Commentary

Missing links — epigenetic regulators of the pancreatic cancer–associated inflammation

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Pancreatic ductal adenocarcinoma (PDAC) features a hostile tumor microenvironment (TME) that renders it remarkably resistant to most therapeutic interventions. Consequently, survival remains among the poorest compared with other gastrointestinal cancers. Concerted efforts are underway to decipher the complex PDAC TME, break down barriers to efficacious therapies and identify novel treatment strategies. In the recent Clinical Science, Li and colleagues identify the long noncoding RNA KLHDC7B-DT as a crucial epigenetic regulator of IL-6 transcription in PDAC and illustrate its potent influences on the pancreatic TME. In this commentary, we introduce epigenetics in pancreatic cancer and put the findings by Li et al. in context with current knowledge.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second leading cause of cancer-related deaths by 2030 [1] and only recently reached an almost 10% 5-year survival rate [2]. For most patients chemotherapy remains the mainstay of treatment; however, with overall poor response rates, the need for novel, personalized treatment has been increasingly acknowledged by clinicians and basic scientists alike. First efforts to uncover an Achilles’ heel of PDAC by means of genomic sequencing consistently yielded mutations in a few genes—the oncogene KRAS, which is mutated in 90–95% of patients, and the tumor suppressors TP53, CDKN2A and SMAD4 [3]. Despite the somewhat uniform genetic landscape, PDAC is underpinned by profound heterogeneity in histopathology and treatment responses. With the advent of high-throughput RNA sequencing, further tumor profiling corroborated the notion of heterogeneity and several transcriptomic classifications of PDAC have been suggested [3], which are better suited to predict patient outcomes and response to chemotherapy. The discrepancy between genotype and the breadth of phenotypes pointed to significant epigenetic alterations both in the epithelial cancer cells and the tumor microenvironment, and recent advances in epigenetic profiling have consistently supported this [4]. Both the epithelial cells and the tumor microenvironment, characterized predominantly by dense stroma, endothelial cells, and dysfunctional immune cells, may undergo epigenetic changes or be affected by epigenetic alterations in epithelial cells.

Epigenetics in pancreatic cancer

Epigenetics refers to changes in the gene expression profile of a cell, without alterations in the actual genetic sequence, to create specific transcriptomic states that can be passed on to cell progeny. At the center of epigenetics stands the chromatin, the composite of DNA, RNA, histones, and other proteins, which can be modified by methylation, acetylation, and sumoylation to activate or repress transcription of a gene. This task is undertaken by chromatin modifying enzymes that act as writers, readers or erasers of epigenetic marks, that in turn create a looser, accessible or a denser, tightly packed chromatin referred to as euchromatin and heterochromatin, respectively [5]. In normal physiology, this strictly regulated process allows cells to embark on a distinct transcriptomic profile at a specific time, based on environmental cues, thereby laying...
Figure 1. The central role of epigenetically driven chronic inflammation in pancreatic cancer

Epigenetic modifiers, such as the long noncoding RNA KLHDC7B-DT, drive IL-6 expression in cancer cells, which subsequently exerts a plethora of effects on the pancreatic tumor microenvironment. IL-6 induces apoptosis in dendritic cells and polarizes macrophages to an immunosuppressive ‘M2’ phenotype, both of which result in restraint T-cell antitumor immunity. M2 macrophages can additionally promote tumor cell intrinsic growth. Cancer associated fibroblasts acquire further epigenetic changes in response to IL-6 and aggravate desmoplasia in the TME. Finally, IL-6 drives a fibrotic reaction by hepatocytes to form a pre-metastatic niche.

the foundation for cell development, differentiation, and proliferation. Therefore, it is not surprising that epigenetic dysregulation is a common feature of cancer and in particular PDAC [5,6].

Indeed, distinct epigenetic changes have been described in pancreatic cancer. They contribute to the stable phenotype of individual compartments of the TME, such as cancer associated fibroblasts (CAFs), perhaps to an even greater extent than their contribution to epithelial cells. Despite some early studies pointing to the contrary [7], CAFs likely acquire a distinct epigenetic profile that is responsible for their carcinogenic phenotype [8,9]. Furthermore, there is emerging evidence that these changes in epithelial cells and CAFs are sufficient to alter the immune microenvironment via paracrine signaling [6,10–12]. The cumulative alterations in the epigenome of the TME results in the recapitulation of two distinct phenotypes [13] that were previously observed with RNA sequencing [14], namely basal/squamous and classical. Consequently, epigenetic drugs targeting master regulators of a specific program pose an interesting field of study that holds promise in first preclinical trials. There are now several approaches of epigenetic priming in PDAC such as the use of the hypomethylating agent decitabine. Decitabine administered at doses well below cytotoxic thresholds, profoundly, alters the tumor microenvironment in a Kras<sup>G12D</sup>/TP53 driven mouse model of PDAC and results in prolonged survival [10,15].

In addition to chromatin modifications, the armamentarium of noncoding RNAs (ncRNAs, RNA transcripts that do not get translated into proteins) encoded in the human genome has been increasingly recognized as an important regulator of epigenomic states. Of the two broad categories of short (~<200 nucleotides) and long ncRNAs (lncRNAs, >200 nucleotides), particularly lncRNAs have a broad impact on gene expression and can partake in cellular differentiation, proliferation and metastasis [16]. Indeed, a compilation of several sequencing studies suggested that roughly
68% of expressed human genes can be classified as lncRNAs [17], further insinuating their biological relevance. While only a minute portion of lncRNAs have been described in more detail, several have been implicated as key players in PDAC [18–20] or other cancers [21,22].

In their work presented in Clinical Science, Li and colleagues [23] identify a novel lncRNA KLHDC7B divergent transcript (KLHDC7B-DT) that was highly expressed in human PDAC and correlated with worse disease-free and overall survival. Interestingly, KLHDC7B-DT expression was positively correlated with increasing tumor size and more advanced stages, suggesting a supportive role during disease progression. Accordingly, overexpression of KLHDC7B-DT in PDAC cell lines in vitro enhanced cell viability, proliferation, and migration. Interestingly, the authors observe a similar effect in an immortalized, nonmalignant pancreatic ductal epithelial cell line, thus suggesting that gain or increasing KLHDC7B-DT expression could offer an evolutionary advantage as cells progress through the dysplasia-intraepithelial neoplasia-cancer sequence.

From a mechanistic standpoint, Li et al. demonstrate KLHDC7B-DT co-localization with the interleukin 6 (IL-6) promoter region where epigenetic modifiers suggested increased transcription. This was further confirmed by increased or decreased levels of IL-6 in the supernatant of KLHDC7B-DT overexpressing or knockdown cells, respectively. In a series of elegant co-culture experiments of KLHDC7B-DT normal or overexpressing PDAC cells with or without macrophages, with or without IL-6 neutralizing antibodies, the authors offer evidence for a KLHDC7B-DT–IL-6–STAT3 signaling axis that functions in an autocrine (cancer cell-to-cancer cell) and paracrine (cancer cell-to-Macrophages) fashion. Macrophages in co-culture with KLHDC7B-DT overexpressing PDAC cells further exhibited an M2-polarized phenotype, as evidenced by higher production of IL-10 and arginase-1 as well as reduction in inflammatory cytokines. Most importantly, when those KLHDC7B-DT ‘educated’ macrophages were transferred to a new co-culture with naïve tumor cells, they supported cell proliferation, migration, and invasion.

### Interleukin-6, chronic inflammation, and the pancreatic TME

The findings by Li and colleagues [23] demonstrate a critical new role for lncRNAs in PDAC by offering mechanistic insight into how PDAC leverages epigenetic mechanisms to create a chronically inflamed TME, which supports tumor growth while priming an immune suppressive milieu. This is of particular relevance since chronic but ineffective inflammation is considered a cornerstone of pancreatic oncogenesis. The first step in this process is catalyzed by epithelial cells, pancreatic stellate cells (PSCs), and CAFs which work in concert to create a dense desmoplastic stroma and a tumor milieu increasingly rich in inflammatory cytokines [24]. Subsequently recruited immune cells are largely skewed toward an immunosuppressive phenotype and provide further support for cancer growth. Interestingly, the parallel process of epithelial cell transformation and increasing inflammation is accompanied by specific changes in DNA methylation patterns, which can already be detected in normal ductal epithelium in the context of pancreatic intraepithelial neoplasia [25]. This suggests that epigenetic changes co-evolve with the pancreatic TME from the earliest stages of PDAC initiation and progression, thus raising the possibility of using epigenetic modifiers for the prevention of pancreatic carcinogenesis.

One of the key mediators of the inflammatory process is IL-6. Indeed, serum IL-6 levels, which are often elevated in PDAC patients, can be correlated with tumor stage, size as well as lymph node and distant metastasis [26]. In preclinical models of mice harboring a KrasG12D driver mutation, genetic ablation of Il6 abrogates progression of dysplastic lesions to PanINs and limits proliferative capacity in tumor cells [27]. Beyond the epithelial compartment, IL-6 has been shown to induce Caspase-3 mediated apoptosis in type 1 classical dendritic cells, which are paramount for priming cytotoxic T cells, thus mounting an effective antitumor response [28]. The ensuing systemic and local DC paucity is further aggravated by STAT3 signaling induced interferon regulatory factor 8 (IRF8) suppression in the bone marrow, resulting in preferential differentiation of macrophage-dendritic cell progenitors into immature granulocytes. Furthermore, IL-6/STAT3 signaling in hepatocytes and subsequent production of the acute phase reactant serum amyloid A facilitates local liver fibrosis and recruitment of myeloid derived suppressor cells, ultimately creating a favorable milieu for metastatic spread [29].

While significant efforts are underway to elucidate the role of epigenetics in chronic inflammation, little remains known about the drivers of these epigenetic changes. However, one can hypothesize that increased release of cytokines, reactive oxygen species and the oxygen deprived TME of PDAC may result in alterations in epigenetic regulators. Particularly, IL-6 mediated DNMT1, a DNA methyltransferase, expression has been implicated as a potential explanation for the distinct epigenetic landscape observed in stromal cells [9]. It is an intriguing hypothesis to now utilize the data provided by Li and colleagues to connect the epigenetic program in pancreatic cancer cells (i.e. the lncRNA KLHDC7B-DT) via the mediator of IL-6 to the epigenome in CAFs and other players in the pancreatic TME. In Figure 1, we summarize in a simplified fashion the effect and regulation of epigenetic changes in the context of IL-6.
mediated inflammatory pathways in PDAC. Beyond pancreatic cancer, the influence of IL-6 signaling during chronic inflammation and shaping of the epigenetic landscape has been described in several cancer types such as breast [30], oral [31], and colorectal cancer [32], pointing to the relevance of further deepening our understanding of epigenetics to develop broadly applicable therapeutics.

In summary, chromatin changes, DNA methylation and now alterations in lncRNA have all been linked to independently drive the tumorigenic inflammation seen in PDAC. Thus, further exploring histone deacetylase (HDAC) inhibitors, hypomethylating agents and their combinations, which can shift myeloid and lymphoid cell polarization and cytokine production, offer an intriguing field to explore in clinical trials. The data by Li et al. [23] add to the growing body of evidence that the epigenome regulates multiple tumor-supporting pathways in different cell compartments, culminating in a dysregulated immune response in PDAC. Leveraging epigenetics to unleash potential therapeutic benefits of immunotherapy in pancreatic cancer may offer an exceptionally effective way to enhance treatment efficacy and improve the dismal prognosis of pancreatic cancer.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
IRF8, interferon regulatory factor 8; LncRNA, long noncoding RNA; ncRNA, noncoding RNA; PDAC, pancreatic ductal adenocarcinoma; PSC, pancreatic stellate cell; TME, tumor microenvironment.

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