Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

Priority Report

Cancer Research

Disruption of the Scaffolding Function of mLST8 Selectively Inhibits mTORC2 Assembly and Function and Suppresses mTORC2-Dependent Tumor Growth *In Vivo*

Yoonha Hwang[1,2], Laura C. Kim[3], Wenqiang Song[1,2], Deanna N. Edwards[1,2], Rebecca S. Cook[3,4,5,6], and Jin Chen[1,2,3,5,6]

Abstract

mTOR is a serine/threonine kinase that acts in two distinct complexes, mTORC1 and mTORC2, and is dysregulated in many diseases including cancer. mLST8 is a shared component of both mTORC1 and mTORC2, yet little is known regarding how mLST8 contributes to assembly and activity of the mTOR complexes. Here we assessed mLST8 loss in a panel of normal and cancer cells and observed little to no impact on assembly or activity of mTORC1. However, mLST8 loss blocked mTOR association with mTORC2 cofactors RICTOR and SIN1, thus abrogating mTORC2 activity. Similarly, a single pair of mutations on mLST8 with a corresponding mutation on mTOR interfered with mTORC2 assembly and activity without affecting mTORC1. We also discovered a direct interaction between mLST8 and the NH_2-terminal domain of the mTORC2 cofactor SIN1. In *PTEN*-null prostate cancer xenografts, mLST8 mutations disrupting the mTOR interaction motif inhibited AKT S473 phosphorylation and decreased tumor cell proliferation and tumor growth *in vivo*. Together, these data suggest that the scaffolding function of mLST8 is critical for assembly and activity of mTORC2, but not mTORC1, an observation that could enable therapeutic mTORC2-selective inhibition as a therapeutic strategy.

Significance: These findings show that mLST8 functions as a scaffold to maintain mTORC2 integrity and kinase activity, unveiling a new avenue for development of mTORC2-specific inhibitors.

Introduction

mTOR is a serine/threonine kinase controlling multiple cellular processes including cell growth, survival, metabolism, and cytoskeletal reorganization (1, 2). mTOR functions in two distinct complexes, mTORC1 and mTORC2, both of which share the mTOR kinase and mLST8, but also contain components unique to each complex (Raptor for mTORC1; Rictor and SIN1 for mTORC2). Although tremendous progress has been made in understanding the regulation and function of mTORC1/2, the specific role of mLST8 in each complex is less well defined. Although originally identified as an mTORC1 subunit (3), increasing evidence suggests that mLST8 is dispensable for mTORC1, but indispensable for mTORC2 activity (4, 5). However, the molecular mechanisms underlying the requirement for mLST8 in mTORC2 activation remain unclear.

In this report, we identify interaction points between mLST8 and mTOR, which when disrupted, specifically impair mTORC2 assembly and activity without affecting mTORC1. Furthermore, we found a direct interaction between mLST8 and SIN1 and further identified the region of the SIN1 NH_2-terminal domain that interacts with mLST8. We found that prostate cancer xenografts expressing the mLST8 mutant incapable of mTOR interaction displayed impaired mTORC2 signaling *in vivo* and reduced tumor growth, suggesting that targeted disruption of mLST8–mTOR interactions could be employed as a therapeutic strategy to selectively target mTORC2, while sparing the activity of mTORC1.

Materials and Methods

Cell lines and cell culture

Human embryonic kidney 293T (HEK293T) cells, BT549, DU159, and PC3 cells were obtained from ATCC and maintained in DMEM containing 10% FBS. HEK293FT were obtained from Thermo Fisher Scientific and maintained in DMEM containing 10% FBS. Primary and immortalized human bronchial epithelial cells BEAS 2B cells were maintained in RPMI media containing 10% FBS. Normal human dermal fibroblasts (NHDF) were obtained from Lonza. NHDF cells were cultured according to the

[1]Veterans Affairs Medical Center, Tennessee Valley Healthcare System, Nashville, Tennessee. [2]Division of Rheumatology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee. [3]Program in Cancer Biology, Vanderbilt University, Nashville, Tennessee. [4]Deparment of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee. [5]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee. [6]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Y. Hwang and L.C. Kim contributed equally to this article.

Corresponding Author: Jin Chen, Vanderbilt University, T-3207E, Medical Center North, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37232. Phone: 615-343-3819; Fax: 615-343-8648; E-mail: jin.chen@vanderbilt.edu.

Cancer Res 2019;79:3178–84

doi: 10.1158/0008-5472.CAN-18-3658

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

manufacturer's protocol using the FGM-2 bullet kit. All cells were cultured in a humidified incubator with 5% CO_2 at 37°C. Cell lines were used between passages 1 and 50 after thaw. Cell lines from ATCC were authenticated using short tandem repeat profiling. *Mycoplasma* testing was performed every 6 months, most recently in January 2019, using the PlasmoTest Kit from InvivoGen.

Generation of the mTOR E2285A knock-in cell line by CRISPR–Cas9 technology

The mTOR E2285A knock-in under *MLST8* knockout background cell line was generated using the CRISPR/Cas9 technology. The sgRNA targeting the genomic sequence close to the codon of the 2285 glutamate residue on mTOR was designed using the CRISPR design tool (http://crispr.mit.edu) and cloned into pSpCas9(BB)-2A-GFP vector. Single-stranded oligodeoxynucleotides (ssODN) were used as the template for the E2285A mutation and a synonymous change to the PAM site. For ease of genotyping, a *Sph*I site near the target was mutated synonymously. The Cas9/sgRNA construct and the ssODNs were cotransfected into *MLST8* knockout HEK293T cells. Forty-eight hours posttransfection, transfected cells were seeded into a 96-well plate by limited dilution. The genomic DNA of individual clones was extracted to amplify the DNA fragment containing the E2285 site. PCR products were digested by *Sph*I-HF to screen for correct clones. Knock-in mutations were verified by the Sanger sequencing of cloned genomic PCR. sgRNA oligos, primers for genomic PCR, and ssODN are listed in Supplementary Table S1.

Xenograft assay

A total of 5×10^6 cells suspended in 100 μL of Matrigel and PBS (1:1) were injected into the hind flanks of 6-week-old athymic nude mice (Foxn1^{nu}; Envigo). Tumor measurements were started 10 days after injection and were monitored for 3 weeks. Tumors were measured every 2–3 days with digital calipers and tumor volume calculated according to the formula $[V = 4/3\pi(l/2)(h/2)(w/2)]$. Data are presented as mean ± SEM ($n = 10$). Two-way ANOVA with Bonferroni Correction was used for statistical analysis. Experiments with mice were preapproved by the Vanderbilt Institutional Animal Care and Use Committee and followed all state and federal rules and regulations.

Additional Materials and Methods are available in the Supplementary Information.

Results

mLST8 is required for mTORC2, but not mTORC1, integrity and function

We generated 293T cells carrying loss-of-function indel mutations in the *MLST8* gene by CRISPR/Cas9-mediated genome editing, using multiple sgRNAs targeting human *MLST8* exon 2, 4, or 7 (Supplementary Fig. S1A) and confirmed loss of mLST8 protein expression (Fig. 1A). mLST8 loss impaired coprecipitation of mTOR with the mTORC2 cofactors RICTOR and SIN1, without affecting the mTORC1 cofactor, RAPTOR. Consistent with the idea that mLST8 loss impairs mTORC2, mLST8-deficient cells displayed decreased pAKT^{Ser473}, the mTORC2 phosphorylation site, under steady state growth conditions (Fig. 1A), or in response to amino acid or serum induction (Fig. 1B). Phosphorylation of other mTORC2 downstream effectors such as PKC and NDRG1 were also reduced (Fig. 1A) with no impact on phosphorylation of the mTORC1 substrate S6K1^{Thr389} or mTORC1 effector S6RP. *In vitro* kinase assays performed using mTOR immunoprecipitates (IP) from 293T cells lacking mLST8 showed strongly diminished phosphorylation of 6His-Akt (Fig. 1C). In contrast, RAPTOR IPs from cells lacking mLST8-phosphorylated GST-S6K1 *in vitro* at similar levels to those from control cells expressing mLST8 (Supplementary Fig. S1B). These findings agree with recent data demonstrating that mLST8 is important for mTORC2 activity, but is dispensable for mTORC1 signaling, despite being a component of the mTORC1 holoenzyme. Importantly, restoration of mLST8 expression in several individually selected mLST8-deficient 293T cell clones (293T-sgMLST8) rescued coprecipitation of RICTOR and SIN1 with mTOR, as well as pAKT^{Ser473} (Fig. 1D). We confirmed our findings in a panel of human and murine cell lines (Supplementary Fig. S1C and S1D).

mLST8 mutations that uncouple binding to LST8 binding element domain of mTOR disrupt mTORC2 complex formation and signaling

Previous analyses of the mLST8-mTOR kinase crystal structure identified structural areas of mTOR and mLST8 that might interact through formation of hydrogen bonds (6). In mLST8, the interaction points may occur across six of seven WD40 repeat domains that cooperate to form a beta-propeller structure (6). We introduced mLST8 point mutations at evolutionarily conserved residues predicted to be involved in hydrogen bonds, where important interactions with mTOR would most likely occur (Supplementary Fig. S2A). These mutations included mLST8-Q44E/N46L (mLST8^{Mut1}) and mLST8-W272F/W274F (mLST8^{Mut2}; Fig. 2A; Supplementary Fig. S2A). mLST8^{WT}, mLST8^{Mut1}, and mLST8^{Mut2} were expressed in an mLST8-deficient 293T-sgMLST8 cell clone, as was mLST8$^{Mut1/2}$, harboring Q44E, N46L, W272F, and W274F mutations. Expression of each mLST8 species was confirmed by Western blot analysis (Fig. 2B). Coprecipitation of mTOR with RICTOR and SIN1 was seen in cells expressing mLST8^{WT}, mLST8^{Mut1}, and mLST8^{Mut2}, but not in cells expressing mLST8$^{Mut1/2}$. RAPTOR coprecipitation with mTOR was not affected by any mutation in mLST8 (Fig. 2B), consistent with the idea that mLST8 is not necessary for mTORC1 assembly. Notably, cells expressing mLST8$^{Mut1/2}$ exhibited far less pAKT^{Ser473} as compared with cells expressing mLST8^{WT}, mLST8^{Mut1}, or mLST8^{Mut2} (Fig. 2B). This was confirmed by *in vitro* kinase assays (Supplementary Fig. S2B).

A reciprocal approach was used to confirm these results, by introducing mutations into mTOR at the predicted site of its interaction with mLST8 amino acids 272 and 274 (Fig. 2A), and thus was expected to mimic the effects of mLST8^{Mut2}. Transfection of 293T cells with mTOR expression constructs confirmed that mTOR^{E2285A} produced a functional mTOR protein capable of coprecipitation with mTORC2 components, including wild-type mLST8 (Fig. 2C). The mTOR^{E2285A} mutation was knocked-in to the endogenous mTOR gene in a 293T-sgMLST8 cell clone, which was confirmed by sequencing of genomic DNA (Supplementary Fig. S2C). Expression of mTOR^{E2285A} with wild-type mLST8 was permissive for mTORC2 assembly, as assessed by coprecipitation of RICTOR with mTOR (Fig. 2D). However, a combination of mTOR^{E2285A} knock-in with mLST8^{Mut1} expression completely abolished mTORC2 assembly, and blocked pAKT^{Ser473}. These data indicate that compromised mTORC2 integrity with mLST8$^{Mut1/2}$ were not due to disruption of overall conformation of mLST8 by introducing the mutations but caused by elimination of the interaction with mTOR.

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

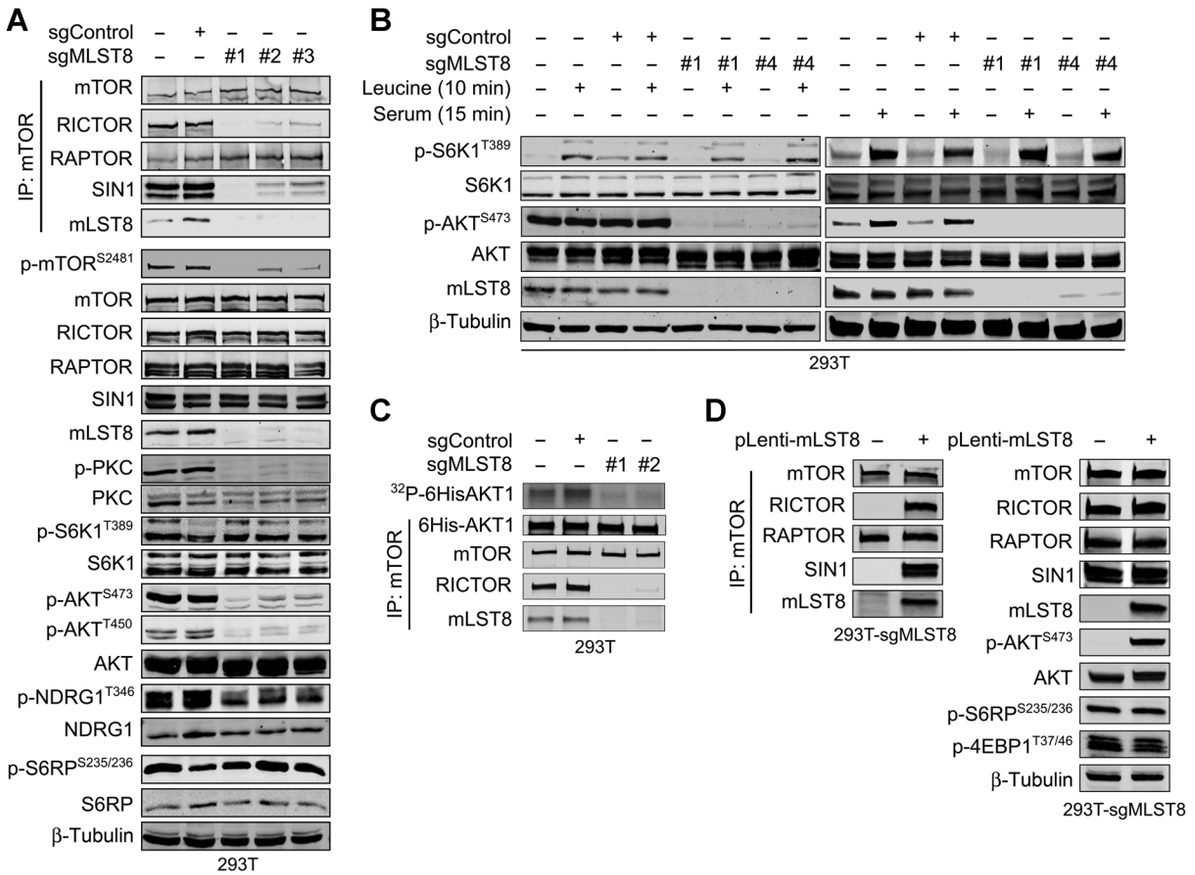

Figure 1.

mLST8 is required for assembly and activity of mTORC2. **A,** mTOR IPs or cell lysates from sgControl or sgMLST8 293T cells were assessed by Western blot analysis. **B,** sgControl or sgMLST8 293T cells were leucine or serum starved overnight, pulsed with L-leucine or 10% serum, and cell lysates were assessed by Western blot analysis. **C,** Immunoprecipitated mTOR complexes were incubated with purified 6His-AKT1 and ^{32}P-γ-labeled ATP. Kinase activity was detected by autoradiography. **D,** Wild-type mLST8 was reexpressed in a 293T-mLST8 knockout cell clone (sgRNA #4). mTOR IPs or cell lysates were assessed by Western blot analysis.

mLST8 serves as a molecular bridge between mTOR and SIN1

Recent Cryo-EM structural analyses of yeast TORC2 revealed that LST8 is sandwiched between Avo1 (the yeast SIN1 homologue) and TOR kinase (7, 8). In contrast, cryo-EM studies of human mTORC1 show that mLST8 is not sandwiched or embedded within mTORC1, but rather protrudes from the structure core, leaving mLST8 highly exposed (6). This does not rule out a potential role of mLST8 in mTORC1 regulation but may explain why mLST8 is critical for assembly and function distinctly of mTORC2. We directly tested this hypothesis by mutational analysis of SIN1 and coimmunoprecipitation of mTORC2 components.

To determine whether mLST8 is a molecular bridge between mTOR and SIN1 in human mTORC2, we first assessed mLST8–SIN1 interactions by co-IP. 293T cell clones deficient for SIN1 (293T-sgSIN1) were generated by CRISPR/Cas9 gene editing of the SIN1-encoding gene, *MAPKAP1* (Supplementary Fig. S3A–S3C). Phosphorylation of AKT^{Ser473} was completely abolished in SIN1-deficient cells (Supplementary Fig. S3B). Next, SIN1 expression was reconstituted in 293T-sgSIN1 cells using expression of a SIN1-GFP fusion construct bearing wild-type SIN1 (SIN1^{WT}), or a SIN1 mutant bearing deletions of the NH_2-terminal 136 amino acids (SIN1$^{\Delta 1\text{-}136}$), conserved CRIM region (SIN1$^{\Delta CRIM}$) or the PH domain (SIN1$^{\Delta PH}$; Supplementary Fig. S3C). mTOR and RICTOR each coprecipitated with GFP in cells expressing SIN1^{WT}, SIN1$^{\Delta CRIM}$, and SIN1$^{\Delta PH}$, but not in cells expressing SIN1$^{\Delta 1\text{-}136}$ (Fig. 3A), suggesting that the SIN1 NH_2-terminal domain is key for interaction with mTORC2 cofactors. Furthermore, cells expressing SIN1$^{\Delta CRIM}$ or SIN1$^{\Delta 1\text{-}136}$ showed decreased pAKT^{Ser473}, consistent with decreased mTORC2 signaling. Notably, the CRIM domain of SIN1 has a known interaction site with AKT and other AGC kinases (9), explaining the reduced pAKT in these cells (Fig. 3A).

Examination of the SIN1 NH_2-terminus revealed that expression of a SIN1 fragment comprised solely of aa 1–136 was sufficient for coprecipitation of mTOR with SIN1 in cells otherwise deficient for SIN1, presumably through a direct interaction between SIN1 and mLST8 (Fig. 3B). Further characterization of the NH_2 terminal domain by expression of sequential deletion mutants (Supplementary Fig. S3C) in 293T cell clones lacking SIN1 expression identified aa 97–127, as the required region for mLST8 binding (Fig. 3C). Importantly, cells expressing

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

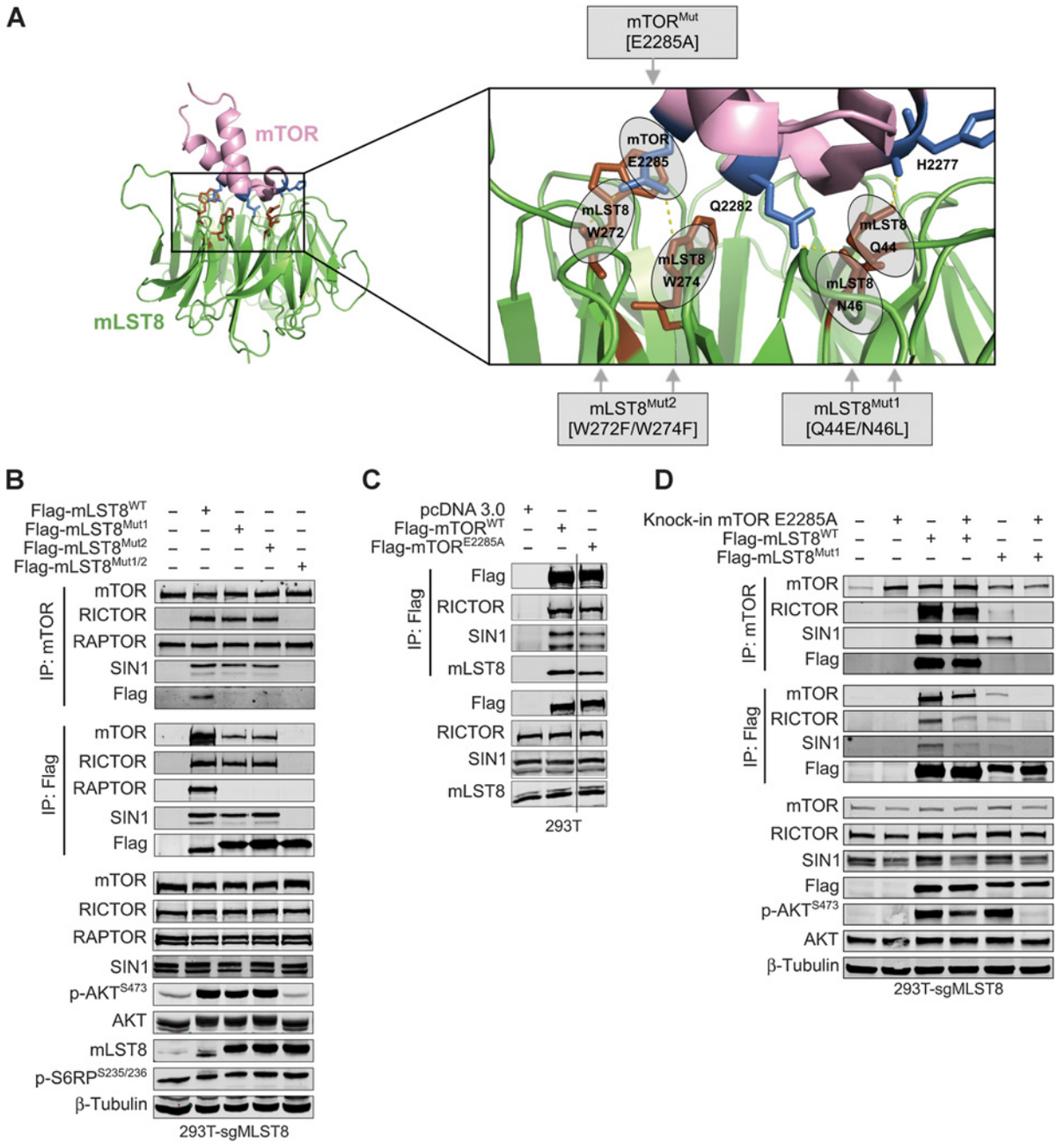

Figure 2.

Mutations disrupting mTOR-mLST8 interface affect mTORC2 assembly and function. **A,** The structure of the mTOR-mLST8 interface generated from the mTOR$^{\Delta N}$-mLST8 crystal structure (PDB ID: 4JSN; ref. 6) using PyMOL software. Conserved residues proposed as hydrogen bonding sites between mTOR (blue) and mLST8 (brown) are shown. **B,** mTOR IPs, Flag IPs, and cell lysates from 293T-sgMLST8 cells (sgRNA #4) expressing wild-type or mutant mLST8 were assessed by Western blot analysis. **C,** Western blot analysis of Flag IPs and cell lysates from 293T cells expressing mTOR^{WT} or mTOR^{E2285A}. **D,** Western blot analysis of mTOR IPs, Flag IPs, and cell lysates from 293T-sgMLST8 cells, with or without the mTOR^{E2285A} knock-in, expressing wild-type or mutant mLST8.

mLST8$^{Mut1/2}$ retain a level of coprecipitation with SIN1 (Fig. 3D), despite the fact that mLST8$^{Mut1/2}$ does not support complex formation between mTOR and SIN1. Interestingly, SIN1 coprecipitation of RICTOR was not affected by disruption of mTOR-mLST8 binding (Fig. 3D), suggesting mLST8 brings SIN1 and RICTOR together with mTOR to build the mTORC2 complex. These data are in agreement with previous studies in yeast describing potential sites of interaction between Avo1 and LST8 within the aa 98–136 region of LST8 (Supplementary Fig. S3D; ref. 7). Collectively, these data support the hypothesis that mLST8 is the key molecular bridge between mTOR and the mTORC2-specific component, SIN1.

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

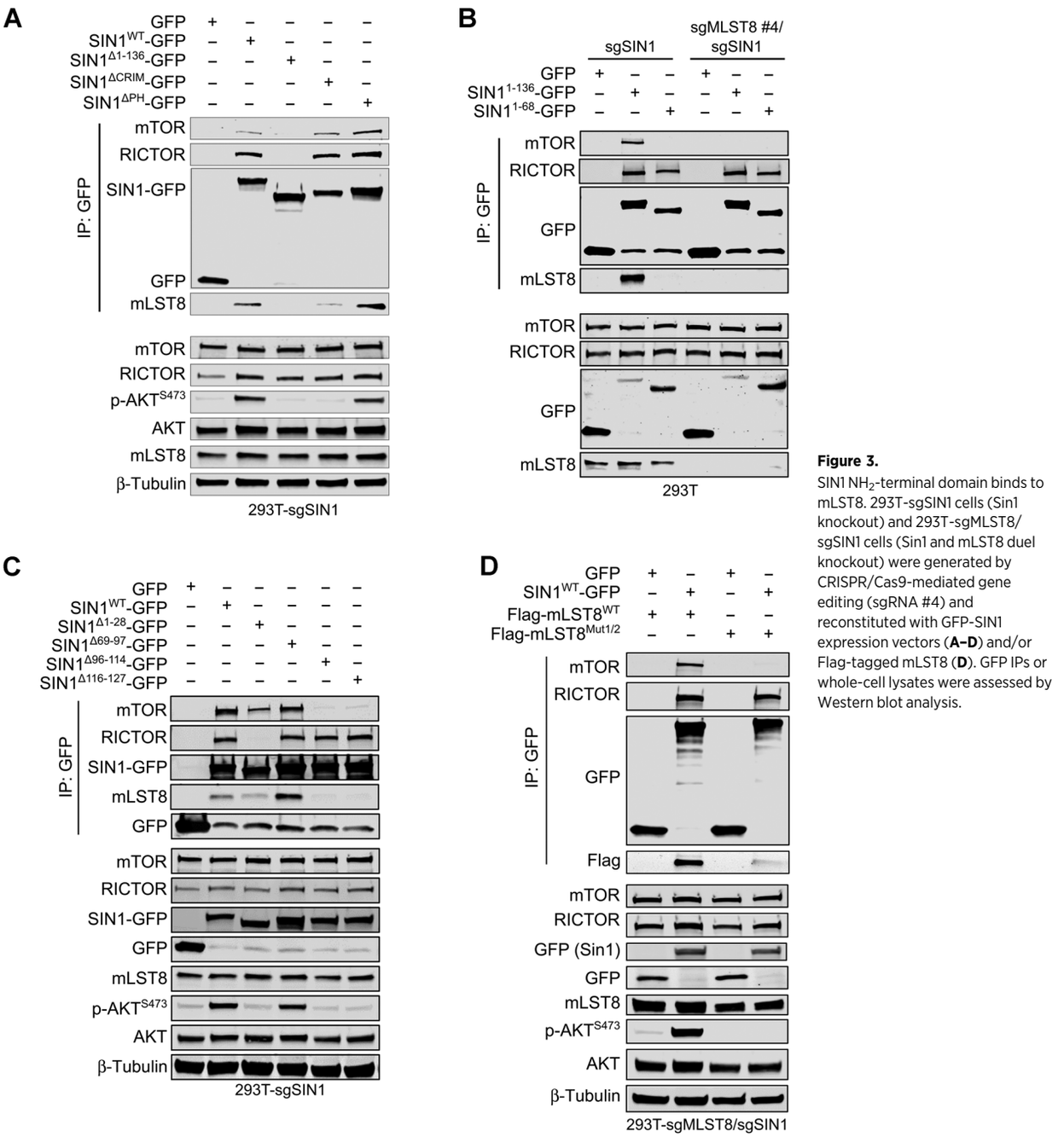

Figure 3.

SIN1 NH_2-terminal domain binds to mLST8. 293T-sgSIN1 cells (Sin1 knockout) and 293T-sgMLST8/sgSIN1 cells (Sin1 and mLST8 duel knockout) were generated by CRISPR/Cas9-mediated gene editing (sgRNA #4) and reconstituted with GFP-SIN1 expression vectors (**A–D**) and/or Flag-tagged mLST8 (**D**). GFP IPs or whole-cell lysates were assessed by Western blot analysis.

mLST8 point mutations that block mLST8–mTOR interactions impair mTORC2 signaling and tumor growth *in vivo*.

Given that mLST8 is required for mTORC2 signaling yet remains dispensable for mTORC1 signaling, it is possible that therapeutic approaches aimed at targeted disruption of mLST8–mTOR interactions might be used for selective targeting of mTORC2 in the setting of cancer. Notably, *PTEN*-null PC3 human prostate tumor cells require PI3K-mTORC2 signaling for cell survival (10), making these an ideal model in which to test this potential strategy for selective mTORC2 inhibition.

We generated mLST8-deficient PC3 cell clones (PC3-sgMLST8), which were confirmed by Western blot analysis to lack mLST8 expression, mTORC2 assembly, and $pAKT^{Ser473}$ (Fig. 4A). A PC3-sgMLST8 cell clone was reconstituted with $mLST8^{WT}$ or with $mLST8^{Mut1/2}$, the mLST8 mutant lacking specific mTOR-interaction points (Fig. 2). Ectopic $mLST8^{WT}$, but not $mLST8^{Mut1/2}$, coprecipitated with mTOR in PC3-sgMLST8 cells, and rescued $pAKT^{Ser473}$ (Fig. 4A), confirming that this approach successfully blocked mTORC2 signaling within the setting of increased PI3K activity driven by *PTEN* loss. Compared with parental PC3 cells, PC3-sgMLST8 cells

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

grew at a markedly reduced rate (Fig. 4B). Although expression of mLST8^{WT} restored the growth rate of PC3-sgMLST8 cells, mLST8$^{Mut1/2}$ did not. The reduction in cell growth caused by loss of mLST8 was mimicked by loss of SIN1, suggesting that mLST8 is an mTORC2-specific regulatory cofactor (Supplementary Fig. S4A and S4B). Furthermore, PC3-sgMLST8 cells reconstituted with mLST8$^{Mut1/2}$ generated tumors that were nearly five times smaller in volume and with a 2.5-fold decrease in final tumor weight (Fig. 4C). pAKT^{Ser473} was markedly diminished in PC3-sgMLST8 tumors expressing mLST8$^{Mut1/2}$, consistent with inhibition of mTORC2 signaling *in vivo*, although pS6K1 was similar in both (Fig. 4D). Tumor cell proliferation, measured by Ki-67 staining, was significantly decreased in tumors expressing mLST8$^{Mut1/2}$, whereas tumor cell death, as measured by cleaved caspase-3 staining, was modestly elevated (Supplementary Fig. S4C). Collectively, these results show that disruption of the mLST8–mTOR interaction can be used for selective inhibition of mTORC2 in cancer.

Discussion

Recent structural analyses of the mTOR complexes revealed several intricacies of mTOR complex stability, substrate accessibility, and sensitivity to rapamycin (6, 7, 11–14). However, the role of mLST8 in assembly of the mTORC2 complex has not yet been clearly defined. Here we provide experimental evidence that distinct interaction points within mLST8 contact mTOR, and at the same time, mLST8 binds with the SIN1-RICTOR complex to

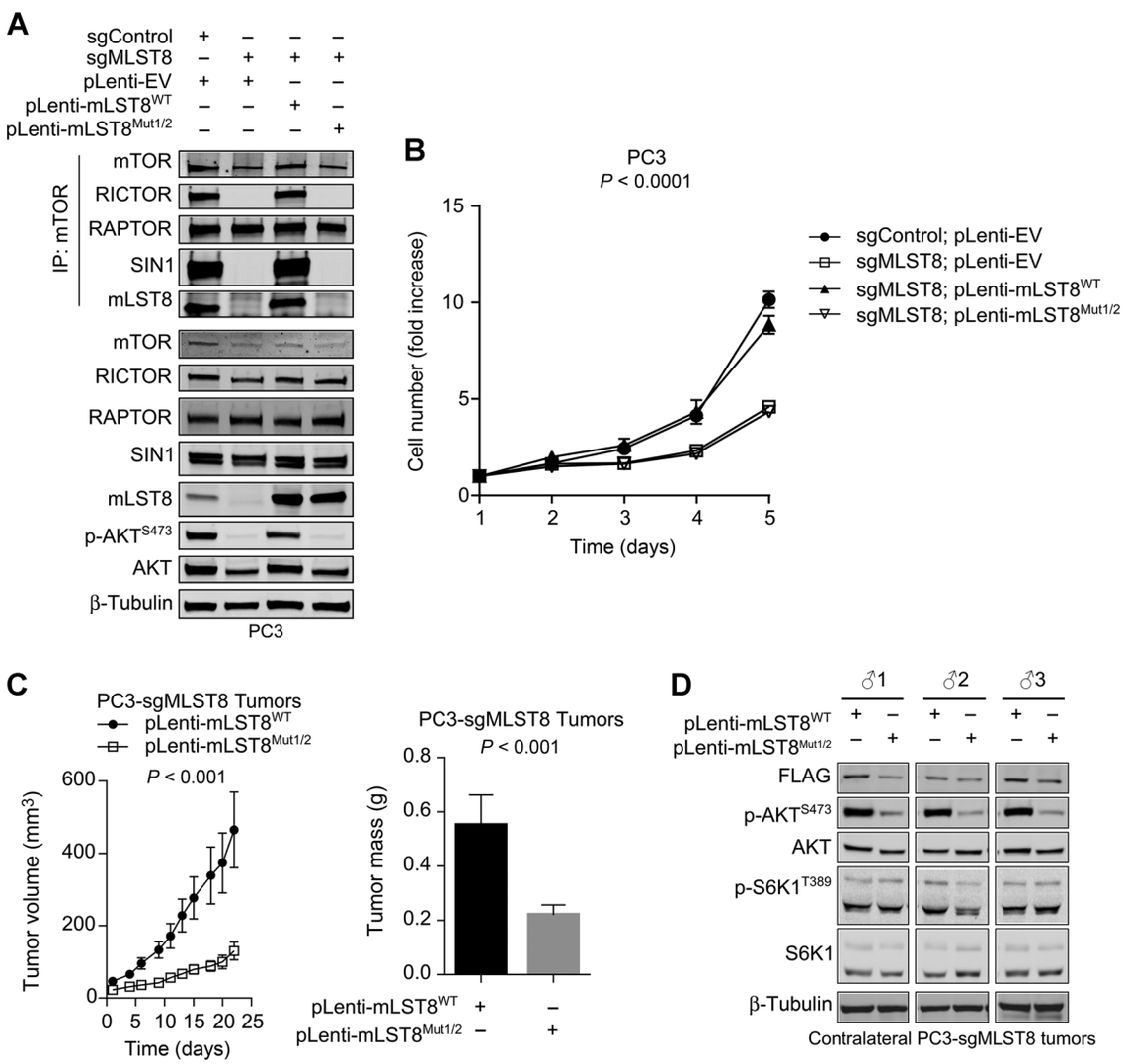

Figure 4.
mLST8-mTOR uncoupling mutations inhibit *PTEN*-null prostate cancer growth. PC3-sgMLST8 cells were generated by CRISPR/Cas9-mediated gene editing using sgRNA #1 and reconstituted with mLST8^{WT} or mLST8$^{Mut1/2}$. **A,** mTOR IPs or whole-cell lysates were assessed by Western blot analysis. **B,** Cell viability was measured by MTT assay ($N = 3$ biological replicates). PC3-sgMLST8 xenografts expressing mLST8^{WT} or mLST8$^{Mut1/2}$ were generated in contralateral flanks of male athymic mice. **C,** Tumor volume and mass was measured. Data are presented as average ± SEM ($n = 10$/group). **D,** Tumor lysates from matched pairs of contralateral tumors were assessed by Western blot analysis.

generate mTORC2. This agrees with previous reports showing that mLST8 does not bind directly to RICTOR (12), but interacts strongly with SIN1, such that SIN1 deficiency abolished mTOR–Rictor interactions (15). We found that mLST8 loss, or even disruption of the mLST8 sequences required for mTOR binding, completely impaired mTORC2 assembly and function in multiple untransformed and cancer cell lines. We propose that mLST8 is the scaffold upon which the molecular components of mTORC2 assemble. As SIN1 is a key regulator of mTORC2 activity (16–18), the stabilizing effect of mLST8 on mTOR and SIN1 becomes critical for mTORC2 function.

Identification of the selective role of mLST8 in mTORC2 has broad implications. mTORC2 has recently been identified as a new effector for oncogenic mutant Ras (19). *RICTOR* is also amplified in several types of human cancer (20, 21). Because several oncogenic mechanisms converge to activate PI3K, including growth factor overexpression, RTK activation or amplification, *PIK3CA* mutation, or *PTEN* loss, mTORC2 is frequently activated across many cancers (16). A growing body of evidence suggests that PI3K-dependent tumors cannot develop in the absence of mTORC2 signaling (10, 21, 22). We have shown herein that targeting mLST8 recapitulates mTORC2-selective inhibition seen in previous studies targeting RICTOR and have identified the discrete interaction points between mTOR and mLST8 that completely abolish mTORC2 signaling when disrupted. Thus, it is possible that drug design strategies aimed at disrupting mTOR–mLST8 interactions would be an effective approach at selective mTORC2 targeting in cancer. Further, currently available mTORC1 inhibitors relieve the potent negative feedback upon the PI3K signaling pathway, thus reviving PI3K signaling and activity of its many downstream effectors that promote tumor cell survival. There is no currently approved mTORC2-selective inhibitor, despite the significant interest in their development. The discoveries herein unveil a new approach for mTORC2-specific inhibitor design, which may hold an impact for patients with mutant Ras and/or PI3K-active cancers.

Disclosure of Potential Conflicts of Interest

J. Chen has received speakers bureau honoraria from NCI intramural program site visit, NIH TPM study section, UT San Antonio T-32 retreat keynote speaker, University of Cincinnati, University of Notre Dame, University of Nebraska, and University of Kentucky. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y. Hwang, J. Chen
Development of methodology: Y. Hwang, L.C. Kim
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Hwang, L.C. Kim, W. Song, D.N. Edwards
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Hwang, L.C. Kim, R.S. Cook, J. Chen
Writing, review, and/or revision of the manuscript: Y. Hwang, L.C. Kim, W. Song, R.S. Cook, J. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Hwang
Study supervision: Y. Hwang, J. Chen

Acknowledgments

This work was supported by a VA Merit Award 5101BX000134 (to J. Chen), NIH grants R01 CA177681 (to J. Chen), R01 CA95004 (to J. Chen), T32 CA009592 (to L.C. Kim and D.N. Edwards), F31 CA220804-01 (to L.C. Kim), and Susan G. Komen Award PDF #17480733 (to D.N. Edwards).

Received November 29, 2018; revised April 5, 2019; accepted May 8, 2019; published first May 13, 2019.

References

1. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168:960–76.
2. Kim LC, Cook RS, Chen J. MTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene 2017;36:2191–201.
3. Kim D-H, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, et al. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol Cell 2003;11:895–904.
4. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Dev Cell 2006;11:859–71.
5. Wang T, Blumhagen R, Lao U, Kuo Y, Edgar BA. LST8 regulates cell growth via target-of-rapamycin complex 2 (TORC2). Mol Cell Biol 2012;32: 2203–13.
6. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTOR kinase structure, mechanism and regulation. Nature 2013;497:217–23.
7. Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, et al. Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. Mol Cell 2015;58:977–88.
8. Karuppasamy M, Kusmider B, Oliveira TM, Gaubitz C, Prouteau M, Loewith R, et al. Cryo-EM structure of Saccharomyces cerevisiae target of rapamycin complex 2. Nat Commun 2017;8:1729.
9. Tatebe H, Murayama S, Yonekura T, Hatano T, Richter D, Furuya T, et al. Substrate specificity of TOR complex 2 is determined by a ubiquitin-fold domain of the Sin1 subunit. Elife 2017;6:e19594.
10. Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH, et al. mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell 2009;15:148–59.
11. Yang H, Wang J, Liu M, Chen X, Huang M, Tan D, et al. 4.4 Å Resolution Cryo-EM structure of human mTOR Complex 1. Protein Cell 2016;7: 878–87.
12. Stuttfeld E, Aylett CH, Imseng S, Boehringer D, Scaiola A, Sauer E, et al. Architecture of the human mTORC2 core complex. Elife 2018;7:e33101.
13. Aylett CHS, Sauer E, Imseng S, Boehringer D, Hall MN, Ban N, et al. Architecture of human mTOR complex 1. Science 2016;351:48–52.
14. Chen X, Liu M, Tian Y, Li J, Qi Y, Zhao D, et al. Cryo-EM structure of human mTOR complex 2. Cell Res 2018;28:518–28.
15. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 2006;127:125–37.
16. Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, et al. PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase Complex. Cancer Discov 2015;5:1194–209.
17. Liu P, Gan W, Inuzuka H, Lazorchak AS, Gao D, Arojo O, et al. Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. Nat Cell Biol 2013;15: 1340–50.
18. Yang G, Murashige DS, Humphrey SJ, James DE. A Positive Feedback Loop between Akt and mTORC2 via SIN1 Phosphorylation. Cell Rep 2015;12: 937–43.
19. Kovalski JR, Bhaduri A, Zehnder AM, Neela PH, Che Y, Wozniak GG, et al. The functional proximal proteome of oncogenic ras includes mTORC2. Mol Cell 2019;73:830–44.
20. Cheng H, Zou Y, Ross JS, Wang K, Liu X, Halmos B, et al. RICTOR Amplification defines a novel subset of patients with lung cancer who may benefit from treatment with mTORC1/2 inhibitors. Cancer Discov 2015;5:1262–70.
21. Morrison-Joly M, Hicks DJ, Jones B, Sanchez V, Estrada MV, Young C, et al. Rictor/mTORC2 drives progression and therapeutic resistance of HER2-amplified breast cancers. Cancer Res 2016;76:4752–64.
22. Tanaka K, Babic I, Nathanson D, Akhavan D, Guo D, Gini B, et al. Oncogenic EGFR signaling activates an mTORC2-NF-κB pathway that promotes chemotherapy resistance. Cancer Discov 2011;1:524–38.

Published OnlineFirst May 13, 2019; DOI: 10.1158/0008-5472.CAN-18-3658

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Disruption of the Scaffolding Function of mLST8 Selectively Inhibits mTORC2 Assembly and Function and Suppresses mTORC2-Dependent Tumor Growth *In Vivo*

Yoonha Hwang, Laura C. Kim, Wenqiang Song, et al.

Cancer Res 2019;79:3178-3184. Published OnlineFirst May 13, 2019.