Title

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Journal

HAEMATOLOGICA, 98

ISSN

0390-6078

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Publication Date

2013-06-12

Table 1. IC50 values achieved after 48h incubation of primary CLL and HD cells and M2-10B4 mouse fibroblasts with cannabinoids. Concentrations in µM, NR=50% viability reduction not reached.

Compound	Action	Primary CLL suspension	Primary HD suspension	Primary CLL co-culture	M2-10B4 fibroblasts
RM*	CNR1 agonist	33.19	60.13	29.27	34.55
CBD§	CNR1 antagonist, CNR2 inverse agonist	21.74	15.09	16.78	13.52
ACEA	CNR1 agonist	31.78	39.01	NR	NR
AM251	CNR1 antagonist	**9.43**	11.44	NR	NR
JWH133	CNR2 agonist	75.68	78.12	NR	NR
AM630	CNR2 antagonist, CNR2 inverse agonist, weak CNR1 agonist/inverse agonist	**12.08**	28.51	27.64	28.27

*RM = R-(+)-Methanandamide
§CBD = (-)-Cannabidiol

Summary and Conclusions: Although some cannabinoids reduce viability in neoplastic cells considerably independent of CNR expression, also healthy cells are affected. The important CXCL12/CXCR4 axis could not be inhibited by cannabinoids in CLL. These data indicate a poor therapeutic potential for cannabinoids in chronic lymphocytic leukemia, although CNR1 mRNA expression could be determined as novel prognostic marker.

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MICRORNA MIR-150 CONTRIBUTES TO THE DISEASE AGGRESSIVENESS AND REGULATION OF B-CELL RECEPTOR SIGNALLING (BCR) IN CLL

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Background: We and others have shown that expression of certain miRNAs associates with disease activity and pathogenesis of CLL (Calin, 2005; Mraz, 2012; Mraz, 2009). However, it remains largely unclear which from the ~1400 described miRNAs in human genome are relevant for the regulation of malignant B cell functions.
Aims: In this study we first screened (TaqMan miRNA Cards-ABI, 750 miRNAs) for abundantly expressed miRNAs in CLL cells hypothesising that miRNAs with strong expression are more likely significantly involved in the regulation of cell functions.
Results: We identified miR-150 as the most abundant miRNA in CLL cells, however, its lower levels associated with stronger calcium mobilization (a marker for active BCR signalling) after stimulation of BCR on CLL cells with anti-IgM ($P<0.05$, N=36). Additionally, miR-150 levels were directly down-modulated after stimulation of BCR with anti-IgM in CLL cells ($P<0.01$). This miRNA has been previously reported to influence the gene expression of normal B cells (Xiao, 2007) altogether suggesting that it might be involved in the BCR-pathway. Several target genes have been described for miR-150 in studies performed on various cell types. However, it is becoming clear that the preferential target(s) of a certain miRNA depend on the context of all mRNAs with potential binding sites and their expression levels in a specific cell type. More importantly, the identification of mRNAs regulated by a certain miRNA can be performed based on the analysis of mRNA expression since most miRNAs affect not only mRNA translation but predominantly decrease target mRNA levels (Guo, 2010). To describe novel targets regulated by miR-150 specifically in CLL cells we performed array-based transcriptome analyses of 110 CLL samples. This identified differential expression of 2 genes with evolutionary conserved binding sides for miR-150 (GAB1 and FOXP1) between CLL cells expressing low *vs.* high levels of miR-150. The immunoblot analysis of GAB1 and FOXP1 in CLL cells confirmed their higher protein levels in cases with low miR-150 expression ($P<0.005$, fold change >10.0) and the transfection of CLL cells with artificial miR-150 led to GAB1/FOXP1 down-regulation. GAB1 is an adaptor molecule that recruits PI3K to the plasma membrane after BCR stimulation and is required for amplification of PI3K signalling and subsequent AKT activation (Ingham, 2001). FOXP1 is a transcription factor uP-regulated by BCR stimulation and implicated in the progression of several B-cell lymphomas (Hu, 2006). We found that, CLL cells with higher expression of *GAB1* or *FOXP1* were more responsive to BCR stimulation *in vitro* and higher expression of each associates with shorter overall survival (OS) (13.9 *vs.* 22.7 years, 13.9 *vs.* 21.1 years; N=168; $P<0.05$). Most notably, a reverse trend was observed for miR-150, where higher levels (>median) were associated with significantly longer OS and TTFT in a multivariate analysis, which included other 6 other routinely used prognostic markers (OS HR: 3.4 [CI 1.4-8.6], P=0.009; TTFT HR: 2.3 [CI 1.3-4.2], P=0.004). Additionally, we have noted differences in the miR-150 expression and methylation profile of its coding region after disease progression (analysis of serial CLL samples).
Summary and Conclusions: We conclude that miR-150 is a novel regulator of genes that control BCR-signalling, which is a factor that prominently affects the biology of malignant B cells.
IGA MZCR NT11218-6/2010, FR-TI2/254.