BET BROMODOMAIN INHIBITORS AFFECT REPLICATION & CELL CYCLE PROGRESSION

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ABSTRACT

Myc family transcription factors contribute to pathogenesis in most cancers and their expression projects a poor prognosis (1). Genetic and pharmacological inhibition of Myc and several Myc targets leads to apoptosis and tumor regression. Recently, inhibitors of BET Bromodomain proteins (BETi) were shown to have anti-tumor properties and this has been attributed to Myc down-regulation (2). In this study, we show that two structurally distinct BETi affect replication and cell cycle progression at low concentrations where the transcriptome remains largely unaltered. At these concentrations, S-phase progression is hindered, as assessed by thymidine incorporation and flow cytometry analyses. However, in a cell-free system replication is not impaired suggesting that BETi-mediated block of replication is linked to effects on chromatin. Furthermore, at higher concentration of BETi, S-phase entry of cells is completely abrogated. Ectopic expression of Myc fails to rescue these phenotypes, suggesting a novel function of BET bromodomain proteins in replication and cell cycle regulation.

KEY FINDINGS

- BET inhibitors regulate cell cycle progression and S-phase entry in concentration dependent manner.
- Cell cycle regulation by BETi at low concentrations when transcriptome and cell cycle regulation genes are unaltered points towards BET's novel role.
- Thus observed effects are independent of Myc.
- mTOR/PI3K inhibitors were identified to synergize with BETi via pharmacogenetic screen in the presence of sub-lethal doses of BETi

RESULTS

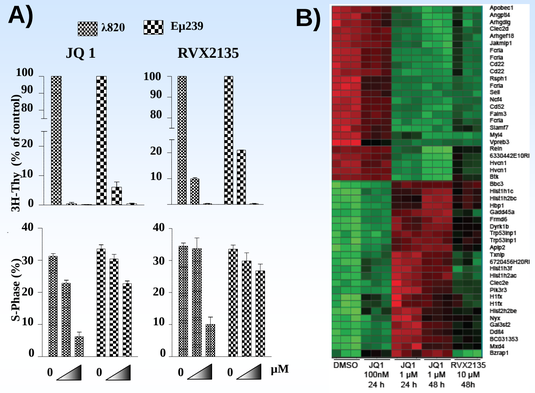


Fig. 1

BETi inhibit progression into and through S-phase though trancriptome remains largely unaltered:

A) Enhanced suppression of 3H-Thy incorporation, while subtle reduction of S-phase was observed following 24h of BETi treatment (0.1μM or 1μM of JQ1 or 1μM or 10μM of RVX2135). Taken together, the data suggests that at lower concentrations of BETi, slower progression of cells through S-phase and at higher concentrations of BETi, the S-phase entry of cells was greatly hindered.

B) Microarray analysis reveals that the transcriptome is largely unaltered when treated with lower concentration of JQ1.

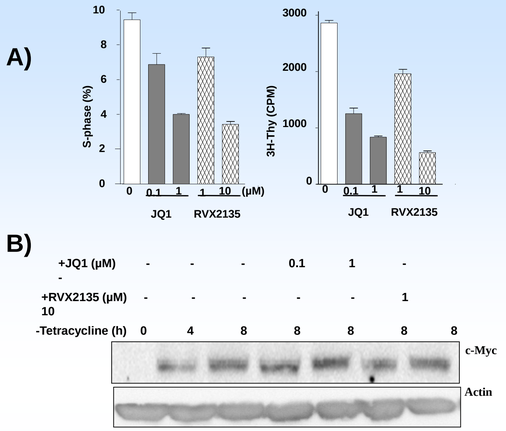
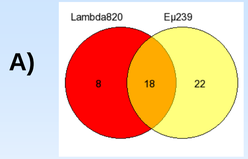


Fig. 2

BETi inhibits S-phase entry in a concentration dependent manner in P493-6 cells: Following tetracycline removal and Myc induction, P493-6 cells were treated with BETi.

A) Flow cytometric analysis and 3H-Ty incorporation assay of these cells following 24hours of BETi treatment shows a concentration dependent decrease of S-phase entry of cells and DNA synthesis respectively.

B) Immunoblot analysis depicting induction of Myc following Tetracycline removal and BETi treatment. Neither of the BETi affects the Myc levels and effects thus observed on cell cycle are independent of Myc. Actin was used as the loading control.



B)

Drug	Target
CYC116	Aurora
ENMD-2076	Aurora
MLN8237	Aurora
VX-680	Aurora
RAF265	Braf/VEGFR
AT7519	CDK
PHA-793887	CDK
TG101209	JAK2
AZD8055	mTOR
KU-0063794	mTOR
BEZ235	mTOR/P13K
GDC-0980	mTOR/P13K
GSK1059615	mTOR/P13K
BKM-120	PI3K
GSK2126458	PI3K
PKI587	PI3K
KX2-391	Src
NPI-2358	VDA

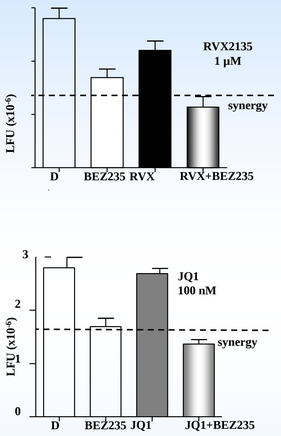


Fig. 3

BETi synergizes with mTOR/PI3K inhibitors:

A,B)List of inhibitors that synergized with sub-lethal doses of BETi in two cell lines identified by Pharmecogenetic screening.

C) NVP-BEZ235 was one of the inhibitors that synergized with BETi. Validation of this finding using cell titer glow analysis on λ820 cell line. (Dashed line represents expected additive value while using the two inhibitors).

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