latter finding, together with two recent reports about the successful application of RNAi in mice (Lewis et al., 2002; McCaffrey et

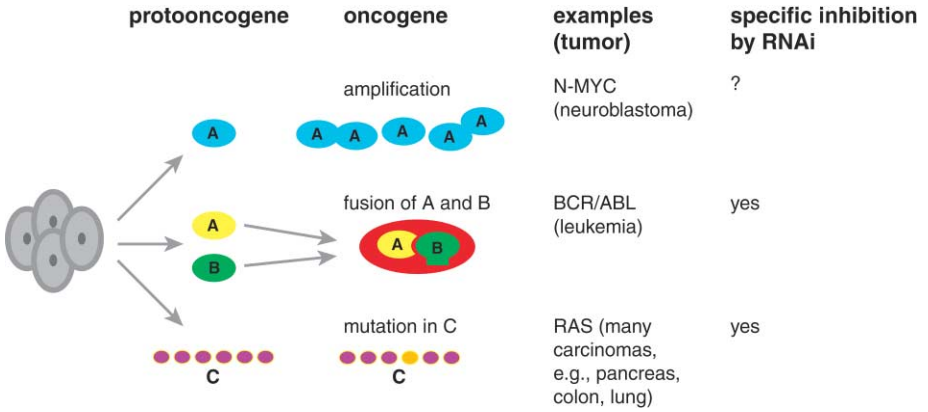

Figure 1. Different mechanisms of oncogene activation in human tumors

In cancer cells, proto-oncogenes have frequently been activated by various mechanisms, producing oncogenes that act in a dominant fashion. In epithelial tumors, point mutations are predominant whereas hematological malignancies often show gene fusions that result from chromosomal translocations.

al., 2002), foretells further studies in which transgenic or knock-in mice carrying such oncogenic alleles will by treated by RNA-based therapeutics.

Besides point mutations, oncogenes can be activated by either gene amplification or chromosomal translocation fusing two parts of unrelated genes to form a chimera (Figure 1). The classical prototype for gene amplification is the N-MYC oncogene, which is amplified e.g., in neuroblastomas, especially at late stages or in very aggressive types of the tumor. Whether and how the RNAi approach can be successfully applied to target amplified oncogenes in human tumors remains to be seen. Obviously, the question is whether the lack of sequence variation between the activated (amplified) oncogene and its normal counterpart in nonmalignant cells causes difficulties with regard to specificity. What about RNAi for targeting oncogenic activation by chromosomal translocation? Recent data from our laboratory suggest that RNAi can also serve as a tool to downregulate chimeric fusion transcripts (Wilda et al., 2002). Leukemic cells with translocation t(9;22) depend on the presence of BCR/ABL oncoprotein and consequently undergo apoptosis when depleted of it.

Nevertheless, some clinicians may still have doubts as to whether RNAi will be given a place in the field of cancer therapy. Initially, there is much enthusiasm about a postgenomic wave of tyrosine-kinase inhibitors, which undoubtedly have the potential to change the treatment of cancer in the future (Shawver et al., 2002). Although success has already been achieved in clinical studies with Trastuzumab and Imatinib mesylate (STI571) in patients with breast cancer or Philadelphia chromosome-positive leukemias, respectively, these are only the first steps on the way to rationally designed target-directed therapies. With regard to ras, farnesyltransferase inhibitors impede oncogenic ras function by inhibition of its posttranslational modifications and have already entered numerous clinical trials (Reuter et al., 2000). Thus, it has to be shown that RNAi-based therapies are as efficacious as small molecule drugs or monoclonal antibodies in targeting kinase enzymes. Second, the main challenge to any gene therapy must also be overcome with RNAi: the double-stranded RNA or the expression vector encoding such molecules needs to reach the tumor cells efficiently. The neutralization of small interfering RNAs by the immune system may also be a foreseeable problem. Third, the advantageous extreme sequence specificity of RNAi may, in turn, form the basis for cancer cells to escape the RNAi-mediated attack. A single point mutation in the targeted region abolishes mRNA degradation and may cause RNAi-resistance in tumors. Recently we had to learn a similar lesson from patients who became resistant to the tyrosine kinase inhibitor STI571 by a variety of mechanisms including point mutations in the kinase domain (Shah et al., 2002; Gorre et al., 2001). Thus, to some extent RNAi-based approaches compete with the kinase inhibitors in the field of molecular therapy. But as is often the case with competitors in the same field: it may be that only their combined efforts will help to overcome the numerous problems associated with drug resistance in cancer treatment. The report by Brummelkamp et al. clearly demonstrates the promising power of RNAi for therapeutic purposes and gives new hope that a potent tool for the reversion of an oncogenic phenotype is now in our hands.

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