PAF1 regulates the stemness of pancreatic cancer stem cells

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Dear Editors,

We read with great interest the article by Karmakar et al., which identified Pol II-associated factor 1 (PAF1) as an important transcription factor regulating the stemness of human pancreatic cancer stem cells[1]. Currently, pancreatic cancer is one of the deadliest human malignancies. With an overall median survival time of 6-9 months and a similarly dismal 5-year survival rate of 6%[2]. The development of many cancers including pancreatic cancer involves a dedifferentiation of cellular identity with the acquisition of a stem cell-like state in a subpopulation of cancer cells. The arising cancer stem cells (CSCs) are exceptionally important because their developmental plasticity allows them to resist conventional therapies, metastasize and give rise to new tumors. The changes in cell identity are caused by transcriptional dysregulation which is a universal feature of tumorigenesis and impacts all cancer hallmarks. Hence, identification of key regulators governing the unique transcriptional landscape of pancreatic CSCs is essential for dissecting the regulatory mechanisms of pancreatic CSCs in pancreatic tumorigenesis and therapeutic resistance, which is implicated for the novel biomarker discovery and drug development in pancreatic cancer.

In this study, the authors found that PAF1, a key transcription factor for transcriptional elongation, is upregulated in pancreatic CSCs while loss of PAF1 reduced the percentage, self-renewal capacity and proliferation of pancreatic CSCs. In addition, the authors showed that loss of PAF1 decreased the formation of tumor spheres in culture, pancreatic tumorigenesis and metastasis in mice. In order to uncover the mechanisms of PAF1 in regulating the features of pancreatic CSCs, the authors knocked down LEO1, CTR9, CDC73 (the components in PAF1 complex) individually and showed that the expression of CSC markers and the ability of tumor spheres of CSCs in culture were not affected and thus proposed that the roles of PAF1 in pancreatic CSC maintenance is independent from PAF1 complex components. Moreover, the authors identified that PHF5A and DDX3 interacted with PAF1 and regulated the stemness of pancreatic CSCs. However, how PHF5A-DDX3-PAF1 complex regulates transcriptional landscape in pancreatic CSCs remains elusive. Recent work from Ali Shilatifard lab found that PAF1 regulated promoter-proximal pause release via enhancer activation[3]. Hence, future work would be of interest to perform H3K27ac (the most important active enhancer/promoter mark) ChIP-seq in pancreatic CSCs with PAF1 knockdown and examine the roles of PAF1 in regulating active enhancers in

pancreatic CSCs. Moreover, in most cases, enhancers control transcriptional output via forming chromatin looping with the promoters of their target genes. Interrogating the 3D chromatin connectome of enhancers/promoters are fundamental to dissect the regulatory mechanism in gene transcription. RNAPII is the key transcription factor in transcription initiation and elongation and involved in genome-wide enhancer/promoter interactions[4]. RNAPII ChIA-PET (Chromatin Interaction Analysis with Paired-End Tag)[4] can capture the RNAPII binding (peak) and interaction (loop) signals in the same sequencing library. Thereafter, performing RNAPII ChIA-PET will enable us to investigate the impact of PAF1 on transcriptional elongation and also the enhancer-promoter interactions in pancreatic CSCs.

In addition, future work should further unravel the roles of PHF5A/DDX3 in regulating the functionality of PAF1 in pancreatic CSCs. For example, PAF1 ChIP-seq followed by PHF5A/DDX3 can validate whether PHF5A/DDX3 regulate the localization of PAF1 on chromatin at genome-wide scale. In the past 2 years, a new frontier in transcription regulation is called liquid – liquid phase separation (LLPS) (i.e. liquid droplets phase separating from the aqueous environment), which can form membrane-less compartments with high-concentrated molecules to facilitate the biochemical reactions for transcription. For example, Richard Young lab uncovered that transcription factors, Mediator and RNAPII can form phased-separated condensates on enhancers (in particular super enhancers) to facilitate transcription activation[5]. As intrinsically disordered regions (IDRs) are considered as a general mechanism for LLPS, whether PHF5A/DDX3 facilitate LLPS via their IDRs and regulate the specific enhancers in pancreatic CSCs should be further investigated in the future.

Collectively, this study uncovered a previously unrecognized transcription factor complex for the maintenance of stemness in pancreatic CSCs. In the future, CRISPR or small molecule compounds library targeting transcription factors or epigenetic modifying enzymes could identify other novel regulators orchestrating the unique epigenome in pancreatic CSCs. Moreover, alteration of 3D chromatin topology, such as topologically associating domains (TADs) (the structures framing enhancer-promoter interactions) or A/B compartment has been implicated in gastrointestinal stromal tumours[6] or colon cancer[7] but not been examined in pancreatic cancer yet. Demystifying the 3D chromatin topology in pancreatic CSCs by CTCF/Cohesin[8] (key architectural proteins) ChIA-PET or HiChIP will help to interrogate the interplay of high-order chromatin architecture and epigenomic landscape in regulating the transcription signature of pancreatic CSCs.

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