Scientists tailor cell surface targeting system to hit organelle ZIP codes

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Scientists who developed a technology for identifying and targeting unique protein receptor ZIP Codes on the cellular surface have found a way to penetrate the outer membrane and deliver engineered particles - called iPhage - to organelles inside the cell.

In a paper published today online in *Nature Communications,* the team led by researchers at The University of Texas MD Anderson Cancer Center reports packaging the phage particles with a peptide called penetratin to reach inside the cell.

This new capacity was used to screen for peptide ligands - binding agents - that connect to receptors on mitochondria, which generate a cell's energy, and ribosomes, which process mRNA to make proteins.

The team found a peptide that binds to a specific ribosomal protein called RPL29 which, when delivered with penetratin, disrupts ribosomal function and kills cells. Cell survival was reduced in both malignant and non-malignant cells and in both mouse and human cell lines.

"We provided proof-of-concept for a direct intracellular ligand-receptor screening technology, which is clearly an unmet need in cancer biology, along with the discovery of an organelle ZIP Code that mediates cell death," said Renata Pasqualini, Ph.D., co-senior author of the paper and a professor in MD Anderson's David H. Koch Center for Applied Research of Genitourinary Cancers.

The RPL29 pathway is a new cell death pathway. The researchers found evidence of three types of cell death caused by disrupting the pathway with the new ligand.

"The molecular tool reported here along with its future ramifications will hopefully be of interest to targeted drug development, gene delivery, and mechanisms of human organelle diseases," said Wadih Arap, M.D., Ph.D., also of the Koch Center.

The iPhage screens for ligands inside the cell

Arap and Pasqualini pioneered a screening technique that exploits the existence of unique ZIP Codes in the vascular system to identify molecular targets and the ligands that can be used to selectively hit them.

They developed engineered viral particles, called phage, and packaged them with massive peptide libraries. When injected, these phage/peptide combinations bind to specific receptors in the blood vessels and organs. Cells are then fractionated and analyzed to discover which ligands bind to specific surface proteins.

Arap, Pasqualini and their colleagues have a number of targeted drugs in various stages of development based on screening and then delivery with the combinatorial particles.

The team wondered whether packaging the particles with penetratin, which is known to cross membranes without requiring a cellular receptor, would allow their technology to work inside of cells. "Penetratin makes a little bubble on the cell surface and the bubble goes in through the membrane," Arap said.

They dubbed the combination of penetratin and phage particles "internalizing phage," or iPhage. In a series of experiments, the team

found:

- iPhage successfully entered normal and malignant cells in both mouse and human cell lines while the engineered phage alone, or phage packaged with mutated penetratin, did not gain entry.
- Connecting iPhage with the mitochondria localization signal (MLS) peptide resulted in a 10-fold concentration of MLS-iPhage in mitochondria compared to simple iPhage, showing that specific organelles could be targeted.
- To screen for new ligands that might target specific organelles, they attached a random peptide library to iPhage particles and treated the KS1767 cells. Subsequent analysis found the peptide that binds to RPL29.
- Packaged with penetratin, this "internalizing homing peptide" with the ungainly name YKWYYRGAA killed 75 percent of cells in culture while the peptide alone or penetratin alone killed virtually none.
- Signs of apoptotic, autophagic and necrotic cell death were found with electron microscopy in cells killed by the YKWYYRGAA-penetratin combination.

Future studies will be needed to understand the complex cell death mechanism caused by the combination.

Provided by University of Texas M. D. Anderson Cancer Center