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REVIEW

Does Antisense Make Sense in Dermatology?

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The concept of antisense technology is elegant but misleadingly simple. Short oligodeoxynucleotides (ODNs) complementary to a target messenger RNA form DNA-RNA hybrids by Watson-Crick base pairing rules, and interfere with expression of the encoded protein. The potential sequence specificity of antisense ODNs makes them attractive as molecular drugs in the treatment of human diseases. The skin is readily accessible and, in theory, is therefore suitable for application of antisense ODNs. Targeted and selective inhibition of keratinocyte gene expression in human epidermis could be an efficient and safe pharmacological approach in a number of skin diseases. Based on recent studies from our group and others, in this review we present our view on the usefulness of antisense ODN technology in skin for the modulation of gene expression related to skin diseases. It has become clear from these studies that practising antisense technology requires careful experimentation and critical data interpretation. Although the antisense technique was applied with success in some skin model systems, we feel that the technology is still in its infancy. The basic questions have been answered, but there are still many more that need to be addressed.

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Antisense oligodeoxynucleotides (ODNs) are believed to show promise as agents in the specific manipulation of gene expression. However, non-specific effects of ODNs often confound the interpretation of antisense studies. Improvements in ODN chemistry and cellular delivery techniques allow for more potent and specific gene inhibition.

Despite the limitations of antisense therapy and the many conditions that must be satisfied for an antisense drug to function, several antisense compounds have already entered phases I and II human clinical trials. Examples are: trials with a Myb-targeted ODN for the treatment of human leukaemias (1), a placebo-controlled trial of ICAM I antisense ODNs in the treatment of Crohn's disease (2), intravenous infusion in HIV-infected patients with an antisense ODN targeted at gag gene mRNA of the human immunodeficiency virus (HIV-1) (3), a dose-escalating trial with systemic administration of antisense p53 ODN to patients with haematological malignancies (4, 5), and phase II clinical trials for the treatment of genital warts induced by human papillomavirus-6 and human papillomavirus-11 (6). Moreover, the first commercial antisense drug, fomivirsen (Vitravene), made by Isis Pharmaceuticals in Carlsbad, California, is already approved for marketing in the US. It specifically inhibits replication of human cytomegalovirus and is being developed for the treatment of cytomegalovirus retinitis (7).

ANTISENSE TECHNOLOGY

Goal and history of the antisense approach

The ultimate objective of the antisense approach is to use a sequence complementary to the target gene to block its expression, thereby selectively reducing or abolishing the level of a single protein. The aim may be to produce a phenotype to investigate the function of the gene in cellular processes, to reduce expression of a gene causing an undesirable phenotype, or to interfere with viral infection.

The development of antisense technology began with early observations that bacteria can regulate gene replication and transcription by the use of small complementary or 'antisense' RNA molecules (for reviews see (8) or (9)). There is evidence that eukaryotic cells also elaborate antisense RNA transcripts, but the physiologic role of such molecules remains controversial (10, 11). Already in 1978, prior to the discovery of regulatory RNAs, P. C. Zamecnik and M. L. Stephenson demonstrated that synthetic ODNs complementary to mRNA sequences could downregulate translation *in vitro* and in cells (12). Subsequently, numerous reports have followed describing the modulation of the expression of various genes by both DNA and RNA antisense techniques. This thesis focuses on the modulation of gene expression by antisense DNA technology.

Concept of antisense DNA technology

The concept of antisense DNA technology is based on the highly specific interaction of base pairs, leading to recognition of one strand of mRNA by the so-called antisense ODN. The well-known Watson-Crick pairing of guanine (G) with cytosine (C), and of adenine (A) with thymine (T), leads to a duplex composed of DNA and a strand of mRNA, oriented antiparallel, as in normal duplex DNA. Below, several factors are discussed that are important for the concept of antisense technology (Fig. 1).

One important consideration is that the ODN must be able specifically to bind the mRNA of interest, and should not get tied up with other mRNAs. Moreover, the ODN must be able to find the target with reasonable frequency at the concentrations employed, and it must bind adequately to its target once it has been found. Based on an estimate of approximately 3 to 4 billion base pairs in the human genome, a number of investigators have calculated the minimum size needed to recognize a single specific sequence in the genome as being between 12 and 15 bases (13). However, there are obviously many more considerations than this. As with other

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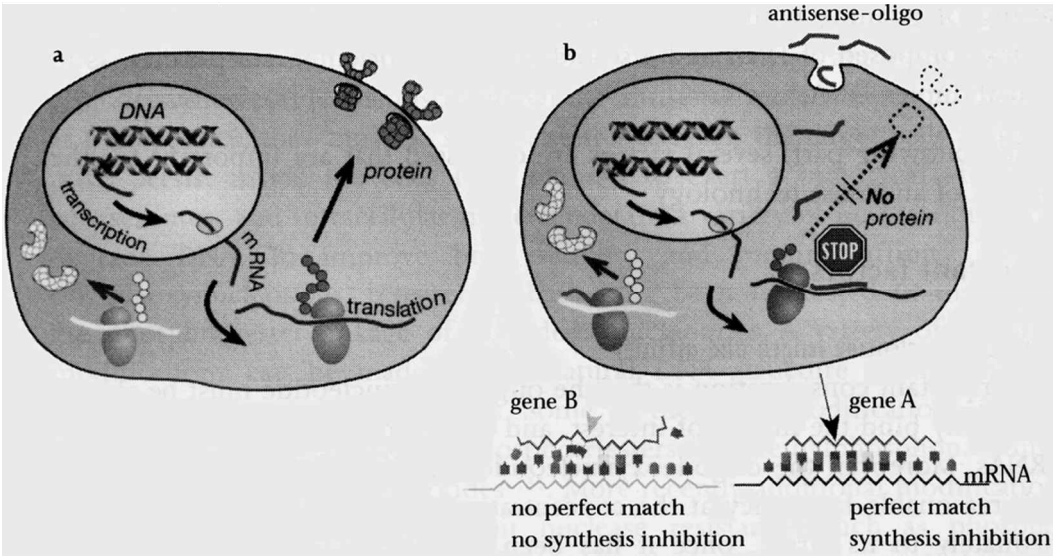

Fig. 1. Concept of antisense technology. (a) Process of normal gene expression. DNA is first transcribed into the intermediary messenger RNA (mRNA). The mRNA is transported from the nucleus to the cytoplasm and serves as a template for translation into protein. Ribosomes read the encoded information and string together the appropriate amino acids to form the encoded protein. This principle is the same for all kinds of proteins (represented here by a cell surface receptor (right) and an intracellular protein (left)). (b) Mechanism of antisense technology. Antisense ODNs are designed to interfere with gene expression. The ODNs enter the cell (top) and bind to the mRNA containing the complementary sequence (gene A). The duplex between gene A and the ODN is strong enough to abort translation of the encoded cell surface receptor. Other mRNAs (e.g. gene B) that do not contain the complementary sequence do not encounter difficulties with protein synthesis because the antisense ODN does not match.

drug-receptor interactions, activity requires a minimum level of affinity. In theory, the affinities for ODN-mRNA interactions are very large. However, in practice, affinity constants are substantially lower, and several factors contribute to this difference. The most important factor is that RNA can fold into a variety of secondary and tertiary structures. Another factor that can influence the affinity of an ODN for its RNA target is that the ODN can form secondary and tertiary structures themselves. Unfortunately, data on ODN-binding kinetics are sparse, especially for modified ODNs. Moreover, the thermodynamic affinity is not measured directly; it is often studied by measuring melting curves, where the fraction of a population of bases that are paired at a given temperature can be determined by spectrophotometry. The melting point is the temperature at which half of the bases are paired. The melting temperature of two complementary strands of natural DNA or RNA is a function of several factors, including length and base composition, as well as the ionic composition of the medium. Generally, the longer the ODN, the higher the melting point, due to the greater number of stabilizing hydrogen bonds; likewise, a higher GC content leads to a higher melting point, since the GC interaction is stronger than that of AT. Higher ionic strength also favours a higher melting point, due to greater neutralization of the repulsion between the two backbones. Again, the *in vivo* situation is considerably more complicated, because the ODN structures are affected not only by the ionic milieu but also by interactions with polycations.

ODN chemistry

Another important consideration for the antisense approach is the ODN chemistry. When the antisense molecules are to be used as therapeutics, they have to be stable enough to reach the target cells and must persist inside the cells long enough to exert a biological effect. Of course, inter- and intracellular milieus contain a variety of nucleases that can degrade DNA. To improve both stability and binding affinity of the ODNs, researchers have long been interested in modifying the phosphodiester linkage of ODNs. A variety of chemical modifications can be made to the natural DNA structure to increase the stability of ODNs. Some of the early modifications include phosphorothioate linkages (14), phosphotriesters, methylphosphonate linkages (15), and α-ODNs. More recently, additional modifications have been identified that exhibit nuclease resistance, such as phosphorodithioate, additional 2'-modifications, 2'-5' linkages, N3'-N5' phosphoramidate, and PNA. Of these modifications, phosphorothioates have progressed the furthest as a class of therapeutic agents. In the phosphorothioate modification, one of the non-bridging oxygens at the phosphorus is replaced with sulphur. This is a conservative substitution that does not change the solubility properties of the ODN. Furthermore, while the phosphorothioate ODNs are still capable of binding to mRNA, they are significantly less susceptible to nuclease digestion (16, 17). However, although this modification prevents nuclease digestion, it leads to other problems. Phosphorothioate ODNs tend to bind non-sequence specifically to proteins, often with much higher affinity than phosphodiester ODNs. In some cases, the phosphorothioate ODNs are very potent enzyme inhibitors (18). This may account for some of the 'non-specificity' observed with the use of phosphorothioate ODNs. These non-sequence-specific effects may lead to additional clinical therapeutic applications for ODNs, but can confound the interpretation of antisense experiments.

So, ODN chemistry is very important in selecting sequences that show potent antisense effects but do not cause non-antisense specific effects.

As described above, the length of an ODN, binding affinity and the ODN chemistry are important factors. Another important factor is the accessibility of the target RNA. In general, most regions of the RNA can be targeted (e.g. 5'- and 3'-untranslated, AUG initiation, coding, splice junctions and introns); however, secondary and tertiary structures of the target RNA molecule ensure that not all sequences are equally accessible. Multiple experiments have already supported this contention (19–27). It is therefore difficult to predict what the chances are that a given ODN will be effective. Consequently, effective antisense target sites must be selected experimentally. However, it is not economical to screen for all possible ODNs, so it will never be known whether the highest affinity ODN possible has been identified.

Uptake and internalization of ODNs

Numerous investigators have characterized ODN internalization in mammalian cells. These studies, not unexpectedly, have yielded conflicting results, because different methods were used for evaluation of ODN internalization, as well as different cell lines and different ODNs. The one consistent conclusion that can be drawn is that all mammalian cells investigated are capable of internalizing phosphorothioate ODNs. The intracellular fate of the ODN is controversial. Some studies suggest that ODNs are internalized by a receptor-mediated or adsorptive endocytosis pathway in which the ODN is retained within membrane-bound intracellular vesicles (18, 28–31). Other studies suggest that the ODN either uses other alternative methods for gaining entry into cells or escapes from the cytoplasmic vesicles (32–35). Crooke et al. (6) suggested that there are multiple competing mechanisms by which ODNs are internalized in the cells. For the majority of cultured cell lines, they observed localization of fluorescently labelled ODNs in cytoplasmic structures and failed to observe antisense effects in the absence of a facilitator such as cationic lipids. However, others have demonstrated that keratinocytes accumulate phosphorothioate ODNs in the cell nucleus without the use of cationic lipids or other transfection methods (33, 34). It is unlikely that there will be a single mechanism by which ODNs become internalized in cells. In addition, there are other parameters that account for the variations of ODN uptake, such as confluency, degree of differentiation, and viability (36). So, for each individual model system, characteristics of ODN uptake must be determined separately. Furthermore, experimental data (including our own) would suggest that behaviour of ODNs in cell culture is not predictive of their behaviour *in vivo*.

Target sequence and mechanism of action

There are multiple theoretical mechanisms by which ODNs can be used to regulate expression of target genes (37, 38). Perhaps the most widely proposed mechanism is cleavage of the targeted RNA by RNaseH, the enzyme that degrades the RNA strand of a DNA-RNA duplex. Although it has not been unequivocally demonstrated that reduction or cleavage of the targeted RNA in cells is mediated by RNaseH, there is a great deal of evidence to support such a conclusion, including direct demonstration of appropriate cleavage products (39–41), and the use of modified ODNs that do not support RNaseH activity (19, 20, 42). In addition to RNaseH, mammalian cells express a variety of other RNases which could be exploited to selectively inhibit expression of a targeted gene. Examples include RNaseL and double-stranded RNA-dependent RNases.

There are also steric mechanisms by which ODNs can prevent expression. The occupancy of the RNA by the ODN could be sufficient, comparable to the working mechanism of classic competitive antagonists. These are thought to alter biological activities because they bind to receptors, thereby preventing natural agonists from binding and inducing normal biological processes. Binding of ODNs to specific sequences may inhibit the interaction of the RNA with proteins, other nucleic acids, or other factors required for its expression. ODNs that bind to sequences required for splicing may prevent binding of necessary factors or physically prevent the required cleavage reactions. This then would result in inhibition of the production of the mature RNA. Other key steps in RNA processing are 5'-capping and polyadenylation. These stabilize pre-mRNA and are important for the stability of mature RNA. They are also important in binding to the nuclear matrix and in transport of mRNA out of the nucleus. Since the structures of the cap and the polyadenylation signal are unique and understood, they present interesting targets. The mechanism for which the majority of the ODNs have been designed is translational arrest by binding to the translation initiation codon or by blocking the movement of the ribosome and subsequent translation of the mRNA. Reduction of targeted protein by an antisense ODN but no reduction in mRNA has been used as evidence for a translation arrest mechanism. Alternatively, demonstration of a selective reduction in target protein by modified ODNs that do not support RNaseH has also been used as evidence for a translation arrest mechanism. However, in both cases other mechanisms of action could account for these observations. Therefore, evidence directly demonstrating a translation arrest mechanism in cell culture or *in vivo* is circumstantial.

Advantage with respect to other drugs

An antisense agent interacts with a target as it does with any other drug, the major difference being the target. Because binding sites in nucleic acids are defined by the primary sequence, the number of potential binding sites is much larger compared to enzyme targets. This inherent feature of an extremely large array of potential targets within any particular gene is a major advantage of employing an antisense agent for pharmacological purposes. If one considers that the majority of currently used drugs interact with protein-based receptors, then the cascade mechanism of gene expression illustrates another advantage of an antisense compound. If a drug which interacts with a protein and an antisense drug which interacts with a nucleic acid coding for that protein both bind irreversibly to their targets (and all considerations of stability, distribution and cell entry are set aside), the antisense compound would be expected to be at least several 100-fold more effective. This is derived from the fact that one gene may code for hundreds of pre-mRNAs, each of which may then be translated into several hundred protein molecules. Thus, one antisense drug specifically interacting with one gene could prevent the

synthesis of a large number of drug receptors. However, it should be noted that even when the expression of a particular gene is already shut off, the previously existing cellular end product of that gene is still available for cellular functions. These considerations suggest that for rapid and effective pharmacological gain, the combination of an antisense agent with a drug that interacts with the end product of the gene may be the most effective.

Limitations of antisense technology

While the idea of antisense technology is simple, the development of antisense ODNs as broadly applicable therapeutics has been slow and arduous (43, 44). In 1992, antisense was proclaimed as one of the top 10 emerging research areas. However, 3 years later, researchers were confronted with significant problems associated with antisense ODNs, ranging from sequence-dependent, non-antisense effects *in vitro* to dose-limiting toxicities in preclinical models (45–48). Like any evolving technology, antisense therapeutics had to overcome several major barriers. One of the first major problems was that natural ODNs containing phosphodiester linkages are readily degraded in serum and fail to demonstrate any specific antisense activity *in vitro* and *in vivo* (49). This has driven the development of ODNs with modified backbones, such as replacement of the phosphodiester backbone linkage by a phosphorothioate backbone, which created a nuclease-resistant, RnaseH-competent ODN. Although these modified ODNs are now in common use, there are still serious problems with the interpretation of data obtained with phosphorothioate ODNs as antisense agents, especially with regard to specific and non-specific interactions with proteins.

Other non-antisense effects of phosphorothioate ODNs that are more often recognized as being problematic involve ODNs that contain four contiguous guanosine residues (the G-quartet). ODNs that contain the G-quartet may be antiproliferative in a sequence-independent manner (50). This means that if the control (sense or scrambled) ODN does not contain the G-quartet sequence, artificial 'antisens' efficacy may be observed. However, it should not be concluded that all ODNs with four contiguous guanosine residues couldn't be sequence-specific antisense agents. For example, several investigators have shown that, under certain circumstances, at least some of the non-antisense effects of CpG containing ODNs may be clinically useful anticancer agents (51, 52). Nevertheless, great care must be taken with ODNs containing G-quartets and CpG motifs. The researcher should keep in mind that some biological effects observed with phosphorothioate ODNs result from combinations of sequence- and non-sequence-specific mechanisms. This must be considered when mechanistic claims are made and each ODN in every system must be examined individually, always employing the proper controls.

Assay design

One of the most important issues facing the antisense community is the question of whether an observed biologic effect is produced by an antisense mechanism. It is therefore important to adhere to several rules in designing an antisense experiment. The most critical part of the experimental plan is the design of the antisense ODN. At this time, it is believed that phosphorothioates are the backbone of choice, despite the various problems. Many examples of successful antisense experiments with phosphorothioates in the literature (22, 53, 54), performed with appropriate controls and critical evaluation of the data, prove this approach to be reasonable. Other backbones, such as methylphosphonates, peptide nucleic acids and phosphotriesters, simply are not sufficiently active. There are several reasons for this, among them (1) the inability of uncharged oligomers to elicit RNaseH activity, (2) the lack of absorptive endocytosis of naked oligomers because of their inability ionically to bind to cell surface proteins (18). Phosphorothioate oligomers are almost certainly binding to cell-surface heparin binding proteins (e.g. Mac-1(55)). However, as discussed earlier, the addition of naked (i.e. not delivered by a vehicle, such as cationic lipids) oligomer may have several important physiologic consequences that must be considered in the context of the experiment being performed.

It is also important to note that screening of ODN activity should be performed by evaluating the ability of the compound to inhibit expression of the target, either on mRNA or protein level, rather than by looking at phenotypic changes (i.e. cell proliferation). To avoid embarrassing mistakes when interpreting and publishing antisense experiments, a few simple rules should be followed. (i) Always demonstrate a reduction in expression of the targeted mRNA or protein. Show that this is a dose-dependent effect. (ii) Determine the effect of the active ODN on another gene product, preferably a closely related one. (iii) Determine the effects of control ODNs in the system; these can include sense sequences, scrambled sequences (same base composition, different sequence) and mismatch sequences. Until these simple rules are followed to demonstrate specificity, the interpretation of the results should be treated with extreme caution. However, when the rules are followed, the usefulness of antisense ODNs in a certain model system can be readily determined.

ANTISENSE TECHNOLOGY IN DERMATOLOGY

In vitro *effects on keratinocytes*

Most of the research on antisense technology in skin that has been done so far has been focused on the uptake of ODNs in keratinocytes. Several groups, including our group, have described intracellular uptake and distribution of ODNs in keratinocytes. One consistent conclusion that can be drawn is that keratinocytes are unique in their ODN-uptake ability. However, there are conflicting results on the site of accumulation of ODNs and keratinocyte permeability in the different reports. The intracellular fate of the ODN and the need for lipidfection reagents is controversial. Some studies have suggested that a receptor-mediated or adsorptive endocytosis pathway is involved, in which the ODN is retained within membrane-bound intracellular vesicles. In these studies, the use of a facilitator such as cationic lipids was recommended (56, 57). However, others have demonstrated that keratinocytes accumulate ODNs in the cell nucleus without the use of cationic lipids or other transfection methods (33–35). Recently, affinity modification studies in keratinocyte cell lines have revealed two high-affinity, cell-specific ODN-binding proteins that might be involved in the recognition and transport of ODNs in keratinocytes (35). In addition, other parameters accounting for the variations in ODN uptake in

keratinocytes are confluency, degree of differentiation and viability (36).

Several papers reporting functional studies with antisense compounds in keratinocytes have revealed antisense ODNs that inhibit the expression of intercellular adhesion molecule-1 (ICAM-1) (38), vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) (58), and protein kinase C alpha (PKC) (59). However, concerns have been raised about the specificity of these compounds, because also complex non-antisense sequence-specific effects have been noted. For example, Hertl et al. (56) found that, besides the antisense ODN, two scrambled control ODNs also inhibited the ICAM-1 expression of keratinocytes. Koike et al. (60) showed that growth enhancement, which was induced by the addition of an antisense ODN complementary to translation initiation codon of retinoblastoma susceptibility gene, was not caused by an antisense mechanism, since it did not result in any appreciable change in transcription of the gene or in the protein expression. One of the most prominent toxic effects observed with phosphorothioate ODNs tested in rodents is immune stimulation. Repeated intradermal administration of phosphorothioates targeted at HPV (human papilloma virus) or HSV (herpes simplex virus) caused local inflammation and epidermal ulceration or necrosis. Furthermore, a study in a model of intact skin revealed a release of IL-1α as a response to administration of these phosphorothioate ODNs (61). We applied a variety of 20-mer antisense and control ODNs designed to hybridize to different regions on the mRNA of the inducible epidermal proteinase inhibitor skin-derived anti-leukoproteinase (SKALP)/elafin that was used as a model target gene (48). When nuclease-resistant fully phosphorothioate ODNs were applied to explant cultures of human skin, they were found to be either ineffective at low doses or severely toxic at higher doses, which could be attributed to the extremely high degree of protein binding found with this type of ODN. When chimeric ODNs with a phosphodiester core and phosphorothioate 5' and 3' ends were applied to intact skin, no toxicity was noted. One of the tested chimeric ODNs that exhibit only minor protein binding was found to inhibit SKALP expression at the protein level in a dose-dependent manner. The observed inhibition on SKALP expression levels was specific as evaluated by application of strict criteria (sense and scrambled ODNs were ineffective, other differentiation-related proteins were not affected). We concluded that topical application of antisense ODNs can be used to modulate epidermal gene expression and could potentially be useful as a therapeutic agent against certain skin diseases.

In order to extrapolate results from *in vitro* findings to human skin *in vivo*, reliable models for epidermal growth and differentiation are crucial. Very suitable is a transplantation model of human skin. In a recent study by Mehta et al., unmodified photodiester ODNs were used in a cream formulation that appeared to enhance the penetration of ODN through stratum corneum and achieve therapeutic concentrations in epidermis and dermis after topical application (62). These results show the ability of the topical formulation to deliver ODNs rapidly to the intended targets within the skin and cause inhibition of target protein.

Application in skin diseases

In the past several years, some investigators have already realized the benefits and attractiveness of local application of the antisense therapy by topical application to affected skin areas. However, there are several questions that must be answered before antisense technology will yield important therapeutic advances in the treatment of skin diseases. For example, are there suitable target genes? SKALP and SLPI are very suitable model genes, but eventually it would be most useful to target genes that are known to significantly contribute to the disease. For the polygenetic skin disease psoriasis, at the moment these target genes are sparse, because they have not yet been unequivocally defined. Possible candidate genes could be the cytokines TGF-α, TNF-α, IL-8 and interferon-γ, because these are produced by keratinocytes and are known to play a role in features of psoriatic skin, such as hyperproliferation, cellular infiltration, increased cytokine production, and expression of HLA class II. Psoriasis is one of a number of diseases with a presumed autoimmune pathogenesis that display significant HLA associations. However, only a small fraction of those who carry the implicated HLA susceptibility alleles develop the disease. This observation suggests that one or more other loci are necessary for the development of psoriasis. These potential candidate antisense targets will hopefully be elucidated through genetic linkage studies. In theory, ODNs targeted against a mutated allele in monogenic diseases that are based on dominant negative mutations would be more amenable for an antisense approach. The antisense ODN targeted against a mutated allele that harbours a mismatch compared to the wild type allele could specifically knockout the expression of the dominant negative protein. Examples of such monogenic skin diseases are congenital bullous ichthyosiform erythrodermas of the Brocq and Siemens types. However, in this case, the chance of finding a good antisense ODN is limited, because the choice of the target region on the mRNA is dictated by the location of the mutation. On the basis of the experiment of Dean et al. (63), for every 7 or 8 ODNs that are screened, one active ODN may result. This arises from the fact that antisense action is dependent on the accessibility of the target RNA. Although there is a report of successful inhibition by particular ODNs with TCCC motifs surrounded by pyrimidine-rich sequences (64), it appears that the only way to generate an active ODN is via brute force: obtain the cDNA sequence and walk it down, meanwhile picking complementary ODNs at random intervals. It is best to screen 30–40 ODNs to obtain one species that is maximally active, but this is almost impossible because of time and cost considerations. An improvement to this method is highly desirable, ideally through predictive computer algorithms. Presumably, combinatorial approaches on arrays (22, 27, 65, 66) will provide solutions for the identification of suitable target sites on the mRNA in the near term.

The next question will be whether there are suitable penetration-enhancing compounds for *in vivo* application on human skin. Use of a vehicle can dramatically reduce ODN concentration and, hence, the potential for non-sequence-specific interactions. However, penetration-enhancing compounds can also induce their own non-specific effects, which may confound interpretation of the data. To complicate matters further, the concentration range over which the antisense ODN/vehicle complex is active may be fairly narrow and determination of the optimum concentration of the ODN/vehicle complex is still an empirical activity. Characterization of the skin grafting mouse model system has provided facilities for this first screening of antisense ODNs and penetration-enhancing

compounds. In addition, subsequent points for attention will be the *in vivo* pharmacologic, pharmacokinetic and toxicologic properties of antisense compounds in skin.

CONCLUSIONS

The antisense technique was introduced to develop drugs that would yield effective therapies for diseases resulting from the production of deleterious proteins. The concept was straightforward: eliminate production of the unwanted proteins by adding antisense ODNs that bind the mRNA, that is coding for these proteins through complementary base pairing. However, antisense ODNs are large, highly charged, complex molecules that interact with a wide variety of cellular components, often causing non-antisense effects. Presently, the antisense strategy appears not to be as simple as previously thought. This is certainly also true for antisense technology in dermatology. Furthermore, because it is both more difficult and less appealing to publish one's experimental failures than one's successes, reports of problems and difficulties with antisense techniques have been slow to appear in the scientific literature. Nevertheless, an understanding of the problems associated with this technology is essential for the achievement of its early promise. Dual problems of toxicity and lack of proper uptake of ODNs confound the use of antisense ODNs in keratinocytes *in vitro*. The development of explant models and skin graft models opens up new avenues for first screening of antisense ODNs.

In conclusion, there is sense in antisense technology in dermatology, but investigators would be wise to be extremely cautious in experimental design and interpretation, and they should be aware that, even then, a good antisense ODN is still hard to find. Much work remains to be done before antisense technology will have an important clinical role in the treatment of skin diseases.

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