Designing new molecular tools to study the life and death of a cancer cell

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Basic and translational research on cancer, and development of new cancer therapeutics, has focused on different aspects of cancer cellular function. One area of focus is the life and death of a cancer cell. Apoptosis, also known as programmed cell death, is a fundamental process of cells including cancer cells. The signal transduction pathways of apoptosis involve many different proteins and their interactions with each other. Protein-protein interactions involved in these apoptotic signals, like those in many other biological processes, are often determined or influenced by a short fragment of protein sequence or even certain key amino acid residues with important functional or structural roles in the protein-protein interface. For biomedical and pharmaceutical scientists, developing new molecular tools to understand and control the functions of these small protein fragments or residues and the biological and pathological processes that they mediate is a task and challenge of both fundamental interest and practical value.

In the work published in the February issue of Experimental Biology and Medicine, Huang, Zhang, Reed, An and their coworkers have developed new synthetic molecules as models to study the structural and functional role of the proline residue and tetrapeptide sequence important for the regulation of cancer cell apoptosis by the XIAP protein. The work was carried out jointly by the laboratories of Ziwei Huang and Jing An, formerly at the Sanford-Burnham Medical Research Institute in La Jolla, California and now the Cancer Research Institute and Department of Pharmacology of the State University of New York (SUNY) Upstate Medical University in Syracuse, New York, Liangren Zhang at Peking

University School of Pharmaceutical Sciences in Beijing, China, and John Reed at Sanford-Burnham Medical Research Institute.

Dr. Huang, who led this international research team, stated "research on protein-protein interactions and their synthetic modulators has become a new frontier for biomedical research and pharmaceutical development. We have chosen a proline containing tetrapeptide as the model to develop new peptidomimetic molecules to study the role of proline and tetrapeptide in the binding of XIAP protein and potential inhibition of XIAP mediated protein-protein interactions critical for apoptotic signaling in cancer cells. Our results suggest that these tetrapeptide analogs can be further developed into new molecular tools to analyze the mechanisms of protein-protein interactions and signal transduction pathways of XIAP in cancer and potential leads to develop anticancer drugs. This study combined the techniques in structure-based drug design, chemistry, and cancer biology and expertise and resources at institutions in America and China. It is an example of international collaboration to apply chemistry to biology and medicine with the long term goal of finding new anticancer therapeutics".

The research team used the crystal structure of a known tetrapeptide AVPI derived from Smac protein bound to XIAP protein as the guide to design a series of peptidomimetic analogs containing a conformationally constrained proline mimetic exo-2-azabicyclo[2.2.1]heptane-3-carboxylic acid. Structural analyses using nuclear magnetic resonance (NMR) and molecular modeling showed that some of these analogs can mimic the conformations of the parent tetrapeptide. Using a fluorescence polarization assay, one of these analogs was shown to be potent like the parent tetrapeptide in binding XIAP protein. This raises the possibility that such an analog may inhibit the antiapoptotic function of XIAP (a protein inhibitor of apoptosis), thus removing the roadblock of the death signal to kill a cancer cell.

Dr. John Reed, who led the binding and biological studies of these molecules in La Jolla, California, said, "The progress made through our collaborations with Dr. Huang and colleagues is a component of a substantial commitment we have made at Sanford-Burnham to discovery and design of small molecule chemical inhibitors of IAP family proteins as potential therapeutics for cancer. We are eager to advance the work towards drug-like leads that might provide renewed hope for those suffering from advanced malignancies."

The design and synthesis of the peptide analogs described in the study are the beginning steps in the long process of research and development of suitable pharmaceutical agents capable of penetrating a cancer cell membrane to reach the XIAP target and triggering the signaling pathway that causes the death of cancer cells in vivo. While further modifications and studies are needed on these peptide analogs in order to show their practical values as cell permeable anticancer agents, the present study of these analogs is of basic research interest for understanding the role of proline and conformation of prolyl peptide bond in mediating the biological function of a protein. It is known that the two different conformational isomers (cis and trans isomers) of the prolyl peptide bond can mediate distinct function of the protein. Many studies in the past of proline and proline mimics show either a mixture of cis and trans isomers or purely cis isomer. In this study, the proline mimic displayed strictly the trans conformation. The interesting conformational and functional effects of the synthetic unnatural mimic of proline discovered here suggest an alternative probe of prolyl isomerization in biology and that such a proline mimic can be applied to study the role of proline and proline containing sequence in other protein-protein interactions involved in a wide range of biological functions.

Steven R. Goodman, Editor-in-Chief of *Experimental Biology and Medicine*, said "This elegant study by Ziwei Huang and colleagues explores the role of proline containing peptides in inhibiting the anti-

apoptotic function of XIAP. This will potentially lead the way to new designer anti-cancer drugs. The article is a wonderful example of the interdisciplinary and international research that is the focus of our journal".

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