Epigenetic modifications as targets to new therapies for Chronic Lymphocytic leukaemia – A preliminary study

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Aim: This study aimed to clarify the involvement of epigenetic modifications in chronic lymphocytic leukaemia development and analyse the therapeutic potential of epigenetic modulators.

Introduction: CLL is the most common type of leukaemia found in adults and is an extremely variable and heterogeneous disease. The CLL aetiology is unknown and it natural history is heterogeneous. However, epigenetic modifications may play an important role in CLL.

Methods: This study enrolled 18 CLL and 7 controls. To perform primary CLL cultures, peripheral blood mononuclear cells from CLL patients were isolated using ficoll gradient and incubated with the hypomethylants, Azacytidine and Decitabine, and decacetylase inhibitors, Panobinostat and Vorinostat, in monotherapy (single dose and daily administration) and in combination for 24 h/48 h. The cytotoxic/cytostatic effect of drugs was evaluated by fluorometric microculture cytotoxicity assay (FMCA). Cell death and cell cycle were determined by flow cytometry using Annexin V and PI/RNase, respectively. CD5 and CD19 antibodies were used to identify normal (CD5−/CD19+) and neoplastic cells (CD5+/CD19+). Methylation pattern was determined by MS-MLPA. Data were analysed using univariate approaches.

Results: Preliminary results show that patients appear to be more sensitive to Azacytidine and Vorinostat than Decitabine and Panobinostat, on single dose administration. Combination of Panobinostat with Azacytidine and Decitabine induced higher cytotoxicity than single dose. For all drugs, daily administration schedule reduced more effectively cell viability/proliferation than the same doses in single administration. These drugs induced cell death mainly by apoptosis with specificity to neoplastic cells. Moreover, CLL patients had a significant higher methylation frequency of PAX5 (70%), KLLN (80%), WT1 (100%), THBS1 (90%) and GATA5 (90%) gene promoters when compared with controls (all genes demethylated, except MSH6). Furthermore, all CLL patients had at least one methylated gene.

Conclusion: The preliminary results suggest that methylation of tumour suppressor genes is a common event in CLL patients and that epigenetic modulators induce a cytotoxic effect, reducing cell viability/proliferation, in a time- and dose-dependent manner. Therefore, these results are promising and encourage further studies in CLL.

References