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A new tool to study an important anti-cancer and immunosuppressive target

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The chemical rapamycin is used clinically as an immunosuppressant and as an anti-cancer agent that works by inactivating a protein named TOR (Target Of Rapamycin). This protein is essential for the growth of normal cells, but is hyperactive in tumor cells. To be able to carry out its various growth-related tasks, TOR needs to assemble into one of two larger protein complexes named TORC1 and TORC2. Curiously, whereas TORC1 is inhibited by rapamycin, TORC2 is unaffected by this drug. The team of Robbie Loewith, professor in biology at the University of Geneva (UNIGE), Switzerland, has just lifted the veil on this mystery.

Their study, published in the journal *Molecular Cell*, describes the structure of TORC2 and explains why rapamycin cannot access the TOR protein in this complex. These results enabled the team to generate a variant of TORC2 in which TOR is unprotected. This variant, which is sensitive to rapamycin, provides researchers in the field with a new tool to study TORC2 function in cells. The elucidation of the structure and function of this complex serves as a crucial step in the ongoing effort to identify clinically important drugs with which this important signaling pathway can be targeted.

When researchers brought back simple soil samples from an expedition to Easter Island in the 1960s, little did they know that they had stumbled upon a real treasure. Back in the lab, it was discovered that this soil contained the bacterium Streptomyces hygroscopicus. It produced a natural compound known as rapamycin (derived from Easter Island's

native name Rapa Nui), that was subsequently found to be a powerful antifungal agent. However, this use was abandoned with the discovery of the immunosuppressive and anti-cancer properties of rapamycin. Nowadays, it is used in transplant patients to prevent the rejection of the new organ. It also shows great promise for the development of new cancer drugs.

How does rapamycin work? The first step was to identify its target: an intracellular protein named TOR (Target Of Rapamycin), which stimulates the growth of cells. As a Post-Doctoral Fellow, Robbie Loewith discovered that TOR needs to assemble into one of two larger protein complexes named TORC1 and TORC2 to carry out its various tasks. Surprisingly, TORC1 but not TORC2 was found to be inhibited by rapamycin. Why the latter is insensitive to this chemical had remained a mystery for more than a decade.

Challenging to study

The ability to inhibit TORC1 with rapamycin made it relatively straightforward to study its functions. In contrast, lacking a rapamycin-equivalent has made it much more difficult to study TORC2 signaling. "In order to more easily study TORC2, we wanted to learn how to make this complex sensitive to rapamycin" says Robbie Loewith, researcher at the Department of Molecular Biology of the Faculty of Science from the UNIGE, Switzerland.

Thanks to an international collaboration, led by the scientist within the National Centre of Competence in Research (NCCR) 'Chemical Biology', the answer to this and several other essential questions are now clear. "First, we determined the structure of TORC2 and were able to visualize how proteins of this complex are organized in three dimensions", details Christl Gaubitz, a PhD student within the Geneva group and co-lead author of the article. "From this we observed which

subunit within TORC2 was obstructing the rapamycin-binding site on TOR".

The researchers thus discovered why rapamycin does not act on TORC2: "By deleting part of this subunit we generated a variant of TORC2 sensitive to rapamycin", explains co-lead author Manoel Prouteau. Equipped with this new tool, the biologists were able to study how TORC2 acts to stimulate cell growth.

Inhibit tumors on both fronts

Due to their fast proliferation, tumor cells have an increased dependency on TORC1 and thus are hyper-sensitive to rapamycin. However, this is not without side-effects. "From our present work we hope to one day identify a specific inhibitor of endogenous TORC2 which could also find use as an effective anti-cancer agent ", concludes Taiana Oliveira, a researcher in the group of Christiane Schaffitzel at the European Laboratory of Molecular Biology, in Grenoble, France, and third co-lead author of the study.

Provided by University of Geneva