Mitosis study finds potential cancer target

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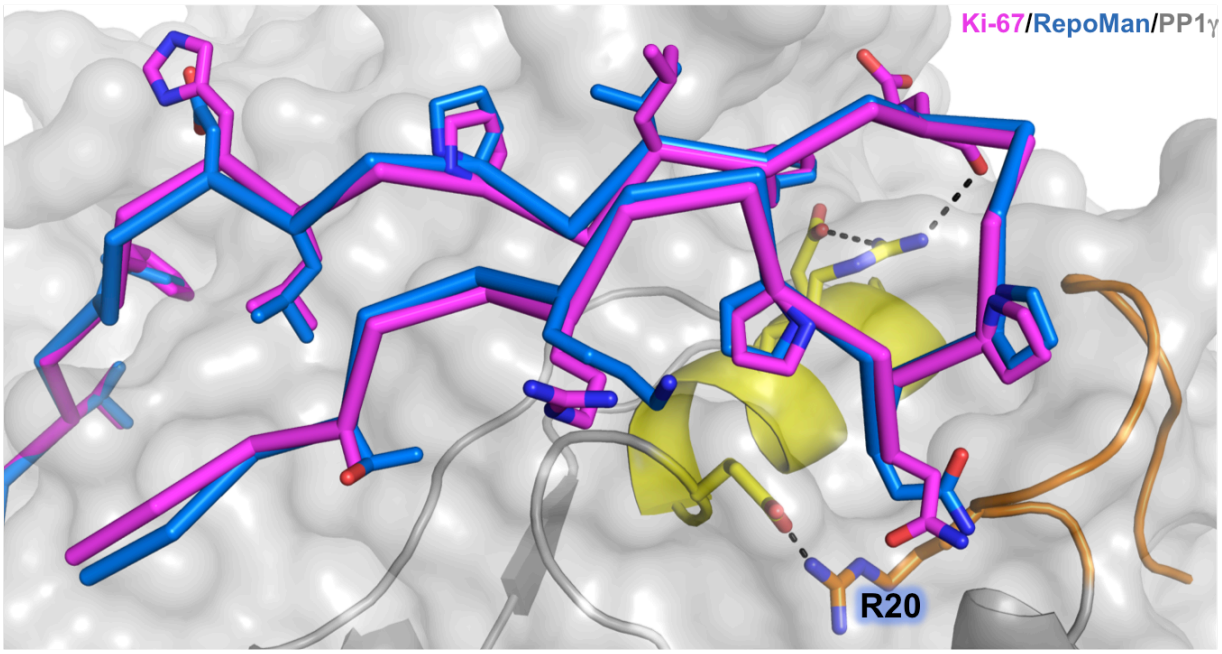

A rendering depicts the specific and unique interaction between proteins PP1-gamma and either RepoMan or Ki-67, which presents a potential target for cancer. Credit: Senthil Kumar/Brown University

Structural biologists show in a new study that an apparently key step in the process of cell division depends on a unique interaction among specific proteins, including one that is strongly linked to cancer. Their hope now is that the detailed new characterization of the interaction will make it a target for exploring a new cancer therapy.

Cell division, or mitosis, is a staple of high school biology classwork, but scientists are still making new discoveries about its intricate workings. Now, researchers have discovered that as copied chromosomes begin to exit mitosis and pull away from their sisters to form a new cell, a stage called anaphase, a protein called Ki-67 brings a protein called PP1 to the chromosomes.

Mitosis is essential to life, but it is also a process that occurs to a runaway degree in cancer. And that made Ki-67 of particular interest to the authors of the new study, which appears in the journal *eLife*, because Ki-67 is highly expressed throughout the various stages of mitosis, said lead author Senthil Kumar, assistant professor (research) of molecular pharmacology, physiology and biotechnology at Brown University.

"Ki-67 is a protein that is widely used as a prognostic marker in cancer biology," Kumar said. "People use this as a marker to study how far cancer has progressed."

Along with fellow Brown faculty members Wolfgang Peti and Rebecca Page and colleagues from other institutions, Kumar therefore wanted to understand exactly how Ki-67 interacts with PP1 in anaphase to bring it to the chromosomes. It turns out that Ki-67 binds to PP1 very tightly and—they also show this to exacting degrees in the new study—that another protein called RepoMan acts just like Ki-67.

Understanding how the proteins and PP1 interact during anaphase, the researchers hoped, could reveal a way to perhaps reduce or slow down mitosis in tumors.

It was particularly important to achieve a precise characterization of Ki-67 and RepoMan's interaction with PP1, Page said, because PP1 interacts with hundreds of proteins in the body, which regulate many key processes that they wouldn't want to hinder. Instead, they wanted to see

if there was something specific in mitosis with these two regulator proteins that they could pinpoint.

"PP1 has this interaction with 200 different regulators, but a number of those regulators use a couple of [binding] sites over and over again," said Page, professor of molecular, cellular biology and biochemistry. "You obviously can't develop an inhibitor for those two sites, because then you'd disrupt PP1 function in a whole array of biological processes. But the really neat thing that Senthil discovered is that this whole interaction is completely unique to these two regulators."

Kumar and Page led the effort by using nuclear magnetic resonance and x-ray crystallography that resolved the proteins and their interactions down to the scale of individual atoms—1.3 tenths of billionths of a meter. What he and the team found was that RepoMan and Ki-67 were binding with PP1 in an unusual way, forming a "hairpin" shape on the surface of PP1 at specific locations. A bioinformatics database search later confirmed that the binding was unique.

Moreover, they identified a novel binding region which is unique only to RepoMan and Ki-67. This novel region could be a potential target for cancer therapy, Kumar said.

Crucial to the research was that in the anaphase of mitosis the binding is even more specific than just either protein linking up with just any form of PP1. Instead they showed that in anaphase, RepoMan and Ki-67 link to a particular form of PP1 called gamma. The proteins' selectivity for PP1-gamma, they found, depended on just one amino acid on the PP1 protein at position 20.

The team, including co-authors at Brunel University in London and the University of Leuven in Belgium, confirmed this in living cells in imaging studies. They also confirmed that preference for Ki-67 and

RepoMan to the gamma form of PP1 happens in the live cells during mitosis. In addition, they showed that substituting the single amino acid at position 20 stopped the function.

The exact role that PP1-gamma or the two regulator proteins may play in cancer is not yet known, Page said, but now they know exactly how they interact and that the interaction is unique. That pushes the door wide open to develop a way to hinder it so they can see what the consequences are for cancer when they do.

"Now we have an approach for trying to dissect what's really happening because we can target this interface in particular," Page said.

More information: Ganesan Senthil Kumar et al, The Ki-67 and RepoMan mitotic phosphatases assemble via an identical, yet novel mechanism, *eLife* (2016). DOI: 10.7554/eLife.16539

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