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LETTER TO THE EDITOR

Targeting stiff stroma by neutralizing LOX-mediated matrix crosslinking in pancreatic cancer

KEY WORDS

Stiffness;
Extracellular matrix;
Pancreatic ductal adenocarcinoma;
Elastic modulus

To the editor:

Pancreatic ductal adenocarcinoma (PDAC) is characterized by extensive desmoplasia, which leads to a dense and fibrotic stroma[1]. The fibrotic milieu observed in PDAC is predominantly attributed to the crosslinking of the extracellular matrix (ECM), a process orchestrated by enzymes such as lysyl oxidase (LOX) and transglutaminase. The LOX family comprises LOX and LOX-like (LOXL) proteins 1−4 that catalyze the initial step in the crosslinking of ECM constituents, including collagens and elastin[2]. The oxidative deamination of selected lysyl and hydroxylysyl residues by LOX and LOXLs produces allysine or hydroxyallysine, which then spontaneously form covalent intra- and inter-molecular crosslinks with other aldehydes or lysine groups in collagen and elastin. As collagen and elastin fibers become more crosslinked, the structural integrity of the ECM is enhanced, resulting in increased tissue stiffness. The stiff stroma can create a more rigid microenvironment that influences various aspects of tumor biology[3].

Recently, a first-in-class pan-LOX inhibitor has been identified, demonstrating the ability to reduce stromal matrix density and potentiate chemotherapy in PDAC[4]. Employing genetically engineered mouse models that enable conditional *Loxl2* knockout and overexpression, Alonso-Nocelo et al.[5] revealed that *Loxl2* ablation significantly diminishes metastasis and improves overall survival, and opposite results are observed with *Loxl2* overexpression[5]. Notably, the oncogenic functions of LOXL2 are potentially associated with non-cell autonomous factors and processes, particularly those involving ECM stiffness and mechanosignalling. In a murine orthotopic syngeneic PDAC model, ECM ablation with anti-LOXL2 resulted in lower tissue stiffness and accelerated tumor progression[6]. Likewise, the deletion of type I collagen in a mouse model of spontaneous PDAC reduced tissue stiffness and accelerated the emergence of pancreatic intraepithelial neoplasia (PanIN) lesions and PDAC[7]. Despite these insights, the precise impacts of LOX-dependent matrix crosslinking on PDAC, particularly in cases that occur spontaneously, still require further investigation.

Here, we generated a LOX neutralizing antibody that only inhibits extracellular LOX activity and verified its impacts in an autochthonous setting. LOX inhibition blocks collagen crosslinking in several preclinical models[8,9]. KPC mice (*LSL-Kras*$^{G12D/+}$; *LSL-Trp53*$^{R172H/+}$; *Pdx1-Cre*), aged around 10 weeks, were employed since they have extensive advanced pancreatic neoplasia at this timepoint[10]. Two different strategies of LOX inhibition were performed: 1) early phase intervention, KPC mice were treated with anti-LOX for 4 weeks, followed by isotype IgG for another 4 weeks and then sacrificed; 2) late phase intervention, KPC mice were first isotype IgG and continued with a 4-week period anti-LOX treatment (Fig. 1A and Supporting Information Fig. S1A). LOX inhibition, at either early or late phases, effectively diminished serum LOX activity in KPC mice (Fig. 1B).

Compared to the control group (isotype IgG), early LOX inhibition had mild effects on the overall collagen matrix abundance but drastically reduced the width, length, and straightness of collagen fibers in KPC mice (Fig. 1C−E). Scanning electron microscope showed that the frequency of tightly attached, intertwined, and thick collagen fibers was substantially reduced by early LOX inhibition (Fig. 1E and F). To strengthen the robustness

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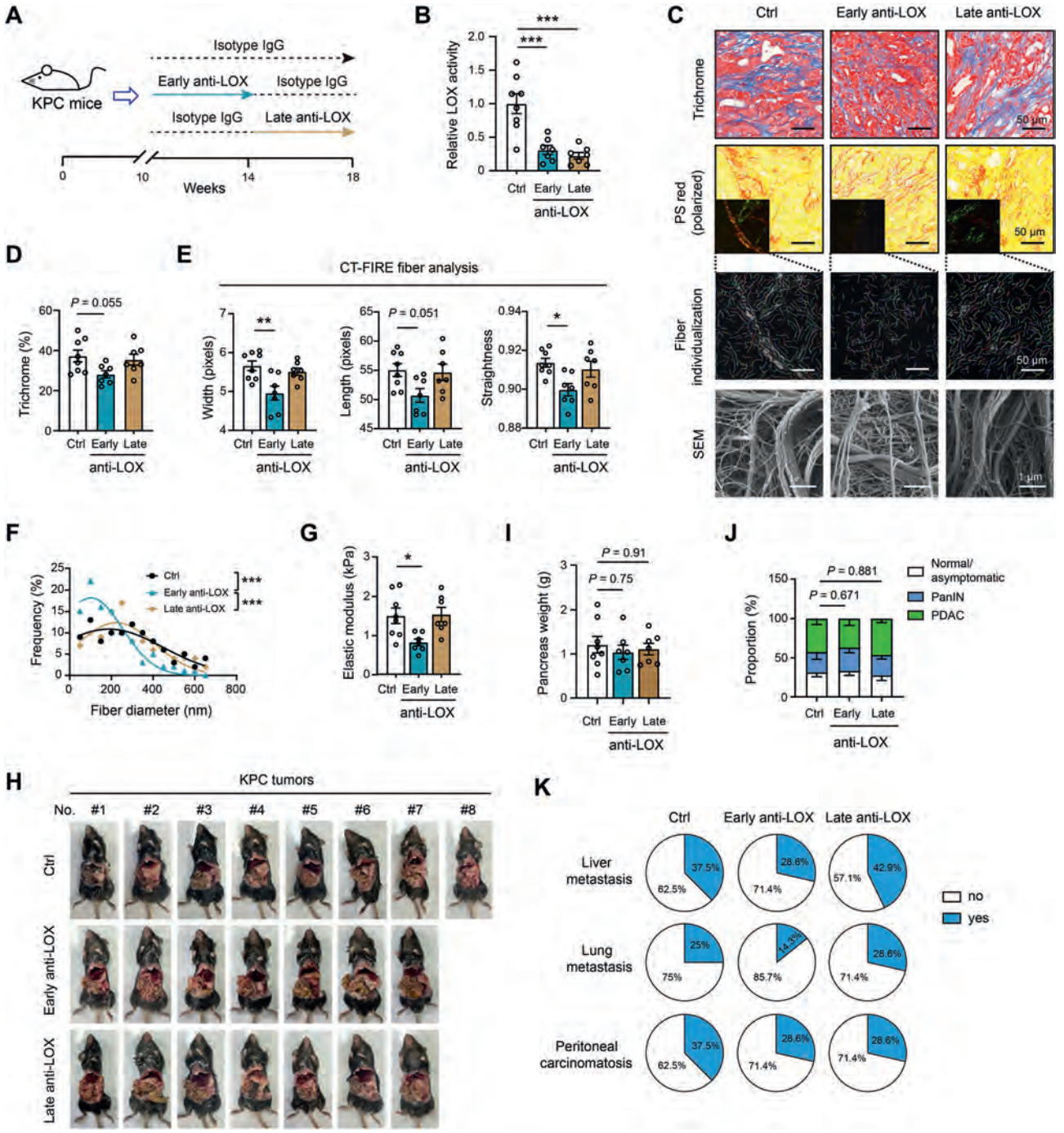

Figure 1 Neutralizing LOX-mediated matrix crosslinking in a murine model of PDAC. (A) Experimental design of anti-LOX treatment in KPC mice. (B) LOX activity after early anti-LOX and late anti-LOX treatment. (C) Representative histology images, obtained by Trichrome stain, picrosirius red (including polarized images and collagen fiber individualization analysis), and scanning electron microscope (SEM). Scale bar, 1 and 50 μm as indicated. (D) Quantification of Trichrome (collagen content). (E) Quantification of collagen fiber width, length, and straightness. (F) Frequency distribution of collagen fiber diameter (selected from 5 fields of SEM images). (G) Young's modulus of fresh tumor samples, measured by atomic force microscopy. (H) Images of KPC mice from each group. (I) Pancreas weight of KPC mice with early anti-LOX and late anti-LOX treatment. (J) The bar graph shows the quantification of normal pancreas, asymptomatic tissue (histologically normal), pancreatic intraepithelial neoplasia (PanIN), and PDAC areas. (K) Pie charts showing the impacts of anti-LOX on liver metastasis, lung metastasis, and peritoneal carcinomatosis. In this experiment, $n = 8$ for control, $n = 7$ for early anti-LOX, and $n = 7$ for late anti-LOX. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Values are represented as mean ± SD and compared by the one-way ANOVA multiple comparisons with Tukey's method among groups or Fisher's exact test.

of this finding, we analyzed the elastic modulus in fresh PDAC tissues and found that LOX inhibition attenuated the elastic modulus in early-phase disease but not late-stage disease (Fig. 1G). However, neither early-phrase nor late-phrase LOX inhibition delayed PDAC malignant transformation (Fig. 1H−J), as evidenced by the area of PanIN and PDAC lesions. Moreover, LOX blocking did not impact body weight, liver metastasis, lung metastasis, and peritoneal carcinomatosis (Fig. 1K and Fig. S1B).

By analyzing the tumor cells and stromal components, we found that inhibition of LOX activity did not substantially affect histological differentiation and the proliferative capacity or apoptosis of tumor epithelial cells (Fig. S1C−S1E). Moreover, early LOX inhibition led to a moderate increase in the number of $CD31^+$ vessels and the populations of overall intratumoral infiltration of $CD45^+$ cells as well as $CD8^+$ T cells in KPC mice (Fig. S1F−S1H). Interestingly, inhibition of LOX activity early but not late resulted in less nerve area in KPC tumors, as demonstrated by $PGP9.5^+$ nerves (Fig. S1I), suggesting a link between ECM stiffness and tumor innervation.

In summary, our findings demonstrate that early LOX inhibition can modify the collagen architecture, leading to a decrease in collagen fiber thickness and an alteration in the tumor's mechanical properties. Interestingly, while this intervention did not delay PDAC progression, it resulted in increased vascularization, immune cell infiltration, and reduced tumor innervation, suggesting a potential shift in the tumor microenvironment dynamics. Thus, these stromal or tumor alterations, whether pro-tumorigenic or anti-tumorigenic, may counterbalance each other and influence PDAC biology. Indeed, the stroma in PDAC can exhibit tumor-restricting potential, with complete ablation of stromal components sometimes leading to enhanced tumor growth. This duality underscores the necessity of understanding the complex crosstalk between tumor cells and their surrounding stroma. The ineffectiveness of late LOX inhibition can be attributed to the advanced stage of the disease, which features significant levels of pre-existing cross-linked collagen. Rather than a one-size-fits-all approach, it is more reasonable to employ combined targeting of signals within the stroma to achieve therapeutic benefits. As illustrated in our study, the implications of targeting ECM stiffness extend beyond merely inhibiting LOX activity. Combining LOX inhibitors with other treatment modalities, such as chemotherapy or immunotherapy, may potentiate more therapeutic efficacy. For instance, the observed increase in immune cell infiltration resulting from LOX inhibition suggests that this approach could sensitize tumors to immunotherapeutic strategies. Given the ECM is highly dynamic[11], a comprehensive strategy that integrates insights from tumor biology, ECM dynamics, and therapeutic interventions will be crucial in the fight against this devastating disease. Last but not least, the reduction of tumor innervation caused by LOX inhibition suggests that matrix stiffness plays a role in driving PDAC innervation, potentially explaining the mechanism through which tumors recruit nerves.

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Author contributions

Shan Zhang: Writing original draft, Methodology, Investigation, Formal analysis, Data curation. Zhiwei Cai: Resources, Methodology, Investigation, Formal analysis. Luju Jiang: Visualization, Investigation. Shuqi Cai: Methodology, Investigation, Formal analysis, Data curation. Zheqi Weng: Validation, Visualization, Investigation. Shu-Heng Jiang: Review & editing, Supervision, Funding acquisition, Project administration, Conceptualization.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.03.010.

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