IQGAP1 is a novel non-degraded substrate of CELMoDs and involved in immune cell motility to the tumor microenvironment

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Introduction

- Cereblon E3 ligase modulating drugs (**CELMoDs**) represent a novel class of immunomodulatory agents that target IKZF1 and IKZF3 for degradation, building upon earlier generations such as Thalidomide and Avadomide (**Ava**).
- CELMoDs have demonstrated potent direct cytotoxicity and efficacy in hematologic malignancies, including multiple myeloma and lymphoma. RNAseq and multiplex immunofluorescence analyses of paired clinical biopsies (screening and on-treatment) from diffuse large B-cell lymphoma (DLBCL) patients treated with Ava revealed increased infiltration of lymphocytes and myeloid cells into the tumor microenvironment (TME)[1].
- Similar findings were observed in patients treated with Golcadomide (**Golca**), a next-generation CELMoD currently under clinical investigation for DLBCL, follicular lymphoma, and T cell lymphoma, and were recapitulated in a genetically engineered humanized cereblon-DLBCL mouse model, highlighting the translational significance of CELMoD-induced immune modulation.
- *In vivo* studies using a human cereblon (hCRBN) DLBCL GEMM model recapitulated the CELMoD mediated increased infiltration of immune cells to the TME[2].
- However, the molecular mechanism responsible for this enhanced leukocyte trafficking remains incompletely understood.
- IQGAP1, a multidomain scaffolding protein, is essential for cellular migration, accumulating at the leading edge of migrating cells and interacting with activated Rac and CDC42 to facilitate actin cross-linking to generate protrusive forces[3-7].
- Here, we present molecular and cellular evidence demonstrating that IQGAP1 is a novel, non-degradable substrate of various CELMoDs that is responsible for these clinical observations.

Methods

- Primary monocytes were purified from buffy coats obtained from normal donors (New York Blood Center) using enrichment kits (Miltenyi).
- CRISPR knockout sgRNA for IQGAP1 and cereblon were designed and built by Cellecta with pRSG17-U6-sg-UbiC-TagGFP2-2A-Puro vector.
- These sgRNAs were transfected into 293FT cells for Lentiviral production using Lenti-X™ Packaging Single Shots (Takara). Viral harvest using Lenti-X concentrator (Takara) was verified using Lenti-X GoStix™ Plus (Takara).
- Monocytic cell line OCI-AML2-Cas9 was transduced using the above Lenti-viral products to generate CRISPR KO cell lines. Five cell lines were generated for either CRISPR IQGAP1 or CRISPR CRBN and generated through puromycin selection.
- Trans-endothelial migration assay (TEM) was conducted using Corning™ HTS Transwell™ Permeable Support System (MilliporeSigma) coated with primary pooled HUVEC cells (Lonza).
- Duolink® Proximity Ligation Assays (PLA) were conducted according to manufacturer's instructions (MilliporeSigma), using either primary human monocytes, or spleen FFPE from hCRBN mouse DLBCL models (BBRC, India).
- Ubiquitinomics on Oci-AML2-Cas9 cells treated with or without Golcadomide were conducted through an automated CST PTMSCAN enrichment of K-GG Peptides followed by LC-MS/MS and advanced AI/ML search algorithm for Peptide ID and quantification.

Results

Figure 1. IQGAP1 is identified as a substrate of CRBN upon Avadomide treatment

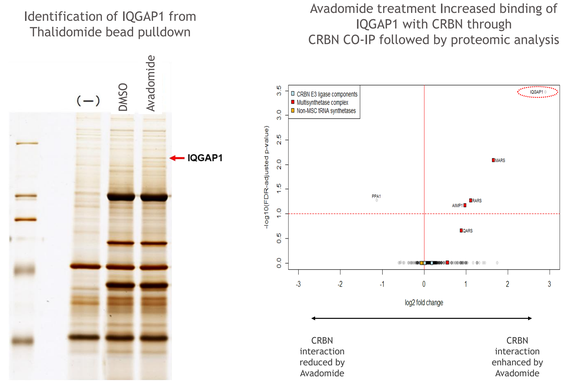


Figure 2. CELMoD treatments enhance IQGAP1 ubiquitination, but not degradation

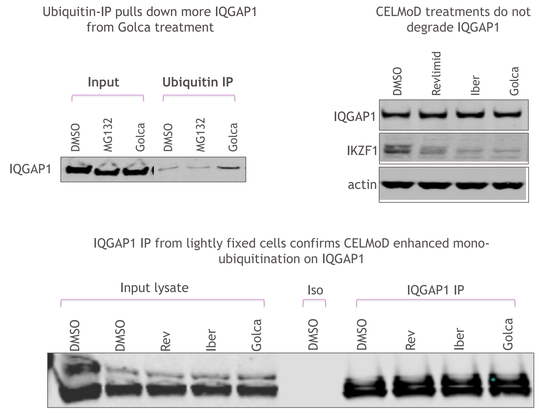


Figure 3. Streptavidin bead pull-down IQGAP1 using transduced CRBN-TurboID Oci-AML2 cells

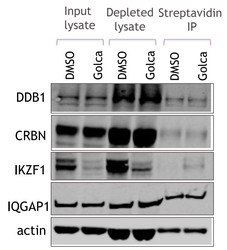


Figure 4 Golca mediates enhanced binding of actin, CDC42 and RAC with IQGAP1

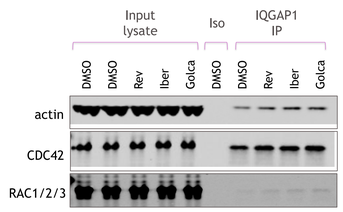


Figure 5. Ubiquitinomics analysis reveals that Golca treatment significantly increases ubiquitination of 3 specific lysine residues on IQGAP1. These residues are all residing at the region where interactions with CDC42 and actin occur (red box)

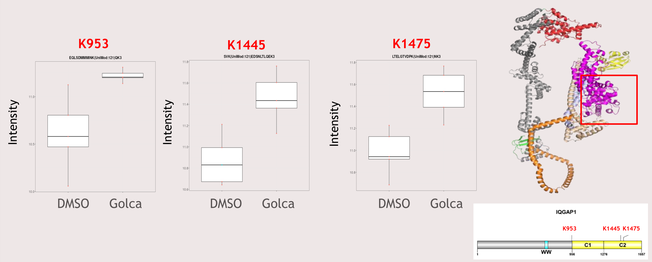
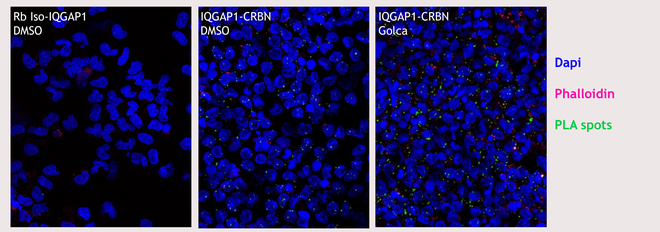
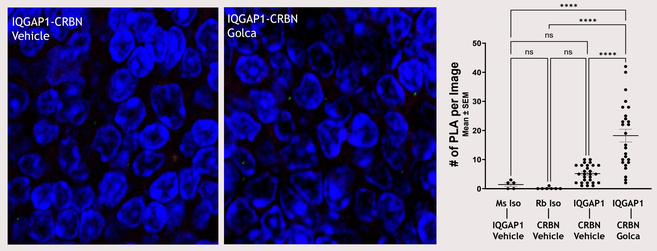


Figure 6. Proximity ligation assays (PLA) confirm that Golca enhances CRBN-IQGAP1 interaction in primary human monocytes (top) and hCRBN mouse spleens (center), as well as IQGAP1-CDC42 interaction (bottom)



Golca treatment increases CRBN-IQGAP1 interaction in spleens of hCRBN mouse DLBCL models



Golca treatment increases IQGAP1-CDC42 interaction in spleens of hCRBN mouse DLBCL models

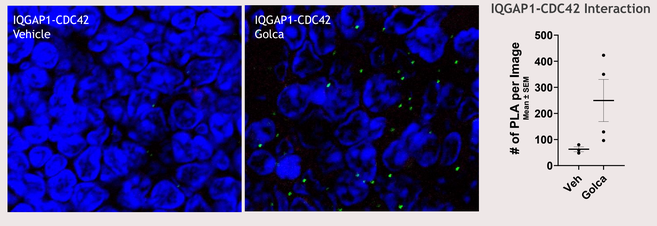


Figure 7. Clinical observations on CELMoD-mediated enhancement in migratory capacity is confirmed using *ex vivo* primary monocytes (n=3)

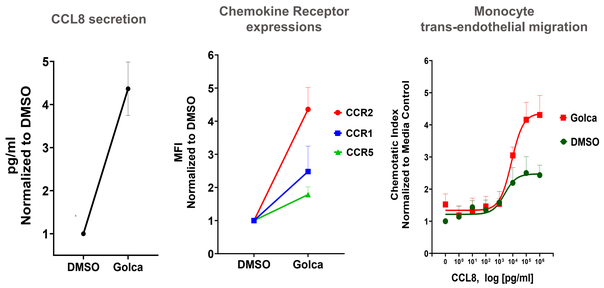
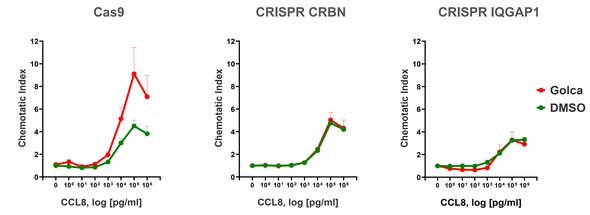
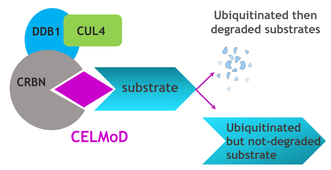


Figure 8. Golca mediated enhancement in migration is dependent on both CRBN and IQGAP1



Conclusion:



- This study provides the first description of a novel, non-degraded CELMoD substrate, IQGAP1, directly linking its biological function to the clinical observation of increased leukocyte infiltration into TME.
- It uncovers a novel mechanism by which CELMoDs binding to CRBN mediates enhanced ubiquitination of three specific lysine residues on IQGAP1.
- This modification increases interaction of IQGAP1 with Rac, CDC42 and actin, promoting directional immune cell migration.
- These insights not only deepen the understanding of CELMoD action but also highlight the potential of CELMoD-based combination therapies to amplify the efficacy of immunotherapies.

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