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Co-targeting of BET proteins and HDACs as a novel approach to trigger apoptosis in rhabdomyosarcoma cells

Julius C. Enßle, Cathinka Boedicker, Marek Wanior, Meike Vogler, Stefan Knapp, Simone Fulda

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Abstract

Histone acetylation marks exert essential functions in regulating gene expression. These marks are written by histone acetyltransferases (HATs), removed by histone deacetylases (HDACs) and read by e.g. BET proteins. While BET inhibitors are promising new anticancer drugs, little is yet known on their antitumor activity in rhabdomyosarcoma (RMS). We therefore investigated the efficacy of the prototypic BET inhibitor JQ1 alone or in combination with other epigenetic modifiers, namely HDAC inhibitors (HDACIs). Here, we discover a synergistic interaction of the panBET inhibitor JQ1 together with various HDACIs, i.e. Quisinostat (JNJ-26481585), Vorinostat (SAHA), Entinostat (MS-275) and Panobinostat (LBH589), inducing apoptosis in RMS cells, whereas JQ1 as single agent exhibits little cytotoxicity. Calculation of combination index (CI) confirmed the synergism of this combination. Importantly, JQ1 and JNJ-26481585 act in concert to suppress colony formation and to trigger apoptosis in an *in vivo* model. Mechanistic studies revealed that combination of JQ1 and JNJ-26481585 cooperatively upregulates BIM and BMF, while downregulating BCL-x_L. This shifted ratio of pro- and antiapoptotic BCL-2 proteins engages activation of BAX and BAK and increases caspases-3 and -7 activity. Individual silencing of BIM or NOXA, overexpression of BCL-2 or MCL-1 as well as addition of the caspase inhibitor zVAD.fmk significantly rescue JQ1/JNJ-26481585-induced apoptosis. Thus, co-targeting of histone acetylation by concomitant inhibition of HDAC and BET proteins synergistically induces mitochondrial apoptosis by shifting the ratio of pro- and antiapoptotic BCL-2 proteins towards apoptosis. These findings indicate that combinatorial use of BET and HDACIs may represent a promising new strategy for the treatment of RMS.