MAGI1-PP2A Facilitates SRC Survival Dependency in IDH Mutant Cholangiocarcinoma

Angela Yu, Caroline Bridgwater, Iris Sze Ue Luk, Aaron Lampano, Supriya Saha, and Sita Kugel

Human Biology Department, Fred Hutchinson Cancer Research Center

Contact:
Angela Yu
Kugel Lab
ayu2@fredhutch.org

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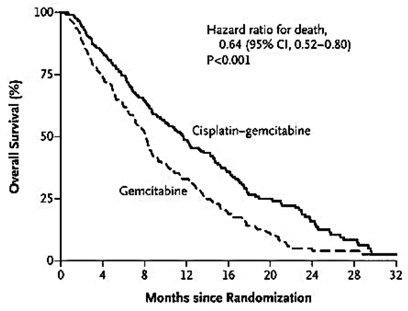
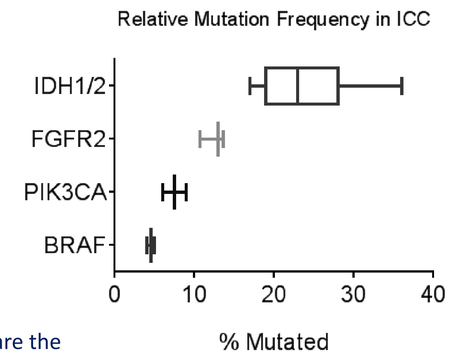
Intrahepatic Cholangiocarcinoma

1. Treatment for Intrahepatic Cholangiocarcinoma (ICC) is limited and ineffective



Cancers of liver and intrahepatic bile ducts (ICC) are the most rapidly rising cancers in the US.

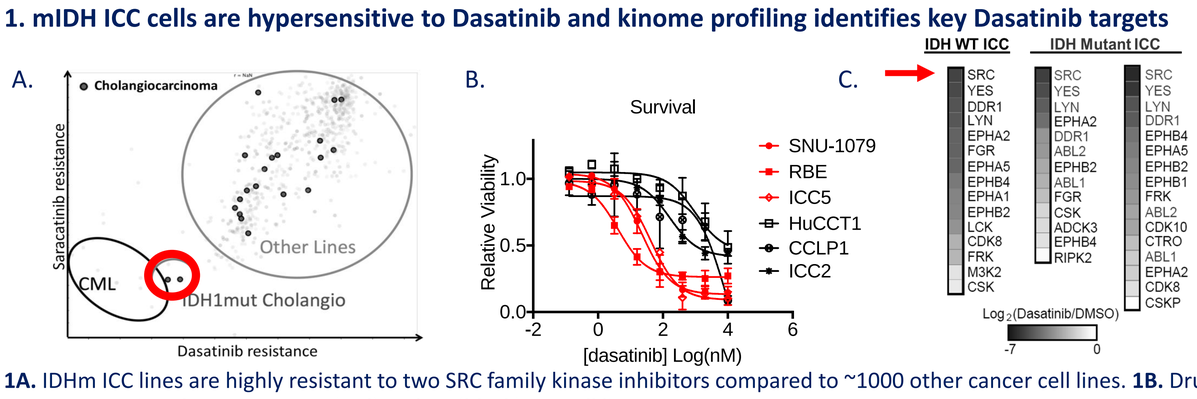
2. Isocitrate dehydrogenase (IDH) mutations: most common mutations in ICC



Mutations in isocitrate binding site of IDH1 (R132) or IDH2 (R172, R140) result in production of R(–)-2-hydroxyglutarate (2-HG), a proposed 'oncometabolite'. 2-HG blocks hepatocyte differentiation, resulting in the accumulation of undifferentiated cells, eventually leading to ICC.

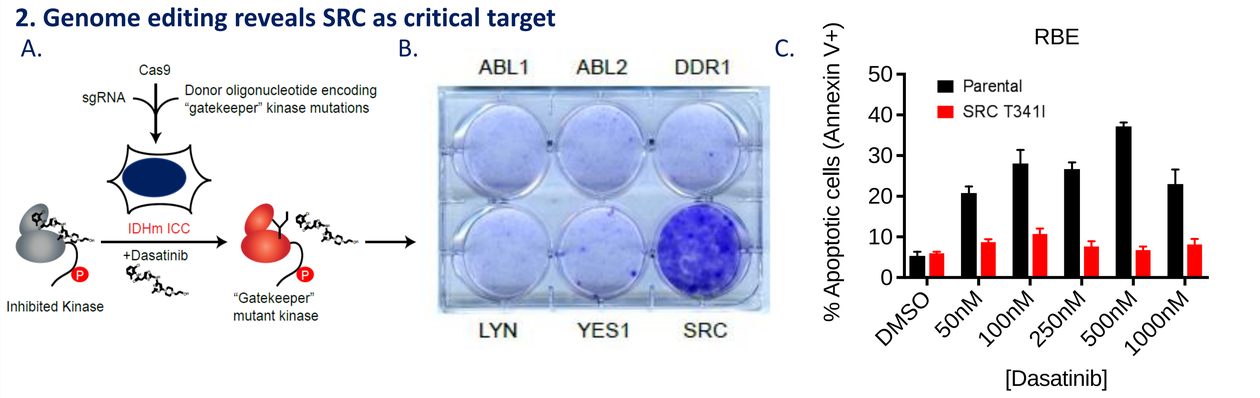
Background

1. mIDH ICC cells are hypersensitive to Dasatinib and kinome profiling identifies key Dasatinib targets



1A. IDHm ICC lines are highly resistant to two SRC family kinase inhibitors compared to ~1000 other cancer cell lines. **1B.** Drug curve comparison between IDHm (red) and wt (black) ICC cell lines.
1C. Kinases enriched in the active kinome of dasatinib-treated cells compared to vehicle treated cells.

2. Genome editing reveals SRC as critical target

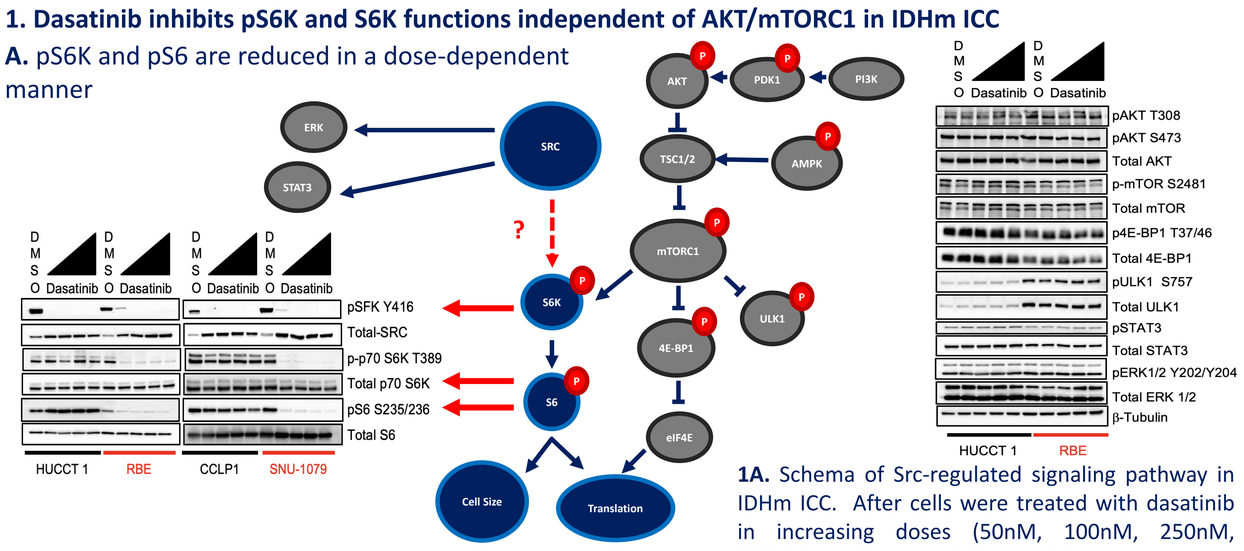


2A. Generation of stable lines with gatekeeper mutations which prevent the kinase from being inhibited by dasatinib. **2B.** SNU1079 cells were treated with dasatinib 50nM for 30days. Only SRC T341I gatekeeper mutation rescues the dasatinib-induced cytotoxicity. **2C.** SRC T341I gatekeeper mutation can rescue apoptosis in IDHm ICC lines.

Dissecting SRC-Regulated Signaling Pathways in IDHm ICC

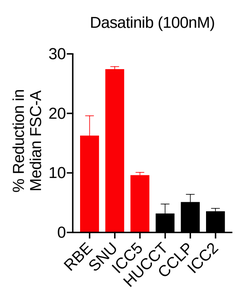
1. Dasatinib inhibits pS6K and S6K functions independent of AKT/mTORC1 in IDHm ICC

A. pS6K and pS6 are reduced in a dose-dependent manner

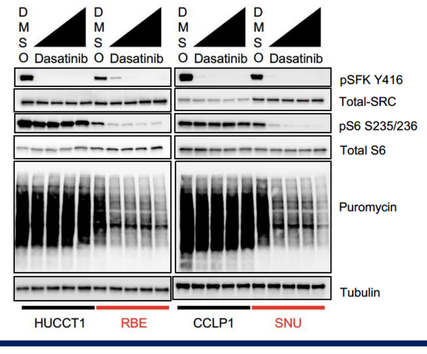


1A. Schema of Src-regulated signaling pathway in IDHm ICC. After cells were treated with dasatinib in increasing doses (50nM, 100nM, 250nM, 500nM) for 6 hours, pS6K and pS6 are reduced in a dose-dependent manner in IDHm cells, but not wild-type cell lines. The effect is specific to S6K and S6 and is independent of mTOR/AKT. Results were consistent across all three IDH mutant human cell lines. **1B.** Cell size measured by flow cytometry is reduced in IDHm cells after dasatinib treatment for 24 hours at 100nM. **1C**. Global translation is reduced dose-dependently in IDHm cells after 6 hours of dasatinib treatment as measured by a puromycin-uptake assay.

B. Cell size is reduced

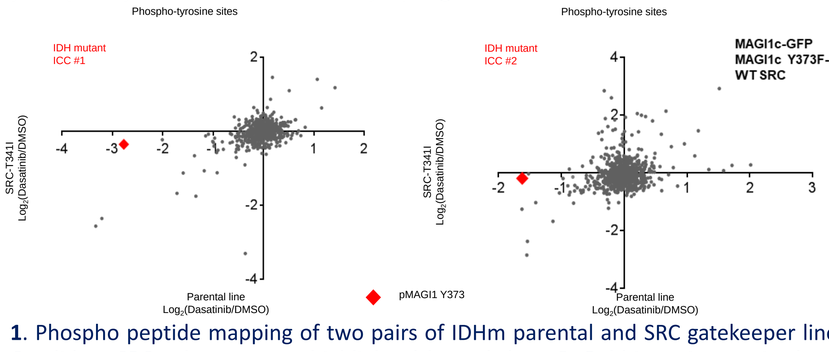


C. Global translation decreases

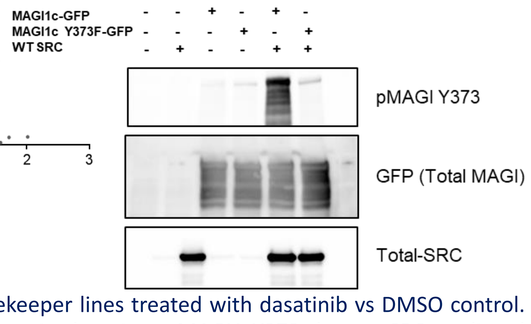


Identification of MAGI1 as Novel SRC Substrate

1. Phospho-proteomics reveals MAGI1 phosphorylation is differentially upregulated



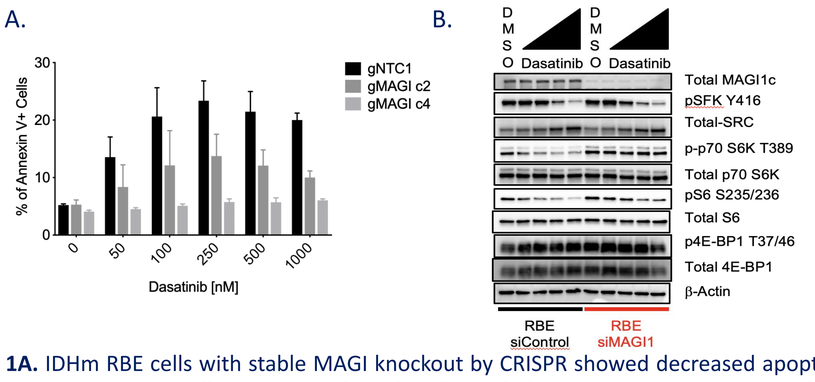
2. Src Phosphorylates MAGI at the Y373 Site



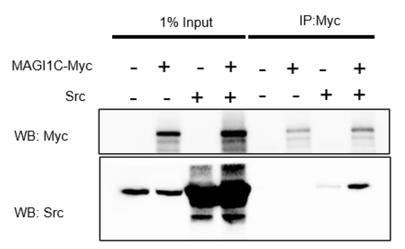
1. Phospho peptide mapping of two pairs of IDHm parental and SRC gatekeeper lines treated with dasatinib vs DMSO control. Candidate SRC substrates are highlighted by red dots. **2.** Rabbit antisera raised against pMAGI1 Y373 detects SRC-mediated phosphorylation of wild-type MAGI1 but not a MAGI1 Y373F mutant (phospho-inactive).

Understanding MAGI1 Functions

1. MAGI knockdown with both siRNA and CRISPR partially rescues Dasatinib induced apoptosis and signaling



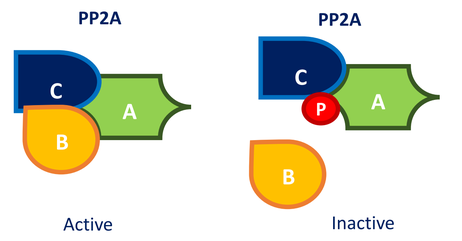
2. SRC and MAGI interact



1A. IDHm RBE cells with stable MAGI knockout by CRISPR showed decreased apoptosis compared to the non-targeting control (NTC1). **1B.** RBE cells were transfected with pooled siRNA targeting MAGI and a non-targeting control and treated with increasing doses of Dasatinib for 6 hours. Signaling blots show partial rescue of pS6 and pS6K marks with MAGI knockdown. **2.** Co-immunoprecipitation in 293T cells confirms the interaction between SRC and MAGI

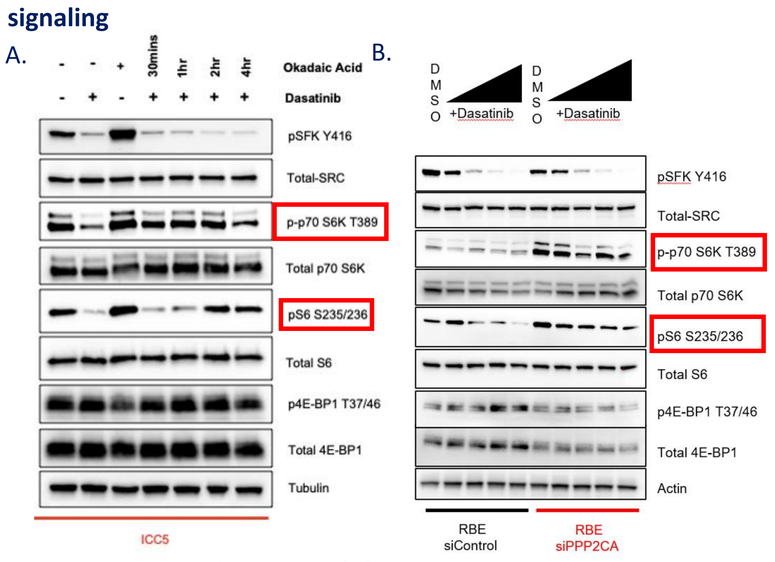
Protein Phosphatase 2A (PP2A)

- Composed of 3 subunits: A (structural), B (regulatory) and C (catalytic)
- mTOR is the only known kinase capable of phosphorylating S6K T389, but mTOR pathway is unaffected by dasatinib treatment
- PP2A targets many substrates including myc, and can bind and dephosphorylate S6K T389
- PP2A phosphorylation is inhibitory



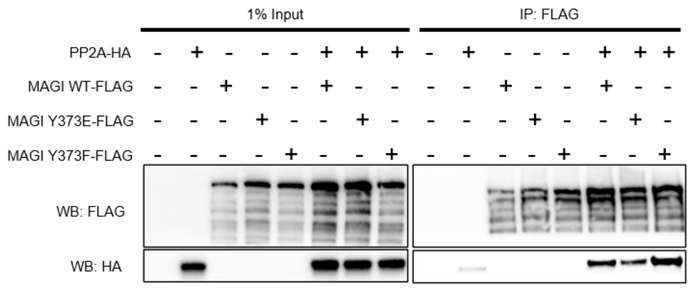
PP2A Facilitates Dasatinib-Induced Dephosphorylation of S6K

1. PP2A knockdown with siRNA and inhibition with okadaic acid partially rescues Dasatinib induced signaling



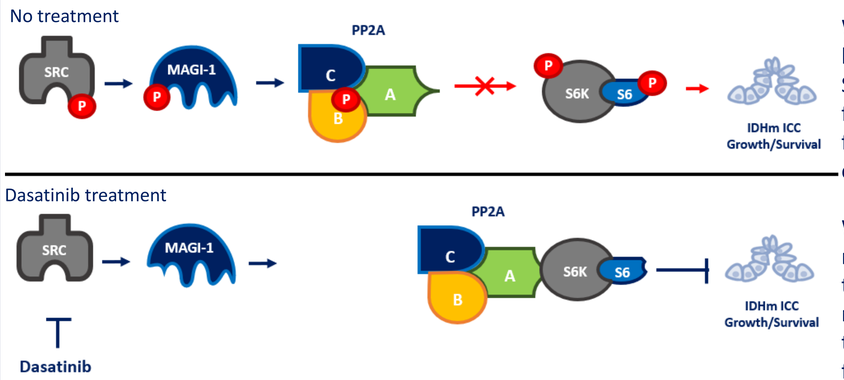
1A. Okadaic acid, a potent and specific inhibitor of PP2A, rescues dasatinib-mediated inhibition of pS6K and pS6 in a time- and dose-dependent manner. Results were consistent across all three IDH mutant human cell lines. **1B.** IDHm cells were transfected with pooled siRNA targeting PPP2CA (the dominant form of PP2A catalytic subunit) and a non-targeting control and treated with increasing doses of dasatinib for 6 hours. Signaling blots show partial rescue of pS6 and pS6K marks with PPP2CA knockdown and this activity was maintained in increasing doses of dasatinib. Results were consistent across all three IDH mutant human cell lines.

2. MAGI and PP2A C subunit interact



2. Co-immunoprecipitation in 293T cells confirms the interaction between PP2A C subunit and MAGI. This interaction is seen in WT MAGI as well as a MAGI Y373E phospho-mimetic mutant and MAGI Y373F mutant which cannot be phosphorylated.

Summary



We have shown that dasatinib selectively kills IDHm ICC cells through inhibiting SRC, S6K and S6 phosphorylation and functions, and presented novel evidence for PP2A involvement in cholangiocarcinoma.

We hypothesize that MAGI1 acts as a negative regulator of S6K/S6 signaling through recruitment of PP2A. MAGI1 has multiple PDZ domains and can bring together various binding partners to facilitate signaling.

When IDHm cells are treated with dasatinib, MAGI1 is not phosphorylated by SRC which leads to S6K dephosphorylation and inactivation due to activation of PP2A.

Future Directions

Characterize the mechanism by which MAGI1 modulates PP2A and suppresses S6K signaling

1. IP-mass spec to determine if SRC/MAGI1 affects PP2A activity by changing the post-translational modification of PP2A catalytic subunit
2. Co-IP of truncated domains of MAGI1 to determine the specific domain in which MAGI1 binds to PP2A complex
3. TurboID proximity labeling assay to identify all binding partners of MAGI1 regulated by SRC
4. Treat patient-derived IDHm organoids with Dasatinib

References and Acknowledgements

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