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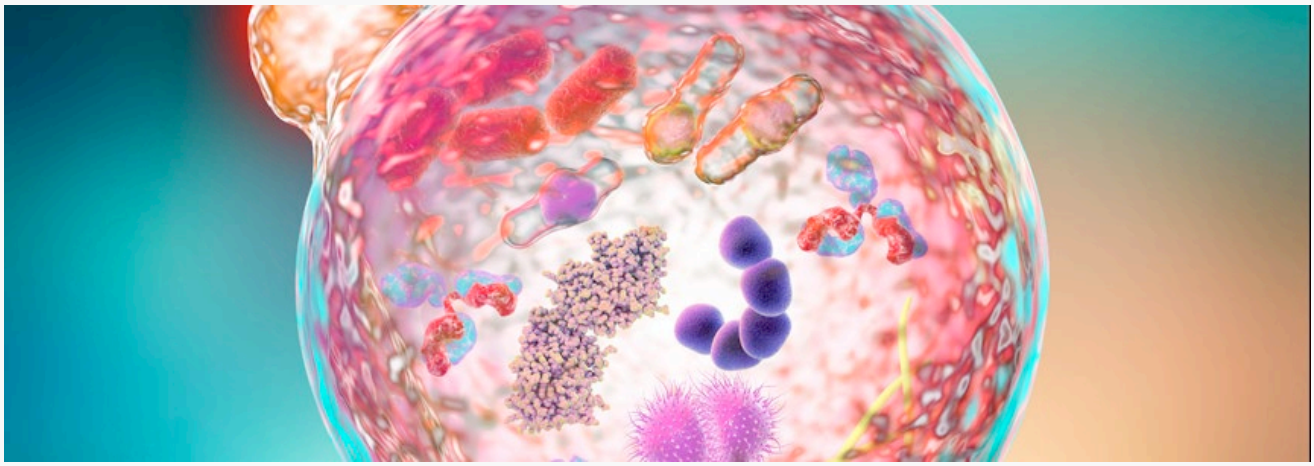


TARGETS & MECHANISMS

MASTERING mTOR

By Lauren Martz, Senior Writer, and C. Simone Fishburn, Editor, *BioCentury Innovations*

The master regulator mTOR has made its mark as the target of dozens of drugs and clinical candidates for diseases ranging from cancer to diabetes, but David Sabatini thinks its full therapeutic value won't be realized until the molecule, which is involved in nearly every physiological process, can be modulated in a tissue-specific way.

Sabatini, who is a member of the Whitehead Institute for Biomedical Research, a professor of biology at Massachusetts Institute of Technology and an investigator at Howard Hughes Medical Institute, discovered mTOR as the target of rapamycin as a Ph.D. candidate at The Johns Hopkins University in the 1990s and has since been credited with the discovery of the two mTOR complexes, mTORC1 and mTORC2.

His focus is now on uncovering the signaling and sensing molecules in the mTOR pathway. Sabatini's lab has identified components of the mTOR complexes and their regulators, including the Ras superfamily Rag GTPases and 23 other proteins involved in the molecule's amino acid-sensing function (see "Modulating mTOR").

Sabatini believes drugging the networks of molecules that interact with mTOR is the answer to achieving tissue-specific signaling. mTOR is involved in sensing intracellular and extracellular levels of a wide variety of molecules that regulate cell growth and other processes, including amino acids, glucose and oxygen.

Because the signaling pathways for the different processes vary by tissue, he thinks there is vast untapped potential in selectively modulating the pathway for different cancers as well as age-related diseases.

In addition, he thinks there are opportunities for combining genetics with mTOR biology, including mTORC2, and labels synthetic lethality as one of the next hot areas in cancer research.

Sabatini is a co-founder of three companies, one of which, Navitor Pharmaceuticals Inc., is exploiting the idea that targeting the mTOR protein network should lead to an mTORC1-specific inhibitor. The others are Raze Therapeutics Inc., which is blocking one-carbon metabolism used by many tumors to treat cancer, and precision functional genomics company KSQ Therapeutics Inc.

This week, Sabatini was selected as the recipient of the Lurie Prize in Biomedical Sciences from the Foundation for the National Institutes of Health (FNIH) for his discoveries related to mTOR.

Sabatini sat down with BioCentury to discuss the progress made in basic and translational research on mTOR over the last two decades and the future for mTOR-modulating therapeutics.

Edited excerpts from the conversation follow.

BioCentury: mTOR lies at the center of several key processes. What are the biggest implications of your initial mTOR discovery for both translational and basic research?

MODULATING mTOR

mTOR pioneer David Sabatini has spent the last two decades interrogating the target and identifying the proteins involved in its signaling. The two complexes that contain the mTOR kinase, mTORC1 and mTORC2, act as master regulators in the cell, coordinating a wide range of input signals to control cellular processes ranging from autophagy to proliferation.

mTOR was discovered as the target of the generic immunosuppressant **rapamycin**, which binds to **FKBP12** to inhibit its activity.

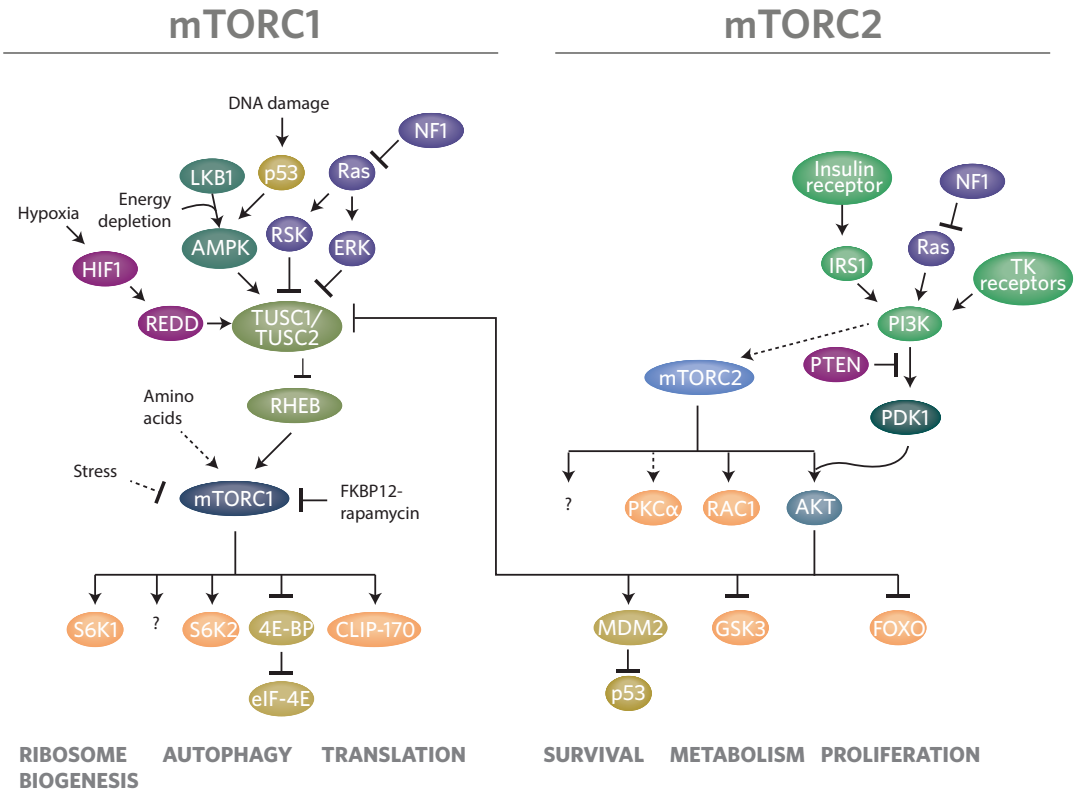
mTORC1: This complex's signaling network is initiated by activation of sensory molecules, including **HIF1**, which detects **hypoxia**, and **p53**, which detects **DNA damage**, and by energy depletion, which results in phosphorylation of **AMPK**. In addition, other input signals such as **amino acids** and cellular **stress** are sensed by proteins in the network that are largely unknown, but Sabatini and colleagues have been working to identify the proteins involved in the amino acid-sensing process. The sensor molecules trigger signaling molecules and ultimately activate or inhibit the **TUSC1/TUSC2** heterodimer, which represses **RHEB**, an mTORC1 activator.

Downstream, activated mTORC1 signals a series of other proteins to regulate cell mass accumulation by activating **ribosome biogenesis** and mRNA **translation**, and by inhibiting **autophagy**.

mTORC2: Although less is known about the specific proteins that regulate mTORC2 **(dotted line)**, pathway inputs include growth factors that are sensed by tyrosine kinase receptors **(TK receptors)**, and insulin, which is sensed by the **insulin receptor**. mTORC2 activates a series of proteins including **AKT** to regulate **cell survival**, **metabolism** and **proliferation**. Additionally, AKT suppresses TUSC1/TUSC2, leading to mTORC1 activation.

While there are many therapeutics in the clinic and on the market that target proteins upstream in the pathway such as p53 and HIF1, there are none in development for many of the downstream targets. *Reproduced with permission from David Sabatini.*

AKT (AKT1; PKB; PKBA) - protein kinase B; AMPK - AMP-activated protein kinase; FKBP12 (FKBP1A) - FK506 binding protein 12kDa; HIF1 - Hypoxia-inducible factor 1; mTOR (FRAP; RAFT1) - Mammalian target of rapamycin; mTORC1 - Mammalian target of rapamycin complex 1; mTORC2 - Mammalian target of rapamycin complex 2; RHEB - Ras homolog enriched in brain; TUSC1 - Tumor suppressor candidate 1; TUSC2 (FUS1) - Tumor suppressor candidate 2



David Sabatini: This protein is connected to almost any physiological process, and modulating mTOR has an impact on almost any pathology around almost any system. That's both good and bad in the sense that there are many opportunities, but it means that most normal tissues also care about mTOR function and it's hard to find a therapeutic window.

What we realize now is that [mTOR], as part of several large complexes and very complicated signaling pathways, is probably one of, if not the, key mediator between two aspects of life that are pretty central, really, to all organisms. And that is the decision whether to grow or not, that is, to add mass or remove mass. And you can think about that at the individual cell level, or you can think about the whole organism level.

And it responds to the right environment, which is basically nutrients. So that's like about as basic a thing as an organism can do. If it has food, it'll put on mass, and get bigger, and develop, and do other things. If it doesn't, it goes down into a pretty quiescent state, trying to survive that period of time until food becomes available.

And mTOR, as part of these complexes, evolved to basically be a sensor of many, many, many different inputs that tell an organism whether it's in the right state to grow or not.

BC: When you come across a protein like mTOR that is so central and connected, how do you find a translational opportunity without disrupting all the other processes?

DS: One opportunity has been cases where there are genetic alterations that lead to hyperactivation of mTOR. And so therefore, drugs bring its level back down to quasi-normal, and that has therapeutic impacts.

That's been the case in certain cancer-prone syndromes, certain forms of epilepsy, hypergrowth processes like cardiac stenosis and immune and organ rejections. They're pretty big cases, but there are certainly many others.

Modulating this system, particularly suppressing it, has really wonderful effects in many, many different disease states but in many of those, the toxicities of taking a general mTOR inhibitor would probably not be something you'd want for long periods of time.

I think what will be the Holy Grail in the mTOR field is getting tissue-specific modulation of this pathway.

BC: How could you achieve tissue-specific modulation?

DS: One of the ways we're interested in doing this, and I think the field is moving in this direction, involves understanding the mechanisms by which mTOR senses different inputs. mTOR basically has antennas for almost anything going on in the environment, both outside and inside the cell. You can imagine that different tissues will care about these inputs differently.

We've worked on the amino acid sensors in particular.

BC: Can you tell us about the Rag proteins that you discovered and whether those proteins, or other sensors in the network, might make good drug targets?

> "These complexes talk to the sensors: the amino acid sensors, glucose sensors and other kinds that we still don't know about. My hope is that those sensors, which are relatively upstream in the pathway, are going to be what we can make drugs against."
>
> David Sabatini, Whitehead Institute

DS: When we first started trying to understand how mTOR sensed amino acids, we had no idea what the proteins were that did the recognition or were involved in the signaling pathway. That's what led us to Rag GTPases.

These are part of the Ras superfamily of GTPases, which as you probably know, are very undruggable because they have incredibly high affinity for GTP, so we focused on trying to find the proteins that regulate the Rags.

This turned out to be very fruitful because there's a very large number of proteins that regulate Rags. In fact, we have four or five large protein complexes whose major role seems to be to regulate the Rags.

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BC: You've also developed technologies to support your research. How did these technologies, such as the system to

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David Sabatini, Whitehead Institute

purify unstable protein complexes, help further your research into mTOR?

DS: We found mTOR in the '90s and it took us until the 2000s to find mTORC1 and mTORC2. The problem just happened to be that the mTOR-containing complexes are very sensitive to a commonly used detergent called Triton X-100. If you were going to lyse a human cell in a dish, you'd typically use something like Triton X-100.

That was just bad luck because most complexes don't seem to care about Triton, but the mTOR complexes do.

When I think back on some of the key milestones for us experimentally, that is it: the idea that maybe we're not finding the complexes because things are just falling apart.

That's how we started developing cross-linking approaches to break open cells in the presence of chemical cross-linkers that stabilize [the complexes].

After that, everything flowed incredibly fast. Once we made that realization, we quickly found mTORC1, then mTORC2, and then another thing that bound to them. That was the inflection point because we had failed for years.

BC: Most of the mTOR inhibitors we've seen indiscriminately inhibit both mTORC1 and mTORC2. What is the challenge to targeting either complex specifically? *DS:* The challenge with discriminating between mTORC1 and mTORC2 is that the catalytic subunit of both of those complexes is mTOR itself, which is a kinase.

The complex that's really been most associated with the most numbers of diseases and with the aging process is mTORC1. This led to the idea that was partially fostered by us that it would be preferable to have specific inhibitors of mTORC1.

One way of doing that is to go after the sensors that feed into the mTORC1 pathway, and to some extent, that's what the company Navitor is interested in doing.

BC: What about targeting mTORC2?

DS: We've shown genetically that it would be quite interesting to inhibit mTORC2. In certain situations, particularly in cancers that have gained hyperactivation of the PI3K pathway either by losing the tumor suppressor PTEN or activating PI3K itself, those cancers seem to be susceptible to loss of mTORC2.

My impression is that there are other companies trying to go after that because the genetic data are quite clear. We'd certainly like to have inhibitors that discriminate between the two, but none of the existing inhibitors can do that.

It's very unclear how we would manage to do that because the only part of the mTORC2 complex that is druggable is the catalytic domain of mTOR.

We did large-scale screens on purified mTORC2 and tried to look for inhibitors of mTORC2 and not mTORC1, and we failed.

BC: In addition to Navitor, you've been involved in two other companies. What have you learned from those experiences about spinning your technologies out into companies?

DS: I think the challenge is that investors want something from an academic that's new and novel, yet it has to be really well validated. Those two things are, to some extent, mutually exclusive because the way the academic world validates things is by putting it out there to let people work on it for years, and it either stands the test of time or it doesn't.

I have always really liked the model of companies that actually do some of their own research. I think that's why Genentech was so amazingly successful.

That's what's been so much fun with Navitor. Because the investors came in relatively early in this amino acid-sensing story, even before we discovered some of the sensors, they had access to information that would be years away from being published. I wish there was more of this.

BC: Some people argue that the pendulum has swung too far in academia toward translational research, at the expense of basic research. What are your thoughts?

DS: I agree. I think there's been lots of political pressures to develop translational science in a lot of academic places, and you see it on review committees for grants.

What I find challenging is that there are relatively few groups that really can make molecules as well as industry can.

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> David Sabatini, Whitehead Institute

When we developed one of the first mTOR catalytic inhibitors with Nathanael Gray [professor of biological chemistry and molecular pharmacology at Harvard Medical School and professor of cancer biology at Dana-Farber Cancer Institute], a molecule called Torin 1, the number of rounds of chemistry that we went through with him blew me away. We did 13 or 14 rounds of medicinal chemistry for a molecule that has a lot of utility in the lab but is nowhere close to being a drug.

BC: What is next for your mTOR research?

DS: In my little world of mTOR sampling, it is definitely figuring out these different sensors and in what tissues they operate in tissue culture systems and at the whole organism level.

BC: What do you think the next big idea will be for cancer drug development in general?

DS: I think the idea of synthetic lethality needs much more investigation.

We had a recent paper with Eric Lander [president and founding director of the Broad Institute of MIT and Harvard] that looked at the idea that if you have an oncogene, you become addicted to something else not necessarily in the same pathway, but some other process that for whatever reason becomes essential in cells that have the oncogene.

Everyone uses PPAR and BRCA1 as a good example. I think the only challenge there is that you're talking about two DNA repair pathways that end up being lost. Those are housekeeping functions. When you think about most oncogenes, they're really driving a signaling pathway. I think there are many different kinds of synthetic lethality.

So I think there's a lot of room to look [in synthetic lethality]. But I'm captivated by how general those ideas will be, and then what kind of impact they're really going to have in cancer therapy.

COMPANIES AND INSTITUTIONS MENTIONED

Broad Institute of MIT and Harvard, Cambridge, Mass.
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Massachusetts Institute of Technology (MIT), Cambridge, Mass.
Navitor Pharmaceuticals Inc., Cambridge, Mass
Raze Therapeutics Inc., Cambridge, Mass.
The Johns Hopkins University, Baltimore, Md.
Whitehead Institute for Biomedical Research, Cambridge, Mass.

TARGETS

BRCA1 - Breast cancer 1 early onset
mTOR (FRAP; RAFT1) - Mammalian target of rapamycin
mTORC1 - Mammalian target of rapamycin complex 1
mTORC2 - Mammalian target of rapamycin complex 2
PI3K - Phosphoinositide 3-kinase
PPAR - Peroxisome proliferation activated receptor
PTEN (MMAC1; TEP1) - Phosphatase and tensin homolog deleted on chromosome ten

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