Synthetic Lethality of CBM Signalosome Inhibition for KRAS^{G12X} Colorectal Cancer

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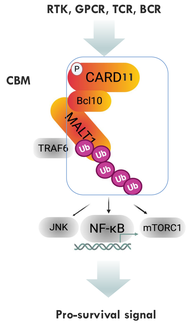
Introduction

The CBM signalosome (CARD11-BCL10-MALT1) is a signaling hub directly regulating multiple oncogenic pathways, including NFκB, JNK, mTORC1 and MYC. This positions the CBM complex as a critical regulator of tumor development and survival. For the first time, we uncovered the essential pro-survival role of the CBM signalosome in KRAS^{G12}-driven colorectal cancers (CRCs). This discovery may provide a therapeutic breakthrough for difficult-to-treat KRAS-driven CRCs.

CBM Signalosome, the Pro-Survival Hub for Cancer Progression



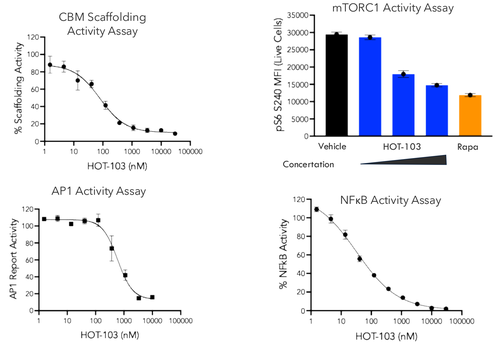
CBM signalosome: CARD11-BCL10-MALT1 complex.

CBM transduces upstream growth & survival signals via a series of phosphorylation and ubiquitination reactions, and activates multiple pro-survival pathways, including canonical NFκB, mTORC1 and JNK.

In cancer, upstream GoFs (BTK, PKC, etc), CARD11 GoF mutations, or CARD11 overexpression activate CBM signalosome, promoting cancer progression.

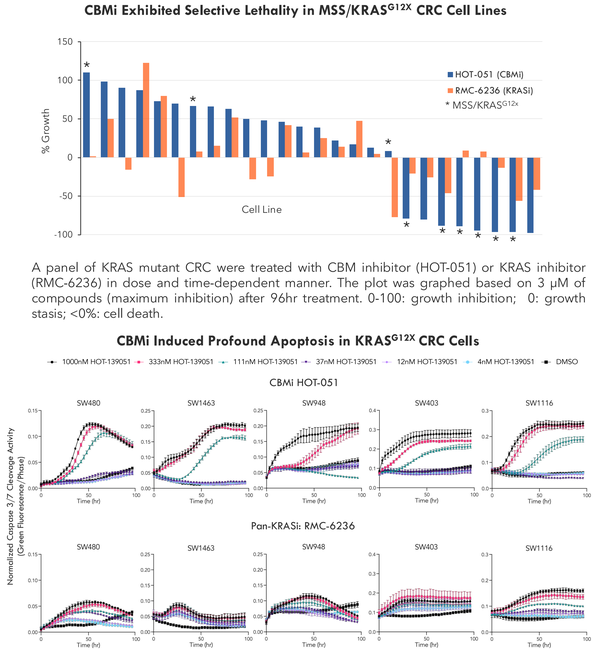
Results

Figure 1. Allosteric CBM Inhibitor Blocked CBM Downstream Signaling Transduction



Scaffolding assay: OCI-LY3 cells were treated with HOT-103 for 24hr, the modulation of p-IκBα was monitored in MSD format. NFκB assay: Jurkat cells were pre-treated with HOT-103 for 1 hr, followed by stimulation with anti-CD3/anti-CD28/PMA for 4 hr, and NFκB reporter activity was measured using a luciferase assay. AP1 assay: HEK293-AP1 cells were pre-treated with HOT-103 for 1 hr, followed by stimulation with 10 nM PMA for 6 hr, and AP1reporter activity was measured using a luciferase assay. mTORC1 assay: OCI-LY3 were treated with HOT-103 (0.3, 1, 3 μM), and rapamycin (10 nM) for 24hrs. The phosphorylated ribosomal protein S6 (Ser240) was analyzed by flowcytometry.

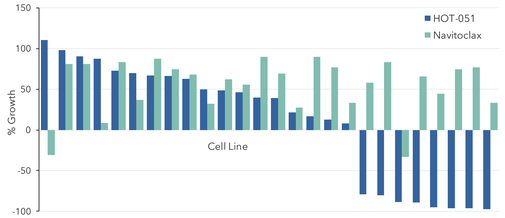
Figure 2. CBM Inhibitor Induced Apoptosis in MSS/KRAS^{G12X} CRC, Superior to KRASi



A panel of KRAS mutant CRC were treated with CBM inhibitor (HOT-051) or KRAS inhibitor (RMC-6236) in dose and time-dependent manner. The plot was graphed based on 3 μM of compounds (maximum inhibition) after 96hr treatment. 0-100: growth inhibition; 0: growth stasis; <0%: cell death.

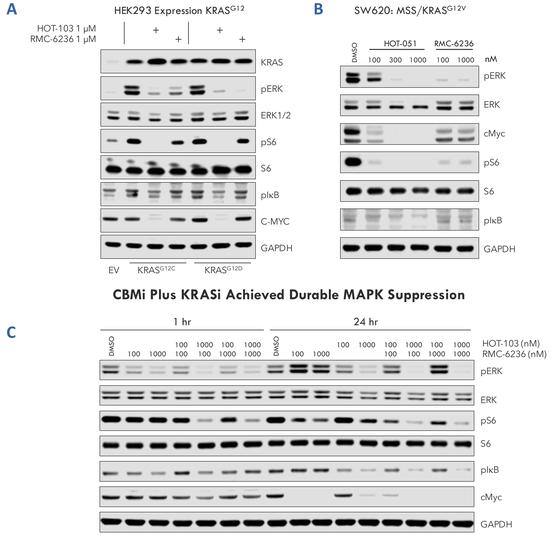
Dose and time dependent apoptosis was measured by caspase3/7 cleavage using Incucyte.

Figure 3. CBM Inhibitor Outperformed BCL2i or BCL2/Bcl-xL Inhibitor in KRAS-Mutant CRC Lines



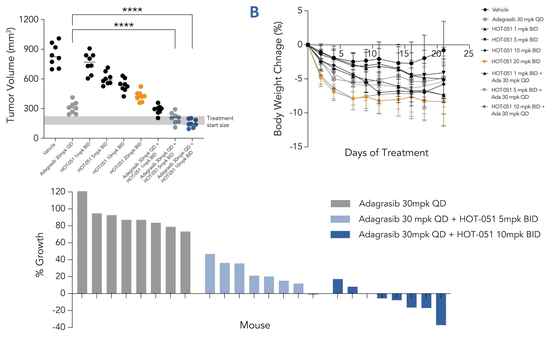
A panel of CRC cell lines were treated with CBM inhibitor (HOT-051) or BCL2/Bcl-xL inhibitor (Navitoclax) in dose and time-dependent manner. The plot was graphed based on 3 μM of compounds (maximum inhibition) after 96hr treatment. 0-100: growth inhibition; 0: growth stasis; <0%: cell death. Depmap CRISPR knockout screen discovered KRAS-active CRC showed modest sensitivity to Bcl-xL knockdown. In head-to-head comparison, CBMi was superior to Navitoclax. Venetoclax had no effect on CRC lines (data not shown).

Figure 4. Combination of CBMi and KRASi Achieved Complete Suppression of Downstream Survival Signaling



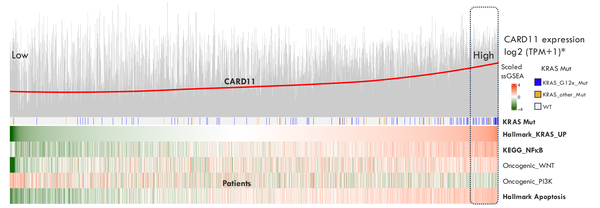
(A) HEK293 cells were transiently transduced with KRAS^{G12C} or KRAS^{G12D}. After 24hr of treatment with 1 μM HOT-051 or RMC-6236, cells were lysed and protein levels were analyzed by Western blot. (B) SW620 (KRAS^{G12V}) cells were treated with HOT-051 or RMC-6236 for 24hr, followed by Western blot analysis of protein expression. (C) SW837 cells (KRAS^{G12C}) were treated with the HOT-103 (CBMi), RMC-6236, or the combination for 1hr or 24hr. Cells were then lysed, and protein expression was assessed by Western blot. **Similar results were also observed in multiple KRAS^{G12X} cell lines**

Figure 5. CBMi Demonstrated Dose-dependent Tumor inhibition Or Regression Alone or Combined with Adagrasib in the SW837 Model



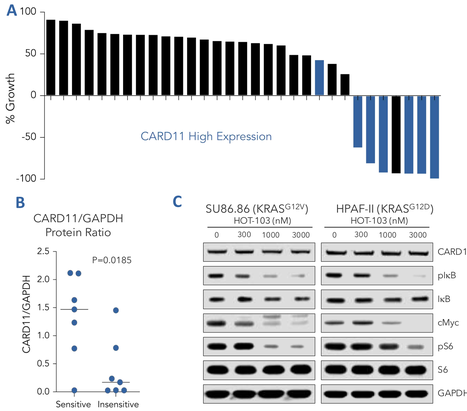
Mice were treated orally with HOT-051, Adagrasib, or the combination at varying doses for 18 days. (A) Tumor volume at the end of study, (B) changes of mouse body weight, and (C) individual tumor growth percentages are shown.

Figure 6. Broader Impact of CBM Inhibition or KRAS Active Cancers – Beyond CRC



Higher CARD11 is significantly associated with high KRAS/NFκB/Apoptosis activities in pan-cancer studies, specifically in high KRASm prevalence cancer indications.

Figure 7. CBMi Selectively Induced Apoptosis/Cell Death in KRAS^{G12X}/CARD11 High PDAC



(A) A panel of PDAC cell lines were treated with CBM inhibitor (HOT-103) in dose and time-dependent manner. The plot was graphed based on 3uM of compounds (maximum inhibition) after 96hr treatment. 0-100: growth inhibition; 0: growth stasis; <0%: cell death. (B) Quantitation of CARD11 protein level in PDAC cell lines. (C) PDAC cells were treated with HOT-103 for 24hr, followed by Western blot analysis of protein expression

Conclusions

- KRAS^{G12X} tumor hijacked CBM signalosome for survival.
- To our knowledge, CBMi is the first molecule that selectively induced apoptosis in KRAS^{G12X} tumor and blocked tumor growth in *in vitro* and *in vivo* preclinical models.
- CBMi +/- KRASi may exert deep and durable response by targeting two cancer hallmarks, proliferation and survival.

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