MedicalXpress

Researchers use new molecular inhibitors to successfully hit difficult cancer target

February 5 2013

Early laboratory tests are the first to successfully use an experimental molecular therapy to block a hard-to-target part of a protein complex linked to several types of invasive cancer.

Scientists report online Feb. 4 in *PNAS Early Edition* the rational design of a small-molecule inhibitor they call Y16. In laboratory tests, the inhibitor helped stop the spread of cultured human breast cancer cells, especially when it was used with another compound known as Rhosin/G04.

The study was conducted by researchers in the Cancer and Blood Diseases Institute at Cincinnati Children's Hospital Medical Center, who developed both of the small-molecule inhibitors.

"We are using the findings from this study to refine our compounds and test them on mouse models of Acute Myeloid Leukemia and certain metastatic tumors – especially breast cancer, where the target pathway of this lead inhibitor is hyperactive," said Yi Zheng, lead investigator and director of Experimental Hematology/Cancer Biology at Cincinnati Children's.

Y16 and Rhosin/G04 appear to successfully target G-protein mediated Rho guanine nucleotide exchange factors (GEFs), which are part of the Rho GTPase complex of cell signaling proteins. Specifically, the compounds inhibit cell signaling that activates part of the protein complex involving a well-known enzyme, RhoA.

Under normal circumstances, the Rho GTPase complex helps maintain a delicate biological balance in regulating cell structure and function, including proliferation and movement. When the complex becomes dysfunctional, it can cause the hyper-activation of invasive cell growth and cancer.

Small molecule inhibitors are tiny organic compounds that attach to proteins to keep them from binding with other proteins. The intent is to block the activation of harmful biological pathways – such as aberrant Rho activity – that fuel disease. Used in a dose dependent manner, these compounds can in theory block cancer-fueling proteins without causing unwanted toxicity to healthy cells.

The challenge is to design a chemical structure that can attach to appropriate binding sites on a given target enzyme. Usually, only proteins with sufficiently deep hydrophobic pockets are considered "druggable." In their paper, Zheng and his colleagues said this "significantly limits the scope of the drug discovery effort." The surface area of many G-proteins, including RhoA, is mostly spherical and lacks obvious binding pockets.

Using computer drug design and high-throughput molecular screening, Zheng and his colleagues looked for molecular structures capable of blocking G-protein Rho GEFs. They came up with a structure that in computer-based tests appeared to work. The result was Y16 and derivatives that bind to a critical junction site of an enzyme called LARG (which stands for leukemia-associated Rho guanine nucleotide exchange factor). The compound prevents the LARG enzyme from activating RhoA.

Computer tests showed that by blocking LARG, Y16 suppressed the activation of the RhoA cell signaling and downstream molecular events that fuel cancer growth. These computer tests were subsequently verified

by laboratory experiments. The degree of suppression was based on the dosage used by researchers.

This suppression was amplified significantly when Y16 was used with Rhosin/G04, which the investigators previously have shown also targets RhoA. Used independently, Y16 and Rhosin/G04 reduced RhoA cell signaling activity by about 50 percent. Used together, the compounds could work synergistically to inhibit RhoA activity and proliferative potential in breast cancer cells, where RhoA signaling is often hyperactive, the researchers said.

When tested on healthy mammary cells not undergoing cancerous transformation, Y16 and Rhosin/G04 did not affect cell function.

The researchers cautioned that an extensive amount of additional research and verification will be needed before determining if Y16 and Rhosin/G04 could be used in clinical settings with human patients.

Provided by Cincinnati Children's Hospital Medical Center