IISER Pune uses nanovesicles to improve cancer drug delivery

The number of colonies reduced signifiicantly in the presence of nanovesicles loaded with the drug, say Manickam Jayakannan (right) and Nagaraj Balasubramanian (second from right).

By encapsulating the poorly water-soluble anticancer drug Alisertib in polysaccharide nano-sized balls or vesicles, researchers at the Indian Institute of Science Education and Research (IISER) Pune have been able to increase its uptake by breast cancer cells and achieve greater ability to restrict cancer cell growth. Better uptake of the drug when encapsulated meant lower concentration (0.02 microM) of the drug was sufficient to restrict cancer growth significantly better than the free drug.

The study, which was started about six years ago, gains importance as the drug by targeting AU`RKA now selectively inhibits RalA protein thereby disrupting cancer cells' ability to grow without anchorage (anchorage independent growth). Anchorage independence in tumors is what allows cells to spread and grow at new sites in the body — metastasis.

Target protein

Since producing inhibitors against RalA protein is challenging and the existing inhibitor is not specific (targets both RalA and RalB), the researchers chose to target Aurora Kinase A (AURKA) instead. Aberrant AURKA activity has been implicated in cancer formation, and its inhibition could potentiate the effect of chemotherapies. When AURKA is silenced, RalA protein, which is present downstream, gets automatically (and selectively) inhibited.

The researchers embedded breast cancer cells in 3D gels (collagen or agarose) and incubated them with nanovesicles containing the drug and a florescent dye. In 15 days the breast cancer cells grew into a colony in the 3D gel. About 75% of the encapsulated drug was retained in the nanovesicle and carried into cancer cells during the 15-day period.

"While the free drug did not inhibit the colony forming ability of cancer cells, the number of colonies was significantly reduced in the presence of nanovesicles loaded with the drug," says Prof. Nagaraj Balasubramanian from the Department of Biology who led the team along with Prof. Manickam Jayakannan from the Department of Chemistry, IISER Pune.

The encapsulated drug produced 94% inhibition of the target AURKA leading to 25% inhibition of RalA; the drug did not affect RalB. As a result, there was 38% inhibition of anchorage independent growth of cancer cells.

In comparison, at 50%, the Ral inhibitor achieved greater inhibition of RalA than the encapsulated drug but the inhibition was not specific — RalB was inhibited by 64%. The inhibition of anchorage independent growth was only 33%.

"What was interesting is that besides being specific to RalA, the encapsulated drug achieved greater inhibition of anchorage independent growth than the Ral inhibitor. This despite the Ral inhibitor achieving double the inhibition of RalA than the encapsulated drug," says Prof. Balasubramanian.

"So the Alisertib drug did better than the existing Ral inhibitor and led to better inhibition of anchorage independent growth. That's the most striking observation of the study," he says. The results were published in the journal *Molecular Pharmaceutics.*

For this study, the nanovesicles were encapsulated with only one drug (Alisertib) in the hydrophobic envelop while a fluroscent dye was loaded in the hydrophilic core. But the 2014 study (published in the journal *Nanoscale*) by the team showed that loading the nanovesicles with two drugs enhances the therapeutic efficacies against cancer cells.

Hydrophobic and hydrophilic anticancer drugs loaded in the envelop and core of the nanovesicle respectively performed better than free drugs and synergistically killed breast and colon cancer cells. The proof-of-concept study was published in 2012 in *Biomacromolecules.*

“We are aiming to start animal studies in mice to study how well the drug contained in nanovesicles is able to inhibit breast cancer tumours. We are also using this drug loaded in nanovesicle to inhibit and study the role of AURKA and RalA in normal and cancer cells. So it also becomes a tool to understand the role of these proteins in cells,” he says.

Fabricating nanovesicles

“To make the nanovesicles self-assemble, we undertook some modifications to the dextran polysaccharide using a molecule from cashew nut shell extract,” says Prof. Jayakannan. “Since both are from bio-based nanosystems, the nanovesicles are not toxic to cells, and possibly human, and are biodegradable.”

Prof. Jayakannan and his team started working on making the nanovesicles self-assemble way back in 2008 and in 2012 they succeeded once they knew the structure that is required to make the nanovesicle with modification.

“The nanovesicle is bilayered and is held together to form a stable vesicle through hydrophilic-hydrophobic interactions,” Prof. Jayakannan says. Since the nanovesicles are about 120 nanometre in size, they are easily taken up by cancer tissue but not normal tissue.

Also, nanovesicles with the drugs loaded become water dispersible thus increasing the uptake by cancer cells. “We are working to make the nanovesicles even more specific to cancer cells,” Prof. Jayakannan says. The drug-loaded nanovesicles are cleaved by esterase enzyme once inside the cell. “In spite of being cleaved, the drug is released slowly in a controlled manner over 8-10 hours” Prof. Jayakannan says. This would be important for how nanovesicles act in tumours.

The new findings show that Einstein’s insights into gravity still hold sway, even in one of the most extreme scenario.

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