New Peptides Could Lead to Better Anti-Cancer Therapies

Scientists are always trying to develop new strategies in the on-going fight against cancer and infectious diseases. Now researchers at the Institute of Human Virology (IHV) of the University of Maryland School of Medicine have synthesized novel compounds that may help specifically kill cancer cells by allowing p53, a key "tumor suppressor", to be activated in them.

p53 is a key regulator of cancer in humans. It helps prevent tumors from developing by blocking the growth and killing of cells with damaged DNA, such as cancer cells. However, to its detriment MDM2 and MDMX are two proteins that bind to the p53 protein and help temporarily inactivate it. This is sometimes useful in preventing unnecessary cell death, but not when the cell has become a cancer cell. In this case, it will promote cancer development and spread.

Dr. Marzena Pazgier

In recent work, IHV researcher Dr. Marzena Pazgier and her colleagues in IHV Senior Investigator, Dr. Wuyuan Lu's laboratory, synthesized 12-amino acid long peptides that help block the binding of MDM2 and MDMX to p53, reactivating this protein and leading to the death of tumor cells. These compounds may eventually lead to the development of novel anti-cancer drugs.

To identify peptides that bind to MDM2 and MDMX, the researchers used a phage display library. Random combinations of 12 amino acids were displayed on the outer capsule of bacteriophages, a virus which infects bacteria. The target protein, in this case MDM2 or MDMX, was incubated with the bacteriophages, and researchers identified the phages and therefore the peptides that bind specifically to the protein.

Amino acids can have two mirror image conformations, L and R. Initially the researchers looked for L-peptides, which are made up of L-amino acids, the conformation that's found in our proteins. The researchers used the phage display library on the L versions of MDM2 and MDMX, and identified a potent inhibitor, PMI, that blocks the binding of p53 to those proteins.

However, "the problem with L-peptides is that they are unstable in human cells and can be degraded by proteases in the cells," says Pazgier. This normally occurs in our cells with our normal proteins when they have been "cut" to the small size we call peptides. However, as a drug they are not very useful. To generate stable compounds that could eventually lead to anti-cancer drugs, Pazgier and her colleagues began to focus on D-peptides. "D-peptides are completely stable," says Pazgier.

Creating these D-peptides was not trivial—the researchers had to screen a phage display library against the D version of the target protein. Unlike L-proteins, D-proteins can't be made by bacteria, and have to be chemically synthesized from scratch, which is both difficult and costly, says Pazgier. "It's only a few labs that can do this synthesis." However, Lu's laboratory at the IHV had the technology and expertise to synthesize these proteins, and the researchers were able to use D-MDM2 to identify peptides that bound to it. They then created mirror images of the L-peptides, to get D-peptides that would work against the L version of MDM2, i.e., the natural state of MDM2..

Chemically synthesizing the proteins had other advantages too. "Synthetic proteins are much, much easier to crystallize than proteins, derived by other means," says Pazgier (Figure 1). And the best way check how a peptide works is to check its crystal structure with the target protein to see how they bind, she adds.

Figure1. Crystals of the p53-binding domain of MDM2 (A) and MDMX (B) in complex with PMI

Both MDM2 and MDMX have a binding pocket where both p53 and the peptides bind, notes Pazgier, and the L-peptides and p53 bind very similarly, since they exist in the L form. However, the D-peptides don't quite bind the same way, so a D version of PMI didn't fit as well

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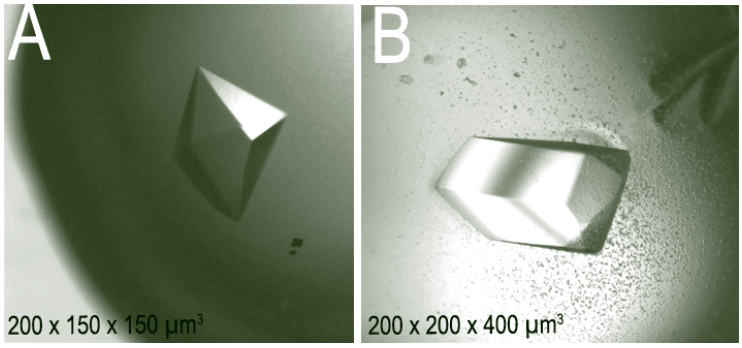

Figure 1

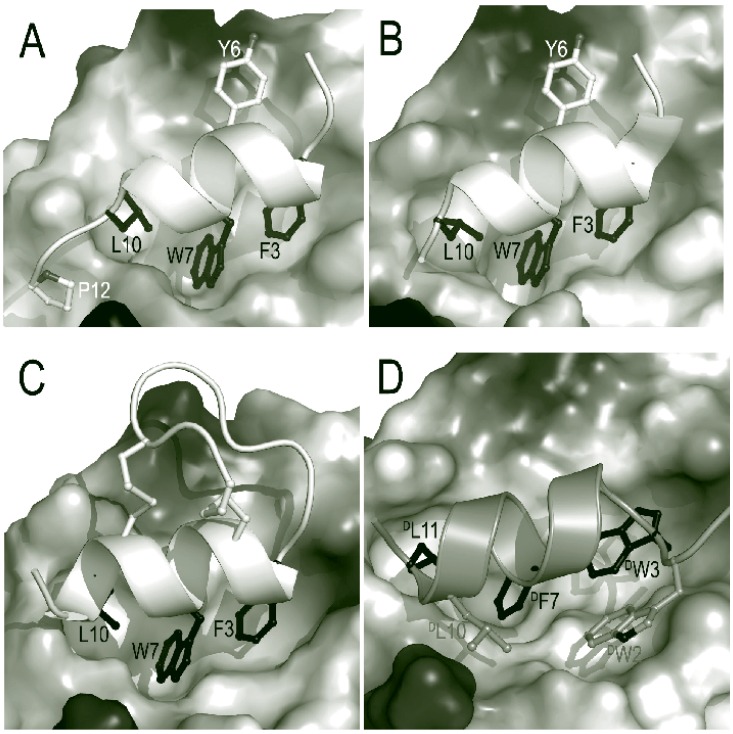

Figure 2

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as the L version. "It's like you have right hand and left hand glove, and they don't fit the same," says Pazgier. By getting a crystal structure of the D-peptide bound to MDM2, and using mutagenesis to modify the peptide to make it fit better in the binding pocket, the researchers were able to make the interaction tighter (Figure 2).

Figure 2. Close-up views of MDM2/MDMX-peptide inhibitor binding sites. The pictures were drown based on the crystal structures determined by IHV researchers of MDMX and MDM2 in complex with identified peptide inhibitors that block the binding of p53 to those proteins. Shown are as follows: (A) MDMX and (B) MDM2 in complex with 12-amino acid long L-peptide inhibitor PMI, (C) MDM2 in complex with 18 amino acid long L-peptide Stingin-1 and (D) MDM2 in complex with 12-amino acid long D-peptide inhibitor D-PMI.

While most previous work had been done on MDM2, blocking both MDM2 and MDMX from binding to p53 would be crucial for releasing p53 fully and helping treat cancer. The D-peptide that the researchers made against MDM2 turned out to be a weak inhibitor of MDMX, for which there aren't many inhibitors. The researchers now plan to screen their phage display library against D-MDMX as well. "By doing that we'll get the best fit for MDMX."

The researchers will also optimize their D-compounds against both MDM2 and MDMX. "This is like the starting point, it's a very exciting story for us right now," says Pazgier. Structural studies provided a detailed insight into D-peptides interactions with MDM2 and validated the binding mode of D-peptides as a novel class of p53 activators; however additional structure-based optimization studies are needed to gain the full benefit from them in the future.

Another issue that is key to developing peptides of any kind of anti-cancer drugs is getting the peptides across cell membranes into the cell. To achieve this, the researchers plan to try newer methods that have shown promise in getting L-peptides across membranes. "If we find a good delivery system, we will have new drug candidates in the future," says Pazgier.

"Wuyuan Lu, Marzena Pazgier and their colleagues have made an exciting, scientific collaboration," said IHV Director Robert Gallo. "There is a real chance that their work will lead to a novel approach for treating many cancers. The technical challenges they still have to face are formidable, but if any group can do it I would bet on them – in fact, I have!"

IHV's Terry Muzzy (left) and Samantha Watts (right) strike a pose with the Baltimore Orioles Bird

IHV Holiday Party

Every December, IHV staff and faculty from each of IHV's HIV/AIDS clinics organize a large holiday dinner for patients and their families filled with gifts for children, local celebrities and entertainment. This year was no different. The Hilton Hotel's Camden Yards Ballroom could barely contain the number of guests and fun-filled activities.

IHV Clinical Faculty and Staff

From left to right, Lydia Cornelius, Jada Carr, Sheila Lee, Jasmine Lee and Carlisle Harvey