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Heparin Prowess: Favorable Vascular–Immune Reprogramming in Pancreatic Cancer

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SUMMARY

Several approaches for overcoming immunotherapy resistance in pancreatic and colorectal cancer syngeneic models were assessed using heparin and immunotherapy. Beneficial responses were attributed to heparin-induced vascular normalization, ensuing $CD8^+$ T-cell infiltration, and M1 macrophage polarization, suggesting the potential for heparin-anchored therapies in cold tumors such as pancreatic cancer.

See related article by Wei et al., p. 2525

In this issue of *Clinical Cancer Research*, Wei and colleagues (1) combined low molecular weight heparin (LMWH) with either adoptive cell transfer (ACT) or an anti–programmed cell death protein 1 (PD-1) checkpoint inhibitor in syngeneic mouse models of pancreatic cancer and colorectal cancer. Three distinct cell lines were used. The Panc02 murine pancreatic cancer cell (PCC) line yielded immune desert (cold) tumors, the MC38 murine colorectal cancer cells formed inflamed (hot) tumors, whereas the CT26 colorectal cancer murine cells generated immunologically intermediate immune-excluded tumors. LMWH alone did not alter subcutaneous tumor growth of any of these cell lines, and did not affect the growth of orthotopically implanted Panc02 cells. ACT, using spleen-derived lymphocytes that were activated with IL2 and concanavalin A, yielded modest results. In contrast, the combination of LMWH with either ACT or an anti—PD-1 antibody exerted beneficial effects that included elimination of a hypercoagulable state, suppression of aberrant angiogenesis with improved blood flow, enhanced penetration of $CD8^+$ T cells into the tumor microenvironment (TME), reprogramming of M2 deleterious macrophages into M1-protective macrophages, a decrease in $FOXP3^+$ T-regulatory (Treg) cells, attenuated tumor growth, suppressed metastasis in the orthotopic pancreatic cancer model, and prolonged survival in mice with orthotopic pancreatic cancer tumors 9 (**Fig. 1**).

Pretreatment, in the subcutaneous models, there were foci of tumor necrosis, leaky blood vessels, and emboli, all of which were most evident in immune desert and excluded tumors where LWMH mitigated their formation without promoting $CD8^+$ T-cell infiltration. In contrast, in the inflamed tumors, LMWH increased the number of $CD8^+$ T cells. Wei and colleagues (1) tested the hypothesis that combining ACT with LMWH will not only eliminate the coagulopathy in the Panc02 immune desert tumors and CT26 immune excluded tumors, but will also enhance $CD8^+$ T-cell tumor infiltration. That is exactly what they observed. Moreover, using multiplex histochemistry they demonstrated that there was a concomitant increase in $CD8^+/PD\text{-}1^+$ T cells, representing a subpopulation of recently activated and immunologically robust pool of $CD8^+$ T cells. In contrast, the number of MHC class II–restricted $CD4^+$ T cells did not increase. Using HALO analysis, they also showed that there was a decrease in the number of Treg cells in the subcutaneous models both at the tumor edge and its center, suggesting a blunting of Treg cell–mediated immunosuppression.

Macrophages are an important component of the TME (2), and Wei and colleagues (1) next tested the hypothesis that LMWH may modulate macrophage polarization. They determined that none of the treatments altered macrophage numbers in the three subcutaneous tumor groups. However, LMWH increased the number of $iNOS^+$ proimmune (M1) macrophages. This effect was enhanced when LMWH was combined with ACT. Moreover, LMWH, but not ACT, markedly decreased $CD206^+$ M2 macrophages. Combining LMWH with ACT caused a further decrease in $CD206^+$ macrophages, decreased cancer cell proliferation and increased cancer cell apoptosis, pointing to the induction of antitumor activity by the combination therapy. Combining LMWH and $CD8^+$ T-cell administration suppressed pancreatic cancer growth in the orthotopic Panc02 model, whereas combining LMWH and non-$CD8^+$ T cells was ineffective. Thus, it was the $CD8^+$, MHC class I–restricted T cells that were mediating a successful therapeutic response in the presence of LMWH.

A dual approach was used to test the hypothesis that LMWH induced vascular normalization and promoted $CD8^+$ T-cell penetration into the tumor. The authors intravenously injected FITC-labeled BSA in mice with Panc02 orthotopic tumors and determined that LMWH caused deeper penetration of FITC–BSA into the tumor and increased functional vascularity, confirmed by using DyLightTM594-labeled tomato lectin. Imaging with *in vivo* two-photon microscopy demonstrated that LMWH enabled $CD8^+$ T cells to efficiently infiltrate into orthotopic Panc02 tumors. These findings are in line with the importance of a normal vasculature and healthy pericyte architecture in decreasing pancreatic cancer growth and enabling efficient drug delivery (3).

The combination of LMWH with either ACT or anti—PD-1 antibody prolonged the survival of mice bearing Panc02 orthotopic tumors because LMWH promoted vascular normalization, thereby allowing $CD8^+$ T-cell penetration into the tumors. LWMH did not alter PD-1 or CD69 expression in immune cells, suggesting that LMWH did not directly modulate immune cell functions. Moreover, LMWH did not directly affect PCC growth nor promote lymphocyte-mediated PCC cytotoxicity. Therefore, LMWH-mediated antitumor activity was not caused through direct immunomodulatory or cytotoxic actions. Instead, LMWH increased the number of pericyte-covered blood vessels while decreasing VEGF-A and basic fibroblast growth factor (bFGF) expression, aberrant tumor angiogenesis, interstitial fluid pressure (IFP), and Fas-ligand (FasL) levels on tumor endothelial cells. The net result was vascular normalization and an

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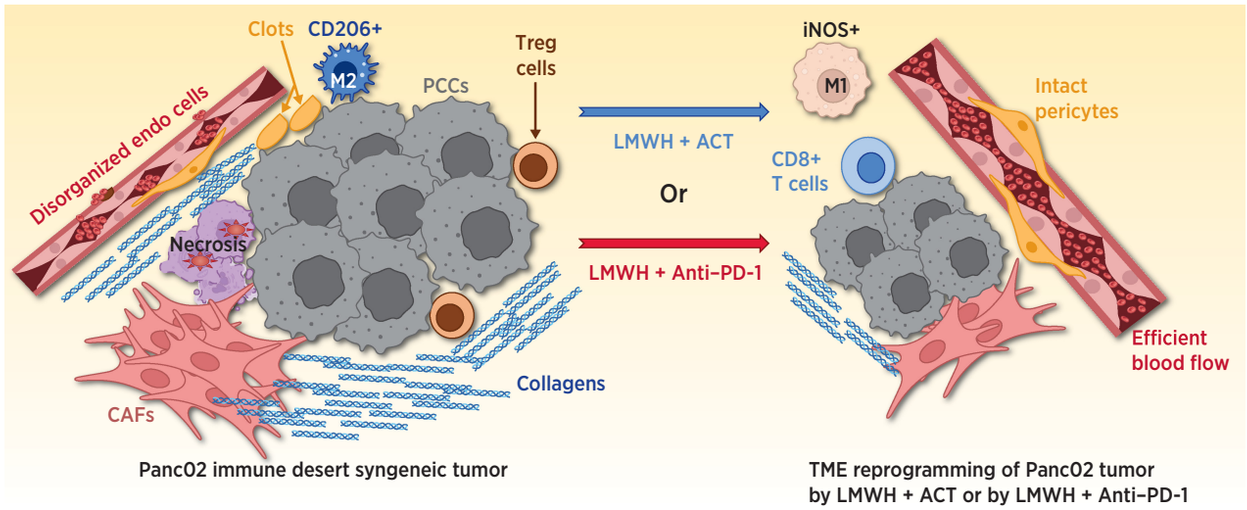

Figure 1.

Beneficial role of LMWH in pancreatic cancer and other cold cancers. Image on the left depicts an orthotopic Panc02 tumor in a syngeneic mouse as an example of a desert-immune tumor that is also known as a cold tumor, in which cancer-directed immune mechanisms are suppressed. The PCCs are surrounded by a desmoplastic stroma that includes CAFs that synthesize collagens, foci of necrosis, regions with micro-emboli, and coagulated blood (clots) that had leaked from the damaged tumor vasculature, M2 macrophages (Macs) that express CD206 ($CD206^+$), $FOXP3^+$ Treg, and a compressed vasculature that exhibits endothelial (Endo) cell damage and disorganization with loss of pericytes. The tumor was not responsive to immunotherapy and exhibited high IFP. Image on the right shows a Panc02 orthotopic tumor that had been treated with LMWH in combination with either ACT or an anti–PD-1 antibody. Both combinatorial strategies produced similar beneficial results. First and foremost, LMWH acted to normalize the tumor vasculature, with reappearance of pericytes, a decrease in IFP, normalization of the hypercoagulable state, and increased functional blood flow. Consequently, there was an influx into the tumor of $CD8^+$ T cells, a decrease in Treg cells, and a switch to beneficial M1 macrophages that expressed inducible nitric oxide ($iNOS^+$). In addition, there was a decrease in IFP, decreased stroma fibrosis, and impressive survival prolongation. These changes support the concept that vascular normalization with LMWH could represent a useful strategy in certain subgroups of patients with pancreatic cancer and other cold tumors and could lead to high-impact combinatorial therapies.

improved immune profile. FasL loss impeded its cytotoxicity toward $CD8^+$ T cells without altering $FOXP3^+/CD8^+$ T-reg cell numbers that express FADD-like IL1β-converting enzyme-inhibitory protein (c-FLIP) that protects Treg cells from apoptosis (4).

LMWH also reversed the fibrotic state in Panc02 tumors, underscoring the difference between this model and pancreatic cancer in genetically engineered mouse models (GEMM) in which fibrosis is not readily reversible. The pancreatic cancer stroma in autochthonous models contains different types of cancer-associated fibroblasts (CAF) some of which exhibit deleterious immune-suppressive properties although other CAFs may be protective (5–8). Depending on the GEMM, stroma targeting in pancreatic cancer may either promote PCC spread and metastasis or improve response to checkpoint inhibition (7–8), underscoring the complexity of the TME that is further burdened by pancreatic cancer heterogeneity, the existence of many CAF subtypes, multitude aberrant signaling and metabolic pathways, marked hypoxia (9), and an immune-modulating microbiome. In contrast, Panc02 cells, originally derived from male C57BL/6 mice injected with 3-methylcholanthrene, do not form such complex tumors and do not harbor *Kras* or *Trp53* mutations (10) that are crucial contributors to immune suppression in cancer (11–12).

Pancreatic cancer is the third-leading cause of cancer-related deaths in the United States, and the 5-year survival rate, while improving, is approximately 12% (13). The study by Wei and colleagues (1) supports the hypotheses that LMWH reprograms the pancreatic cancer TME and impedes its coagulopathy, promotes vascular normalization, decreases IFP, enhances $CD8^+$ T-cell influx into immune desert tumors while decreasing deleterious Treg cells, reverses immune suppression when administered with either ACT or an anti–PD-1 antibody, and promotes M1 macrophage polarization (**Fig. 1**). Thus, the less complex Panc02 model highlighted the potential utility of LMWH in solid cold tumors. These findings could impact pancreatic cancer therapeutics by stimulating studies to assess LMWH benefits in novel combination therapies for enhancing immune surveillance such as combining PD-1 and CTLA4 blockade, or targeting ligands such as TGFβ and CXCL12 to block their immune exclusion actions. LMWH could also be administered with FDA-approved therapies such as gemcitabine together with nab-paclitaxel, targeted therapies based on gene mutations (such as mutant-specific KRAS inhibitors), gene amplifications or fusions, and perhaps in conjunction with chemoradiotherapy in the primary and neoadjuvant settings. LMWH may also facilitate targeting deleterious immune cells, such as targeting pancreatic cancer macrophages that express V-domain Ig suppressor of T-cell activation (VISTA), a potent suppressor of $CD8^+$ T-cell proliferation and activation (14). Given the heterogeneity of pancreatic cancer in humans (15), it will also be important to establish clinically validated biomarkers to help guide therapeutic combinations with LMWH.

Author's Disclosures

No disclosures were reported.

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References

1. Wei F, Su Y, Quan Y, Li X, Zou Q, Zhang L, et al. Anticoagulants enhance molecular and cellular immunotherapy of cancer by improving tumor microcirculation structure and function and redistributing tumor infiltrates. Clin Cancer Res 2023;29:2525–39.
2. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. Nat Rev Drug Discov 2022; 21:799–820.
3. McCarty MF, Somcio RJ, Stoeltzing O, Wey J, Fan F, Liu W, et al. Overexpression of PDGF-BB decreases colorectal and pancreatic cancer growth by increasing tumor pericyte content. J Clin Invest 2007;117: 2114–22.
4. Plaza-Sirvent C, Schuster M, Neumann Y, Heise U, Pils MC, Schulze-Osthoff K, et al. c-FLIP Expression in Foxp3-Expressing cells is essential for survival of regulatory T cells and prevention of autoimmunity. Cell Rep 2017;18:12–22.
5. Guerra C, Barbacid M. Genetically engineered mouse models of pancreatic adenocarcinoma. Mol Oncol 2013;7:232–47.
6. Collisson EA, Bailey P, Chang DK, Biankin AV. Molecular subtypes of pancreatic cancer. Nat Rev Gastroenterol Hepatol 2019;16:207–20.
7. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell 2014;25:719–34.
8. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 2014;25:735–47.
9. Biffi G, Tuveson DA. Diversity and biology of cancer-associated fibroblasts. Physiol Rev 2021;101:147–76.
10. Logsdon CD, Arumugam T, Ramachandran V. Animal models of gastrointestinal and liver diseases. The difficulty of animal modeling of pancreatic cancer for preclinical evaluation of therapeutics. Am J Physiol Gastrointest Liver Physiol 2015;309:G283–91.
11. Ischenko I, D'Amico S, Rao M, Li J, Hayman MJ, Powers S, et al. KRAS drives immune evasion in a genetic model of pancreatic cancer. Nat Commun 2021;12: 1482–597.
12. Ghosh M, Saha S, Bettke J, Nagar R, Parrales A, Iwakuma T. Mutant p53 suppresses innate immune signaling to promote tumorigenesis. Cancer Cell 2921;39:494–508.
13. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48.
14. Blando J, Sharma A, Higa MG, Zhao H, Vence L, Yadav SS, et al. Comparison of immune infiltrates in melanoma and pancreatic cancer highlights VISTA as a potential target in pancreatic cancer. Proc Natl Acad Sci U S A 2019;116: 1692–7.
15. Connor AA, Gallinger S. Pancreatic cancer evolution and heterogeneity: integrating omics and clinical data. Nat Rev Cancer 2022;22:131–42.