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ANTISENSE STRATEGIES IN LEUKEMIA

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Several studies indicate that protooncogene products play a regulatory role in basic cellular functions such as proliferation, differentiation and apoptosis because of their interference with several signal transduction pathways.[1] These results have been obtained using different methodologies such as microinjection of specific MoAb[2] or *in vitro* transcribed antisense RNA,[3] transfection of plasmids or retroviral vectors expressing antisense RNA sequences,[4] antisense oligodeoxynucleotides[5] and more recently gene locus disruption by homologous recombination.[6] One of the opportunities offered by these methodologies is to obtain a specific and possibly complete inhibition of target gene at the DNA, mRNA and protein level and then to look at the biological implications of the specific gene inactivation. Therefore these experimental approaches allow the discrimination between genes which are simply related to a specific gene function and genes regulating or limiting that cell function. The methodology of the antisense oligodeoxynucleotides is based upon the concept of using basically mature mRNA as the primary target. The hybrid duplex formed between mRNA and the complementary oligodeoxynucleotide will lead to a decreased half life of the messenger by RNase H degradation[7] or to a translational arrest.[8] Furthermore, antisense oligonucleotides targeted to donor-acceptor sites for splicing pre-mRNA can inhibit gene expression at the post-transcriptional level[9,10] and oligonucleotides complementary to genomic DNA can interact with it, by means of Hogness base pairing in the major groove, to form a triple-helical structure and inhibit the transcription of the target gene.[11,12] A continous evolution of this methodology has led to a technical improvement because of the possibility to synthesize modified oligodeoxynucleotides which are more stable[13] and the coniugation of these oligomers with different chemical compounds that can improve the cellular uptake. In fact it has been recently described a delivery system based on receptor mediated endocytosis to introduce the oligos complexed with transferrin-polylysin conjugate into the cells.[14] Using this technology it has been reported that protooncogenes such as c-fos,[15] c-myc,[16] c-myb[17] and normal genes such as cdc2,[18] PCNA,[19] IL-6,[20] and CSF1[21] play a regulatory role in cell proliferation since their inactivation inhibits proliferation in several different cell lines and in normal hematopoietic cells. These results offer new insights of the molecular mechanisms underlying and controlling the genetic program of cell proliferation. In the normal hematopoietic tissue, when the myeloid differentiation commitment of a normal precursor cell occurs, the G1 phase of the cell cycle progressively increases in lenght and terminally differentiated granulocytes are practically arrested in this phase of the cycle and cannot be triggered along the proliferative pathway.[22] This observation rises the question of the possible correlation between the proliferative and differentiative pathways. It has to be pointed out by Wichstrom that spontaneous myeloid differentation has been obtained after inhibition of HL60 cells proliferation with a c-myc antisense oligomer.[23] Furthermore, experiments carried out in our laboratory indicate that G1 arrested cells, after the inhibition of cell proliferation with a specific antisense c-

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myb oligomer, are capable to differentiate only along the monocytic differentiation pathway even if treated with all-trans retinoic acid,[24] a specific granulocytic differentiation inducer. These results strongly support the existance of different *differentiative windows* in myeloid differentiation and particularly that the genetic program underlying granulocytic differentiation is activated only when proliferation and differentiation occur simultaneously, whereas monocytic and macrophagic differentiation can be activated also when the cell population is G1 arrested.

The treatment of normal bone marrow cells with an antisense c-myb oligomer allows the monocytic-macrophagic colonies formation but not granulocytic colonies in methyl cellulose assay. On the other hand the overexpression of several cell-cycle related genes such as c-myc,[25] c-myb,[26] c-erbA[27] and c-jun[28] in different myeloid and erythroid cell lines suppresses almost completely the differentiation capability of these cells. The overexpression of a normal p53 gene can induce spontaneous granulocytic differentiation in HL60 cells.[29] The preliminary conclusion of these experiments is that several oncogenes are involved primarily in cell proliferation and that cell differentiation requires permanent withdrawal of these gene products and the permanent exit from the cell-cycle. On the other hand, the knowledge of the mechanisms which control cell differentiation is still quite primitive.

The study of the function of oncogenes product during hematopoietic differentiation has provided critical insights on the molecular mechanism underlying this process.[30] In fact, particularly interesting are the results obtained by using antisense oligos to inactivate the receptor for CSF1, the cellular homologue of the fms oncogene, which is a receptor involved in the monocytic differentiation. The inactivation of the c-fms oncogene mRNA inhibits completely the capability of HL60 cells to differentiate along the monocytic pathway.[31] Another oncogene which we studied in our laboratory, related to granulocytic differentiation, is the c-fes.[32] In fact this protooncogene, encoding a p93 c-fes tyrosine kinase protein, is expressed only in myeloid cells and its abundance increases in polymorphonuclear cells.[33,34] The inactivation of the c-fes mRNA with a specific antisense oligomer in HL60 and spontaneous promyelocytic blast cells doesn't interfere with the proliferation of these cells but completely inhibits the granulocytic differentiation, inducible by all-trans retinoic acid, because of the activation of the apoptotic program.[35] The same antisense oligomer doesn't interfere with monocytic differentiation induced in HL60 cells by vitamin D3 and only marginally interferes with the macrophage differentiation inducible in these cells by phorbol esters. Therefore, our observations indicate that the function of the c-fes protooncogene product is essential during myeloid differentiation and during the mature granulocytes life. Its function might therefore be similar, in granulocytic cells, to that of the bcl-2 oncogene in pre-B cells.[36]

Other studies showed that several hematopoietic cell lines expressing bcl-2 underwent to proliferation arrest and apoptosis when treated with specific antisense oligos.[37] All the results above mentioned lend support to the fact that proliferation, differentiation and apoptosis are strictly genetically related processes.[38] This is particularly evident in normal myelopoiesis where a dinamic balance occurs between quiescent, proliferating, differentiating and apoptotic cells. This balance is altered in acute myeloid leukemias. In fact in blast cells of acute leukemia, independent from their type, both proliferation and differentiation are arrested, their half life is remarkably longer than that of normal maturating myeloid cells so that their inhability to mature and undergo apoptosis plays a major role in leukocytosis. It is thus evident that the growth advantage of these leukemic blast cells is originated not by an increased cell proliferation activity, but mainly by the maturation arrest arrest leading to a prolonged survival.[39] This biological behaviour is common to the vast majority of acute myeloid leukemia independently from their type and from their genetic abnormalities. It is clear however that when the proteins encoded by these oncogenes are in some way altered (point

mutations, gene rearrangements, gene amplifications) the leukemic transformation can occur.[40] The selective inhibition of the expression of activated oncogenes, which often underlies an abnormal cellular function, is one of the desired goals of cancer therapy.

Several studies describe the inactivation of oncogene products using the antisense oligonucleotides technology. In this regard particularly interesting are the studies concerning the possible role of the BCR/ABL chimeric mRNA in chronic myeloid leukemia (CML) carrying the t(9;22) translocation.[41,42] Kinetic studies performed in CML indicate that these abnormal cells do not proliferate or mature faster than the normal counterpart. Instead the basic defect underlying the abnormal degree of granulocytopoiesis in CML appear to reside in the expansion of the myeloid progenitor pool in bone marrow and in peripheral blood. Nevertheless the generation of terminally differentiated cells indicates that the process of hematopoiesis retains several normal features.

The biological behaviour of CML is therefore different from that characterizing AML and therefore we can consider the chronic phase of CML basically as an hyperplastic rather than a neoplastic disease. The c-abl protooncogene encodes a cytoplasmic protein with tyrosine kinase activity[43,44] which can be also involved in the survival of hematopoietic cells.[45] The tyrosine kinase activity is increased in cells carrying bcr/abl hybrid gene encoding a novel phosphoprotein. An antisense oligonucleotide inhibiting specifically the bcr/abl chimeric mRNA leads to a suppression of CML leukemic cells colony formation althought the mechanism of inhibition of CML leukemic cells is not yet clarified.[46] Using the antisense strategy it has been found that other oncogenes are involved in hematological malignancies, i.e. the bcl-2 oncogene in follicular lymphomas[37] and c-myc in Burkitt lymphomas.[47]

Several laboratories are involved in the study of the possible role of PML-RARa and RARa-PML chimeric mRNAs, transcribed specifically in acute promyelocytic blast cells carrying the t(15;17), in the pathogenesis of this type of leukemia.[48-50] The overexpression of these proteins in U937 cells showed a strong interference with the differentiative program of these monoblastic cells.[51] It has been also reported that bone marrow purging of leukemic cells can be efficiently obtained using specific c-myb[52] and bcr/abl[53] antisense oligonucleotides. In conclusion we can affirm that the antisense methodology is a promising approach to control the neoplastic cell *growth* in hematological malignancies where the genetic alterations are well characterized.

The experimentation of antisense oligos is recently been carried out in laboratory animals and in clinical trials of patients having different hematological neoplastic diseases. In the future it will be probably possible to reach one of the more desired goal in cancer therapy: specificity of action and selective cell death of cancer cells.[54]

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