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Keep Quiet and Stay in Line! Smart Polymers to Keep an Eye on Pancreatic Tumors

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Pancreatic cancer is among the deadliest epithelial malignancies and one of the few solid neoplasms with a rising incidence worldwide. Pancreatic cancer is the 12th most common cancer in the US[1] and, paradoxically, is predicted to become the second most common cause of cancer-related deaths within the next decade or so.[2] Pancreatic cancer prognosis is largely dependent on the stage of diagnosis. Unfortunately, pancreatic tumors are rarely detectable at early stage, and the vast majority of patients (80%–90%) are diagnosed with local and/or distant metastasis, with only 3% of patients surviving to 5 years.[3] Improving survival will definitely require better therapeutics for late-stage disease. In this issue of *Molecular Therapy*, Naqvi et al.[4] describe a new strategy to impair the metastatic spread of experimental pancreatic cancer tumors using nucleic acid binding polymers (NABPs). They show that a third-generation polyamidoamine dendrimer called PAMAM-G3 was efficient in treating a well-calibrated aggressive experimental mouse model of pancreatic cancer dissemination.

The authors took advantage of the fact that PAMM-G3 binds pro-inflammatory extracellular nucleic acids and nucleic acid-protein complexes to skew toll-like receptor (TLR) activation.[5] These compounds, originally developed as a tool for gene delivery, proved to be safe and effective in treating experimental models of lupus, acute liver failure, and influenza infection.[5,6] Interestingly, circulating nucleic acids such as cell-free DNA (cfDNA), pathogen associated molecular patterns (PAMPs), and damage associated molecular patterns (DAMPs) are hallmarks of various carcinomas and usual suspects in TLR-dependent metastatic dissemination, notably of pancreatic cancer cells.[7] Blunting the dialog between cancer cells and their immediate microenvironment may not only directly impair primary tumor metastatic spread, but also jeopardize the pre-conditioning of distant pre-metastatic sites for remote cancer cell implantation.

With this in mind, the authors of the new study found that PAMAM-G3 behaves at least as a two-edged sword to abrogate TLR activation and nuclear factor κB (NF-κB) nuclear translocation induced by cell-free DNA (cfDNA) in cancer cell lines from pancreatic origin. The preferred mechanism of action, which still needs to be clarified in these particular cells, is that PAMAM-G3 decreases the cellular uptake and the subcellular localization of TLR9 agonists from the endosome to the nucleus to prevent TLR9 activation.[5] Interestingly, as this NABP exerts its function by depleting DAMPs and PAMPs upstream of TLR9,

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PAMAM-G3 also has the potential to impair the pro-metastatic effect of many more TLR and non-TLR receptors that are activated by cfDNA, RNA, and associated proteins and to escape drug resistance to treatment often seen with compounds targeting single pathways. Moreover, in pancreatic cancer models, PAMAM-G3 also titers extracellular microvesicles to neutralize the pro-invasive effects of exosomes on cancer cells.

Moving to *in vivo* studies with a novel syngenic immunocompetent murine model of pancreatic cancer metastasis, they next brilliantly demonstrate the therapeutic benefit of PAMAM-G3 on tumor burden and also other clinical markers of liver disease and acute illness. Despite the fact that cationic polymers are notoriously toxic upon repeated administration, mice treated with PAMAM-G3 showed no laboratory abnormalities in hepatic or renal function and blood cell counts as compared with the vehicle-treated controls, addressing one of the main concerns of this novel approach.

Systemic approaches to defeat experimental pancreatic tumors have been investigated before, with only a handful of successes. Given the propensity for KRAS mutations in pancreatic cancer, genetic approaches to RAS inhibition (since this target has historically been described as "undruggable") have been developed using lipid nanoparticles or liposomes. Recently, Kamerkar et al.[8] took a step forward by generating inhibitory exosomes (iExosomes) derived from human foreskin fibroblasts, loaded with specific small interfering RNA (siRNA) against KRAS. The authors demonstrated the therapeutic efficacy of specific siRNAs against KRAS delivered by iExosomes in a large panel of preclinical models of pancreatic cancer. Interestingly, iExosomes harbor CD47, which prevents their non-specific uptake by the reticuloendothelial system, and enter cells through macropinositosis, an endocytic pathway exacerbated following oncogenic KRAS activation in cancer cells. As for PAMAM-G3, the path from preclinical studies to clinical trial could be arduous because it requires scaling up and further studies in relevant experimental models. PAMAM-G3 may have an edge on iExosomes because sibling, clinical grade NABPs have already been produced and evaluated clinically as gene transfer vectors for siRNA delivery in cancer patients with minimal evidence of toxicity, a precedent that advocates for the rapid translation to the bedside of patients suffering from pancreatic cancer.

The study by Naqvi et al.[4] made use of a tumor model based on primary cultures isolated from genetically engineered mice that are genetically homogeneous. The next step will be to address the antitumoral efficacy of this promising molecule in experimental models that better mimic the molecular and cellular heterogeneity of pancreatic cancer. Indeed, clinical heterogeneity is frequently seen in pancreatic cancer between cells within a single tumor (intratumoral heterogeneity) and between tumors of the same histologic type in different patients (intertumoral heterogeneity).[3] The degree of intratumoral heterogeneity is now well recognized as an important prognostic factor in this disease. This heterogeneity can be plural, (epi) genetic, transcriptomic, metabolic… and may explain why the increasing knowledge of the mechanisms behind the natural history of pancreatic cancer has not yet translated into effective treatments. In addition, pancreatic cancer is characterized by a dense stromal reaction that can account for anywhere from 20% to 80% of the tumor mass and which fuels and protects tumor cells from therapeutic attack. Several cutting-edge experimental models, such as patient-derived xenografts (PDXs) or spontaneous tumors arising in genetically engineered mouse models driven by KRAS activation in the pancreas, could help better appreciate how PAMAM-G3 may be effective in preventing metastatic spread in relevant human models and in mouse models recapitulating the complex architecture of human neoplasms.

Based on the mode of action, it is tempting to speculate that a NABP-based therapeutic strategy would likely benefit patients with locally advanced pancreatic tumors that are not eligible for curative surgery, but not patients with already detectable local and/or distant metastasis (albeit this must be validated experimentally). Patients with locally advanced tumors represent almost 40% of newly diagnosed pancreatic cancer patients; for these patients, PAMAM-G3 may address a major unmet need as a therapy to prevent metastasis from occurring. Using PAMAM-G3 as a monotherapy seems unrealistic; in the future, PAMAM-G3 should be combined with drugs or treatments that impair the growth of primary tumors but that are also suspected to predispose tumors to metastatic spread. Such risk of metastatic dissemination might be considerably reduced in the presence of PAMAM-G3. Therapeutic combinations based on PAMAM-G3 may have significant clinical impact by increasing the number of surgical candidates in the patient population with locally advanced tumors. Other inflammatory tumors, including breast cancer or soft tissue sarcoma, could be next.

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