REVIEW

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JQ1: a novel potential therapeutic target

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The bromo- and extra-terminal domain (BET) signaling pathway plays an important role in cell proliferation, immune responses, and pro-inflammatory events. JQ1 as a first-in-class potent and selective inhibitor of the BRD4 signaling pathway is widely used for tumor biology studies. It was found that JQ1 could potently reduce cancer cell viability *in vitro* and *in vivo*. The underlying mechanisms include an effect on cell cycle arrest in the G1 phase and a decrease in the percentage of cells in the S phase. Furthermore, JQ1 could alter cytokines expressions not only in T cells but also in dendritic cells (DCs). Apoptosis of tumor cells was induced by JQ1 through downregulation of E2f1 protein expression. In addition, JQ1 exhibited a potent suppressive effect on ERα and androgen receptor (AR) signaling pathways in breast and prostate cancers. Accumulating evidence supports the notion of BRD4 suppression as a target of therapeutic intervention in clinical oncology. Our present review article advances the understanding of the role of the JQ1 / BRD4 protein.

1. Introduction

In recent years, inhibition of bromo- and extra-terminal domain (BET) protein function is being highlighted as an important therapeutic target for metabolic disorders, leukemia, cancer, inflammatory diseases, and autoimmune diseases (Alghamdi et al. 2016). The BET protein family comprises four members including BRD2, BRD3, BRD4, and BRDT in mammals. They are implicated in immune responses during cancerous cell growth (Alghami et al. 2016). Therefore, a couple of small molecules (I-BET 762, OTX- 015, TEN-010, and CPI-0610) have been developed to inhibit BET protein function. Consequently, JQ1 (Fig.1) ((*S*)-*tert*-butyl2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetate) was shown to act specifically against the BET protein (Alghamdi et al. 2016). It is a first-in-class potent and selective BET bromodomain inhibitor, competitively binding to the acetyl-lysine recognition hydrophobic pocket of BRD4 with highest affinity (Zuber et al. 2011; Baud et al. 2014; Delmore et al. 2011). BRD4 as a transcriptional regulator recruits transcriptional regulatory complex to the acetylated chromatin to govern the expression of a series of proteins such as c-Myc (Zhang et al. 2015). On the other hand, JQ1 displaces BET bromdomains from chromatin and interfere with BRD4 function leading to cell cycle arrest and promotion of apoptosis. Subsequently, its downstream signaling event to RNA polymerase II (Pol II) is suppressed and expression of translocated c-MYC is downregulated (Alghamdi et al. 2016; Filippakopoulos et al. 2010). As a result, JQ1 causes a significant reduction of tumor cell viability (Althoff et al. 2015). Therefore, JQ1 as a potential therapeutic agent is widely studied in various cancerous cell lines and multiple animal models. Furthermore, it is utilized as a small molecular probe tool to selectively test different biological functions and therapeutic potential of identified proteins. These proteins are involved in transcriptional regulation, epigenetics, and cancer, which would help identify the individual roles of various proteins in human physiology and disease (Baud et al. 2014).
Herein, we attempt to review the role of JQ1 / BRD4 protein and their related biology in order to deepen our understanding of their potential role in clinical oncology.

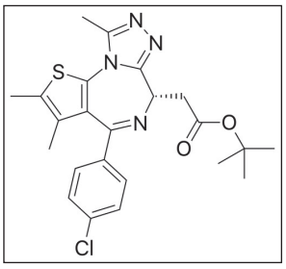

Fig.: Molecular structure of the BET bromodomain inhibitor, JQ1

2. Suppressive effect of JQ1 on cell proliferation of mesenchymal stem cells

It was observed that JQ1 could suppress the growth of mesenchymal stem cells (MSCs). After JQ1 treatment, the percentage of cell cycle arrest in G1 phase was increased, whereas the percentage of cells in the S phase was decreased. To explore the underlying mechanisms, microarray analysis was performed. The research data showed that JQ1 could cause downregulation of key genes controlling cell cycle (such as CCND1, CCNA2, CCNB2, c-MYC, CDK1, CDK6, and E2F2), and upregulation of genes related with apoptosis. These findings were consistent with other studies, in which G1 cell cycle arrest might be induced by JQ1 in many tumor cells such as leukemic cell lines and nerve sheath tumors (Alghamdi et al. 2016).

3. Impact of JQ1 on immune and pro-inflammatory responses

BET proteins are closely associated with the immune system. Accounting for a link between chromatin signaling and interleukin (IL) 17-producing T helper (TH17) cells, suppression of BET protein with JQ1 was studied in autoimmune disorders. It was displayed that the BET family of chromatin adaptors governed differentiation from naive human or murine CD4(+) T cells to

TH17 in a bromodomain-dependent manner. Furthermore, they controlled the activation of previously differentiated TH17 cells through regulating various TH17-associated cytokines such as IL-21, IL-17, and granulocyte-macrophage colony-stimulating factor (GMCSF). Mouse autoimmune disease model revealed that use of therapeutic dose of BET inhibitor could efficaciously prevent progress of autoimmune disorder *in vivo* (Mele et al. 2013). JQ1 was capable of altering cytokine expression not only in T cells but also in dendritic cells (DCs). It reduced surface molecular expression on DCs and prevented T-cell expansion through a disruption of the association between acetyl-310 RelA and BRD4. After LPS, CD3, and CD28-stimulated-DCs were treated by JQ1, expression of featured cytokines, IL-6 and TNF-a, was evidently decreased, whereas expression of T-cell receptors (TCRs) and TCR signaling transduction was not altered in parallel (Sun et al. 2015).

BET proteins play important roles in inflammatory responses of macrophages. Both BRD4 and BRD2 are physically involved in the interaction with the promoters of inflammatory cytokine genes in macrophages. Blockade of BET proteins' signaling pathway with JQ1 would quench hyper-inflammatory conditions resulting from high levels of cytokine production. Furthermore, JQ1 treatment blunted subsequent "cytokine storm" in LPS-induced endotoxemic mice. Serum TNF-α and IL-6 are major indicators of septic and LPS-induced shock. The findings revealed that both TNF-a and IL-6 promoters could be potently inhibited by JQ1, as evidenced by an obvious downregulation of IL-6 and TNF-a expression levels. JQ1 was then suggested as a next-generation anti-inflammatory therapeutic agent to treat inflammatory diseases such as human systemic inflammatory response syndrome and sepsis (Belkina et al. 2013).

4. Role of BRD4 signaling pathway in tumor biology

JQ1 as an acetylated lysine analog has been used to treat a range of malignancies such as multiple myeloma, acute myeloid leukemia, tamoxifen-resistant breast cancer (Feneg et al. 2014). The underlying mechanism involved is an induction of apoptosis of tumor cells and inhibition of proliferation of tumor cells after JQ1 treatment. At a molecular level, downregulation of E2f1 protein expression was observed although MYCN or BRD4 protein expression was not suppressed in the tumors. Immunohistochemical analysis exhibited that JQ1 could remarkably decrease Mib-1 (Ki-67) expression, which is an important marker of cell proliferation. Apoptosis of tumor cells was significantly induced, as evidenced by an apparent increase of cleaved caspase 3 level (Althoff et al. 2015). In addition, BET proteins could regulate c-Myc transcription. Inhibition of BET proteins caused downregulation of c-Myc transcription and subsequently led to a decrease of expression of c-Myc-dependent target genes. In an experimental model of Myc-dependent hematologic malignancy (multiple myeloma), JQ1 manifested a potent anti-proliferative effect and promoted cell cycle arrest and cellular senescence. Enhancer-bound BRD4 was depleted by BET inhibition, which immediately suppressed c-Myc transcription in a time- and dose-dependent manner. Afterwards, the c-Myc oncoprotein was depleted (Delmore et al. 2011). In addition, JQ1 could dramatically potentiate the anti-cancer activity of combined chemotherapy with As_4S_4 (arsenic sulfide), cisplatin, irinotecan or celecoxib in both colon and gastric cancer cell lines. Protein expression of BRD4 was potently inhibited by JQ1. c-Myc was suppressed, whereas p53 protein expression was activated (Zhang et al. 2015). The predominant c-Myc isoform was selectively downregulated partially through superenhancer functional perturbation. The transcriptional regulation of key lineage-specific survival genes and oncogenes were intervened. Therefore, BRD4 inhibition was suggested as a new therapeutic strategy for c-Myc-overexpressing high-grade serous ovarian carcinoma (HGSOC) (Baratta et al. 2015).

Owing to the inhibitive effect of JQ1 on the classic ERα signaling pathway, *in vivo* anti-breast cancer activity was observed in a tamoxifen-resistant breast cancer xenograft mouse model. This strong long-lasting effect of JQ1 could enhance the ER (estrogen receptor) degrader fulvestrant. The underlying mechanism was that JQ1 significantly downregulated expression levels of ERα and its target genes GREB1, pS2, and cyclin D1. Nevertheless, expression of other breast cancer genes such as Her2, FoxA1, and SRC-3 was not affected (Feng et al. 2014).

It is known that castration-resistant prostate cancer (CRPC) is a challenging disease. After androgen ablation therapy, progression to CRPC is predominantly compelled by downregulation of androgen receptor (AR) signaling. It was found that human AR-signaling-competent CRPC cell lines were sensitive to inhibition of the BET signaling pathway. JQ1 was capable of disrupting the physical interaction between BRD4 and N-terminal domain of AR. Thereafter, BRD4 localization to AR target loci was potently abrogated and AR-mediated gene transcription was blocked. *In vitro* experimental studies exhibited that the efficacy of BET bromodomain inhibition was significantly higher than that of direct AR antagonism in mouse model of CRPC xenograft. These aforementioned findings suggested that JQ1 as a novel epigenetic approach might be utilized to synergistically block oncogenic drivers in patients with advanced prostate cancer (Asangani et al. 2014).

Apart from the abovementioned tumors, other Hedgehog-driven tumors such as medulloblastoma, basal cell carcinoma, and atypical teratoid rhabdoid tumor can respond to JQ1 treatment (Tang et al. 2014). However, the clinical use of JQ1 for various cancers remains uncertain so far. One important issue is the dose of JQ1 and its consequence. JQ1 concentration ranging from 100 nM to 1 μM could exert anti-cancer effects on cancer cell lines *via* suppression of BET proteins, subsequently influencing transcription of target cancer-related genes (Alghamdi et al. 2016). However, administration of high doses of JQ1 might result in experimental mouse death based upon our own observations (unpublished data).

5. Anti-leukemic effects of JQ1

JQ1 could affect cell morphology and suppress proliferation of cord-derived mesenchymal stem cells. This observation is line with growth inhibition by JQ1 in other cancer cell lines such as multiple myeloma, neuroblastoma, and leukemia (Alghamdi et al. 2016). It was reported that the mixed lineage leukemia 3 (MLL3)-suppressed leukemias were not sensitive to conventional chemotherapy but to JQ1 (Chen et al. 2014). Suppression of BRD4 by JQ1 could cause robust anti-leukemic effects *in vitro* and *in vivo* through triggering cell-cycle arrest and apoptosis (Zuber et al. 2011; Chen et al. 2014). Furthermore, terminal myeloid differentiation appeared and leukemia stem cells were eliminated. This similar phenomenon was observed in various human AML (acute myeloid leukemia) cell lines and diverse AML-subtype patients. The underlying mechanism was at least involved in the role of MYC gene. JQ1 suppressed MYC gene expression, which was capable of promoting aberrant self-renewal (Zubet et al. 2011). Indeed, a rapid decrease of Myc mRNA and protein expression was observed in human AML lines (Chen et al. 2014).

6. Conclusive remarks

The BRD4 signaling pathway is widely involved in various cancerous scenarios including breast cancer, prostate cancer, leukemia, multiple myeloma, and neuroblastoma. Its underlying mechanisms are based upon its, effects against proliferation, cytokine expression, and blockade on ERα and androgen receptor (AR) signaling pathways. Accumulating evidence supports the notion of BRD4 suppression as a target of therapeutic intervention in clinical oncology.

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References

Alghamdi S, Khan I, Beeravolu N, McKee C, Thibodeau B, Wilson G, Chaudhry GR (2016) BET protein inhibitor JQ1 inhibits growth and modulates WNT signaling in mesenchymal stem cells. Stem cell res Ther 7: 22.

Althoff K, Beckers A, Bell E, Nortmeyer M, Thor T, Sprussel A, Lindner S, De Preter K, Florin A, Heukamp LC, Klein-Hitpass L, Astrahantseff K, Kumps C, Speleman F, Eggert A, Westermann F, Schramm A, Schulte JH (2015) A cre-conditional MYCN-driven neuroblastoma mouse model as an improved tool for preclinical studies. Oncogene 34: 3357-3368.

Asangani IA, Dommeti VL, Wang XJ, Malik R, Cieslik M, Yang RD, Escara-Wilke J, Wilder-Romans K, Dhanireddy S, Engelke C, Iyer MK, Jing XJ, Wu YM, Cao XH, Qin ZHS, Wang SM, Feng FY, Chinnaiyan AM (2014) Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 510(7504): 278-282.

Baratta MG, Schinzel AC, Zwang Y, Bandopadhayay P, Bowman-Colin C, Kutt J, Curtis J, Piao HY, Wong LC, Kung AL, Beroukhim R, Bradner JE, Drapkin R, Hahn WC, Liu JF, Livingston DM (2015) An in-tumor genetic screen reveals that the BET bromodomain protein, BRD4, is a potential therapeutic target in ovarian carcinoma. P Natl Acad Sci USA 112: 232-237.

Baud MGJ, Lin-Shiao E, Cardote T, Tallant C, Pschibul A, Chan KH, Zengerle M, Garcia JR, Kwan TTL, Ferguson FM, Ciulli A (2014) A bump-and-hole approach to engineer controlled selectivity of BET bromodomain chemical probes. Science 346(6209): 638-641.

Belkina AC, Nikolajczyk BS, Denis GV (2013 BET protein function is required for inflammation: Brd2 genetic disruption and BET inhibitor JQ1 impair mouse macrophage inflammatory responses. J Immunol 190: 3670-3678.

Chen C, Liu Y, Rappaport AR, Kitzing T, Schultz N, Zhao Z, Shroff AS, Dickins RA, Vakoc CR, Bradner JE, Stock W, LeBeau MM, Shannon KM, Kogan S, Zuber J, Lowe SW (2014) MLL3 Is a Haploinsufficient 7q Tumor Suppressor in Acute Myeloid Leukemia. Cancer Cell 25: 652-665.

Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi JW, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS (2011) BET Bromodomain Inhibition as a Therapeutic Strategy to Target c-Myc. Cell 146: 903-916.

Feng Q, Zhang Z, Shea MJ, Creighton CJ, Coarfa C, Hilsenbeck SG, Lanz R, He B, Wang L, Fu XY, Nardone A, Song YC, Bradner J, Mitsiades N, Mitsiades CS, Osborne CK, Schiff R, O'Malley BW (2014) An epigenomic approach to therapy for tamoxifen-resistant breast cancer. Cell Res 24: 809-819.

Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang YC, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE (2010) Selective inhibition of BET bromodomains. Nature 468(7327): 1067-1073.

Mele DA, Salmeron A, Ghosh S, Huang HR, Bryant BM, Lora JM (2013) BET bromodomain inhibition suppresses TH17-mediated pathology. J Exp Med 210: 2181-2190.

Sun Y, Wang Y, Toubai T, Oravecz-Wilson K, Liu C, Mathewson N, Wu J, Rossi C, Cummings E, Wu D, Wang S, Reddy P (2015) BET bromodomain inhibition suppresses graft-versus-host disease after allogeneic bone marrow transplantation in mice. Blood 125: 2724-2728.

Tang YJ, Gholamin S, Schubert S, Willardson MI, Lee A, Bandopadhayay P, Bergthold L, Masoud S, Nguyen B, Vue N, Balansay B, Yu FR, Oh S, Woo P, Chen S, Ponnuswami A, Monje M, Atwood SX, Whitson RJ, Mitra S, Cheshier SH, Qi J, Beroukhim R, Tang JY, Wechsler-Reya R, Oro AE, Link BA, Bradner JE, Cho YJ (2014) Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition. Nat Med 20: 732-740.

Zhang L, Tong YY, Zhang XL, Pan MG, Chen SY (2015) Arsenic sulfide combined with JQ1, chemotherapy agents, or celecoxib inhibit gastric and colon cancer cell growth. Drug Des Dev Ther 9: 5851-5861.

Zuber J, Shi JW, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR (2011) RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 478(7370): 524-528.