Epigenetic regulation of BCL6 in B-cell lymphoma: effects of Romidepsin, a Histone Deacetylase Inhibitor.
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INTRODUCTION

BCL6 is a transcriptional repressor essential for germinal centre formation and T-dependent antibody responses. The deregulation in the expression of this gene is associated with the development of B-cell lymphomas (1). This deregulation is frequently caused by genetic modifications like translocations or point mutations and recently it has been demonstrated that epigenetic mechanisms are also involved. Hypermethylation of promoter CpG islands and altered patterns of histone modification are common in cancer. These abnormalities affect the structure of the chromatin and gene expression, resulting in the silencing of genes important for the regulation of normal cell proliferation (2). For this reason, therapy with chromatin modifying drugs has enormous potential for cancer treatment. Histone deacetylases (HDAC) are important targets for epigenetic treatment in cancer (3). Romidepsin (from Celgene) is a HDAC inhibitor used in cutaneous T-cell lymphoma treatment, but potential effects in B-cell lymphoma are not known in detail (4).

In the present study, we analyzed the effects of Romidepsin in different human B-cell lymphoma cell lines, including its influence on BCL6 expression.

MATERIALS AND METHODS

1) Lymphoma cell lines
Raji, Ramos, and Dp75 Burkitt's lymphoma. BCL6 positive.
Mutu III: Burkitt's lymphoma. BCL6 negative.
Bjab: a B-cell lymphoma line. BCL6 positive.
Cells were grown in RPMI media + 10% FCS.

2) Treatment with Romidepsin
[Romidepsin] - 0, 2 and 5nM [cells] - 3x10^5 cells/ml

RESULTS AND DISCUSSION

PREVIOUS RESULTS

Our group has recently shown BCL6 gene epigenetic regulation in lymphoma B cells. BCL6 expression was associated with the presence of active histone marks, mainly histone acetylation, on its promoter. On the contrary, in non-expressing BCL6 cells, a predominant enrichment of repressive histone marks was observed (5).

AIMS

- To study the effects of the HDACi Romidepsin in proliferation, cell cycle, cell death and differentiation of lymphoma B cells from different origin
- To analyze the effect of Romidepsin on BCL6 expression.

1) Romidepsin effect on cell proliferation

Raji and Dp75 cells lines were resistant to Romidepsin treatment as assessed by WST1 method.
Ramos, Mutu III and Bjab cell lines were sensitive to Romidepsin in time and dose dependent manner.
Similar effects were observed when cell proliferation was measured using Guava Via Count (data not shown).

2) Romidepsin effect on cell cycle

A) Romidepsin treatment induced cell cycle arrest in G0/G1 phases in Raji, Ramos and Mutu III. This effect was very prominent in Raji cells (see cell cycle profiles).
B) CDKs inhibitors were analyzed by RT-PCR and Western-Blot in Raj and Ramos cells upon Romidepsin treatment.
No changes in p16 and p21 expression were found (not shown).
p27 mRNA and protein expression dramatically increased in Raj and Ramos cells, in agreement with Romidepsin effects on cell cycle.

3) Romidepsin effect on cell death.

Romidepsin induced apoptotic cell death in Ramos and Mutu III cells but not in Raji, as assessed by sub-G0/G1 fractions (A) and Annexin-V binding assay (B).

C) Western-Blot analysis of PARP protein confirmed apoptotic cell death in Ramos, but not in Raji cells.

4) Romidepsin effect on BCL6 expression and cell differentiation.

A) A decrease in BCL6 mRNA and protein expression was found in Raji and Ramos cells upon Romidepsin treatment. This could be associated with induction of differentiation.
B) BLIMP1 is critical for B-cell differentiation. Romidepsin induced an increase of BLIMP expression, indicating differentiation to plasma cells.

CONCLUSIONS

- Treatment with the HDACi Romidepsin causes differential effects on B cell lymphoma cells.
- Romidepsin induces cell cycle arrest in G0/G1 phase accompanied with increased p27 levels.
- Romidepsin induces apoptosis and/or cell cycle differentiation in a cell-dependent manner.
- Romidepsin causes downregulation of BCL6 mRNA and protein levels in BCL6 positive cell lines.
- Downregulation of BCL6 and upregulation of BLIMP1 induced plasma cell differentiation.

REFERENCES

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FUTURE WORK

- To analyze Romidepsin effects on cell cycle, death and differentiation in other B-cell lymphoma cells.
- To study the effects of Romidepsin in primary cells from lymphoma patients.
- To gain further insight on the BCL6 regulation by Romidepsin.

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