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SPOTLIGHT

To escape or not to escape, that is the question

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Marco et al. (https://doi.org/10.1083/jcb.202507217) identify a tumor-selective endocytic route in which CD44 and EPHA2 co-traffic antisense oligonucleotides (ASOs) to nuclear-proximal, recycling-endosome–like compartments that become leaky through lipid peroxidation and show that stress granule–mediated repair of these compartments limits productive ASO escape.

Antisense oligonucleotides (ASOs) are short, chemically modified nucleic acids that silence disease-relevant transcripts by recruiting RNase H1 to degrade complementary mRNAs in a sequence-specific manner. Despite their clinical promise—exemplified by approved therapies for spinal muscular atrophy, Duchenne muscular dystrophy, and hereditary transthyretin amyloidosis—their therapeutic potential remains constrained by poor cytosolic delivery. Quantitative NanoSIMS imaging in vivo has shown that only 1–2% of GalNAc-conjugated ASOs escape endosomes in hepatocytes (1), and independent estimates suggest that as little as 0.3% of other small RNA therapeutics are present in the cytoplasm at any given time (2), figures broadly consistent with single-organelle imaging in cell culture (3, *Preprint*). Attention has largely focused on membrane permeabilization as the primary escape mechanism, but a recent preprint proposes an additional, intraorganellar barrier: upon endolysosomal damage, luminal proteins may undergo pH-dependent condensation that immobilizes ASOs within the damaged compartment even when the limiting membrane is breached (4, *Preprint*). Further studies will help elucidate the molecular mechanisms driving this condensation and its quantitative contribution across different cellular models.

Against this backdrop, Marco et al. (5) present evidence for a cell type–specific endocytic pathway in pancreatic ductal adenocarcinoma (PDAC) (Fig. 1) that routes ASOs to a compartment permissive for escape. The pathway begins at the plasma membrane, where the scavenger receptor CD44—highly expressed in the squamous subtype of PDAC—binds ASOs directly (apparent K_D ~10.8 μM) and activates the ERK–p90RSK axis. This kinase cascade phosphorylates EPHA2 on Ser897, licensing its endocytosis and RAB17-dependent trafficking to the nuclear surface (6). The resulting nuclear-captured endosomes, which share features with recycling endosomes—a compartment previously associated with higher escape probability (7)—then undergo lipid peroxidation, become leaky, and release ASO cargo into the nucleus-proximal cytoplasm. KRAS-mutant PDAC cells constitutively express high levels of EPHA2 pSer897, a modification largely absent from adjacent non-transformed tissue, providing a potential basis for tumor selectivity. Loss of EPHA2 reduces ASO efficacy by more than 100-fold, and nuclear capture–defective EPHA2 mutants phenocopy the knockout, supporting the idea that the specific trafficking destination—not merely uptake—determines productive delivery.

One of the main findings of the study is that stress granule (SG; RNA-protein condensate) assembly at damaged endosomes limits ASO escape. As ASO-loaded endosomes become leaky, G3BP1-positive SG condensates form at these sites together with galectin-9 and canonical SG components including EIF3b and polyadenylated mRNAs, plugging membrane lesions. This SG-mediated repair function was previously shown at chemically, physically, and mycobacteria-damaged endosomes in human macrophages (8) and appears here to restrict the therapeutic window. Notably, neither ESCRT-III components, annexins, nor PITT pathway proteins were detectably recruited to ASO-containing endosomes in G3BP double-knockout (DKO) cells, suggesting SG condensates act upstream of or independently from canonical repair machinery in this context. Genetic ablation of G3BP1 and G3BP2, or pharmacological inhibition of SG assembly with ISRIB, strongly enhanced ASO-mediated KRAS knockdown in KPC cells without reducing uptake.

A key strength of the study is its use of disease-relevant models. Most mechanistic work on ASO trafficking has relied on generic cancer lines or non-transformed cells. Here, the authors use primary KPC cells derived from autochthonous mouse PDAC tumors, 3D spheroid models, and immunofluorescence of human PDAC surgical specimens confirming EPHA2 pSer897 expression in patient tumor nodules. Isogenic $Epha2^{+/+}$ and $Epha2^{-/-}$ KPC lines, combined with structure-function rescue using $EPHA2^{S897A}$ and $EPHA2^{NLS}$ mutants, provide

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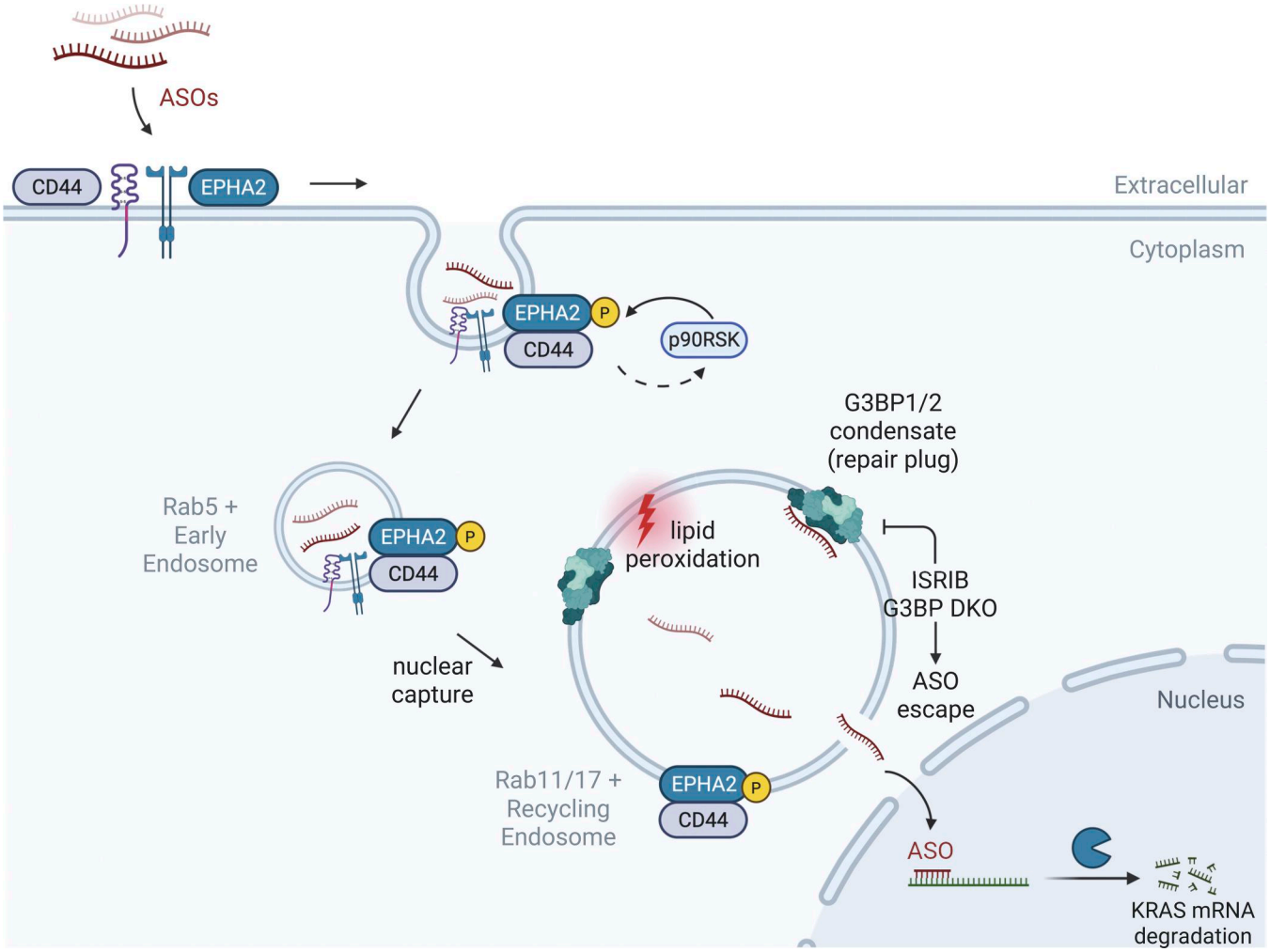

Figure 1. **The EPHA2/CD44 nuclear capture pathway and SG–mediated endosomal repair in KRAS-mutant PDAC cells.** ASOs (red) bind CD44 at the plasma membrane, triggering p90RSK-dependent phosphorylation of EPHA2 on Ser897 (P). The EPHA2–CD44 complex is co-endocytosed into RAB5-positive early endosomes and trafficked via RAB11/17-positive recycling endosomes to the nuclear surface (nuclear capture). At this nuclear-proximal compartment, lipid peroxidation renders the endosomal membrane leaky, enabling ASO escape into the perinuclear cytoplasm and subsequent nuclear entry, where ASOs direct KRAS mRNA degradation. G3BP1/2-nucleated condensates are observed at membrane rupture sites as repair plugs, limiting productive ASO escape. Pharmacological (ISRIB) or genetic targeting of SG assembly suppresses this repair response and enhances ASO-mediated KRAS knockdown. Figure generated using BioRender.com (https://www.biorender.com/).

a comprehensive mechanistic dissection of the pathway.

The role of G3BP1 in this context, however, raises questions worth debating. Lysosomal damage is known to induce SG formation in U2OS cells (9) and human macrophages (8), and G3BP proteins have been shown to bind LAMP1-positive lysosomes as part of the TSC–mTORC1 regulatory axis (10). In line with these studies, Marco et al. found a twofold - to 3.5-fold enhancement of KRAS mRNA knockdown when inhibiting SGs with ISRIB or in G3BP1/2 DKO cells. In contrast, in a recent preprint, Sitarska et al. (3, *Preprint*) found no recruitment of G3BP1 to damaged endosomes in U2OS cells. However, G3BP1/2 DKO in that context did induce a twofold to threefold increase in ASO effectiveness—a gain that may carry practical weight given that fewer than 4% of internalized ASOs reach the nucleus even under conditions of >90% target knockdown (3, *Preprint*). Importantly, pharmacological SG inhibitors did not phenocopy the DKO in U2OS cells but did so in KPC cells, pointing to a genuine mechanistic difference between the two systems (3, *Preprint* 5). Formal in vivo validation in autochthonous KPC models with ISRIB cotreatment, acknowledged by the authors as a priority, will be needed to determine whether these cellular gains translate to a meaningful therapeutic window. Heterogeneity in the molecular response between the present manuscript and other cellular models could reflect several nonexclusive possibilities: G3BP1 may have SG-independent roles in endolysosomal trafficking specific to the cellular context in which the EPHA2/CD44 pathway operates; the nuclear-captured endosomal compartment described by Marco et al. may be a distinct, more permissive subpopulation not engaged in commonly used cell lines; or the SG response itself may be heterogeneous enough that relying on a single marker is insufficient to capture its full diversity (11). Additional technical considerations include the reliance of many SG localization studies on overexpression rather than endogenous knock-in tagging, which can alter condensate

dynamics and recruitment thresholds, and the possibility that repair condensates at rupture sites are sub-diffraction structures not readily detected by the same means used for micrometer-scale condensates.

These considerations point to several open questions. Whether the EPHA2/CD44 nuclear capture pathway operates in other KRAS-driven tumors or is specific to PDAC remains open. Whether targeting other repair pathways synergizes with SG inhibition and how these pathways are coordinated at the same damaged membrane are questions that cannot yet be answered. More broadly, understanding the biophysical principles that govern how biomolecular condensates wet and plug damaged membranes, and characterizing the diversity of repair condensate types across different cellular models, will be important for interpreting these results in a wider framework (12). The in vivo data presented here are encouraging, but we are still far from a clear picture of how these mechanisms interact quantitatively in physiologically relevant settings.

What Marco et al. do establish is that the endosomal compartment reached by ASOs matters as much as the escape mechanism itself and that the cellular machinery responding to endosomal damage is a tractable target in at least one disease-relevant context. Whether this principle generalizes remains to be addressed.

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