Leveraging CRISPR-Cas9 screening platform for discovery of novel tumor intrinsic phagocytosis modulators

SITC 2022

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BACKGROUND

Targeting “don’t eat me” signaling has achieved profound responses in clinal trials for hematologic malignancies, highlighting the potential therapeutic value of macrophage-mediated phagocytosis for cancer treatment. In recent years, CRISPR-Cas9 based *in vitro* co-culture screens have significantly expanded our knowledge of the key tumor-intrinsic mechanisms mediating cytotoxic lymphocyte killing. However, the interactions between tumor cells and macrophages remain elusive. To illuminate the tumor-intrinsic regulator of macrophage phagocytosis, we developed both positive and negative screening strategies, and deployed multiple primary bone marrow-derived macrophages (BMDM) or macrophage cell line-based co-culture screens.

METHODS

DLD1 and MDA-MB-231 cancer lines were infected with a whole-genome CRISPR-Cas9 library and co-cultured with Raw264.7 or J774A.1 in the presence or absence of the therapeutic antibodies magrolimab or cetuximab. MC38 syngeneic cancer cells were infected with a library of 3000 immune-related genes and co-cultured with primary BMDM. For negative selection, the macrophages were selectively killed off post co-culture and the tumor cells were collected for next generation sequencing (NGS) and statistical analysis. For positive selection, macrophages and tumor cells were fully dissociated from the plate post co-culture, and the mixture of cells was subjected to a CD11b positive selection column. Macrophages and tumor cells were enriched separately for downstream analysis.

RESULTS

For the negative screen, bioinformatic analysis revealed that CD47 knockout in tumor cells strongly sensitized macrophage-mediated clearance of target cells both from naïve and cetuximab-treated conditions. Furthermore, CD47 or EGFR knockout conveyed growth advantage in co-culture upon treatment with magrolimab or cetuximab, respectively. For the positive screen, we developed a method to sequence bar-coded DNA from tumor cells engulfed by macrophages. Consistently, CD47 sgRNAs were significantly enriched inside macrophages in the naïve condition and were depleted upon treatment with magrolimab. Together, these findings demonstrated the robustness of our phagocytosis-based CRISPR screening platform. In additional to validating the role of CD47 in this system, we discovered and validated a group of novel tumor intrinsic regulators of macrophage-mediated phagocytosis.

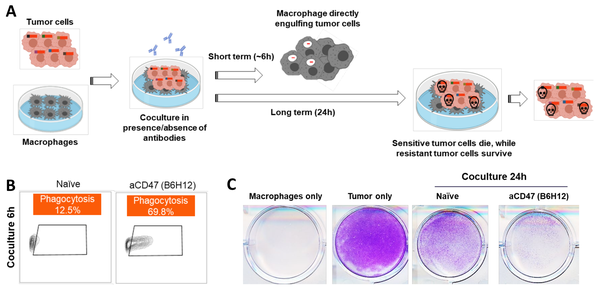

Figure 1: Development of *in vitro* tumor/macrophage co-culture platform. (A) Schematic diagram showing *in vitro* tumor/macrophage coculture and its two different stages; (B) Flow cytometry detecting tumor cell-engulfing macrophages after 6h of co-culture; (C) Crystal violet staining of remaining tumor cells after 24h of co-culture.

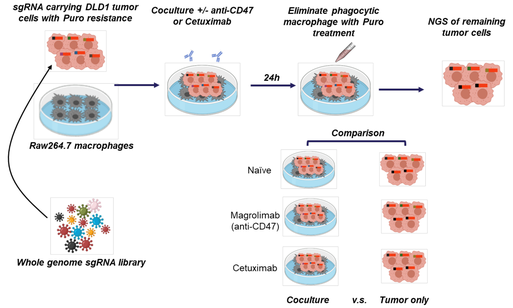

Figure 2: Negative selection screen strategy for *in vitro* DLD1/Raw264.7 co-culture. Comparison analysis of NGS data was made between remaining tumor cells from coculture and tumor only across 3 treatment conditions.

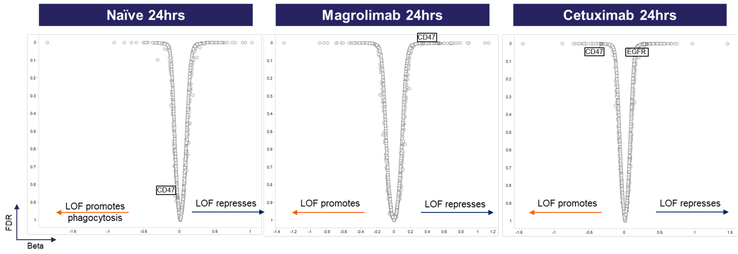

Figure 3: Genome-wide CRISPR negative screen identifies tumor intrinsic phagocytosis regulators in DLD1/Raw264.7 co-culture. Hits on the left side indicate depletion of these genes promotes phagocytosis; Hits on the right side indicate depletion of these genes repress phagocytosis.

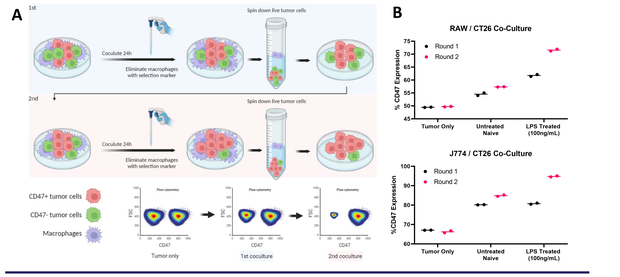

Figure 4: Development of *in vitro* tumor/macrophage two-round competition co-culture platform. (A) Schematic diagram of 2-round co-culture; (B) CD47+ tumor cells are further enriched after 2-round co-culture.

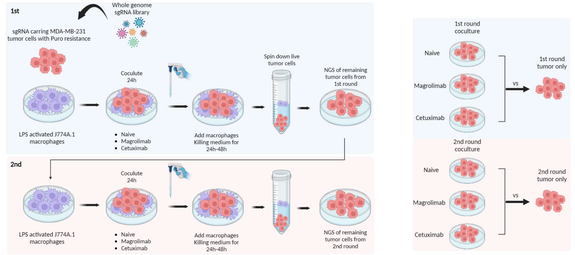

Figure 5: Negative selection screen strategy for *in vitro* MDA-MB-231 two-round co-culture with LPS-activated J774A.1

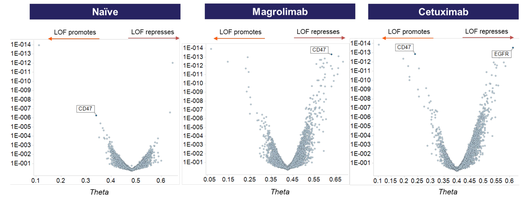

Figure 6: Genome-wide CRISPR negative screen identifies tumor intrinsic phagocytosis regulators in MDA-MB-231 two-round co-culture with LPS-activated J774A.1

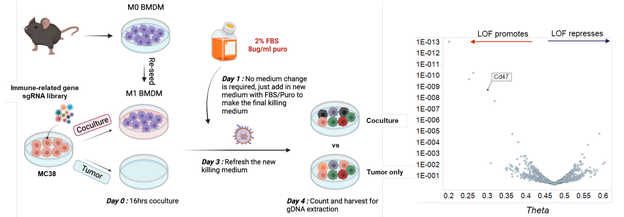

Figure7: Negative selection screen strategy for *in vitro* MC38/Primary BMDM co-culture and tumor intrinsic phagocytosis regulators identified

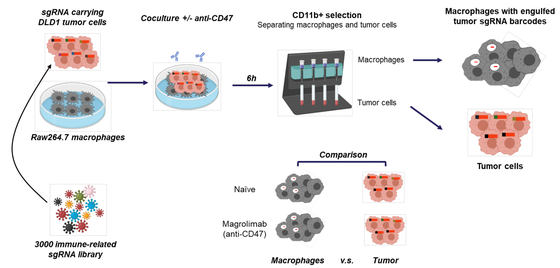

Figure 8: Positive selection screen strategy for *in vitro* DLD1/Raw264.7 co-culture. Comparison analysis of NGS data was made between macrophages and tumor cells from co-culture.

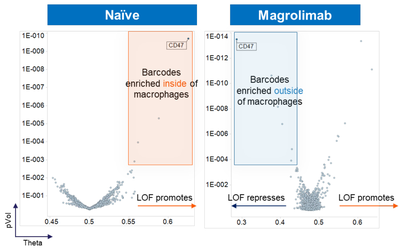

Figure 9: CRISPR positive screen identifies tumor intrinsic phagocytosis regulators in DLD1/Raw264.7 co-culture

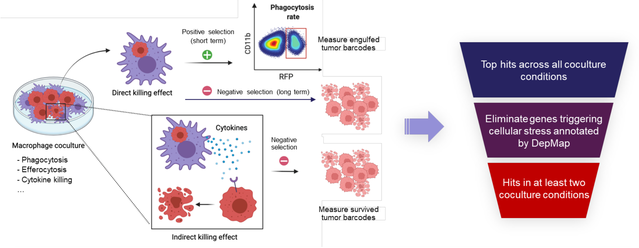

Figure 10: Strategy for phagocytosis regulator hits interrogation. Short term positive selection identifies targets directly involved in enhancing macrophage engulfment of tumor cells; Long term negative selection identifies targets involved in tumor cells resistant to phagocytosis and/or macrophage-derived cytokines-mediated killing.

CONCLUSION

We have successfully established and implemented multiple macrophage and tumor cell line co-culture screening platforms, and systematically revealed tumor intrinsic regulators of phagocytosis, uncovering multiple known and novel therapeutic targets regulating vulnerability of tumor cells to macrophage-mediated phagocytosis. Our proprietary high-throughput phagocytosis CRISPR-based screening platform provides an unbiased and rapid solution to study macrophage and tumor cell interactions and discover novel targets for cancer therapy.