FUNCTIONAL INVESTIGATION AND CHARACTERIZATION OF THE INVOLVEMENT OF NOVEL TARGET GENES AND NEW THERAPEUTICS FOR MYELODYSPLASTIC SYNDROMES AND LEUKEMIA LINEAGES

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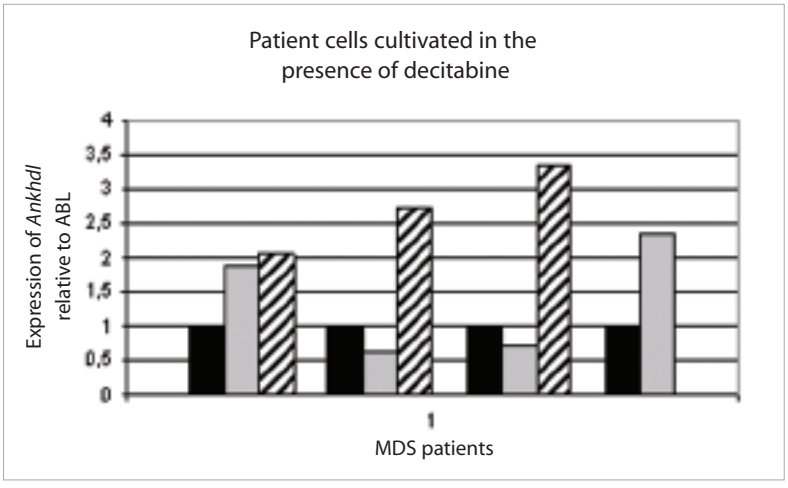

Myelodysplastic syndromes (MDS) are a group of heterogeneous hematopoietic disorders characterized by inefficient hematopoiesis. Little is known regarding MDS pathogenesis and the processes that mediate their frequent transformation into leukemia. During the last years it has become evident that composition and /or function alterations of the cellular microenvironment may be implicated in the progression of several hematological disorders, mainly MDS. New therapies have been proposed based on the biological characteristics of this type of tumor, however the molecular events responsible for the maintenance and dissemination of the anomalous clonal population remain unknown, frequently leading to the use of therapeutic agents that are not target specific. Therefore, the characterization of important molecular targets for differentiation processes and myeloid tumor progress could provide information, that would contribute for the creation of new specific drugs with greater and better action. During the Human Genome Project, several novel genes were identified, many of which presented great potential for therapeutic targets. This project proposes to characterize the regulation of novel gene expressions, specifically *Arhgap*10, *MasK*, and *Formin*, as well as other proteins, in myelodysplasias, submitted to different treatments, with the purpose of investigating molecular mechanisms of this type of tumor and the creation of new strategies for anti-tumoral therapy. Due to the nonexistence of cell or animal models with myelodysplasia, in order to fulfill some of the aims, we will use leukemia lineages as models. Furthermore mutations will be searched in genes that can associate evolving to leukemia such as *Ptpn*11, *Flt*3, *Aml*-1, *Gata*-1.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Apoptosis has a crucial role in myelodysplastic syndromes (MDS) and acute myeloid leukemia. Early disease MDS is associated with excessive apoptosis; apoptosis rate diminishes during disease progression. Cytochrome c/ APAF-1/ CASP-9 pathway is the main pathway involved in the apoptosis initiation by several stimuli. Original APAF-1 comprises of three functional domains; APAF-1XL and APAF-1LN isoforms have an insertion between CARD and ATPase domains and APAF-1XL has also an additional WDR. It was previously described that only the isoforms with the extra WDR activate pro-caspase 9. We hypothesized that APAF-1XL expression could be related to the higher rates of apoptosis found in early-stage disease of MDS and we showed , for the first time, that APAF-1XL is highly expressed in bone marrow cells of low risk myelodysplastic syndrome and in cells of patients with acute myeloid leukemia (AML) responsive to remission induction therapy. We are also interested in investigating the role of two new cytoskeletal genes (*Arhgp*21 and *Ankhd*1), previously described by our group in MDS and acute leukemia. Many interesting results were obtained: our data showed that *Arhgp*21 is overexpressed in AML and ALL (acute lymphoid leukemia) cells and is associated with FAK in leukemia cell lines and in normal peripheral blood. These findings raise the hypothesis that *Arhgp*21 may be involved in leukemogenesis, aiming this gene as a candidate for anti-tumor therapy. We also observed that *Arhgp*21 is upregulated by decitabine treatment in bone marrow mononuclear cells from patients with myelodysplatic syndromes and there is a positive correlation with □-catenin expression. Alfa-catenin deletion causes abrogation of cell differentiation and MDS. Ecitabine is an important agent for treatment of high risk MDS. Regarding the *Ankhd*1 gene, we observed that its expression is modulated by therapeutic agents in myeloid cell lines. The ankyrin repeat and KH domain containing 1 protein (ANKHD1) is protein homologue of Drosophila MASK (Multiple ankyrin repeats KH domain), which is known for its crucial role in photoreceptor differentiation, cell survival, and proliferation. We have demonstrated an upregulation of the splice variant *Ankhd*1 mRNAs expression during HL-60 and erythroblast differentiation. We showed that *Ankhd*1 is upregulated in KG1 and HL60 leukemia cell lines after treatment with α-Interferon or G-CSF in a dose-dependent way, suggesting its involvement in the regulation of differentiation and proliferation. Thalidomide is a drug used to treat low risk MDS. Our study also showed that thalidomide increases the number of CFU-GM and alters the cytokines expression profile in long-term bone marrow cultures from patients with myelodysplastic syndromes.

MAIN PUBLICATIONS

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