# Lipid Carriers in Cancer: Context Matters

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Investigating immune suppression mechanisms in cancer may inform on strategies to overcome resistance to current immunotherapies, common across solid tumor types but near ubiquitous in pancreatic ductal adenocarcinoma (PDAC). A recent study by Kemp and colleagues in *Cancer Research* identified an immunomodulatory axis originating in tumor-associated macrophages whereby macrophage-derived apolipoprotein E (APOE) activates NF-κB in tumor cells in a paracrine manner, inducing expression of immunosuppressive chemokines. In contrast, APOE promotes antitumor immunity in other cancer types including melanoma, highlighting the context dependency of APOE signaling and its impact on the tumor microenvironment. As new immunotherapy approaches increasingly aim to modulate both the myeloid and lymphoid compartments of the PDAC immune milieu, identification of specific mechanisms that foster macrophage-mediated immune suppression may facilitate the development of effective strategies that enable the immune system to tackle these tumors.

*See related article by Kemp et al., p. 4305*

Pancreatic cancer is a highly aggressive disease characterized by immunosuppression and resistance to immunotherapies, and elucidating the features of the immunosuppressive pancreatic ductal adenocarcinoma (PDAC) microenvironment could reveal new therapeutic targets. To identify putative mediators of PDAC immune suppression, Kemp and colleagues analyzed single-cell RNA sequencing data from human PDAC as well as benign adjacent tissue and noted pervasive expression of cholesterol carrier encoding apolipoprotein E (*APOE*) among tumor-associated macrophages (TAM), with higher *APOE* expression in TAMs than in macrophages from benign pancreas (1). As APOE is a secreted protein, the authors measured plasma APOE levels that stratified PDAC patient survival, suggesting its potential utility as a biomarker as well as a potential role in disease progression. *APOE* was induced in macrophages by tumor cell–secreted factors, dependent on oncogenic KRAS signaling in the epithelial compartment. In $Apoe^{-/-}$ hosts, orthotopically transplanted PDAC cells displayed reduced tumor growth. The absence of APOE in the host reduced immune suppression, evidenced by increased $CD4^+$ and $CD8^+$ T cells, $GZMB^+CD8^+$ T cells, and $CD11b^+CD11c^+$ myeloid cells, and fewer regulatory T cells ($T_{reg}$), immature myeloid cells, and specifically monocytic myeloid-derived suppressor cells (MDSC). Host APOE promotes PDAC progression at least in part by suppressing antitumor T-cell responses, as T-cell neutralizing antibodies restored tumor growth in $Apoe^{-/-}$ mice. T-cell neutralization also reversed alterations in immature and $CD11b^+CD11c^+$ myeloid cell frequencies, consistent with reciprocal cross-talk between intratumoral T cells and myeloid cells facilitated by macrophage-derived (and potentially fibroblast-derived) APOE. Pancreatic cancer cells express four of the major APOE receptors: LDLR, VLDLR, LRP1, and LRP8. Functional studies implicated LDLR as the predominant receptor linking APOE signaling to immune suppression, though roles for the other receptors warrant further investigation perhaps with respect to other stroma-derived ligands. APOE-LDLR signaling induced expression of chemokines with established roles as myeloid cell chemoattractants including CCL2 and CXCL5. This axis also induced expression of CXCL1, an immunosuppressive secreted factor recently implicated as a cancer cell-intrinsic signal that restricts T-cell abundance in the PDAC microenvironment (2). Mechanistically, APOE-LDLR signaling activated canonical NF-κB signaling in PDAC cells, with implications for transcriptional regulation of the immunosuppressive chemokines examined in this study and for the broader transcriptional program regulated by this key inflammatory pathway.

These findings add meaningfully to our understanding of the functions of APOE in cancer. Prior work on APOE has focused predominantly on its role in cardiovascular and Alzheimer disease, and on its lipid homeostatic function in these contexts. While Kemp and colleagues convincingly implicate APOE as a modulator of the PDAC immune microenvironment via activation of NF-κB and induction of immunosuppressive chemokines such as CXCL1 and CXCL5, APOE may more broadly impact PDAC TAM function by regulating additional immunomodulatory nodes, as well as local cholesterol trafficking and lipid metabolism. *APOE* expression in PDAC TAMs positively correlated with expression of alternatively activated macrophage markers *MARCO* and *TREM2*. A population of $TREM2^{hi}$ macrophages was recently identified as a predicter of resistance to immune checkpoint therapy (3). Though not a focus of the study, single-cell differential expression analysis of this $TREM2^{hi}$ macrophage cluster revealed high expression of *APOE* as well. Whether macrophage *APOE* and *TREM2* expression predict response to immune checkpoint therapy in PDAC remains unclear, but it may gain relevance as combination therapeutic strategies come to light that foster efficacy of checkpoint inhibitors in PDAC.

As APOE functions as a lipid carrier and facilitates cellular uptake of cholesterol and lipoproteins, $Apoe^{-/-}$ mice have high plasma cholesterol, which may impact PDAC progression via multiple mechanisms. As noted by Kemp and colleagues, a recent study showed that genetic or pharmacologic means to reduce plasma cholesterol promoted the development of basal-like PDAC, the more aggressive subtype (4), such that the suppression of PDAC growth observed in $Apoe^{-/-}$ mice may be due in part to elevated plasma cholesterol promoting the less aggressive, classical subtype. In addition, a recent investigation of the role of sterol *O*-acyltransferase 1 (SOAT1) in PDAC revealed that cholesterol esterification is essential to prevent the negative feedback of cholesterol on the mevalonate pathway and to support PDAC cell proliferation *in vitro* and tumor growth *in vivo* (5). The hypercholesterolemia characteristic of $Apoe^{-/-}$ mice may be sufficient to

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suppress the mevalonate pathway and its diverse outputs required for PDAC progression, including the isoprenoid species needed for proper membrane localization and activity of RAS and RAS-related GTP-binding proteins. Interestingly, a recent study used a reductionist approach to demonstrate that cholesterol supplementation induces acinar-to-ductal metaplasia, an initiating step of pancreatic carcinogenesis, via generation of second messenger cyclic adenosine monophosphate (cAMP) and induction of downstream protein kinase A (PKA; ref. 6). However, cholesterol had no impact on cAMP/PKA signaling in PDAC cells, consistent with context-dependent roles for cholesterol signaling throughout pancreatic tumorigenesis and suggesting that systemic APOE inhibition may differentially impact PDAC initiation versus progression of invasive disease.

The context dependency of APOE signaling in cancer extends to anatomic site as well. A recent analysis of clear cell renal carcinoma (ccRCC) identified a TAM subpopulation marked by *APOE, TREM2*, and *C1Q* that is enriched in ccRCC compared with healthy kidney and was strongly prognostic of disease recurrence following surgical resection (7), suggesting a potential tumor-promoting role in the ccRCC microenvironment as in PDAC, though this remains to be validated functionally. However, in other cancer types, APOE signaling impacts the immune microenvironment to suppress tumor progression. *APOE* is a well-established transcriptional target of the liver-X receptors (LXRα and LXRβ), transcription factors in the nuclear receptor superfamily. Endogenous ligands for LXRs include oxysterols and other cholesterol metabolites, such that *APOE* induction upon ligand engagement by LXRs supports systemic cholesterol homeostasis. A pharmacologic LXR agonist reduced tumor growth and significantly prolonged survival in mouse models of melanoma, glioblastoma, and lung cancer; these therapeutic benefits were lost in $Apoe^{-/-}$ mice (8). The LXR/APOE axis reduced intratumoral MDSC abundance and increased antitumor T-cell activity, the opposite of APOE function in PDAC. Further mechanistic studies will be important to parse cancer cell-intrinsic (i.e., mutational signature) and/or tissue-specific (i.e., basement membrane or other architectural components) features that determine APOE function in cancers. Lessons learned from investigation of APOE function in metabolic disease may provide important mechanistic clues with respect to the interaction between APOE and the immune system. In the severe hyperlipidemia model featuring genetic loss of both *Apoe* and *Lxrb*, cholesterol accumulation in $CD11c^{+}$ antigen-presenting cells promotes T-cell priming in lymphoid organoids (9). Though this results in autoimmunity in the context of severe metabolic syndrome, cholesterol loading of myeloid cells and increased T-cell priming with APOE disruption in the context of cancer may potentially be beneficial. Consistent with this notion, acute APOE inhibition and associated hypercholesterolemia induced expansion of splenic $T_{FH}$, $T_{H1}$, and $T_{regs}$, as well as germinal center formation, plasma cell abundance, and autoantibody formation (10). While this systemic immune response is undesirable, therapeutic targeting of APOE in a manner that less profoundly increases cholesterol levels may be a potent way to promote immune responses against cancer. Additional mechanistic studies across different tumor types will be needed to assess this therapeutic potential.

In addition to studies of APOE-immune system interactions in cancers of diverse tissue contexts, this study by Kemp and colleagues inspires further investigation of APOE in the PDAC microenvironment. *Apoe* is expressed by PDAC TAMs, but also by cancer-associated fibroblasts (CAF). The relative significance of APOE production by each cellular compartment remains to be determined. Expression of *Apoe* specifically within the inflammatory CAF (iCAF) compartment of the heterogeneous CAF population raises the possibility that APOE derived from TAMs versus CAFs may preferentially impact distinct cell types in the tumor microenvironment. Spatially, iCAFs have been reported in stroma-dense regions relatively far from tumor cells, while classically activated, myofibroblastic CAFs more commonly juxtapose tumor cells. These spatial relationships, together with the findings of Kemp and colleagues, suggest that APOE secreted by iCAFs may act on cell types in the stroma including neighboring CAFs and immune cells, whereas APOE from TAMs may act on tumor cells to induce immunosuppressive chemokines. The distinct roles of APOE receptors in the PDAC microenvironment also warrants further investigation, as the particular arrays of lipoprotein receptor expression on cells of different tumor types would likely result in distinct downstream signaling responses and functional outputs, and may underlie the disparate effects of APOE signaling in cancer. Together, the recent studies described here motivate additional dissection of cholesterol signaling and mediators of cholesterol homeostasis in PDAC, in hopes that this improved understanding may identify new combination therapeutic strategies for this disease.

## Authors' Disclosures

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