Bromodomain and Extra-Terminal Domain Epigenetic Reader Proteins as a Therapeutic Target for Metastatic Melanoma
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Background
In the US, skin cancer is the most common cancer, and melanoma is the deadliest form of skin cancer.

Over 192,000 Americans are expected to be diagnosed with melanoma in 2019. About 96,000 will be diagnosed with metastatic melanoma, making it a key area of interest for research and drug development.

The introduction of BRAF inhibitors and cancer immunotherapy has greatly improved treatment of melanoma, however, the problem of tumor relapse and therapy resistance persists.

The Bromodomain (BRD) and Extra-Terminal Domain (BET) family of epigenetic readers is represented by three members in humans: BRD2, BRD3, BRD4, and have been shown to exert key roles in chromatin remodeling and transcriptional regulation.

Specific BET inhibitors have been developed and are already in dose escalation clinical trials to investigate safety, tolerability and pharmacokinetics.

Methods

**MTT assay**
Melanoma cells (A375, A375mA2, A2058, WM164) were plated 5x10⁴ per well and treated with various concentrations of JQ1 (1, 5 and 10 µM) in triplicate and incubated for 72 hours at 37°C. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, followed by 37°C incubation for 4 hours. After addition of stop solution (isopropanol 0.04N HCL), absorbance readings were taken at 570 nm using spectrophotometer.

**Migration and Invasion assay**
Uncoated (migration) and matrigel-coated (invasion) trans-well inserts were seeded with 1x10⁶ melanoma cells (A375, A2058, A375mA2) with and without JQ1 treatment to the top chamber in 100 µL of serum free RPMI 1640 media. The lower chamber contained 600 µL of medium with 15% fetal bovine. After 24 hours (migration) or 48 hours (invasion), noninvasive cells on the upper surface were removed by cotton swabs and inserts were fixed in 4% paraformaldehyde. Crystal violet staining was then used to quantitate the number of cells that had invaded or migrated.

**Western blotting**
For electrophoresis, 3x10⁶ melanoma cells were lysed using RIPA lysis buffer for each sample (A375, A2058) with and without 10 µM JQ1 treatment. Protein concentration was determined using BCA assay. Cell lysates were resolved on 10% polyacrylamide gels, then transferred to nitrocellulose membranes, and incubated overnight at 4°C with primary antibodies (anti-MMP2 and anti-Actin). Signals were detected using appropriate HRP-conjugated secondary antibodies and chemiluminescent substrate on a blot imager.

**Quantitative RT-PCR**
RNA was isolated from 1x10⁶ melanoma cells (A375, A2058, WM164) with and without 10 µM JQ1 treatment using column extraction. Total RNA was then converted to cDNA using reverse transcription. qPCR was performed on triplicate wells using SYBR Green master mix. MMP2 expression was normalized to GAPDH expression levels.

Conclusions
- Although cancer immunotherapy and BRAF inhibitors have aided in the treatment of melanoma, tumor relapse and therapy resistance is still an ongoing issue.
- Our results suggest that BET proteins play an important role in the growth of melanoma cells.
- Both, migration and invasion were found to be reduced in JQ1 treated cells relative to the control cells in all examined cell lines, supporting the role of BET (BRD4 or BRD2) proteins in melanoma metastasis.
- Further studies are ongoing to delineate the mechanistic role of BRD proteins in the metastatic melanoma.

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