Targeted Nanoparticle Delivery of Bifunctional RIG-I Agonists to Pancreatic Cancer

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In this issue of *Molecular Therapy*, Das et al.[1] harness surface-receptor targeting nanoparticle delivery to combat pancreatic ductal adenocarcinoma with bifunctional short interfering RNA (siRNA), which activates the innate immune receptor retinoic acid-inducible gene I (RIG-I) and silences the anti-apoptotic protein Bcl-2. Nucleic acid receptor agonists are gaining importance in cancer therapy[2] as combination therapies employing checkpoint inhibitors open up new opportunities in immunotherapy and add momentum to the field. A key challenge for nucleic acid therapeutics, however, is delivery, as they cannot freely traverse cell membranes and need to be formulated in ways that enable them to reach intracellular compartments. Das et al.[1] add a further layer to this approach and use surface-modified nanoparticles to deliver RIG-I agonists to specific cancer cells *in vivo*.

RIG-I detects 5′-tri- or diphosphorylated double-stranded RNA, which is generated in the cytosol during viral infection,[3–5] and signals via the adaptor protein MAVS (mitochondrial antiviral-signaling protein), leading to the production of type I interferon and pro-inflammatory cytokines.[6] Stimulating this response with agonistic RNAs can be exploited to direct the adaptive immune system against cancer cells and generate an immunogenic tumor microenvironment.[7] In addition, RIG-I activation can induce expression of the pro-apoptotic Bcl-2 family members Puma (p53 upregulated modulator of apoptosis) and Noxa, which leads to efficient induction of apoptosis in many tumors, but not in primary cells.[8] Antagonizing anti-apoptotic Bcl-2 can further enhance tumor cell-specific apoptosis. As the minimal required ligand size for RIG-I is very similar to that of siRNA, these two functions can be readily combined in the same molecule, termed bifunctional siRNA. This approach has been previously used to effectively treat mouse models of cancer types that are largely resistant to conventional chemotherapy, such as melanoma[9] and pancreatic cancer.[10] So far, most preclinical *in vivo* applications have been conducted using conventional intravenous or intraperitoneal delivery with transfection reagents such as *in vivo*-JetPEI, and, even though many preclinical studies have shown this non-specific approach to be effective,[11] more specific delivery methods are desirable to improve treatment and reduce side effects. When administered intravenously, the majority of particles end up in the liver, spleen, and lung. However, both RIG-I-induced apoptosis and siRNA-mediated targeting ideally require efficient delivery to tumor tissue. In solid, topically accessible tumors, this can be achieved by intratumoral injection, as is currently pursued in a clinical trial using the RIG-I activating lead-compound RGT100 by MSD/RIGontec (ClinicalTrials.gov: NCT0306502).

There is, however, a lack of methods to specifically deliver RIG-I agonists to surgically inaccessible or widely distributed tumors and metastases. Developing innovative solutions for tumor cell-specific delivery, therefore, holds great promise to improve clinical applicability of nucleic acid therapeutics in cancer therapy.

Here, the authors tackle this issue by exploiting sigma-receptor expression on pancreatic ductal adenocarcinoma (PDAC) and melanoma for uptake of anisamide-conjugated nanoparticles containing bifunctional siRNA. Sigma receptors are highly expressed on many tumors[12] and, although their natural ligands and function are incompletely understood, the Sigma1-receptor ligand anisamide is frequently used as a nanoparticle surface modification to enable tumor targeting *in vivo*.[13] In an orthotopic allograft mouse model of PDAC, Das et al.[1] show that two low doses (5 μg per mouse and injection) of nanoparticles encapsulating a RIG-I-activating Bcl-2 siRNA are sufficient to induce tumor regression. They demonstrate a reduction of immunosuppressive M2 macrophages in tumor tissue as well as cytotoxic CD8 T cell infiltration and a pro-inflammatory tumor microenvironment, characterized by a reduction of interleukin 10 (IL-10)-expressing cells and increased numbers of interferon-γ positive cells. Anisamide-conjugated nanoparticles enhanced delivery to tumor tissue approximately 3-fold when compared to non-nonjugated particles and exhibited no long-lasting systemic effects. Nevertheless, despite strongly delayed tumor growth, the treatment was not sufficient to cure animals or prolong their lives. It will be interesting to see how anisamide nanoparticle delivery performs in more drastic treatment regimens, e.g., repeated high-dose injections of 50 μg/mouse as used previously with non-targeted JetPEI delivery in similar models.[10]

The results of Das et al.[1] represent an important step in the development of targeted immunotherapies, which will hopefully spark further development in the field. So far, the majority of particles still end up in the liver and lung while, even with anisamide targeting, only about 2% reach their destination in the tumor. Here, future improvements are mandatory to enable clinical application of targeted nanoparticle delivery. On the other hand, it is not entirely clear how large the individual contributions are of RIG-I activation inside tumor cells versus the local induction of tissue and peripheral immune cells to ultimate therapeutic success. In

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addition to genetic experiments with RIG-I-deficient cell lines and animals, selective targeting with nanoparticles might shed light on this topic.

If cell-specific targeting is consistently developed and more specific ligands are identified in the future, it might soon be possible to simultaneously deliver different siRNAs and innate receptor agonists within the same injection volume for a truly patient-tailored immune response.

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