Removal of BFL-1 sensitises some melanoma cells to killing by BH3 mimic drugs

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Metastatic melanoma is an aggressive form of skin cancer with <20% 5-year survival rate when detected at advanced stages [1]. BRAF mutations occur in 60% while NRAS mutations occur in 20% of melanoma patients both causing constitutive activation of the MAPK pathway, thereby driving uncontrolled cell proliferation and increasing resistance to cell death [2]. In patients with melanoma, the activated BRAF-mutated kinase can be inhibited by BRAF-targeting drugs, and its downstream protein mitogen-activated protein kinase (MEK) can be inhibited by a MEK-targeting drug [3]. However, there is no targeted drug for mutant NRAS, hence in the case of NRAS mutant melanomas the current approaches are largely concentrated on downstream signalling pathways such as using MEK-targeted inhibitors [4]. Yet, nearly all melanoma patients eventually relapse. Hence, improved therapies for patients with melanomas are urgently required. One such approach could be to use cell death-inducing BH3 mimetic drugs that inhibit the pro-survival proteins of the BCL-2 family (BCL-2, BCL-XL, BCL-W, MCL-1 and BFL-1) [5, 6]. So far, there are no publications describing BH3 mimetic drugs targeting the pro-survival protein BFL-1, whose gene is frequently amplified and whose mRNA is highly expressed in melanoma [7–9]. We revealed by western blot analysis that BFL-1 protein can be readily detected in >50% of human melanoma-derived cell lines. BCL-XL and MCL-1 could also be detected (Supplementary Fig. 1). It has previously been shown that treating some of these cell lines with the BCL-2 inhibitor ABT-199 alone or in combination with the mutant BRAF inhibitor does not kill the cells [10]. In addition, we were able to detect BFL-1 protein in several human melanoma-derived xenograft samples (Supplementary Fig. 2).

We treated several melanoma-derived cell lines for 72 h with the MCL-1 inhibitor S63845 [6], the BCL-XL inhibitor A1331852 [11] or the BRAF inhibitor PLX4032 [12]. While the MCL-1 inhibitor efficiently killed UACC257, SKMEL2 and HMCB cell lines, the BCL-XL inhibitor or the mutant BRAF inhibitor had almost no effect on the survival of all cell lines tested (Supplementary Fig. 3a, b). Reduction of BFL-1 by RNA interference was reported to lead to spontaneous killing and to enhanced sensitivity to 5-fluorouracil in melanoma cells [13]. To test the role of BFL-1 in sensitivity of melanoma cells to BH3-mimetic drugs, we deleted BCL2A1 in three BRAF mutant melanoma cells (M14 and UACC257 show high BFL-1 expression; LOXIMVI show medium BFL-1 expression) and one NRAS mutant cell (SKMEL30 show high BFL-1 expression) by using our inducible CRISPR/Cas9 platform [14]. Western blot analysis of the CRISPR/Cas9 engineered melanoma cells confirmed loss of the BFL-1 protein (Supplementary Fig. 4a). BFL-1 deletion did not increase the spontaneous death of these melanoma cells (Supplementary Fig. 4b), nor their sensitivity to single-agent treatment with any of the BH3-mimetic drugs tested or PLX4032, when used as single agents (Fig. 1a–d). Of note, removal of BFL-1 increased the death of M14 and SKMEL30 melanoma cells treated with a combination of the MCL-1 and the BCL-XL inhibitors (Fig. 1a, b). The removal of BFL-1 had no additional impact on the response of the UACC257 and LOXIMVI melanoma cells to any of the drug combinations tested (Fig. 1c, d).

Since the loss of BFL-1 increased the killing of M14 melanoma cells when combined with BH3 mimetic drugs that target MCL-1 and BCL-XL, we next tested the response of these cells to combination treatments that also include an inhibitor of MEK1/2, trametinib, or an inhibitor of ERK, ulixertinib, respectively (Fig. 1e). These inhibitors target the constitutively activated MAPK pathway in BRAF mutant melanomas. Combined inhibition of MEK1/2, MCL-1 and BCL-XL resulted in the stronger killing of BFL-1 knockout cells compared to the parental cells. No increase in cell killing was observed with ulixertinib, comparing the parental to the BFL-1 knockout M14 melanoma cells.

Our findings identified BFL-1 as a factor that mediates resistance to combined MCL-1 and BCL-XL inhibition in certain melanoma cells. However, out of the four cell lines tested this was mostly evident in M14 cells, suggesting that their survival is safeguarded by three pro-survival BCL-2 proteins, BFL-1, MCL-1 and BCL-XL. Thus, to achieve efficient killing of these malignant cells, all three of these pro-survival proteins need to be inhibited. This could be achieved either by combinations of BH3 mimetic drugs or via additional anti-cancer agents (e.g. inhibitors of MEK1/2) that cause up-regulation of pro-apoptotic BH3-only proteins that can neutralise the pro-survival BCL-2 protein(s) that is/are not targeted by the BH3 mimetic drugs [15].

DATA AVAILABILITY

All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from the corresponding author.
Fig. 1  Testing the responses of diverse parental and BFL-1 knockout human melanoma cell lines to different drug regimens. The survival of parental and BFL-1 knockout melanoma cells was determined by FACS analysis after staining with Annexin V-AF647 and DAPI after 72 h of treatment with the indicated drugs at the indicated concentrations. a–d 1 μM MCL-1 inhibitor S63845, 1 μM BCL-XL inhibitor A1331852 and 1 μM BRAF inhibitor PLX4032 were used alone or in combination. e 1 μM MCL-1 inhibitor S63845, 1 μM BCL-XL inhibitor A1331852, 0.5 μM ERK1/2 inhibitor Ulixertinib and 5 nM MEK1/2 inhibitor Trametinib were used alone or in combination. Data represent mean ± SEM of three independent experiments. P values were calculated by performing two-way ANOVA followed by multiple comparisons testing. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. DMSO was used as the vehicle control.
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AUTHOR CONTRIBUTIONS

LG performed and designed most experiments and wrote the manuscript; RLS, LT, PS and JGC helped to perform experiments. MS and GL helped with discussions and advice on experiments and to write the manuscript; AS and MJH planned the project, were involved in experimental design and helped write the manuscript. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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