The Application of Antisense Technology to Medicine

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In a seminal biological insight 50 years ago, James Watson and Francis Crick proposed a structure for the genetic material deoxyribonucleic acid, or DNA. They proposed that DNA consists of two deoxyribonucleotide molecules each having a 5´-end and a 3´-end, which define a polarity for each deoxyribonucleotide strand. The two strands, when bound together in antiparallel orientation (that is, the 3´-end of one being juxtaposed to the 5´-end of the other) compose a complete DNA molecule. The two strands are bound together by pairing among the four complementary bases in each strand such that adenine in one strand binds to thymine in the second, and cytosine in one binds to guanine in the second. Thus, all the genetic information is contained in each strand, written in what might be called a mirror image in each strand. This structure permits the transmission of genetic information by allowing a complementary strand to be produced for either single strand. One half (one strand) can therefore produce an entire DNA molecule, as occurs during cell division (1).

Because each triplet set of nucleotides on a strand of DNA encodes an amino acid, this structure is also the basis of protein synthesis (Figure 1). In this process, one or another portion of one strand (a gene) is copied by ribonucleic acid polymerase II producing a complementary molecule of ribonucleic acid, or RNA. This messenger RNA (mRNA), therefore, contains essentially the same information that is contained in the gene that has been transcribed. The RNA molecule is processed in the nucleus such that certain sequences (introns) are excised leaving a smaller, mature RNA molecule. After intron excision and additional processing, the RNA molecule is exported to the cell cytoplasm where it directs protein synthesis at the ribosome. Because RNA is relatively unstable in the cell, the amount of protein synthesized is dependent on not only the rate and duration of RNA production from the gene but also on factors that stabilize or lengthen the lifespan of the RNA in the cytoplasm.

Formulation of Antisense Technology

Over the last several decades, this knowledge of DNA/RNA physiology has been applied in a variety of ways. One of the more productive applications is the development of antisense technology. The basic idea is that if an oligonucleotide (a short RNA or DNA molecule complementary to a mRNA produced by a

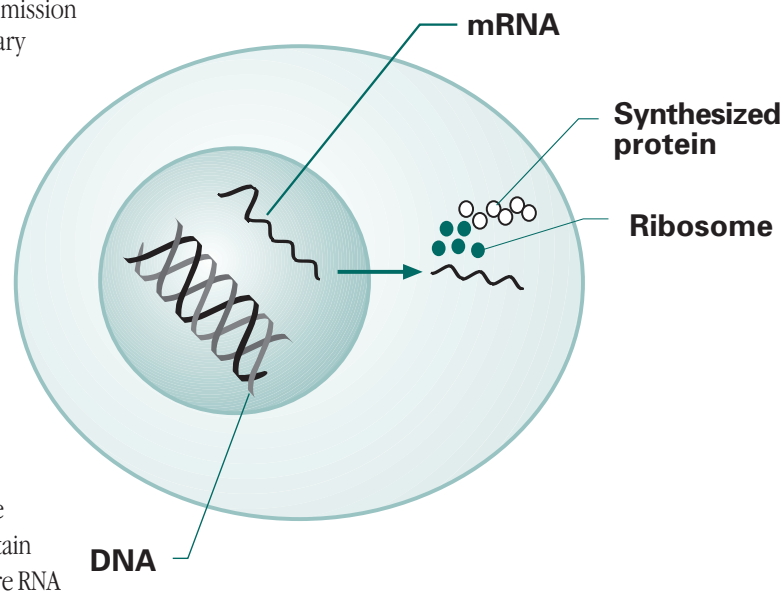

Figure 1. Gene directed protein synthesis.

gene) can be introduced into a cell, it will specifically bind to its target mRNA through the exquisite specificity of complementary-based pairing—the same mechanism which guarantees the fidelity of DNA replication and of RNA transcription from the gene. This binding forms an RNA dimer in the cytoplasm and halts protein synthesis (Figure 2). This occurs because the mRNA no longer has access to the ribosome and because dimeric RNA is rapidly degraded in the cytoplasm by ribonuclease H. Therefore, the introduction of short chains of DNA complementary to mRNA will lead to a specific diminution, or blockage, of protein synthesis by a particular gene. In effect, the gene will be turned off.

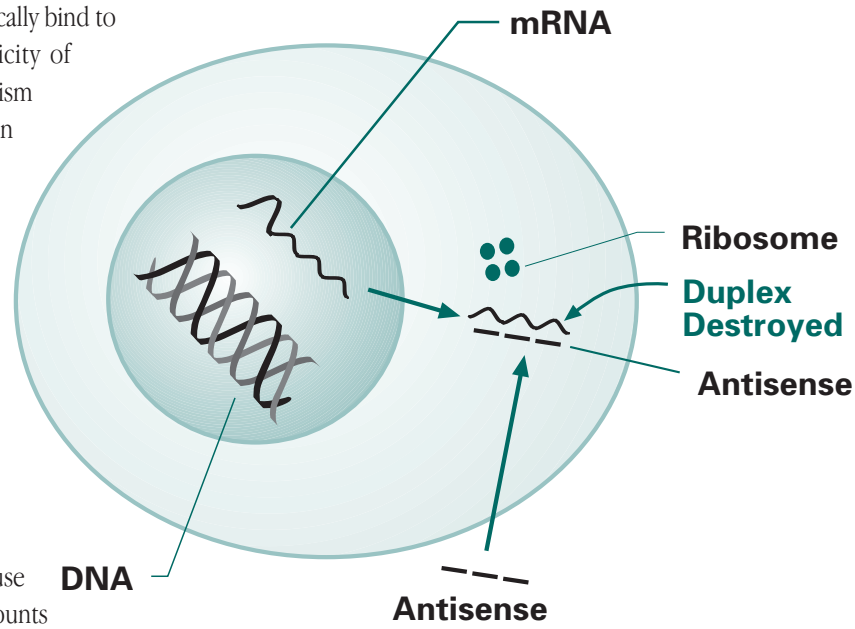

Figure 2. Antisense inhibition of protein synthesis.

The technical problems associated with the use of this technology are many. First, sufficient amounts of antisense oligonucleotide must be administered to the vicinity of target cells and, more importantly, must be taken up by those cells. Second, the antisense oligonucleotide should, ideally, have a long enough half-life within the cell to successfully impair mRNA translation into protein over a significant period of time. Finally, the oligonucleotide must also be nontoxic and sufficiently specific so as not to interfere with other cellular functions. In many applications, these hurdles have been overcome and antisense technology has developed into a productive branch of biology.

These technical challenges can be overcome in various ways depending on the specific application at hand. Oligonucleotides can be mixed with a variety of lipids to form complexes that are more easily incorporated by cell membranes, facilitating the entry of associated oligonucleotides into the cells. A number of other techniques have also been developed to facilitate the uptake of oligonucleotides by cells. Chemical modification of the antisense oligonucleotides can render them more stable in cells and blood by increasing their resistance to ribonuclease digestion. Also, complementary DNAs or fragments of complementary DNAs can be incorporated in reverse sense in order to generate antisense RNA products in the host cell itself. This results in a long-term inhibition of the synthesis of the target protein.

Application of Antisense Technology in vitro

Antisense technology has been applied successfully in two general areas. The first is in fundamental research where the introduction of antisense oligonucleotides can help determine the role of a specific gene in a specific physiological process (2-4).

For example, our laboratory has been interested for some time in the idea that local components of the renin angiotensin system can be produced by specific cells. We hypothesized that the production of angiotensin II by cells feeds back on those cells resulting in cell growth and other changes. In our view, this tissue (cellular renin angiotensin system) could therefore potentially play a role in a wide variety of cardiovascular disorders, including atherosclerosis and vascular hypertrophy. It is difficult to demonstrate that a cellular system is operative in any given process, however, because a circulatory renin angiotensin system exists that produces angiotensin II in tissue culture medium as well as in tissues. To approach this problem we developed oligonucleotides to inhibit the synthesis of angiotensinogen, the substrate from which cells make angiotensin II. We were able to demonstrate that the application of these oligonucleotides, but not of scrambled oligonucleotides (control oligonucleotides of identical composition but scrambled sequence), resulted in a decline in cell growth. The introduction of angiotensin II to the cells restored this growth (5). Thus, through the application of antisense technology, we were able to demonstrate the biological principle that cells can make their own angiotensin II with growth promoting effects. We extended this antisense work to certain cancers and demonstrated, for the first time, that neuroblastoma cells, for example, also possess similar cellular renin angiotensin systems that regulate their growth (5). These studies, and many like them, represent the application of antisense technology to research.

Therapeutic Application of Antisense Technology

A second application of this technology, and one that is potentially of more immediate relevance to the practicing physician, is the use of this technology in therapy (6-13). In principle, antisense oligonucleotides complementary to viral RNAs can suppress a wide variety of viral infections; a tremendous amount of research is ongoing in this area. Similarly, antisense oligonucleotides directed towards the products of oncogenes can play a role in reducing the growth of cancer cells, and this lead is being hotly pursued.

Perhaps the most widely discussed application of antisense technology lies in its applications to gene therapy. In this case, a variety of vectors is used to introduce antisense-encoding genes into a large number of cells in a patient or animal to produce long-term inhibition of a protein. For example, in animal models the introduction of vectors encoding antisense angiotensin II receptor sequences results in long-term normotension in spontaneously hypertensive animals (14).

These are but a few of the possible applications of antisense technology. As familiarity with the relevant chemistry increases, it is likely that more effective oligonucleotides and gene vectors will be developed, thereby providing the ability to interfere at will with the translation of specific mRNAs.

Triplex Antisense Technology

In the face of all this progress, still newer technologies are being developed based on concepts related to antisense biology. For example, it is known that oligonucleotides can, in certain instances, bind to duplex DNA molecules through an unusual kind of base pairing. In this triplex binding mode, oligonucleotides insert themselves into the major groove of the DNA double helix on a reasonably specific basis determined by the nucleotide sequence of the target DNA (15). This triplex technology provides the opportunity to reduce gene transcription itself rather than to destroy mRNA once it is produced. Because the triplex oligonucleotides can be made to permanently alter the DNA after localizing to specific target sites, the technology actually has the potential to permanently silence genes.

In our laboratory, we have used this technology in an effort to produce triplex binding to one of the consensus binding sites of the antitumor protein p53. The protein p53 is in normal cells and is turned on when cells become damaged or malignant, impairing their growth and preventing the reproduction of faulty cells. Certain cancer cells lack p53 function, which allows continued growth and reproduction. The p53 protein binds to specific DNA sequences, and we speculated that triplex oligonucleotides designed to bind to those sequences might mimic p53 effects. We subsequently demonstrated that the use of triplex oligonucleotides will suppress cell growth in certain cancer cells lacking normal p53 function, thus raising the possibility that triplex biology may lead to the development of effective anticancer agents (15).

RNA Inhibition

It has recently been shown that double-stranded RNA in the cytoplasm triggers an as yet poorly understood cascade of events leading to the suppression of the transcription of the gene producing the specific mRNA involved in the cytoplasmic RNA duplex (16). This could potentially lead to the development of new pharmacological agents.

Perspective

Antisense technology is a formidable tool for investigating physiologic and pathologic processes. In addition, it is soon likely to become a mainstay of therapy, particularly in infectious diseases, with wider applications in the future as gene therapy techniques are developed further. Antisense pharmaceuticals will soon be available for the routine care of patients and are expected to prove to be effective, specific agents with favorable therapeutic profiles.

References

1. Watson JD, Crick FH. Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid. 1953. Ann N Y Acad Sci 1995;758:13-14.
2. Feeley BT, Poston RS, Park AK, et al. Optimization of ex vivo pressure mediated delivery of antisense oligodeoxynucleotides to ICAM-1 reduces reperfusion injury in rat cardiac allografts. Transplantation 2000;69:1067-1074.
3. Lu R, Serrero G. Inhibition of PC cell-derived growth factor (PCDGF, epithelin/granulin precursor) expression by antisense PCDGF cDNA transfection inhibits tumorigenicity of the human breast carcinoma cell line MDA-MB-468. Proc Natl Acad Sci USA 2000;97:3993-3998.
4. Koller E, Gaarde WA, Monia BP. Elucidating cell signaling mechanisms using antisense technology. Trends Pharmacol Sci 2000;21:142-148.
5. Cook J, Chen L, Bhandaru S, et al. The use of antisense oligonucleotides to establish autocrine angiotensin growth effects in human neuroblastoma and mesangial cells. Antisense Res Dev 1992; 2:199-210.
6. Engelhard HH. Antisense oligodeoxynucleotide technology: potential use for the treatment of malignant brain tumors. Cancer Control 1998;5:163-170.

7. Murano M, Maemura K, Hirata I, et al. Therapeutic effect of intracolonically administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. Clin Exp Immunol 2000;120:51-58.
8. Scala S, Portella G, Fedele M, et al. Adenovirus-mediated suppression of HMGI(Y) protein synthesis as potential therapy of human malignant neoplasias. Proc Natl Acad Sci USA 2000;97:4256-61.
9. Bochot A, Couvreur P, Fattal E. Intravitreal administration of antisense oligonucleotides: potential of liposomal delivery. Prog Retin Eye Res 2000;19:131-147.
10. Moon IJ, Choi K, Choi YK, et al. Potent growth inhibition of leukemic cells by novel ribbon-type antisense oligonucleotides to c-myb1. J Biol Chem 2000;275:4647-4653.
11. Pagnan G, Stuart DD, Pastorino F, et al. Delivery of c-myb antisense oligodeoxynucleotides to human neuroblastoma cells via disialoganglioside GD(2)-targeted immunoliposomes: antitumor effects. J Natl Cancer Inst 2000;92:253-261.
12. Agrawal S, Kandimalla ER. Antisense therapeutics: is it as simple as complementary base recognition? Mol Med Today 2000;6:72-81.
13. Mani S, Gu Y, Wadler S, et al. Antisense therapeutics in oncology: points to consider in their clinical evaluation. Antisense Nucleic Acid Drug Dev 1999;9:543-547.
14. Martens JR, Reaves PY, Lu D, et al. Prevention of renovascular and cardiac pathophysiological changes in hypertension by angiotensin II type 1 receptor antisense gene therapy. Proc Natl Acad Sci USA 1998;95:2664-2669.
15. Re RN, Cook JL. Suppression of cellular proliferation using p53 DNA recognition site-related oligonucleotides. Am J Med Sci 1996;311:65-72.
16. Hammond SM, Bernstein E, Beach D, et al. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 2000;404:293-296.

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