Early progress reported in designing drugs that target 'disordered' proteins

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St. Jude researchers (From left) Jian Zuo, Ph.D., member of the Developmental Neurobiology Department, Anang Shelat, Ph.D., assistant member of the Chemical Biology and Therapeutics Department, Luigi Iconaru, Ph.D., from the Developmental Neurobiology Department and Richard Kriwacki, Ph.D., member of the Structural Biology Department, discuss research goals next to one of the NMR spectrometers at St. Jude Children's Research Hospital. Credit: St. Jude Children's Research Hospital / Peter Barta

St. Jude Children's Research Hospital scientists have identified a small, drug-like molecule that inhibits the function of a "disordered" protein in research that may advance a novel approach to hearing restoration. The study appeared recently in the journal *Scientific Reports*.

The protein, p27, is among the estimated one-third of human proteins called intrinsically disordered proteins (IDPs) that do not spontaneously fold into specific 3-D shapes. p27 helps to regulate cell division; reduced levels of the protein are associated with the spread of breast and other cancers. This study, however, was sparked by evidence of the possible benefits of inhibiting p27, particularly to aid regeneration of sensory hair cells of the inner ear to combat hearing loss.

The results also raise broader hopes regarding drug development targeting disordered proteins. Disordered proteins are implicated in a wide range of diseases, including diabetes and neurodegenerative disorders, but so far drug-development efforts have failed. Most drugs work by binding to proteins' stable 3-D shape, which disordered proteins lack.

The p27 protein works by binding to an enzyme and forming a complex that blocks cell division. This type of regulation is necessary to keep cells in check when not otherwise instructed to divide. In this study St. Jude researchers used NMR spectroscopy to identify 36 small molecules that bind to two different but partially overlapping regions of p27 where the protein binds to the enzyme. NMR spectroscopy uses magnetic properties of atoms to discover structural details of different molecules and especially how they interact with one another. Most drugs are small molecules. One of the small molecules in this study inhibited p27 function in biochemical assays, demonstrating in principle that small molecules can disrupt and possibly regulate function of disordered

proteins.

"The thought had been that small molecules would not bind specifically to disordered proteins," said co-corresponding author Richard Kriwacki, Ph.D., a member of the Department of Structural Biology. "This study demonstrates that small molecules identified by screening a library of compounds not only bind to a disordered protein, but sequester and inhibit the protein's activity."

Scientists have begun work to engineer a compound that forms a stronger bond and encompasses the p27-enzyme binding site.

"The concept of p27 inhibition as a possible strategy for hair cell regeneration has been around for more than 15 years, but until now no one has been able to do it," said co-corresponding author Jian Zuo, Ph.D., a member of the St. Jude Department of Developmental Neurobiology, who studies hair cell regeneration. "I knew Richard was an authority on intrinsically disordered proteins like p27 so I approached him; and he came up with the innovative, some would say crazy, idea of screening small molecules for inhibition of p27."

Hair cells in the inner ear convert sound vibrations into electrical signals that travel to the brain via the auditory nerve. In chickens, fish and amphibians, hair cells regenerate from the surrounding cells called supporting cells, but human hair cells lost to injury, disease or age do not. Such damage is a leading cause of hearing loss.

Laboratory experiments have shown that supporting cells from mice can be coaxed into becoming hair cells in part by blocking production of p27.

Using NMR spectroscopy, researchers screened a library of small molecules for evidence of p27 binding that may disrupt protein function.

Investigators were specially looking for small molecules that bind p27 where the protein normally binds its enzyme partner and blocks cell division. First author Luigi Iconaru, Ph.D., a St. Jude postdoctoral fellow, led the effort. Co-author Anang Shelat, Ph.D., assistant member of the St. Jude Department of Chemical Biology and Therapeutics, developed the small-molecule library using molecules that were slightly larger and more complex than traditionally used for drug- development screening.

Surprisingly, researchers found two distinct groups of small molecules that bind distinct, but overlapping segments of p27. The small molecules provided insight into how disordered proteins bind, including the dynamic interaction between small molecules and short-lived binding sites created by different arrangements of the amino acids that make up p27.

"The next step is to link the small molecules and binding sites identified in this study together to form larger compounds that bind p27 at multiple sites with greater affinity and specificity," Zuo said. "While small-molecule compounds are still a long way from the clinic, these results are another small step on the long road to a drug for hearing loss that could be infused into the cochlea to generate new hair cells."

More information: Luigi I. Iconaru et al. Discovery of Small Molecules that Inhibit the Disordered Protein, p27Kip1, *Scientific Reports* (2015). DOI: 10.1038/srep15686

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