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Preview

Senescence Inflames the Pancreatic Tumor Microenvironment

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Pancreatic adenocarcinomas (PDACs) are scarcely vascularized and thus virtually insensitive to chemotherapy and immunotherapy. In a recent issue of *Cell*, Lowe and collaborators[1] have demonstrated that senescence induction by MEK plus CDK4/CDK6 inhibitors favors PDAC revascularization coupled to infiltration by therapeutically actionable $CD8^+$ T cells.

Pancreatic adenocarcinomas (PDACs) are aggressive tumors with exceptionally dismal prognosis, at least in part owing to the robust stromal reaction that generally accompanies pancreatic oncogenesis. Indeed, growing PDACs establish a densely fibrotic and poorly vascularized tumor microenvironment (TME) that supports disease progression and resistance to treatment by limiting access to immune cells and chemotherapeutics.[2] Genetic alterations common in human PDACs, including activating mutations of KRAS proto-oncogene, GTPase (*KRAS*), and loss-of-functions mutations in tumor protein p53 (*TP53*), have been mechanistically linked to pancreatic oncogenesis amidst an intricate paracrine network that co-opt pancreatic stellate cells (PSCs) to drive fibrosis and local immunosuppression.[3] Such a stromal component has attracted attention as a potential target for the development of novel treatments.[2] However, no therapies targeting components of the pancreatic TME have been approved for use in patients with PDAC so far. Lowe and collaborators have recently demonstrated that the TME of PDACs undergoing cellular senescence[4] in response to trametinib, an inhibitor of mitogen-activated protein kinase kinase 7 (MAP2K7, best known as MEK), plus palbociclib, a cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitor, supports an angiogenic response that enables the recruitment of therapeutically actionable $CD8^+$ cytotoxic T lymphocytes (CTLs) (Figure 1).[1]

Lowe and colleagues set to investigate the efficacy of trametinib plus palbociclib (T/P) *in vivo*, in immunocompetent mouse models of PDACs driven by *Kras* and *Trp53* (the mouse ortholog of human *TP53*) mutations. At odds with previous findings in models of KRAS-driven lung carcinomas,[5] T/P had limited therapeutic efficacy as it failed to recruit natural killer (NK) cells. Instead, T/P administration favored the generation of $CD31^+$ blood (but not lymphatic) vessels harboring open lumens coupled to a reduction in stromal hyaluronic acid. This vascularization response was preserved in immunodeficient mice and depended on the secretion of pro-angiogenic factors including vascular endothelial growth factor A (VEGFA) by PDAC cells responding to T/P with a permanent proliferative arrest known as cellular senescence.[4] Such a senescence-associated secretory phenotype (SASP) originated from NF-κB signaling in PDAC cells, as demonstrated with a short-hairpin RNA (shRNA) targeting RELA proto-oncogene, NF-kB subunit (RELA, best known as p65). Vascularization depended on VEGFA signaling in endothelial cells (ECs) via kinase insert domain receptor (KDR), as shown with the KDR-specific antibody DC101. Conversely, EC activation linked to expression of markers such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) was mainly driven by interleukin 6 (IL6), C-C motif chemokine ligand 5 (CCL5) and C-X-C motif chemokine ligand 1 (CXCL1).[1]

T/P pre-administration improved the delivery of gemcitabine (a common chemotherapeutic) to mouse PDACs, an effect that (1) relied on KDR-mediated vascularization, (2) extended the survival of immunocompetent PDAC-bearing mice, and (3) could be recapitulated in immunodeficient mice bearing human PDAC xenografts. Depletion experiments confirmed that NK cells are not involved in the partial therapeutic efficacy of T/P in immunocompetent mice. Moreover, no differences were detected in the abundance of tumor-infiltrating neutrophils, myeloid-derived suppressor cells (MDSCs), and immunosuppressive $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells. Conversely, T/P-treated PDACs efficiently recruited $CD8^+$ CTLs downstream of NF-κB signaling in PDAC cells, KDR-dependent vascularization, and VCAM1 expression by ECs. Supporting some degree of direct immunostimulation,[6] PDAC cells responding to T/P exposed increased levels of MHC class I molecules on their surface, which favored CTL activation in a diverse experimental setting harnessing the model antigen ovalbumin. However, CTL depletion failed to affect the partial therapeutic activity of T/P in immunocompetent PDAC models, largely reflecting the exhausted phenotype of CTLs infiltrating T/P-treated PDACs, which encompassed high surface levels of the co-inhibitory receptor

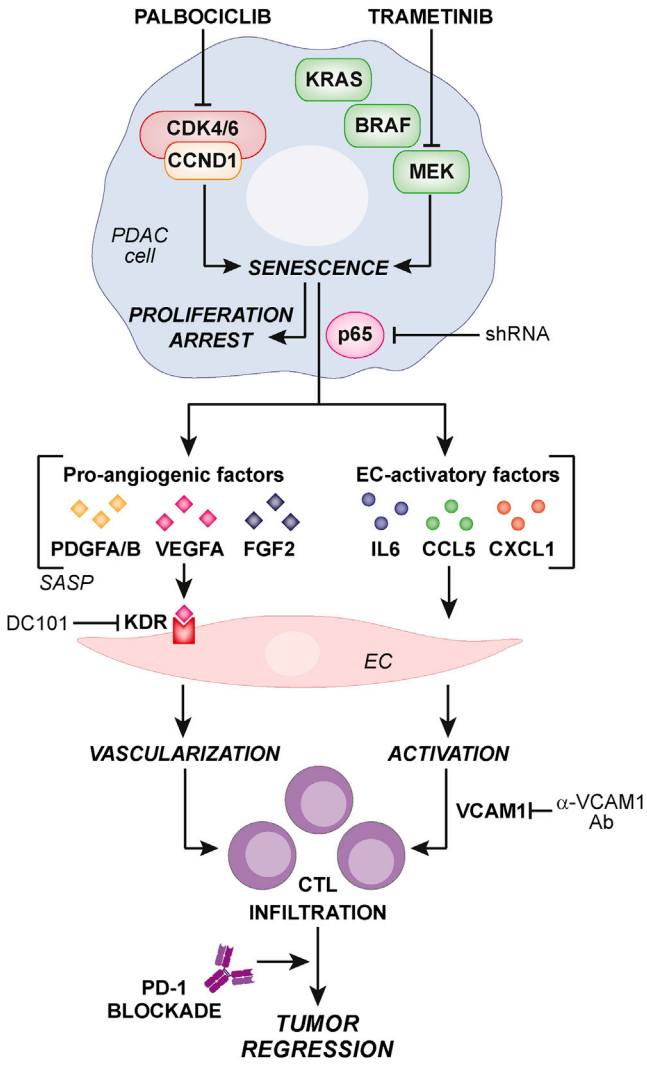

Figure 1. Cellular Senescence Renders the Microenvironment of PDACs Actionable with Immunotherapy

Palbociclib plus trametinib cause senescence in PDAC cells, resulting in the secretion of bioactive factors that induce EC activation and neovascularization. Such functional blood vessels support the delivery of chemotherapeutic agents (e.g., gemcitabine), as well as tumor infiltration by CTLs. Although the CTLs that infiltrate palbociclib- and trametinib-treated PDACs are exhausted and hence fail to mediate therapeutic effects per se, addition of a PD-1 blocker efficiently restores CTL effector functions to enable superior therapeutic activity. Ab, antibody; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CCND1, cyclin D1; FGF2, fibroblast growth factor 2; PDGF, platelet derived growth factor.

programmed cell death 1 (PDCD1, best known as PD-1). Consistent with this, co-administration of a PD-1 blocker favored the activation of PDAC-infiltrating CTLs, correlating with superior therapeutic activity *in vivo*. Globally corroborating the working model from Lowe and collaborators (Figure 1), the therapeutic effects of T/P plus a PD-1 blocker were compromised when p65 was downregulated in PDAC cells, KDR or VCAM1 were antagonized, or CTLs were depleted.[1]

Interestingly, pharmacological inhibition of $KRAS^{G12C}$ has been reported to mediate immunostimulatory effects linked to immunogenic cell death (ICD)[7] in a variety of $KRAS^{G12C}$-driven tumor models.[8] Consistent with this, the $KRAS^{G12C}$ inhibitor AMG 510 synergized with a PD-1 blocker in the eradication of $KRAS^{G12C}$-expressing mouse colorectal tumors established in immunocompetent syngeneic mice,[8] hence resembling T/P in mice bearing KRAS-driven PDACs.[1] Further supporting a role for ICD, PDACs exposed to T/P exhibited poor caspase 3 (CASP3) activation, and recruited high amounts of (exhausted) CTLs,[1] whereas (1) neoplastic lesions undergoing non-immunogenic cell death are characterized by robust CASP3 activation and poor infiltration by immune cells,[7] and (2) cellular senescence per se preferentially recruit NK cells.[5,9,10] However, whether PDAC cells exposed to T/P *in vitro* elicit prophylactic immunity (once inoculated in tumor-naive, immunocompetent, syngeneic mice) against living cells of the same type (which would be indicative of ICD induction) remains to be determined.

Irrespective of these unknowns, the findings by Lowe and collaborators suggest that combining T/P and a PD-1 blocker (all of which are approved by the USFDA for cancer therapy) stands out as a promising approach for patients with PDAC. Clinical trials investigating the safety and efficacy of this combinatorial regimen are awaited.

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