Cancer

RHOJ protein helps skin cancer resist chemotherapy

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By Mar de Miguel

RHOJ, a small GTPase, could hold the key to the survival of tumor cells during cancer treatments. When epithelial cells transformed into mesenchymal cells, the Rho-related GTP-binding protein RhoJ regulated their resistance to chemotherapy. The scientists observed this mechanism in mouse models of skin squamous cell carcinoma, but their results could go beyond just one type of cancer.

When the team started its experiments, which were published March 23, 2023, in *Nature*, "I had never heard about RHOJ before," the lead author Cédric Blanpain told *BioWorld*. Blanpain, a professor and director of the Laboratory of Stem Cells and Cancer at the Université Libre de Bruxelles (ULB), explained that his group was studying the differences in all the genes during the epithelial-mesenchymal transition (EMT) when they found that RHOJ was one of the top hits differentiating upregulated genes between EMT tumor cells.

A previous study had associated RHOJ with chemoresistance in melanoma. And another publication suggested that it could control the activation of the DNA repair pathway. However, the mechanism was not fully described. "We went step by step and checked what could be the mechanism by which RHOJ controls the DNA repair pathway in the DNA damage response," he said.

After finding high levels of minichromosome maintenance protein (MCM), an enzyme that regulates DNA replication, the researchers established a collaboration with Juan Méndez, an expert on this topic and group leader at the Spanish National Center of Oncology Research (CNIO), who discovered the relation of RHOJ and the origins of replication.

The origins of replication, specific DNA sequences, are the places where genetic material begins its duplication. They are critical sites for cells, which must prevent replication from starting outside of these regions, as occurs in some types of cancer. In addition, replication must be correct to avoid mutations that favor tumor formation.

“In EMT tumor cells there are dormant origins of replication that are recruited upon DNA damage in a RHOJ-dependent manner. But that still does not explain how exactly RHOJ can control the origin of replication,” Blanpain remarked.

The researchers used a mouse model of skin squamous cell carcinoma and in vitro assays to demonstrate that RHOJ was preferentially expressed in EMT cancer cells and controlled the resistance to different therapies. Later, they developed proteomic and mass spectrometry analyses to identify the proteins that interact with RHOJ in EMT tumor cells.

They found that, after chemotherapy, RHOJ controlled the formation of long nuclear actin filaments that activated latent origins of replication, promoted DNA repair, and enabled cell survival after the DNA damage induced by the treatment.

“When we block actin polymerization and the formation of new actin, we could impair the DNA repair pathway and chemotherapy in EMT cells. The novelty it is indeed mechanistically. RHOJ is regulating nuclear actin in the formation of the novo origin of replication,” he said.

Melanoma or skin squamous carcinoma are not the only EMT tumor cells in which RHOJ is mediating DNA repair. In human breast cancer cell lines that present EMT, RHOJ also regulated the response chemotherapy. “We did find a role of RHOJ in these. We also found a role of RHOJ in glioblastoma, where EMTs might not be the right word,” he said. The scientist said that RHOJ is active in many cancer types, marking a direction for a new line of research.

Targeting RHOJ

Translationally, one key question is to what extent targeting RHOJ leads to on-target, off-tumor toxicities. “The question is if you inhibit RHOJ function everywhere in the body, is it tolerable?” Blanpain wondered. Since RHOJ is expressed in endothelial cells and blood vessels, “do you maintain blood vessel integrity if you inhibit the function of RHOJ?”

He added, “sometimes, molecules such as RHOJ that are important during development, might not be required during homeostasis later on. One way to assess that is to induce the knockout of RHOJ in every cell of the body” and see whether this is viable or is it leading to side and secondary effects.

RHOJ is a Rho GTPase that belongs to the family of Ras proteins, encoded by RAS genes in cell signaling pathways to control cellular growth and death. KRAS, HRAS and NRAS genes are part of the same family, with various mutations associated with cancer. Ras mutations are found in 30%-40% of cancer, which the scientific community considered untreatable.

“For decades it was said that RAS was undruggable because it was complicated. But now the pharma industry had found a way of targeting RAS…. For the moment, it is a covalent inhibitor, an inhibitor that interacts with the protein and makes a disulfide bond with the cysteine,” Blanpain said.

“This would be potentially doable for RHOJ, because it has some cysteines accessible close to the theoretical binding pocket and the active compounds of this active part of the GTPase. In theory it is indeed possible these days to imagine making a small molecule that could inhibit the function of RHOJ,” he explained. “But, of course, drug development takes a lot of time and needs to be done in a stepwise manner. So, we should go step by step, to see what kind of marketable RHOJ inhibitor would be, if RHOJ is targetable, if it is safe and effective, and then find the drugs that would target RHOJ.” (Debaugnies, M. et al. Nature 2023, Advanced publication).

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