The BET bromodomain inhibitor OTX015 (MK-8628) affects the expression of microRNAs involved in the pathogenesis of lymphoma: *in vitro* and *in vivo* evidence

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Abstract #271

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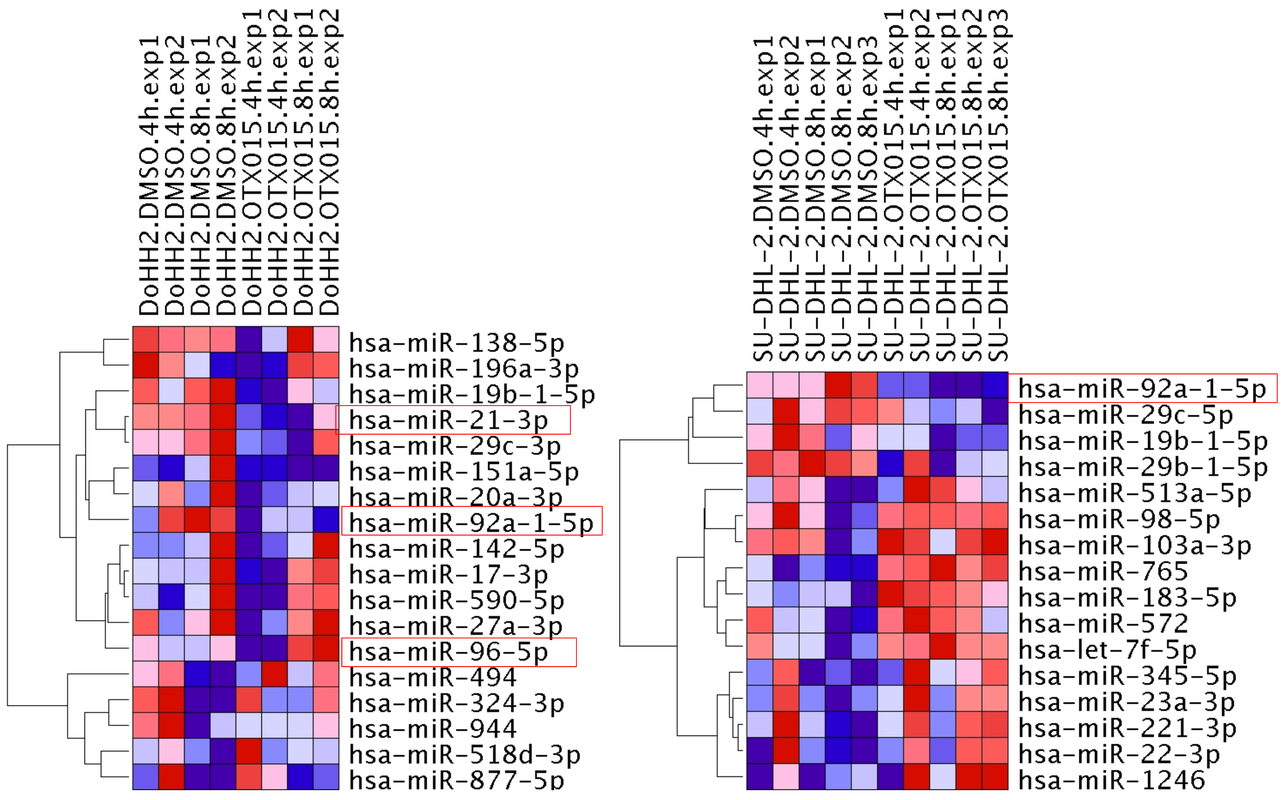
Introduction, Results and Conclusions

INTRODUCTION: Aberrant changes in histone modifications, DNA methylation and expression levels of non-coding RNA, including microRNAs (miRNAs), contribute to lymphoma pathogenesis and represent potential therapeutic targets. BET bromodomain inhibitors such as OTX015 (MK-8628), have demonstrated promising preclinical activity in haematological and solid tumour models[1, 2] and more recently in an ongoing phase I study (NCT01713582). To better understand the mechanism of action of OTX015 we studied its effects on relevant miRNAs in several lymphoma models, comprising diffuse large B-cell lymphoma (DLBCL), splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma (MCL) cell lines.

RESULTS: miRNA profiling of germinal center B-cell (GCB)-DLBCL DoHH2 and activated B-cell-like (ABC)-DLBCL SU-DHL-2 exposed to DMSO or 500 nM OTX015 (4, 8 h) identified four miRNAs with decreased expression ranging from -0.37 to -2.01 log2 FC (miR-21-3p, miR-92a-1-5p, miR-196a-3p and miR-29b-1-5p) and seven with increased expression ranging from 0.36 to 0.64 log2 FC (miR-96-5p, miR-630, miR-935, miR-1181, miR-765, miR-345-5p and miR-1246). We focused on miR-92a-1-5p (log2 FC, -2.01; P=0.004) and miR-21-3p (log2 FC, -0.37; P=0.0045), and the tumor suppressor miR-96-5p (log2 FC, 0.39; P=0.041) (Figure 1). Changes in these oncomirs matched variations in validated target genes (e.g., miR-92a-1-5p: *CDKN1A*, log2 FC, 0.81, *CDKN2A*, log2 FC, 0.26; miR-96-5p: *MYC*, log2 FC, -0.57, *MYD88*, log2 FC, -0.35) (Table 1). In mice carrying SU-DHL-2 xenografts and treated with OTX015 or vehicle alone for 3 days, miRNA expression was similarly up- or downregulated in tumour tissue, but only changes in miR-92a-1-5p were statistically significant (P=0.03) (Figure 2). miR-92a-1-5p was also decreased by OTX015 (500 nM, 8h) in 1/3 SMZL and 3/3 MCL cell lines, including REC1 and JeKo-1 with high expression of this oncomir due to DNA amplification. miR-21-3p was decreased in 2/3 MCL but none of the SMZL lines. miR-96-5p was increased in 2/3 SMZL but none of the MCL lines (Figure 3).

CONCLUSIONS: Changes in miRNA expression may contribute to OTX015 antitumour activity, particularly for the oncomir miR-92a-1-5p, which was strongly and systematically downregulated by OTX015. Specific oncomirs may represent pharmacodynamic biomarkers for BET bromodomain inhibitors in different lymphoma types as well as in the clinical setting.

Figure 1



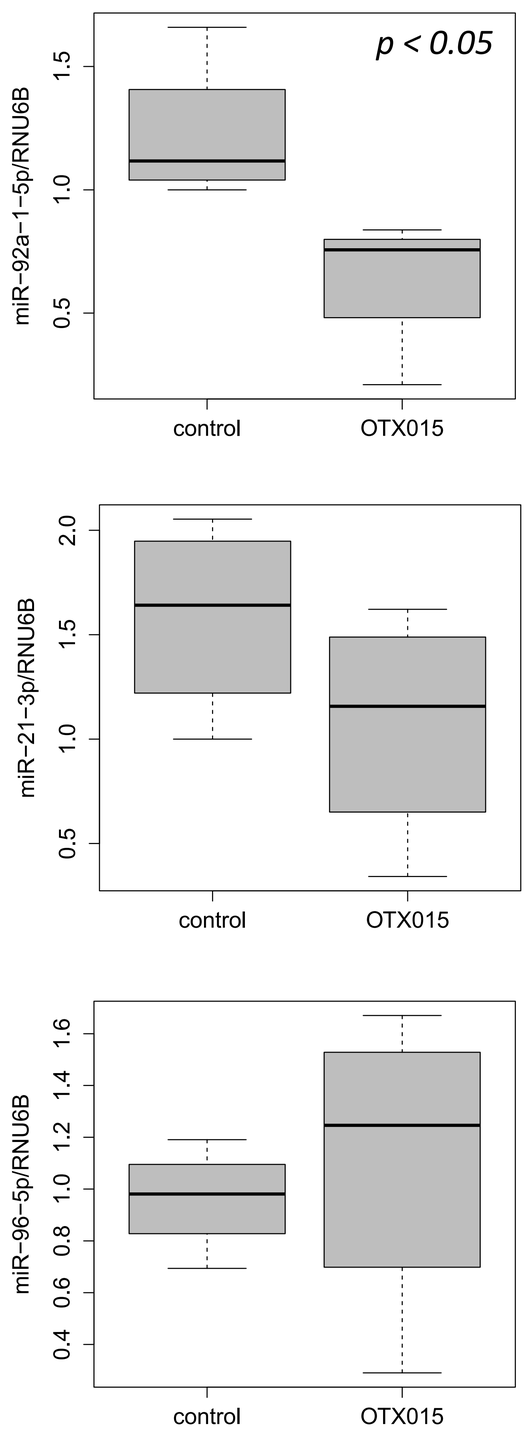
OTX015 modulates miRNA expression in DLBCL cell lines, as shown by miRNA expression profiling. The degree of red and blue represent upregulation and downregulation, respectively.

Table 1

Target gene (miRNA)	DMSO (average expression)	OTX015 (average expression)	p-value
CDKN1A (miR-92a-1-p)	10.29	10.95	<0.001
CDKN2A (miR-92a-1-5p)	7.17	7.33	<0.001
MYC (miR-96-5p)	10.46	9.89	<0.001
MYD88 (miR-96-5p)	8.43	8.08	<0.001
Target gene (miRNA)	**DMSO (average expression)**	**OTX015 (average expression)**	**p-value**
CDKN1A (miR-92a-1-p)	9.01	9.82	<0.001
CDKN2A (miR-92a-1-5p)	7.66	7.92	<0.001
MYC (miR-96-5p)	10.60	9.26	<0.001
MYD88 (miR-96-5p)	8.43	8.08	<0.001

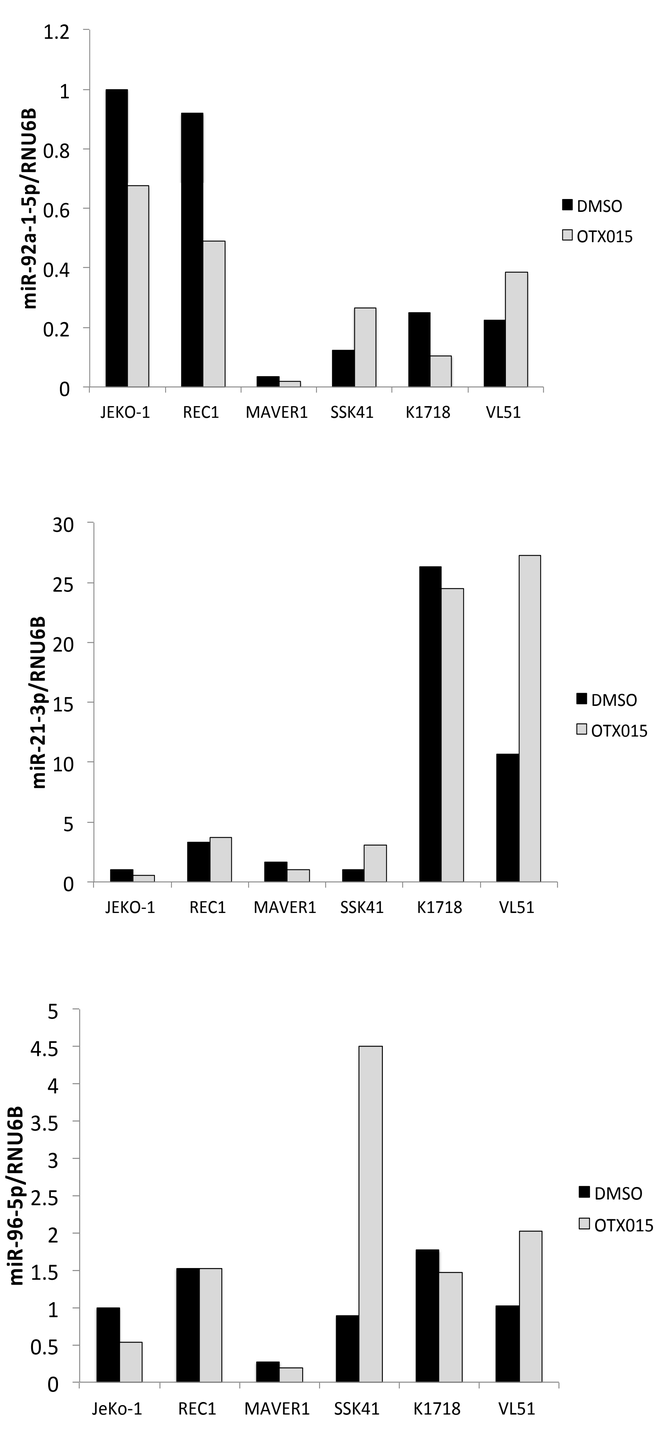
Changes in expression levels of miRNAs correspond to variations in target genes in DoHH2 (upper panel) and SU-DHL-2 (lower panel).

Figure 2



OTX015 modulates miRNA expression in DLBCL xenografts.

Figure 3



OTX015 modulates miRNA expression in MCL and SMZL cell lines

Methods

Trizol Reagent was used to isolate RNA from DLBCL (SU-DHL-2, DoHH2), SMZL (SSK41, Karpas1718, VL51) and MCL (JeKo-1, REC1, MAVER1) cell lines following 500 nM OTX015/DMSO for 4 and 8 h and from DLBCL SU-DHL-2 xenografts treated for 3 days with OTX015 25mg/kg, bid, po. Samples were analyzed with the Agilent Human microRNA microarray v.3 and the Illumina HumanHT-12 v4 Expression BeadChip. Limma was used to detect differentially expressed transcripts. Selected miRNA changes were validated by real-time PCR using TaqMan miRNA assays. All fold-changes are represented in log2 scale and a p-value of <0.05 is considered statistically significant.

References

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2. Stathis A *et al, European Journal of Cancer 2014; 50(suppl. 6): 196.*

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Pre-clinical studies

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