Cancer therapy

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An oncolytic circular RNA therapy

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In vitro-transcribed RNA is a promising emerging class of therapeutic, but the poor specificity of cargo RNAs so far has limited their application in cancer immunotherapy. A new study reports the delivery of a synthetic circular RNA with inline cis-acting translational elements — encoding an engineered, mitochondrion-specific oncolytic protein — that shows both therapeutic and prophylactic potential against adenocarcinoma.

Protein-coding RNA cargoes produced via in vitro transcription have taken the world by storm in the wake of the successful global deployment of vaccines against COVID-19 consisting of mRNA encapsulated in lipid nanoparticles (LNPs)[1,2]. The widespread application of these RNA cargoes has been enabled in the past decade through advancements in nanotechnology and drug delivery, which have resulted in the development of sophisticated drug nanocarriers such as LNPs[3]. These carriers have been used to deliver RNA to cancer cells for immunotherapy applications; however, poor delivery, limited transcriptional specificity and the immunogenicity of exogenous mRNA continue to curtail these approaches[3–6].

Several classes of therapeutic oncolytic protein have been investigated and delivered as coding RNA transcripts to cancer cells, including caspases, PUMA, MLKL and tBid[5,6]. However, given that these proteins result in the irreversible cell fate of apoptosis, and that the specificity of existing delivery methods is far from perfect, these cargoes are something of a sledgehammer: able to kill tumor cells effectively, but not without potential off-target damage in non-malignant tissues. Oncolytic cargoes with better specificity and less irreversible, all-or-nothing behavior are therefore desired. Now, reporting in *Nature Cancer*, Feng et al. describe a new oncolytic RNA cargo for treatment of EIF4G2$^+$PTBP1$^+$ panadenocarcinoma[7].

The RNA cargo developed by Feng et al.[7] centers on an engineered protein derived from human gasdermin D (hGSDMD), a pore-forming effector protein that can induce cell death through pyroptosis by disrupting ion homeostasis across biological membranes[8]. Through multilayered bioengineering approaches, the authors were able to develop a cargo with the potent ability to lyse mitochondrial membranes in tumor cells, causing the release of tumor antigens and thereby driving strong adaptive anti-tumor immune responses. The specificity of this cargo for mitochondrial membranes — rather than cell membranes — dramatically reduces concerns about irreversibility and off-target effects compared with concerns about previously investigated oncolytic cargos.

In this work, Feng et al.[7] sidestepped the ubiquitous eukaryotic cap-dependent translation process and instead opted for cap-independent translation mediated by an internal ribosomal entry site (IRES). This approach has the potential to achieve more-specific translation activity than cap-dependent translation, owing to differences in the expression of transcriptional cofactors across tissue and cell types and across cell states[9]. Specifically, the authors used an IRES derived from human rhinovirus type 2. Transcriptional initiation from this IRES requires the presence of the IRES-transacting factor PTBP1 and the transcription factor eIF4G2[10,11]. By using this approach, the authors were able to produce RNA sequences that were translated only in cells expressing the genes encoding PTBP1 and eIF4G2 — a gene combination reported by the authors as being characteristic of adenocarcinoma. As circularization greatly extends the half-life of RNA and reduces adverse reactions to exogenous RNAs, the authors further used ribozymatic permuted intron–exon splicing based on the common T4 bacteriophage thymidylate synthase gene to produce a protein-coding circular RNA (circRNA) cargo[12,13].

To improve the targeting specificity of wild-type hGSDMD, Feng et al.[7] produced an engineered GSDMD protein with a carboxy-terminal peptide sequence, derived from the vaccinia virus F1L protein, to drive protein localization to the mitochondrion[14]. To further restrict the activity of the transcribed protein to the mitochondrial space and avoid off-target disruption of other subcellular compartments, the authors modified the wild-type caspase-sensitive region of the protein to instead be sensitive to the mitochondrial processing peptidase subunit PMPCB, found only in the mitochondrial matrix. Through these two augmentations, the authors produced an engineered protein product with the ability to localize to mitochondria and with activity that depends on mitochondrial factors, improving both the potency and the specificity of hGSDMD-mediated membrane toxicity.

The authors next encapsulated the optimized, mitochondrion-specific GSDMD circRNA in LNPs and assessed the anti-tumor efficacy of these LNPs in a humanized mouse model of adenocarcinoma. The authors observed ablation of several adenocarcinomas and reduced growth of gliomas and hematological tumors, and also recorded GSDMD expression in eIF4G2$^+$PTBP1$^+$ tumors, as expected. After adoptively transferring ovalbumin-specific transgenic T cells (CD8$^+$ OT-I T cells and CD4$^+$ OT-II T cells) into a transgenic mouse model with mitochondria-localized ovalbumin expression, the authors observed that their LNPs induced the production of IFN-γ and the persistent proliferation of OT-I and OT-II cells in tumor-bearing mice. This observation confirmed effective tumor ablation, as well as adaptive immune responses to antigens produced through mitophagy.

The authors further evaluated the potential for prophylactic use of their circRNA LNPs in an LSL-Kras^{G12D}p53^{R172H} mouse model, in which mice develop a variety of solid adenocarcinomas during adulthood. The authors observed a substantial increase in 12-week survival rate and decrease in 16-week combined tumor incidence for mice that received weekly intraperitoneal injections of circRNA LNPs, as well as a strong tumor-specific cytotoxic T cell response — demonstrating the protective ability of their circRNA LNPs against adenocarcinogenesis.

The findings of Feng et al.[7] show the feasibility of using differential expression of translation factors in cancer cells to regulate therapeutic RNA expression, which results in preferential protein

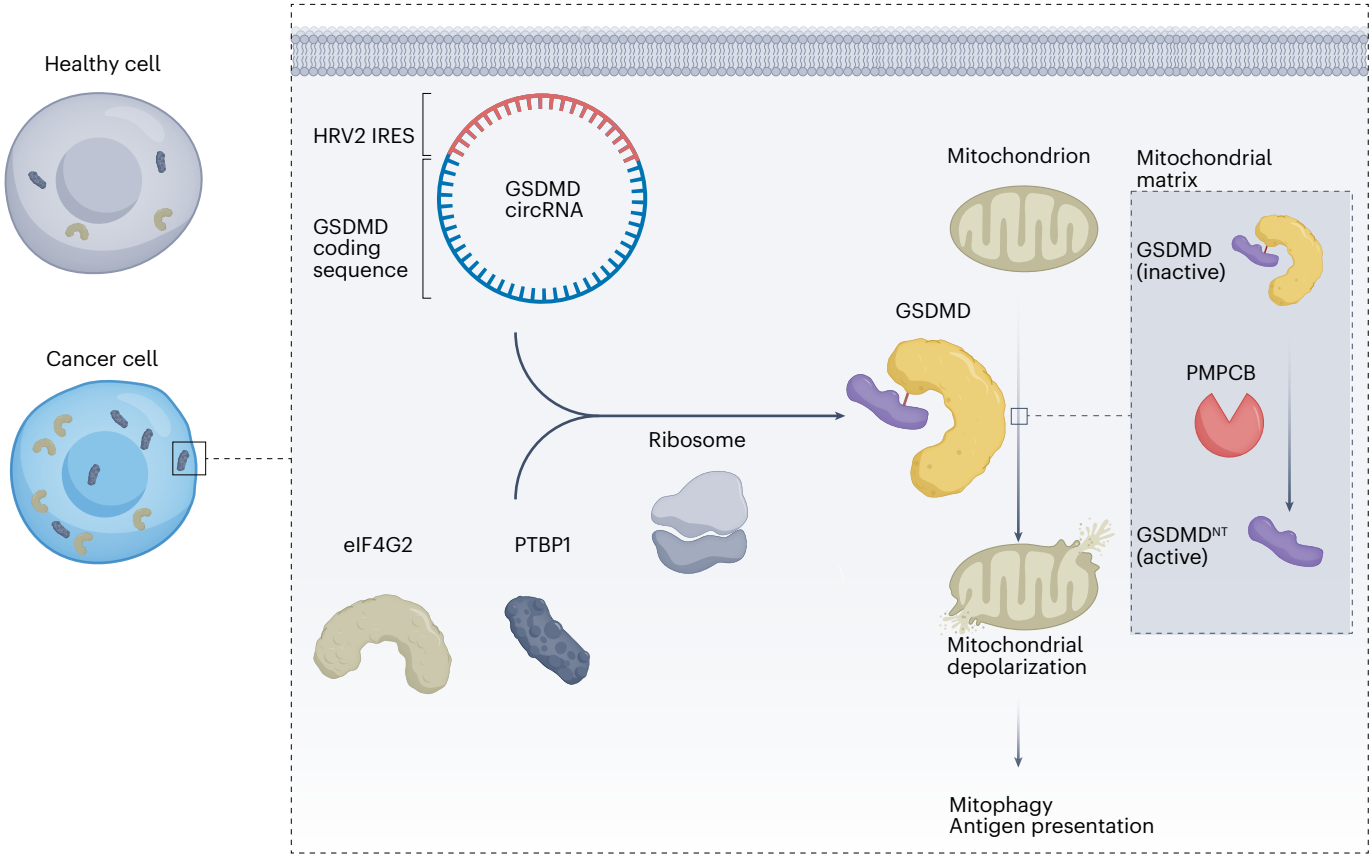

Fig. 1 | Overview of adenocarcinoma treatment using circular RNA. Feng et al.[7] have developed a circRNA that encodes engineered GSDMD for potential deployment in treating adenocarcinomas. GSDMD circRNA — mainly comprising an IRES from human rhinovirus 2 (HRV2) and an engineered GSDMD sequence — is delivered intratumorally into mice. Within a cancer cell, eIF4G2 and PTBP1, which both have high expression in adenocarcinoma, coordinate with the human rhinovirus 2 IRES to initiate translation of GSDMD, which translocates to the mitochondrion owing to a carboxy-terminal peptide sequence derived from the vaccinia virus F1L protein. The PMPCB in the mitochondrial matrix then cleaves a linker sequence in the engineered GSDMD protein to produce active GSDMD^{NT}, which perforates mitochondrial membranes by disrupting ion homeostasis. Membrane disruption leads to mitophagy and presentation of tumor-associated antigens, which ultimately results in recruitment of the adaptive immune system and induction of an anti-tumor response.

production in malignant cells. This generalizable approach could be used to restrict the expression of a wide variety of anti-tumor proteins to malignant tissues, which is highly attractive for cancer therapy. Furthermore, in-depth transcriptomic analysis of translation-factor expression across a range of malignant and healthy tissues could potentially identify factors other than eIF4G2 and PTBP1 that can be used for either broad anti-tumor nucleic acid therapies or therapies for specific drug-resistant cancer types. Additionally, the control of translation-factor-based expression is attractive beyond the field of cancer therapy for achieving improved spatial control of RNA therapies.

Feng et al.[7] have also developed a promising new engineered oncolytic protein with high mitochondrial specificity. Notably, they show that this oncolytic protein results in mitophagy and antigen presentation that leads to anti-tumor adaptive immune responses, which suggests that this construct could prove an attractive transgene for the treatment of other cancer types, either alone or in combination with other therapies such as immune-checkpoint blockades. However, further validation of the anti-tumor efficacy and immunostimulatory properties of this cargo is needed before the widespread deployment of this new construct for other cancer types. Furthermore, the therapeutic animal model used by Feng et al. relied upon local intratumoral injection, but systemic administration of therapeutic circRNA could be an attractive route to explore if sufficient translation specificity could be achieved. All told, this new work represents a substantial technological advance, and adds valuable tools to the cancer therapy toolbox.

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Competing interests

The authors declare no competing interests.