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Mini Review

Deciphering Neoadjuvant Therapy Resistance in Pancreatic Cancer: Single-Cell and Spatial Transcriptomic Insights into Cancer-Associated Fibroblasts

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Abstract

Cancer-Associated Fibroblasts (CAFs) constitute the predominant stromal component in Pancreatic Ductal Adenocarcinoma (PDAC) and orchestrate adaptive resistance mechanisms that limit Neoadjuvant Therapy (NAT) efficacy. Despite recognition of CAF heterogeneity as a critical determinant of treatment outcomes, the molecular mechanisms governing CAF-mediated chemoresistance remain incompletely understood. Recent advances in single-cell RNA sequencing and spatial transcriptomics have illuminated the remarkable plasticity of stromal populations and their dynamic interactions within the tumor microenvironment. This commentary synthesizes emerging evidence demonstrating that a distinct myofibroblastic CAF subset characterized by elevated P4HA1 expression establishes spatially defined resistance niches that compromise NAT responses in PDAC. We discuss how P4HA1$^+$ mCAFs exploit paracrine signaling through TIMP1 to engage CD63/ITGB1 receptor complexes on malignant cells, thereby activating PI3K/AKT pro-survival pathways that sustain proliferation and confer gemcitabine tolerance. Furthermore, we examine the therapeutic implications of precision nanomedicine approaches designed to simultaneously disrupt stromal P4HA1 expression while enhancing chemotherapeutic delivery. By integrating multi-omics profiling with functional validation studies, this work establishes P4HA1$^+$ mCAFs as central mediators of NAT resistance and proposes that targeting the P4HA1-TIMP1-CD63/ITGB1 signaling axis represents a promising strategy to overcome stromal-mediated chemoresistance in PDAC.

Keywords: Cancer-associated fibroblasts, Pancreatic cancer, Neoadjuvant therapy, Single-cell RNA sequencing, Prolyl 4-hydroxylase subunit alpha 1

Highlights

a) P4HA1^{+} myofibroblastic CAFs are enriched in neoadjuvant therapy-resistant PDAC tumors.

b) P4HA1^{+} myofibroblastic CAFs establish chemoresistant niches through TIMP1-mediated stromal-epithelial crosstalk.

c) S-SNACs@GEM@Cas-P4HA1 nanoplatform targets stromal P4HA1 to reverse chemoresistance.

d) P4HA1+ mCAFs expression acts as a biomarker for monitoring PDAC treatment response and evaluating postoperative prognosis.

Background

PDAC remains among the most lethal malignancies, with projected escalation to the second leading cause of cancer mortality by 2040 and a persistently dismal 5-year survival rate of approximately 13% [1]. The therapeutic landscape for PDAC has evolved considerably with the implementation of neoadjuvant therapy strategies, which demonstrate potential to improve rates of R0 resection and prolonging event-free survival in patients with borderline resectable and locally advanced disease [2]. Recent clinical investigations, including the PREOPANC-1 and Prep-02/JSAP05 trials, have validated the clinical utility of preoperative chemotherapy regimens, reporting improvements in overall survival and progression-free intervals compared to upfront surgical resection [3,4]. However, the considerable heterogeneity in patient responses to standardized NAT protocols underscores fundamental gaps in our understanding of resistance mechanisms operating within the Tumor Microenvironment (TME) (Figure 1).

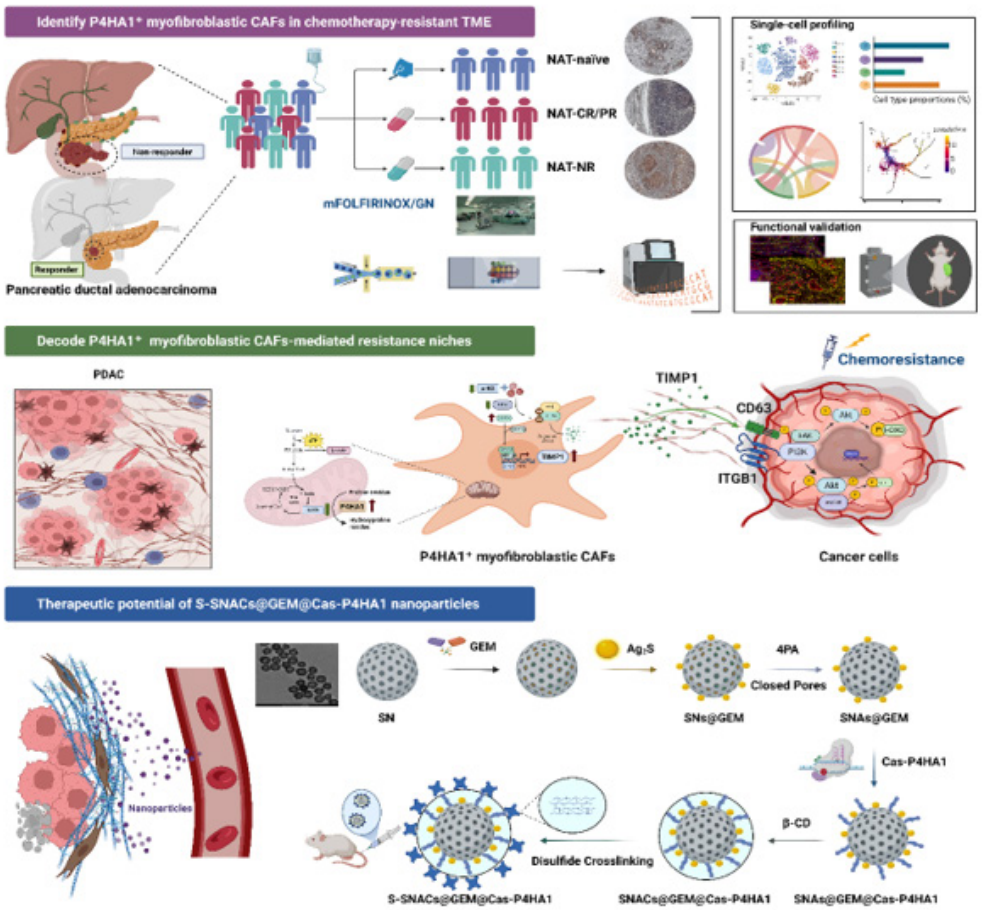

Figure 1:

The intractable TME of PDAC represents a formidable barrier to therapeutic efficacy, with CAFs emerging as principal architects of treatment resistance [5]. CAFs constitute the predominant stromal population and orchestrate diverse protumorigenic functions including extracellular matrix remodeling, immunosuppressive signaling, and metabolic reprogramming that collectively impede drug delivery and promote cancer cell survival [6]. The remarkable phenotypic plasticity of CAF populations, encompassing Myofibroblastic CAFs (mCAFs), Inflammatory CAFs (iCAFs), and Antigen-Presenting CAFs (apCAFs), reflects distinct functional specializations that dynamically evolve in response to therapeutic pressures [7,8]. While direct CAF depletion strategies have demonstrated detrimental outcomes in preclinical models, with genetic ablation of FAP$^+$ mCAFs precipitating immunosuppression and accelerated tumor progression in PDAC, contemporary therapeutic paradigms prioritize stromal reprogramming over ablation, emphasizing pharmacological normalization of CAFs toward quiescent phenotypes as a more rational approach to modulate TME architecture without disrupting tumor-restraining stromal functions [9]. Single-cell transcriptomics and spatial profiling technologies have revolutionized our capacity to dissect CAF heterogeneity with unprecedented resolution, revealing that CAF subpopulations exhibit context-dependent phenotypic switching that influences therapeutic responses [10]. Despite these advances, the identity of CAF subsets mediating NAT resistance, their paracrine effectors, and spatial distribution within resistant tumors remain incompletely defined. This commentary synthesizes evidence establishing P4HA1$^+$ mCAFs as principal mediators of NAT resistance in PDAC and examines the mechanistic basis for therapeutic targeting to overcome chemoresistance.

Main Text

P4HA1$^+$ mCAFs Orchestrate NAT Resistance Through Secretory Reprogramming

P4HA1 functions as the catalytic component of collagen prolyl 4-hydroxylase, an α-ketoglutarate dependent dioxygenase essential for collagen biosynthesis and extracellular matrix assembly [11]. Beyond its canonical role in collagen modification, accumulating evidence implicates P4HA1 in metabolic reprogramming through competition with prolyl hydroxylases for α-ketoglutarate, thereby attenuating HIF-1α degradation and stabilizing hypoxic signaling cascades [12]. This metabolic crosstalk establishes a positive feedback loop wherein HIF-1α transcriptionally upregulates P4HA1 expression, perpetuating both stromal collagen deposition and protumorigenic paracrine signaling.

The spatial enrichment of P4HA1$^+$ mCAFs in NAT-resistant PDAC tissues suggests these cells occupy privileged positions within the TME that facilitate stromal-epithelial communication. Unlike conventional mCAF populations characterized solely by α-SMA and FAP expression, P4HA1$^+$ mCAFs exhibit enhanced biosynthetic capacity for collagen and matricellular proteins, establishing dense fibrotic barriers that physically impede drug penetration while simultaneously activating survival signaling in adjacent malignant cells. Transcriptional profiling reveals that P4HA1$^+$ mCAFs upregulate gene networks associated with extracellular matrix organization, inflammatory cytokine secretion, and epithelial-mesenchymal transition, positioning them as multifunctional coordinators of therapeutic resistance. P4HA1$^+$ mCAF enrichment inversely correlates with NAT response and survival across multicenter retrospective PDAC cohorts. Furthermore, molecular subtyping based on P4HA1$^+$ mCAF transcriptional signatures effectively stratifies patients by prognosis and predicts differential sensitivities to conventional chemotherapies, suggesting potential utility as a biomarker for treatment selection.

Paracrine TIMP1 Signaling Mediates P4HA1$^+$ mCAF-Cancer Cell Communication

Tissue inhibitor of metalloproteinases 1 (TIMP1) emerges as a critical secreted effector through which P4HA1$^+$ mCAFs confer chemoresistance and enhance cancer cell survival. Spatial profiling reveals preferential TIMP1 enrichment in NAT-resistant niches where P4HA1$^+$ mCAFs interface with malignant epithelium, establishing this paracrine pathway as essential for chemoresistance [13]. Mechanistically, TIMP1 secreted by P4HA1$^+$ mCAFs engages a receptor complex comprising CD63 and integrin β1 (ITGB1) on cancer cell membranes, initiating downstream activation of Phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) pro-survival signaling cascades. This tripartite signaling architecture, wherein CD63 simultaneously binds TIMP1 and ITGB1, enables integration of stromal cues with integrin-mediated mechanotransduction pathways. The consequent activation of PI3K/AKT signaling promotes cancer cell proliferation, suppresses apoptosis, and enhances resistance to gemcitabine-induced cytotoxicity, establishing a paracrine resistance axis that operates within spatially defined stromal niches [14].

The dependence of TIMP1 signaling on CD63 expression provides mechanistic insight into stromal-epithelial communication in chemoresistance. CD63 depletion disrupts TIMP1-ITGB1 interactions and markedly attenuates PI3K-AKT pathway activation, sensitizing cancer cells to chemotherapy despite continued TIMP1 exposure. This observation indicates that receptor availability on cancer cells, rather than ligand abundance alone, represents a critical determinant of stromal-mediated resistance. The spatial restriction of TIMP1-CD63 interactions to P4HA1$^+$ mCAF-enriched regions further demonstrates that resistance mechanisms operate locally within discrete tumor microenvironmental niches rather than systemically, underscoring the importance of spatial context in therapeutic resistance.

Precision Nanoplatforms Reverse Chemoresistance Through Stromal P4HA1 Depletion

Targeting P4HA1$^+$ mCAF-mediated resistance requires

strategies beyond conventional CAF depletion, which paradoxically accelerates tumor progression by disrupting tumor-restraining stromal populations.9 Stromal reprogramming approaches that selectively modulate protumorigenic CAF functions while preserving tissue architecture provide superior therapeutic rationale. Precision nanomedicine platforms address this challenge through dual-payload delivery systems comprising CRISPR-Cas12 targeting stromal P4HA1 and encapsulated gemcitabine within mesoporous silica nanocarriers. These nanocarriers incorporate hypoxia-responsive, glutathione-responsive, and pH-responsive release mechanisms that enable TME-triggered drug release while minimizing systemic exposure [15].

This dual-targeting strategy dismantles resistance niches through coordinated stromal reprogramming and cytotoxic therapy. P4HA1 depletion in CAFs suppresses TIMP1 secretion, disrupting paracrine resistance signaling and sensitizing cancer cells to gemcitabine. Concurrent gemcitabine delivery exploits enhanced chemosensitivity to achieve synergistic tumor regression unattainable with either monotherapy, demonstrating that multi-mechanism coordination surpasses dose escalation in precision oncology.

Translational opportunities and mechanistic frontiers in stromal-targeted therapy

The identification of P4HA1$^+$ mCAFs as principal orchestrators of NAT resistance provides immediate clinical opportunities. Stromal P4HA1 expression serves as a predictive biomarker for treatment response, enabling patient stratification for therapy intensification. The TIMP1-CD63/ITGB1 axis offers multiple therapeutic intervention nodes, including CD63-ITGB1 inhibitors and TIMP1-neutralizing antibodies. Our S-SNACs@GEM@Cas-P4HA1 nanoplatform exemplifies precision nanomedicine that simultaneously depletes stromal P4HA1 and delivers gemcitabine, effectively dismantling resistance niches and restoring chemosensitivity. This dual-targeting approach represents a paradigm shift from conventional CAF depletion strategies toward selective stromal reprogramming that preserves tumor-restraining subpopulations.

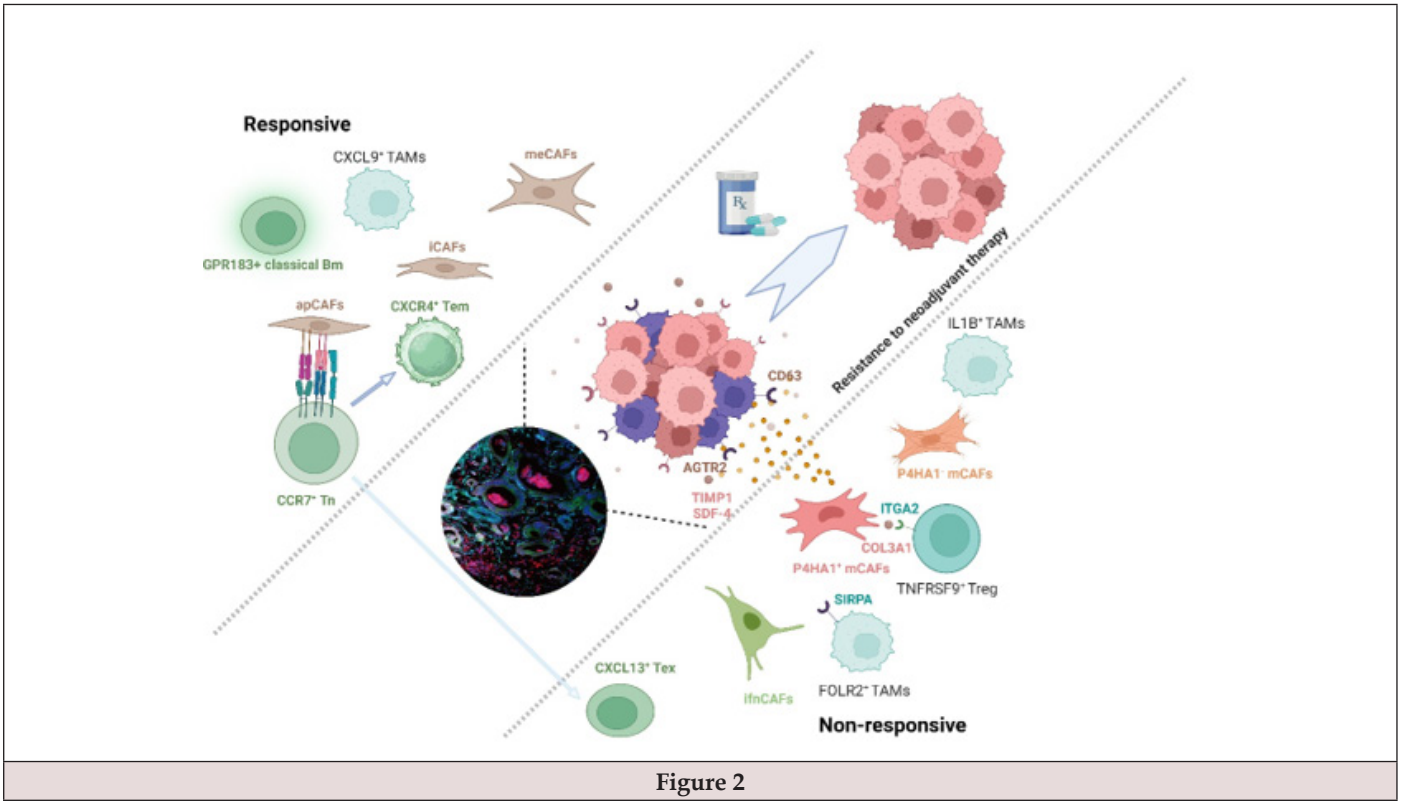

Figure 2

Several limitations warrant consideration. Lack of pre-treatment paired samples reflects technical challenges in obtaining sufficient tissue from fine needle aspirations for single-cell profiling, though multi-cohort validation comparing untreated and differentially responsive samples robustly characterized NAT-induced TME remodeling. The absence of flow cytometry sorting prevented direct P4HA1$^+$ CAF isolation, mitigated by mechanistic studies focusing on highest P4HA1-expressing patient-derived lines to minimize heterogeneity confounds. Future investigations should employ lineage tracing to define P4HA1$^+$ mCAF developmental origins, longitudinal profiling to elucidate temporal expansion dynamics during NAT, and systematic assessment of compensatory

mechanisms following P4HA1 targeting. Comparative analyses across desmoplastic malignancies will establish whether P4HA1$^+$ mCAF-mediated resistance represents a universal mechanism or requires tumor-specific therapeutic adaptation (Figure 2).

Conclusions

The identification of P4HA1$^+$ mCAFs as principal mediators of NAT resistance elucidates stromal mechanisms governing therapeutic failure in PDAC. These fibroblasts establish chemoresistant niches through TIMP1-mediated activation of CD63/ITGB1-PI3K/AKT signaling in cancer cells. Mechanistically, P4HA1 stabilizes HIF-1α via α-ketoglutarate competition, sustaining CAF secretory function and ECM remodeling. Precision nanoplatforms co-delivering P4HA1-targeting CRISPR and gemcitabine dismantle resistance niches while circumventing paradoxical effects of CAF depletion. Multi-omics subtypes derived from P4HA1$^+$ mCAF signatures stratify patient outcomes across independent cohorts, establishing this axis as both a predictive biomarker and therapeutically actionable target. Disruption of this pathway through stromal reprogramming represents a tractable strategy to overcome stroma-mediated chemoresistance in PDAC.

Declarations

Ethics Approval and Consent to Participate

All studies involving human participants were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Henan University (approval number: HUSOM2025-193). Written informed consent was obtained from all participants prior to sample collection. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the First Affiliated Hospital of Henan University.

Consent for Publication

All patients provided verbal or written informed consent, and all sites were approved by institutional review boards.

Availability of Data and Materials

Data generated in this study have been deposited in the Figshare repository and are publicly available (https://figshare.com/articles/dataset/S23062901/26963734/1).

Competing Interests

The authors declare that they have no competing interests, financial or otherwise, that could be perceived as influencing the objectivity of this research.

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Authors' Contributions

W.F.: Study concept and design. W.F., and ZC.D.: Obtained funding. ZC.D.: Acquisition of clinical and pathological data. ZC.D., YR.Z. and YB.L.: Analysis and interpretation of the data. JD.X.: Molecular biology experiments. X.C.: Animal experiments. QR.Z.: Nanoplatform design and characterization. H.W.: Pathological experiments. ZC.D.: Drafting of the manuscript. R.H.L.: Editing and critical revision of the manuscript for important intellectual contents. W.F.: Study supervision. All authors: Approval of the final version of the manuscript.

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