Deliver nucleic acid therapeutics to solid tumors? RNAi Can!

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Antibody targeted nanoparticle delivers siRNA sites of metastatic ovarian tumor cells. (A) Model of polymeric micelle used to conjugate antibodies and siRNA for functional, intracellular delivery. Antibodies are bound through biotin-streptavidin linkage, while siRNA is immobilized by charge interaction with a cation polymer. (B) SKOV3 are HER2 positive human ovarian cancer cells that model metastatic disease when injected into the peritoneal space. SKOV3 cells are luciferase positive, localize to body cavity, and minimally to liver and spleen. Unpackaged siRNA (Cy5.5 labeled) is filtered by the kidney, while antibody targeted siRNA localizes to site of tumor cells.

RNA interference (RNAi) technology has revolutionized molecular biology, particularly in understanding the cellular mechanisms of human disease. With RNAi researchers can target nearly any cellular RNA for destruction, including protein-coding mRNA. This reversible destruction is an incredibly powerful therapeutic approach, as evidenced by the massive number of preclinical studies employing RNAi to treat human diseases. However, very few of these RNAi targets progress to clinical relevance. The first challenge in developing this technology is that RNA compounds are rapidly degraded and excreted when in the blood. We now know that if RNA is deposited in a lipid based nanoparticle, it is protected from degradation. Moreover, these nanoparticles facilitate cellular uptake. The second challenge has been to correctly target these nanoparticles to disease tissue. Most unmodified nanoparticles circulate in the blood until the liver and spleen metabolize them for excretion. Thus RNAi therapeutics have only been developed for cancers affecting the blood and liver. More recently, it was found that nanoparticles could be attached to a naturally occurring protein, transferrin, and this would target the RNA to cells expressing the transferrin receptor. In all
of these studies RNAi processing did occur in human tissue, as the RNAi target mRNA was significantly down regulated in patient biopsies. However, expanding our repertoire of targeting agents is essential to bring RNAi therapies to the clinic.

To address this need researchers in the Press Lab (Clinical Research Division) previously developed an interchangeable nanoparticle for the delivery of siRNA. This polymer can bind any siRNA molecule independent of its sequence, then using a common and simple linkage between biotin and streptavidin, the nanoparticle can be decorated with antibodies targeting any disease-specific cell receptor. The antibody-targeted nanoparticle is internalized into cells through receptor-mediated endocytosis. Many drugs that enter cells through this mechanism remain trapped in endosomes where the pH is reduced and drugs are destroyed. Instead, the nanoparticle developed in the Press lab is activated at low pH and is released into the cell, allowing the siRNA to function. This therapeutic strategy was developed and tested with human cells grown in the lab, leaving the possibility that these nanoparticles would still be processed and destroyed in the liver and spleen. In a recent publication in Oncotarget, Dr. Corinna Palanca-Wessels, a former Fred Hutch researcher, now working at Seattle Genetics, tested the ability of these nanoparticles to deliver functional siRNA to tumor cells in a mouse model of metastatic ovarian cancer.

To utilize antibody targeting, cancer cells must express a cell surface marker at much higher levels than any healthy tissue. A large success in this field has been in breast cancers that dramatically overexpress the HER2 receptor. An antibody targeting this protein, trastuzumab (Herceptin), has been extremely effective in patients with HER2 amplification. Interestingly, other solid tumors also amplify HER2, however, response to trastuzumab has had mixed results. In gastric cancers this therapy has been successful, while in ovarian cancer this monotherapy showed no therapeutic effect. A possible approach is not to simply bind HER2 and stop its activity, but rather to bind HER2 and deliver another drug to the HER2-expressing cell, such as siRNA. To test this approach researchers generated trastuzumab coated nanoparticles containing siRNA against multiple targets. These targeted agents were first evaluated using cultured ovarian cancer cells. Under these conditions a fluorescently tagged siRNA could be visualized in cells, and more importantly, the siRNA successfully depleted mRNA for three unique targets. Targets included GAPD, a common control target for RNAi and PCR technologies, Bcl-xL, a gene commonly amplified in chemotherapy resistant ovarian cancers, and STAT3, a transcription regulator in the same pathway as Bcl-xL. All of the targets were evaluated at the mRNA level, and Bcl-xL depletion was also verified at the protein level.
With this combination of antibody and siRNA validated in cultured cells, researchers then tested their activity in mouse models. Metastatic ovarian cancer first spreads locally within the abdominal cavity, thus a xenograft model can be generated by intraperitoneal injection of ovarian cancer cells. Bioimaging demonstrated that these mice form tumors within the body cavity, spleen, and liver. Mice were then treated with siRNA alone or trastuzumab-targeted nanoparticles containing siRNA. A fluorescent tag on the siRNA revealed that non-targeted molecules are filtered by the kidney, while siRNA in the targeted nanoparticle localize to sites of tumor cells. Excitingly, the siRNA was also functional within the tumor mass. This was evaluated by measuring GAPD mRNA levels.

These promising results will need to be followed up in two main ways. First, these in vivo studies suggest nanoparticles may elicit an immune response that must be mitigated. Second, new antibody targets must be explored. Luckily, targets may have already been identified. Dr. Corinna Palanaca-Wessels highlighted one such target, "Other attractive target combinations include pairing internalizing antibodies that recognize antigens expressed specifically on B-cells, for example CD22 or CD19, with siRNAs that target genes such as c-Myc or Bcl-2 which are overexpressed in difficult-to-treat lymphoid malignancies including the so-called "double hit" diffuse large B-cell lymphomas." Alternatively, this technology can take advantage of work in the immunotherapy field such as the recent finding that mesothelin is a specific marker for pancreatic cancers.

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