Review Article

Nuclear Factor of Activated T Cells Operates as Conductor of the Epigenetic Orchestra in Pancreatic Carcinogenesis

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Key Words

Nuclear factor of activated T cells · Pancreatic carcinogenesis · Gene activation and silencing

Abstract

Recent years have witnessed an explosive increase in our knowledge about the epigenetic features in human cancers. It became apparent that pancreatic cancer, additionally to its genetic background, is an epigenetic disease characterized by widespread alterations in DNA methylation, histone modification and recruitment of microRNAs. Although more and more in-depth research is performed and epigenetic modifications are shown in healthy and de-generated cells, we still lack insight into how epigenetic modifications are orchestrated during carcinogenesis. By introducing nuclear factor of activated T cell proteins and their impact on gene activation and silencing in pancreatic cancer, this review cites an example of how de-regulated oncogenes modulate epigenetic alterations in epithelial cancers.

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Introduction

Within the last decade, epigenetic modifications shifted into the center of attention in the field of developmental and medical research. Once it became clear that DNA is not deleted from somatic cells after the completion of development, the question remained how somatic cells express different phenotypes although they retain the same DNA [1]. Due to its stability and strong conservation, additional features linked to the DNA sequence must exist that are more dynamic and can account for the alterations between the genotype and the phenotype of a cell.

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The mechanisms linking the stability of the DNA sequence with the wide range of cellular phenotypes are summed up under the term of ‘epigenetics’. Epigenetics is defined as any heritable genomic mechanism unrelated to changes in the DNA sequence [2]. There are several well-known epigenetic mechanisms subdivided into DNA methylation, histone modification and microRNAs, all of them affecting the cell by induction or suppression of gene expression [3–7]. For instance, posttranslational modification of histone tail residues highly influences the structure and activity of chromatin. While acetylation of histones is primarily associated with transcriptional activation, methylation of histones can lead to both, activation and repression, depending on the modified residue [8, 9]. Epigenetic modifications are applicable to normal cellular development and maintenance, but they are also responsible for the deregulation of gene expression, resulting in diseased cellular phenotypes. Most notably, the deregulation of epigenetic mechanisms can contribute to cancer development [10–16].

One of the most aggressive malignancies is ductal adenocarcinoma of the pancreas (PDAC) [17]. Due to the late diagnosis in advanced stages, early invasion and metastases as well as extreme resistance to conventional therapy, pancreatic cancer has a 5-year mortality rate of 97–98% [18]. As a result of its dismal prognosis, many concerns were expressed in the last few decades over understanding the background of pancreatic cancer biology. In 2008, Jones et al. [19] discovered 1,561 somatic gene mutations within more than 20,000 analyzed genes, yielding an average rate of 63 genetic abnormalities per pancreatic cancer, clustered in 12 regulatory pathways. Conceptually, these data suggest that pancreatic cancer is substantially a disease of pathways. However, research into these pathways made it clear that these cascades must ultimately take on the function of epigenetic regulators to silence tumor suppressors and activate oncogenes in a heritable manner. Thus, studies into epigenetics in pancreatic cancer are a logical extension to the genetic paradigm of this malignant disease.

Many excellent reviews exist about epigenetic mechanisms active in pancreatic cancer [1, 2, 20–22] but less is known about the pathways and transcription factors orchestrating histone modifications, DNA methylation and recruitment of microRNAs. Based on the oncogenic transcription factor NFAT (nuclear factor of activated T cell) and its implication in histone modifications in pancreatic cancer, this review will focus on transcription factors that initiate epigenetic changes and influence the assembly of chromatin on key genes responsible for pancreatic cancer development.

NFAT Transcription Factors in Pancreatic Carcinogenesis and Established Pancreatic Cancer

Pancreatic cancer evolves through a series of histopathological changes, referred to as acinar-to-ductal metaplasia and progressive pancreatic intraepithelial neoplasia, which are accompanied by a recurrent pattern of genetic and epigenetic alterations, the earliest and most prevalent of which is oncogenic activation of Kras. Constitutive activation of Kras (Kras^{G12D}) is generally accepted to represent the initial key event in pancreatic carcinogenesis and found in virtually all invasively growing pancreatic tumors [23].

The relevance of Kras^{G12D} mutation to pancreatic carcinogenesis has been elegantly demonstrated in genetically engineered mouse models with conditional activation of this oncogene [24]. These studies revealed that constitutive activation of Kras alone is not sufficient for neoplastic progression; instead additional abnormalities and signals from the inflammatory environment are necessary to promote pancreatic cancer [25]. This type of proinflammatory environment can be provided by chronic pancreatitis, the most relevant risk factor for PDAC development in human beings [26]. Chronic pancreatitis supports the initi-

ation and progression of this malignancy by direct modification of gene expression networks in pancreatic epithelial cells, predominantly of inflammatory transcription factors.

One of these inflammatory transcription factors is the NFAT family (isoforms NFATc1–c5), originally identified as a group of inducible nuclear proteins, which regulate the transcription during T lymphocyte activation [27]. However, a multitude of studies quickly established that NFAT proteins are also expressed in other cell systems than the immune system, where they participate in the regulation of the expression of genes influencing cell growth and differentiation. Ectopic activation of NFAT members is now recognized as an important aspect of oncogenic transformation in several human malignancies, most notably in pancreatic cancer [28].

Upon calcium-dependent activation of calcineurin, the phosphatase targets and dephosphorylates NFAT, which leads to its nuclear translocation. In the nucleus, NFAT proteins form nucleoprotein complexes with other transcription factors and DNA-binding proteins for subsequent target gene regulation [29].

In pancreatic cancer, NFAT proteins take on highly oncogenic qualities downregulating tumor suppressor genes and promoting oncogenes. A significant amount of NFAT-dependent oncogenic processes with implications in pancreatic carcinogenesis and established pancreatic cancer comprise or result in epigenetic alterations, predominantly histone modifications. For this purpose, NFAT transcription factors cooperate with chromatin-remodeling proteins to induce the activation or silencing of target genes.

NFATc1 and p300 Cooperate for c-Myc Activation in Pancreatic Cancer

As an immediate early transcription factor, proto-oncogenic c-Myc functions as a master regulator of G1-S cell cycle progression and growth promotion in pancreatic cancer [29, 30]. In pancreatic cancer cells, serum-induced c-Myc expression and cancer growth are mediated by fast and sustained stimulation of the Ca^{2+} /calcineurin/NFAT signaling and transcription pathway. Mechanistically, NFATc1 binding to the serum-responsive element within the proximal c-Myc promoter initiates p300-dependent histone acetylation rendering the promoter transcriptionally active. Hyperacetylation of the c-Myc promoter is required for recruitment of ELK1, a protein signaling downstream of Kras, responsible for maximal activation of c-Myc. The functional significance of this pathway is emphasized by impaired c-Myc expression, reduced tumor growth and G1 arrest following the pharmacological or genetic inactivation of NFATc1 (fig. 1a) [30].

NFAT and STAT3 Cooperation Regulates Key Genes in Pancreatic Carcinogenesis via Enhancer-Promoter Communications

Recent investigations by our group established that NFATc1 cooperates with the inflammatory transcription factor STAT3 to promote Kras^{G12D}-driven carcinogenesis. NFATc1 activation accelerates pancreatitis-mediated carcinogenesis in Kras^{G12D} mice, whereas its genetic or pharmacological inactivation attenuates this effect. From the mechanistic point of view, NFATc1 interacts with STAT3 to form enhancer-promoter communications at jointly regulated genes involved in inflammation and oncogenesis, e.g. EGFR and Wnt family members. The NFATc1-STAT3 transcription pathway is operative in pancreatitis-mediated carcinogenesis as well as in established human pancreatic cancer (fig. 1b) [Baumgart et al., in press].

Fig. 1. **a** NFAT proteins interact with histone acetyltransferase p300 for histone 3 acetylation inducing a chromatin structure allowing Elk1 binding to the c-Myc promoter. **b** NFAT cooperates with STAT3 via enhancer-promoter communications to activate target genes. **c** NFAT binding to target genes leads to the formation of local heterochromatin by sequential recruitment of the histone methyltransferase Suv39H1 and HP1 proteins. **d** NFAT acts in concert with the Polycomb group member EZH2 to silence target genes.

NFAT Recruits the Histone Methyltransferase Suv39H1 for Silencing the Tumor Suppressor p15

Activation of NFAT proteins does not only lead to target gene activation in pancreatic cancer, but also contributes to gene silencing. Being a member of the Ink4 family, p15^{Ink4b} impedes the activation and function of cyclin-dependent kinases 4 and 6, which leads to cell cycle inhibition and diminished G1-S phase transition [31]. Therefore, p15^{Ink4b} incorporates important functions such as being a tumor suppressor in numerous malignancies, most importantly in pancreatic cancer. p15^{Ink4b} inactivation by genetic or epigenetic events occurs in over 90% of all tumors [19]. NFATc2 targets p15^{Ink4b} for inducible and sequential heterochromatin formation and gene silencing. Sequential chromatin immunoprecipitation revealed that NFATc2 binding to its putative binding site on the p15^{Ink4b} promoter leads to recruitment of the histone methyltransferase Suv39H1. Local trimethylation of lysine 9 on histone 3 (H3K9trime) allows docking of heterochromatin protein 1γ, which results in the stabilization of the heterochromatin complex on the p15^{Ink4b} promoter. Conflicting with that, the inactivation of NFATc2 disrupts the repressor complex and results in the restoration of p15^{Ink4b} expression and function (fig. 1c) [32].

NFAT Participates in the Polycomb Group Protein Function by Cooperating with EZH2 in Established Pancreatic Cancer

The term ‘Polycomb’ was first coined almost 80 years ago to describe additional sex comb formation in *Drosophila melanogaster* resulting from a loss of homeotic (Hox) gene expression [33]. Polycomb protein complexes induce gene repression via H3K27 trimethylation [34].

Polycomb proteins can be divided into two functional biochemical categories: Polycomb repressive complexes (PRC) 1 and 2. While members of the PRC2 complex initiate gene repression by the catalyzation of H3K27 trimethylation, proteins belonging to PRC1 maintain the repressive state [35, 36]. Under physiological conditions, the activity of Polycomb proteins is crucial in the development as well as in the maintenance and proliferation of pluripotent progenitor cells in a variety of tissues. Overexpression of these proteins may promote tumorigenesis by fostering a self-renewing population of cells [37, 38]. Indeed, overexpression of Polycomb proteins is responsible for malignant progression and poor prognosis in breast [39], bladder [40] and prostate [41] cancer, and shows a strong association with hallmarks of cancer, including induced cellular proliferation [42], angiogenesis [43], survival [44] and migration [45].

EZH2 is the only PRC2 protein member thus far studied in pancreatic cancer. Strong nuclear accumulation of EZH2 was found in 55% of well-differentiated tumors and in 98% of poorly differentiated samples in a comprehensive immunohistochemical analysis of PDAC, indicating a significant correlation between EZH2 expression and dedifferentiation in pancreatic cancer [46]. Additionally, EZH2 overexpression participates in epithelial mesenchymal transition and invasion through the repression of epithelial proteins like E-cadherin [47].

As the core PRC1 and PRC2 proteins lack any DNA binding capabilities, the question remains, how Polycomb complexes modify the chromatin of target genes. Recent evidence from our group investigating pancreatic cancer cells clearly shows that NFAT induces EZH2 on the transcriptional level and partners with methyltransferase to control the expression of target genes involved in pancreatic cancer (fig. 1d) [unpubl. data].

Perspective

These examples of NFAT-mediated epigenetic changes in pancreatic cancer only demonstrate a small insight into how oncogenic transcription factors contribute to pancreatic cancer development by orchestrating chromatin-remodeling programs.

In addition to the striking number of genetic changes, epigenetic alterations constitute another layer of complexity and contribute to the heterogeneity of pancreatic cancer. The era of epigenetics in pancreatic cancer has emerged strongly within the last few years and deepened our understanding of pancreatic cancer biology. One of the most important characteristics of epigenetic mechanisms, which clearly demarcate them from genetics, is their reversibility. This feature provides new targets for novel therapeutic interventions in pancreatic cancer and other epithelial tumors.

However, the challenge still remains to distinguish between physiological epigenetic mechanisms operative in every single cell and the deregulation of histone modification, DNA methylation and recruitment of microRNAs, contributing to cancer development, especially when it comes to targeting epigenetic alterations in new therapeutic cancer approaches. Therefore, the careful analysis of cancer-specific deregulated programs with an impact on the induction of epigenetic changes might open new doors in cancer cell-specific therapy.

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