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REGULATION OF BCL6 IN AGGRESSIVE B-CELL LYMPHOMA: EFFECTS OF EPIGENETIC DRUGS

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INTRODUCTION

BCL6 is an important transcriptional repressor considered one of the master regulators of the germinal center reaction. BCL6 controls the exit of the B-cells from the germinal center in order to differentiate toward plasma cells. In some lymphomas deregulated expression of BCL6 is detected. This deregulation is frequently caused by genetic modifications like translocations or point mutations and epigenetic mechanisms are also involved. Previous results of our group have demonstrated that CTCF regulates BCL6 expression through epigenetic mechanisms in lymphoma cells (Batlle-López et al. Oncogene 2015). Therapy with epigenetic drugs has an enormous potential for cancer treatment. Romidepsin is an inhibitor of histone deacetylases (HDACi) approved for the treatment of some T-cell lymphomas, but its role on B-cell lymphoma has not been thoroughly investigated. JQ1 is a BET bromodomain inhibitor that represses the expression of some genes as MYC, often found deregulated in lymphomas. In this study, we analyze the effects of Romidepsin and/or JQ1 treatment in different aggressive B-cell lymphoma cells where BCL6 and/or MYC are deregulated.

OBJECTIVES

To analyze the effects of the HDACi Romidepsin and the BET bromodomain inhibitor JQ1 on cell cycle, apoptosis and differentiation of B-cell lymphoma cell lines.

To study the regulation of BCL6 mediated by these drugs and the epigenetic regulation of BCL6 by CTCF.

METHODS

Lymphoma-B cells derived from Burkitt Lymphoma or from Diffuse Large B-Cell Lymphoma (DLBCL) were treated with Romidepsin and/or JQ1 drugs. BCL6 and MYC loci status were analyzed by Fluorescence *in situ* hybridization (FISH). Cell metabolic activity (WST-1 assay), cell proliferation (cell counting), cell cycle (propidium iodide staining and p27 and/or p21 protein expression) and apoptosis (Annexin-V binding and PARP cleavage protein expression) analysis were performed. B-cell differentiation was studied by analyzing BCL6 protein expression by Western-Blot and plasmatic cell surface markers expression measured by flow cytometry. Luciferase assays were used to measure the changes on the BCL6 repression activity upon HDACi treatment. BCL6 acetylation after Romidepsin treatment was assessed by protein immunoprecipitation. Chromatin immunoprecipitation (ChIP) experiments were performed to study the role of CTCF on BCL6 epigenetic regulation.

RESULTS AND CONCLUSIONS

In this study, we analyzed the effects of the treatment with Romidepsin and JQ1 alone or in combination in different human B-cell lymphoma cell lines. A decrease in the metabolic activity and inhibition on cell proliferation were found with the different treatments. Romidepsin treatment alone and in combination with JQ1 induced apoptosis in lymphoma cells. Apoptosis was demonstrated by PARP cleavage and an increase in the number of annexin-V positive cells measured by flow cytometry. All cell lines analyzed treated with JQ1 alone showed cell cycle arrest in G0/G1 phase and an increase in p21 and 27 protein expression, which is consistent with the cell cycle arrest. Protein levels of BCL6 were found downregulated in BCL6 expressing cell lines upon Romidepsin and/or JQ1 treatments. Simultaneously, an increase in genes of the plasmatic differentiation program as PRMD1/Blimp1 was observed. This effect was accompanied by the increase of plasmatic surface markers. In presence of Romidepsin

an increase of BCL6 acetylation and a decrease of its repressor activity was observed. CTCF positively regulates BCL6 by its binding to the exon1 of BCL6, accompanied by active chromatin marks. In the presence of Romidepsin, this binding is reverted and repressive chromatin marks are incorporated. Altogether, our results show differential effects of the treatment with Romidepsin and/or JQ1 in lymphoma B cells. Finally, histone acetylation is important in the epigenetic regulation of BCL6 mediated by CTCF.