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MEETING REPORT: ANTISENSE OLIGONUCLEOTIDES

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organizers of the Fourth Meeting of the Italian Society of Experimental Hematology "Discutiamone Insieme"

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ABSTRACT

The use of antisense oligonucleotides as a therapeutic tool in modulating gene expression represents a newly established strategy for treating diseases. Such oligomers may be designed to complement a region of a specific gene or messenger RNA. Using this approach, oligonucleotides can serve as a potential block of transcription or translation through sequence-specific hybridization with targeted genetic segments. In the Fourth Meeting of the Italian Society of Experimental Hematology "Discutiamone Insieme", authors reported the use of *in vitro* synthesized oligonucleotides to inhibit normal and chimeric gene expression of bcl-2 in normal and neoplastic cell lines, respectively, that carry the t(14;18) translocation. The roles of c-myb and B-myb in the control of the proliferation and differentiation of normal hematopoietic cell lines have been investigated by selective inhibition of the expression of specific transcripts. To get some insight into the correlation between proliferation and differentation in myeloid cells, some authors studied and reported the differentation potential of G1-arrested cells obtained by a specific oligodeoxynucleotide complementary to the 5' region of the c-myb mRNA. The use of anti-P53 antisense oligos in the modulation of the growth of normal and neoplastic bone marrow progenitors was presented and confirmed the pivotal role of this gene in cell cycle control. The role of abl gene expression in normal and chronic myelogenous leukemia (CML) cells is not yet completely understood. Selective inhibition of this proto-oncogene and of the abl-bcr oncogene have been achieved by using of c-abl sequence specific antisense oligonucleotides; this approach sheds new light on the function of this gene in CML. Furthermore, recent reports describe direct DNA interaction with oligonucleotides resulting in intermolecular triple helix (triplex) formation, or the use of a full antisense message in the inhibition of gene expression. The multidrug resistance (MDR1) gene was targeted by sequence specific oligonucleotides capable of forming a triplex helix with genomic DNA. The level of expression of the MDR1 gene was reduced in presence of these oligonucleotides, showing the usefulness of this new approach in the modulation of gene transcription. Finally, the use of an antisense messenger RNA (asRNA) strategy to study UL44 gene function (DNA polymerase accessory protein) in human cytomegalovirus (HCMV) was reported. Strong viral inhibition was been observed at various times after infection a sensitive cell line. The papers presented at this meeting, demonstrate the multiplicity of ways antisense RNA technology can be utilized for studying gene functions and offer a model for future specific gene therapy in normal, neoplastic and infective hematological diseases.

Key words: antisense oligonucleotides

The Fourth Meeting of the Italian Society of Experimental Hematology "Discutiamone Insieme" was hosted in Florence, Italy, on July 5, 1993. These meetings were originally proposed to review progress in the clinical application of new technologies, and to update results in the experimental research of various Italian hematologic groups.[1] The

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Fourth Meeting was convened to review the expanding opportunities for applying antisense-oligonucleotide synthetic probe technology to biology and clinical medicine.

Use of in vitro oligonucleotides for the inhibition of normal and chimeric gene expression of bcl-2 in normal and neoplastic cell lines carrying the t(14;18) translocations

Several participants reported antisense oligonucleotide studies based on inhibition of the proliferation of normal and chimeric gene expression of bcl-2 in normal and neoplastic cell lines carrying the t(14;18) translocations.[2] Many reports show that bcl-2 overexpression is associated with prolonged survival through inhibition of programmed cell death. By contrast, treatment with a specific oligomer complementary to bcl-2 mRNA leads to activation of apoptosis. In fact Dr. Domenico Delia and co-workers demonstrated that several hemopoietic cell lines expressing bcl-2 undergo proliferation arrest in addition to apoptosis if treated with AS-bcl-2 oligomer. Cytofluorimetric analysis by PI staining shows a large decrease in the percentage of S/G2-M cells in the AS-treated cells and the appearance of a hypodiploid peak. This is a very specific effect because Northern blot and RT-PCR analysis showed significant inhibition of bcl-2 mRNA. In addition, these AS oligomers can inhibit the bcl-2 protein in an *in vitro* translation assay. These data clearly demonstrate that As-bcl-2-specific oligomers can change the distribution of the cells in the cell cycle phase and activate of apoptosis in sensitive hemopoietic cell lines. Nicolin reported some preliminary data on selective and specific oligonucleotide inhibition of expression of the bcl-2 gene translocated near immunoglobulin heavy-chain gene sequences (IgH) on chromosome 14. This translocation gives rise to a neoplastic transcript containing the fused sequence bcl-2-IgH.[3] The fusion point between bcl-2 and the IgH transcript is similar to the physiological rearrangement involving JH-DH-VH sequences,[4,5] with the presence of junctional specific sequences called *N* sequences or *extra* sequences.[6] These junctional bcl-2-IgH-specific sequences are clone specific and mark the leukemic cells. The authors sequenced the junctional transcripts of two human lymphoid cell samples carrying the t(14;18) translocation: the junctional bcl2-IgH sequences obtained were used to design sequence-specific anti-sense oligonucleotides. The aim was to block the expression of the neoplastic bcl2-IgH gene but not that of normal bcl2 gene expression.

Role of c-myb and B-myb in the control of the proliferation of normal hematopoietic cell lines

The oncogene v-myb was originally identified in two retroviruses (AMV and E26) which give rise to two different leukemias in the chicken.[7,8] A new gene, called B-myb (myb-related) has recently been cloned[9] and found to have a high degree of sequence homology with c-myb. Furthermore, some of this transcriptional functions are similar to c-myb.[10] Very few data are available on the role of the inhibition of the expression of the these genes in the regulation of hematopoiesis.

Dr. Introna summarized the results from his group on the role of c-myb and B-myb in the control of the proliferation of hematopoietic cell lines. This point was investigated by selective inhibition of the expression of the specific transcripts by different antisense oligonucleotide probes complementary to distinct regions. Oligonucleotide dose-dependent selective inhibition of proliferation was tested on human myeloid cell line U937 and HL60. Inhibition of proliferation in these myeloid cell lines was not followed by differentiation to macrophages or to granulocytes.

The blast cells of acute myeloid leukemia are unable to progress through the cell cycle and mainly stop in the G1 phase. Moreover, these blast cells are also characterized by differentation arrest.[11] To get some insight into the correlation between proliferation and differentation in myeloid cells, Dr. Rossella Manfredini and co-workers studied the differentiation potential of G1 arrested cells. Blockage of the cell cycle progression was affected by a specific oligodeoxynucleotide complementary to the 5' region of c-myb mRNA, as already described.[12]

The authors used the HL60 myeloid cell line as the *in vitro* differentiation model. These cells can differentiate to granulocytes when treated with all-trans retinoic acid (ATRA), to macrophages when treated with TPA, and to monocytes when treated with vitamin D3. The results show that G1 arrested HL60 cells are capable of differentiating only along the monocytic pathway, even if treated with ATRA.

These results strongly support the existence of different *differentiation windows* in myeloid maturation. The genetic program underlying granulocytic differentation is in fact activated only when proliferation and differentiation occur simultaneously, whereas monocytic differentiation can be activated even when the cell population is G1 arrested. The molecular basis for this different biological behavior is still unknown, but the c-myb protooncogene may also play an important role in the control of granulocytic differentiation.

The use of anti-P53 antisense oligos to modulate of the growth of normal and neoplastic bone marrow progenitors

Dr. Lanza reported the results of using different size (18-24 mer) antisense oligonucleotides based on p53 protein coding mRNA sequences. A role for mutated forms of p53 protein in the progression of chronic myelogenous leukemia (CML) has been reported.[1] The *wild* type p53 protein may lose its anti-proliferative and oncosuppressor effect[14,15] on hematopoietic CML cells when mutation occurs. The authors synthesized different oligonucleotides based on *wild* type or mutated type p53 transcript sequences. They used these anti-sense oligos to study the inhibition of CML stem cell and myeloid progenitor cell proliferation. They found that only neoplastic CML cells with a *wild* type p53 transcript can be selectively inhibited for protein expression, and that this inhibition increases cellular neoplastic proliferation and clonogenic capacity, which was tested by CFU-GM dosage. These data on CML cells carrying the *wild* type p53 transcript, focus attention on the possible disadvantages of using antisense oligonucleotides on neoplastic cells against genes with possible oncosuppressor and anti-proliferative activity.

Use of c-abl sequence specific antisense oligonucleotides to inhibit the proto and the abl-bcr oncogene

The role of abl gene expression in normal and CML cells[16] is not jet completely understood.[17] The c-abl gene is localized on chromosome 9 (band q34) and codes for a tyrosine-kinase protein.[18,19] It has been reported that suppression of c-abl expression in human normal hematopoietic progenitors is associated with reduced growth of granulocytic-macrophage forming colony progenitors cells (CFU-GM), but with normal growth of erythroid-forming colony progenitors cells (BFU-E and CFU-E).[20]

In order to explore the role of c-abl gene expression on normal hematopoiesis, Dr. Rosti and co-workers used an 18 mer sequence-specific antisense oligonucleotide, complementary to part of exon Ia and part of exon Ib of c-abl gene mRNA, in an attempt to obtain selective inhibition of the proto and the abl-bcr oncogene in CML patients.[21,22] They used CD34-positive cells obtained from bone marrow samples of CML patients. Progenitor and staminal cells were obtained and purified from other bone marrow cells by means of immune conjugate beads and magnetic separation.[23] Inhibition of c-abl antisense oligonucleotides was measured by counting the number of cloned colonies on a methylcellulose colony forming assay. The authors reported a significant difference and reduction in the number of CFU-GM colonies in the cultures with anti-c-abl oligonucleotides. CFU-GEMM and BFU-E were not influenced by inhibition of c-abl expression obtained with the antisense oligonucleotides. This approach sheds new light on the function of this gene in CML.

Direct DNA interaction with oligonucleotides resulting in intermolecular triple-helix (triplex) formation

Recent reports describe direct DNA interaction with oligonucleotides resulting in inter-

molecular triple helix (triplex) formation. Following the early discovery of the triple helix structure in synthetic polyribonucleic acids,[24] the more recent demonstration of oligonucleotide-directed triple helix formation within natural DNA sequences has shown the potential usefulness of this structure in a number of applications, including therapeutics. Oligonucleotide-directed triplex formation has been used for sequence-specific DNA cleavage, to block specific DNA binding proteins, and to repress specific gene transcription.[25] Dr. Scaggiante and co-workers presented the possibility of modulating multidrug resistance gene type 1 (MDR1) expression with a 27 mer synthetic oligonucleotide that forms a triplex with a DNA region of the MDR1 gene.[26] They studied and reported *in vitro* dose-dependent oligonucleotide-DNA MDR1 triplex formation in LoVo DX and CEM-VLB 100 cell lines. In the presence of these oligonucleotides a DNA triplex was produced and the level of expression of the MDR1 gene was reduced, illustrating the usefulness of this new approach in modulating gene transcription.

Viral inhibition of human cytomegalovirus (HCMV) protein by antisense RNA

Human cytomegalovirus (HCMV) can cause a broad range of clinical illnesses in immunocompromised subjects, particularly in those transplanted.[27] The functions of many CMV gene products have not yet been characterized.[28] Dr. Ripalti and co-workers reported a new strategy for understanding function of one CMV DNA-binding protein (DBP), UL44, with a molecular weight of 52kd (DBP52). It has been suggested that this protein might be essential for any minimal replication system of HCMV origin. The authors inserted the sequence coding for a fragment of UL44 (n. 604-1302) in an antisense orientation, under the control of the IE1 promoter/enhancer region of HCMV in an eucaryotic vector expressing the neomycin resistance gene pRC/CMV. The construct was used to transfect U373-MG cells permanently, and the resulting cell lines U373-asUL44 were studied for viral replication. Inhibition of viral replication was observed together with a strong decrease in DBP52 expression in U373-asUL44. This work demonstrates the usefulness of antisense RNA technology for studying viral gene function and offers a model for gene therapy in other human viruses that is based on autologous stimulation of antisense RNA expression.

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