BET bromodomain inhibitors abrogate cell cycle progression and induces apoptosis in Myc-induced mouse lymphoma cells without affecting MYC transcription

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Abstract

Overexpression of one of the three *MYC* genes is a hallmark of many human cancers. They encode transcription factors that regulate expression of a number of genes critical for tumor development. Conditional transgenic mouse models have shown that Myc inhibition causes tumor regression, Myc is therefore an attractive target for therapy and effective pharmacological Myc inhibition has been a long-standing goal in cancer research. Recent publications have shown that selective inhibitors of BET bromodomain and extra-terminal (BET) domain family of proteins, that bind to acetylated lysines on histones, show promise as potent anti-cancer drugs via down-regulation of the *MYC* oncogene. Here we confirm that two structurally different BET protein inhibitors (BETi), JQ1 (1) (proto-type) and RVX2135, inhibit the proliferation and induce apoptosis of lymphoma cells arising in Myc-transgenic mice. BETi have been reported to directly inhibit *MYC* transcription. Surprisingly, in our system BETi inhibition had no effect on *MYC* transcription, despite exhibiting broad transcriptional effects evident from expression profiling. Our data challenge the prevailing view that BETi operate primarily via suppression of *MYC* transcription in hematological malignancies (1-6). Instead we suggest that BET proteins have Myc-independent pleiotropic effect that should be exploited to treat a wide range of cancers. Myc still remains a challenging target for therapy to date.

Results

Figure 1. Features of RVX2135, a novel BET inhibitor. **A)** Structure of the chemical scaffold from which RVX2135 was developed. **B)** FRET assay showing displacement of BET proteins by RVX2135.

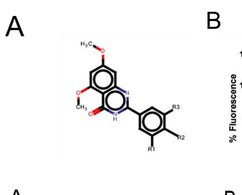
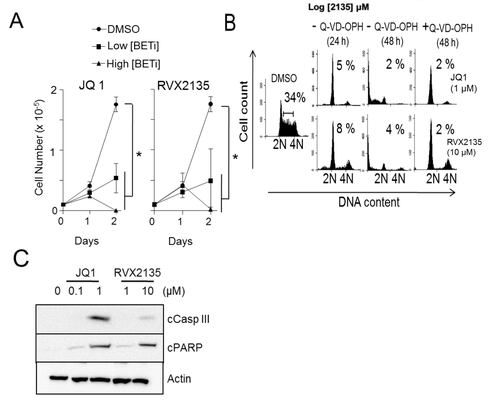


Figure 2. BET inhibitors induce cell cycle arrest and apoptosis of Myc-transgenic mouse lymphoma cells. A) λ820 cells were treated for 48h with BETi and were counted. Low concentrations of BETi were defined as 100 nM JQ1 and 1 µM RVX2135, whereas high concentrations as 1 µM JQ1 and 10 µM RVX2135. **B)** λ820 cells were treated for 24h and 48h with BETi in the presence or absence of the pan-caspase inhibitor Q-VD-OPH. Cell cycle analysis by flow cytometry analysis of 7-AAD-stained cells, shown are cells in S-phase. **C)** Western blot analysis of λ820 cells were treated for 24h with the indicated concentrations of BETi.



Results

Figure 3. BETi does not suppress Myc transcription in Myc induced murine lymphoma cells. A) qRT-PCR analysis of Myc RNA expression in indicated cell lines treated with different concentrations of BETi. **B)** Western blot analysis of c-Myc protein levels. **C)** Western blot analysis of λ820 cells treated for 24h with CHIR99021(Selleck chemicals) in the presence or absence of the indicated concentrations of BETi.

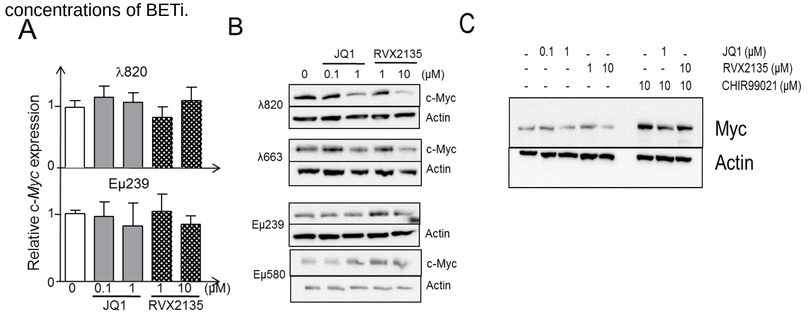
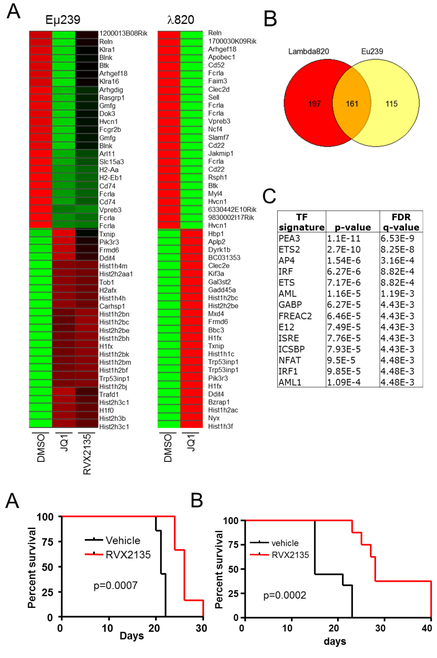


Figure 4. BETi induce broad transcriptional effects affecting several growth promoting gene signatures. A) Supervised hierarchical clustering of Illumina beadchip microarray data in λ820 and Eμ239 cells treated with BETi. Shown are the 50most down- and up-regulated genes (fold-change). **B)** Venn diagram of the genes down-regulated by treatment with λ820 and Eμ239 cells with 1 µM JQ1 for 24h. **C)** GSEA of genes co-regulated (down) by JQ1 in both λ820 cells and Eμ239 cells. Shown are top transcription factors (TF) associated with the gene signatures.

Figure 5. **RVX2135 causes potent therapeutic responses in mouse models of aggressive Myc-induced lymphoma. A)** λ820 cells were transplanted into syngeneic B6 mice via tail vein injection. Four days after injection, mice were dosed with 75 mg/kg (b.i.d; five days per week) RVX2135 (n=6) or vehicle (n=7). **B)** A lymphoma arising in a *λ-Myc* mouse (ID#2749) was transplanted into recipient B6 mice via tail vein injection, accompanied by treatment with either vehicle or RVX-2135. Four days after injection, mice were dosed with 75 mg/kg b.i.d. RVX2135 (n=8) or vehicle (n=9).



Results

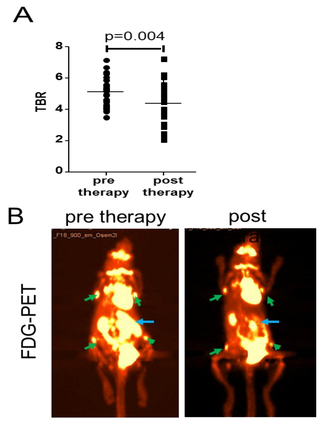


Figure 6. RVX2135 causes rapid therapeutic responses in mouse models of aggressive Myc-induced lymphoma. A) Four mice were transplanted with lymphoma cells from a λ-*Myc* mouse (ID#2749). Twelve days after transplantation, when mice were yet to show manifest disease, they were injected with 18-FDG and scanned with a PET/CT imager. Mice were treated with four doses of 75 mg/kg b.i.d. RVX2135 and imaged post-therapy. The tumor PET signal to background ratio (TBR) of all measured PET-positive sites (n=23), representing the spleens of all mice and 4-5 lymph nodes, is shown. **B)** Representative PET image of a mouse treated with RVX2135. PET-positive lymphoid tissues used in the TBR calculation in A are shown.

Conclusions

RVX2135 blocks cell cycle progression and induces apoptosis in *Myc*-induced mouse lymphoma cells *in vitro* and in mice. Our data strongly suggests that the effect of BETi is likely not monogenic since several transcription factor networks are altered. Previous studies in hematological malignancies and some solid tumors have suggested that the main target of inhibition by BETi is *MYC* (1-6). By using GEM models of lymphoma it has here been possible to uncouple BETi effects on *Myc* transcription from the effects of BETi on other transcription factor networks. Interestingly, despite maintaining c-Myc expression, the murine B-cell lymphoma cells are as sensitive to BETi as the human lymphoma cells previously studied (2-6). Therefore the fact that there is no genetic rescue in the mouse model demonstrates that BETi block lymphoma cell proliferation and induces apoptosis independent of inhibiting *Myc* transcription in B-cell lymphoma. This finding underscores the complexity of epigenetic regulation of transcription factor networks in cancer cells.

Acknowledgement

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Conflict of interest: KGM, EMG, and HCH are employees of Zenith Epigenetics Corp. The rest of the authors declare no conflicts of interest