ROLE OF PDX1 IN PANCREAS DEVELOPMENT

PDX1 (pancreatic and duodenal homeobox 1) is often termed a “master regulator” of pancreas development [1-3]. In this work, the terms “master gene” and “master regulator” imply that this protein initiates (or participates in the initiation of) a hierarchic sequence of events during development [4]. The mammalian pancreas is a mixed exocrine and endocrine gland organ that plays a crucial role in food digestion and glucose homeostasis. The exocrine compartment consists of acinar cells, which produce and secrete digestive enzymes into ducts formed by pancreatic ductal cells. Acinar cells make up the vast majority of pancreatic epithelial cells. The endocrine compartment consists of islet cells, which produce and secrete hormones into the bloodstream. Islet cells are responsible for maintaining blood glucose levels. Endocrine cells are organized as clusters of cells, known as islets of Langerhans, and are responsible for hormone production, including insulin and glucagon.

Embryonic development of the pancreas is initiated via specialization and proliferation of a small group of endodermal cells. All further cell types originate from these multipotent progenitor cells present in epithelium at the early stages of development of the prospective pancreas (figure).

Much of what we know about successional relationship between different cell types has been established using transgenic mice, which allowed labeling certain types of cells during development. These studies provided important information on the stepwise progression of endodermal cells first to the development of certain multipotent lineages of progenitor cells (figure, panel (a)), and next to fully differentiated cells with specialized functions in the mature organ [6-8] (figure, panel (b)).

Several key transcription factors participate in maintenance and proliferation of progenitor cells and form a regulatory network with the majority of nodes (points of interaction of transcription factors with their functional binding sites) still poorly understood [9]. The identity of the cells is preserved via a combination of the expression of transcription factors specific for this type of cells with a definite pattern of chromatin modification, and it is dependent on external signals [7, 10-13].

PDX1 is a unique product of the developing pancreas and crucial for its development. Deletion of the gene encoding this protein in mice and humans results in the absence of pancreas (agenesis), which places this gene at the very top of the regulatory hierarchy of pancreas development [3, 8]. A high level of PDX1 is characteristic of...
Overlapping of genes expressed at different stages of pancreas embryogenesis and during pancreatic adenocarcinoma (modified from [5, 6]).

Several transcription factors regulate the cascade of events during the development of the pancreas, which leads to the transition of progenitor cells into fully differentiated cells. Many of these factors (underlined) participate in cell dedifferentiation during tumor initiation and progression. a) Four stages of pancreas differentiation. DE, definitive endoderm; FE, foregut endoderm; DF, distal foregut; PE, pancreatic-specified endoderm. The stages of early embryogenesis are denoted with numbers: 1) formation of foregut endoderm; 2) induction of pancreatic cell lineage. Key transcription factors are indicated for each stage (master regulators). b) Differentiation stages of pancreatic cells.
the epithelium of the developing gland, whereas this level is low in postnatal acinar, ductal, and the majority of endocrine cells, except for β-cells that maintain a significant level of PDX1 [5, 8, 14, 15]. Maintenance of the identity of pancreatic cells depends on the functioning of the pancreatic gene regulation network that includes numerous cross-section interactions between individual transcription factors. PDX1 is one of the central points in this network, and maintenance of the correct functioning of the pancreas cell lineage requires its high concentration. This is achieved through binding of the PTF1a factor with the enhancer controlling the PDX1 gene [12] and/or through autoregulation [9].

In early embryogenesis, pancreas, kidney, and lungs are developed from the endodermal gut tube (figure, panel (a)). However, transcriptional programs that define further stages of the pancreas development are not fully understood. Four stages have been outlined based on differentiation of human embryonic stem cells in vitro [6] – definitive endoderm, foregut endoderm, dorsal foregut endoderm, and pancreatic-specified endoderm. Based on epigenetic modifications, potential enhancers for each of this stage were identified. According to the available data, a large number (119,795) of the potential enhancers identified [6] may at these stages interact with such factors as PDX1, PROX1, ONECUT-1 (OC1), NKX6.1, PTF1a, FOXA1, TCF2, SOX9, GATA4/6, and HES1 [2, 3, 6, 9]. Due to these interactions, pancreatic progenitor cells are amplified, and then the identity of pancreatic cells is maintained [2]. More than 350 enhancers are activated directly during differentiation of embryonic pancreatic cells [3].

It has been shown [6, 16] that many of these enhancers are initially inactive (closed chromatin), but their interaction with the pioneer factors FOXA1 and FOXA2 [17, 18] makes them primed for interaction with lineage-specifying transcription factors (such as PDX1) [4] that define the following pathway of progenitor cells. This interaction leads to activation of the enhancers and, hence, of the genes that they control [6].

The interaction of these factors determines the choice of the differentiation direction at early stages. For example, the expression of SOX9 master-regulator before the beginning of pancreatic endoderm differentiation (figure, panel (b)) coincides with an increase in expression of the PDX1 gene. SOX9 and PDX1 reciprocally enhance expression of each other due to interaction of PDX1 with regulatory sequences of SOX9, and vice versa, due to interaction of SOX9 with regulatory sequences of PDX1. Hence, PDX1 and SOX9 cooperatively induce the beginning of differentiation towards pancreatic lineage and, at the same time, inhibit intestinal lineage [1, 19]. It should be mentioned that multifunctionality is a feature characteristic of PDX1. Apart from the activation of pancreatic lineage and suppression of intestinal lineage, PDX1 also inhibits the development of hepatic lineage [3].

Modified versions of these combinatorial interactions of master regulatory factors affect the emergence and development of various pathological processes, including the development of cancer [13]. One of the most therapy-resistant cancer type is pancreatic cancer [20] with its most widespread form – pancreatic ductal adenocarcinoma.

MAJOR GENETIC CHARACTERISTICS OF PDAC

The finding that mutations in KRAS are found in ~90% of patients and that the activity of oncogenic KRAS is required both for initiation and maintenance of a primary tumor as well as for metastatic damage to the pancreas represents a distinctive feature of pancreatic cancer [15, 21, 22]. Also, the CDKN2/INK4A tumor suppressor is commonly inactivated in PDAC. Inactivating mutations in the TP53 gene have been identified in 75% of PDAC cases, and in ~55% cases deletions or mutations in the SMAD4 gene were observed. The regulatory signaling pathways involved in embryonic pancreas development, such as Notch, Sonic Hedgehog, and Wnt are often activated in PDAC [23-25]. Other changes occur less often and are described in detail in recent reviews [26-28].

These genetic changes cause a critical disruption of the fundamental control processes in the normal pancreas cell, which finally initiates formation and directs the growth of very aggressive tumors. However, it is not quite clear whether the abovementioned genes are necessary and sufficient for tumor initiation and development. New genes supposed to have an important role in PDAC development are constantly described in scientific literature. Moreover, the data that even such fundamental changes as above are insufficient for malignant transformation of pancreatic cells are increasingly reported. In particular, it was shown that pancreatic cells were resistant to malignant transformation by the oncogenic form of KRAS even when these cells already had non-active forms of tumor suppressors p53 or INK4A (CDKN2A). An exception is a subpopulation of pancreatic cells expressing the PDX1 gene [15, 25].

CANCER AS A RECAPITULATION OF EMBRYOGENESIS PROCESSES – THE PDAC CASE

Cancer is often considered a problem of developmental biology. There is an evident similarity between cancer and embryonic cells [29]. Along with other authors, we have repeatedly reported that genes operating in embryonic cells that are switched off in cells of mature tissues are often switched on in malignant tumor cells, and vice versa [30, 31]. Accordingly, the master regulators identified during investigation of pancreas embryogenesis
are candidates for the role of key genes responsible for initiation and progression of PDAC. It is important to remember that quite often, and perhaps always, master factors play their roles by interacting with each other.

The figure (panel (b)) illustrates how closely master regulators participating in the development and pancreatic cancer are overlapped. The finding that the same genes and regulatory networks are used during embryonic and carcinogenesis permits us to hope that identification of the elements of these networks in any of these processes will provide the missing information for better understanding of the mechanism of the other processes. Here, it should be kept in mind that this complementarity requires the knowledge of which cells in the development process give rise to cancer and ensure further cancer progression. Today it is often not quite clear. Identification of progenitor cells for any cancer is a complicated problem. In general, tumors are classified according to their histological characteristics, but morphological properties are not always indicative of the cell origin [32]. This, in turn, makes identification of master genes involved in etiology of cancerous tumor even more difficult.

In particular, initially PDAC has got its name due to histological similarity with duct structures. However, recent studies have shown that tumors with ductal morphology can emerge during activation of a mutant KRAS in duct-like progenitor cells or in pancreatic acinar cells via the process known as acinar-to-ductal metaplasia [31, 33]. In this process, acinar cells are reprogrammed into embryonic progenitor ductal cells. A large amount of data suggest this mechanism, although it cannot be taken as conclusively established [34]. Actually, dedifferentiated cells possess properties similar to both ductal and progenitor cells present during pancreas development (expressing the SOX9, HNF6, HES1, PDX1, and NESTIN genes). However, unlike progenitor cells, they do not express HNF1β and NKx6 and were therefore termed duct-like cells [8]. PDAC tumors retain features of highly differentiated epithelial (ductal) cells in the early stages of development, while a phenotype similar to mesenchymal — the so-called quasi-mesenchymal phenotype — is characteristic of rapidly progressing tumors. Substantial changes in the cell phenotypes that occur in pancreatic carcinogenesis are probably caused by both somatic genetic and epigenetic changes in tumor cells and signals from the stromal environment. This implies the existence of specific transcription factors that control the regulatory programs in each of histological phenotypes by accepting these signals [35]. Master regulators participating in specifying the pathway of cell differentiation during development (lineage-specifying transcription factors) play a key role in this process [4, 5].

Whereas these factors and their interactions are mostly unknown, some of them have been recently identified using modern high-throughput genome and epigenome analysis. For example, acinar cell transdifferentiation probably includes: a) loss of cellular identity (dedifferentiation), and b) subsequent acquiring of ductal properties. The loss of cellular identity is related to switching off the expression of such transcription factors as NR5A2, MIST1, and PTF1A, which maintain the identity of acinar cells and prevent KRAS-induced oncogenesis. Deletion of the NR5A2 or PTF1A genes from pancreatic cells facilitates rapid acinar-to-ductal metaplasia. The MIST1 factor is also essential for maintaining functions, stability, and identity of acinar cells [5].

Acquiring of ductal phenotype includes upregulation of the ductal master regulators, such as SOX9 and HNF6. Artificially induced in acinar cells expression of the SOX9 transcription regulator, which maintains identity of ductal cells, leads to acinar-to-ductal metaplasia. The PRRX1 transcription factor, expression of which is upregulated during acinar differentiation, binds the SOX9 promoter and positively regulates SOX9 expression. The ductal-specific transcription factor HNF6 suppresses expression of acinar factors and activates that of ductal factors, including SOX9.

Considering the role of PDX1, it should be mentioned that the onset of PDX1 expression occurs already in ductal carcinoma progenitor cells such as PanIN [36] and that artificial induction of its expression in acinar cells causes acinar-to-ductal metaplasia [5]. Some authors suggest that PDX1 can function as a gatekeeper of acinar cell dedifferentiation, because the deletion of the PDX1 gene promotes dedifferentiation of acinar cells [31]. Nevertheless, the data on the role of PDX1 in maintenance of acinar cells identity seem to be rather contradictory (see a recent paper [37] as an example).

Thus, the initiation of the cascade of transdifferentiation of acinar cells to ductal ones is achieved probably through activation of several transcription factors at a top position in the hierarchical signaling cascade (figure, panel (b)). However, the complete reprogramming requires the presence of oncogenic KRAS mutations [5].

Despite recent progress in understanding genomic and epigenomic changes of human embryonic pancreatic progenitor cells, so far there is no clear understanding of the complete regulatory system underlying differentiation of ductal cells in normal and tumor pancreas tissues. This is also true for other types of cancer.

**PDX1: A MULTIFACETED MASTER GENE IN PANCREATIC CANCER**

PDX1 changes its function at least three times during pancreas oncogenesis: (i) it has been suggested [31] that

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1 A normal cell can either change its cellular identity and become a different cell type (transdifferentiation) or lose functionality specific for this type of cells and return to the state of progenitor cell — dedifferentiation. Transdifferentiation can proceed directly or via a dedifferentiated state [8].
PDX1 plays a crucial role in maintenance of acinar cell identity and prevention of so-called pancreatic intraepithelial neoplasia (PanIN) that precedes PDAC. Hence, at this stage, PDX1 plays a role of suppressor; (ii) the role of PDX1 changes to oncogenic during neoplastic transformation as it stimulates cell proliferation and inhibits apoptosis; and, finally, (iii) when the expression of the PDX1 gene in cancerous cells ceases during the process of epithelial—mesenchymal transition (EMT), metastasizing begins. Therefore, at this stage PDX1 plays a role of an EMT suppressor [31].

This change of functionality from stage to stage is likely caused by substantial changes in the epigenetic context of the PDX1 binding sites in chromatin during different stages of PDAC progression. This hypothesis seems justified on the background of the known data on epigenetic differences between acinar and PDAC cells [31].

According to the alternating function, there is only minimal overlapping of the genome regions that bind PDX1 in normal acinar cells and in PDAC cells [31], as shown in experiments using the chromatin immunoprecipitation (ChIP) technique with antibodies against PDX1 combined with high-throughput sequencing of chromatin DNA from acinar cells and mouse cell lines derived from PDAC mice. This implies that PDX1 regulates different sets of genes in primary and transformed cells. In particular, in acinar cells PDX1 interacts with genes associated with the differential state of acinar cells (such as CELAI, CELA2, CPA2, and AMY1) and with embryonic development and differentiation of epithelial cells (e.g. NR5A2, FOXA2, and ONECUT1). In tumor cells, PDX1 is bound to the genes involved in carcinogenesis, including EMT and cellular response to TGF-β signal. The enhanced binding of PDX1 in these genome regions correlates with active transcription of the associated genes and activation of epigenetic modifications of chromatin.

The data demonstrating that regions of PDAC-specific genes (MAP3K1, MAP4K3, MAPKAPK2, and CDKN2B) in acinar cells contain an elevated level of the repression label (H3K27me3) as compared to PDAC cells are also associated with the variation in functions.

Finally, the data demonstrating that PDX1 binding genome regions in acinar cells are enriched in binding sites of transcription factors that define a normal differentiated state of pancreas, such as HNF4A, NKKX6.1, HNF1A, and HNF1B, are also in agreement with different functions. In contrast, PDX1 binding sites in some PDAC cells are enriched in motifs of oncogenic transcription factors, such as GSC2, PRRX2, STAT5, C-JUN, and C-FOS.

Expression of the genes controlled by PDX1 is significantly different in acinar and PDAC cells [31]. However, a more detailed analysis of each stage of PDAC progression is required for more substantiated conclusions.

MULTIFUNCTIONALITY IS CHARACTERISTIC OF TRANSCRIPTION FACTORS AND OTHER REGULATORS OF ONCOGENESIS

Multifunctionality is actually not surprising, considering that otherwise it would be difficult for nature to solve the problem of creating complex organisms when having only a restricted number of genes. Nevertheless, the situation when products of the same gene have antagonistic functions intuitively seems not quite logical. For example, cancer genes are historically classified as oncogenes or suppressors, but there is increasing information that there exist genes that can play one or another role “depending on the context”. This phenomenon was first reviewed by our late colleague Vadim Moiseevich Kavsan with coauthors [38]. Further, we briefly summarized examples of this antagonistic dualism. We used the data published after 2013 and refer the reader to a review [38] for earlier publications. Also, we did not touch here the dual oncogene/suppressor role of microRNA; instead the reader is referred to recent papers [39, 40] and references therein.

1) The TGF-β factor of the tumor microenvironment may cause apoptosis of cancer cells or initiate the epithelial—mesenchymal transition, which facilitates invasion and metastasis of cancer cells [41].

2) In colorectal cancer, the UVRAG gene, in particular, responsible for maintenance of chromosome stability, due to a mutation in the reading frame generates a strongly truncated version of the UVRAG protein, which counteracts the suppressor function of the wild type UVRAG. At the same time, this truncated UVRAG facilitates carcinogenesis, EMT, and metastasis [42].

3) The insulin-like growth factor binding protein (IGFBP2) in cancer is commonly considered an oncogene, and its expression is often upregulated in tumor cells. Nevertheless, there are quite a few papers reporting that IGFBP2 also acts as a suppressor [43].

4) It was recently reported that the SRPK1 splicing kinase could function as an oncogene or as a suppressor, modulating activity of Akt protein kinase [44].

5) It was found that depending on the context the NOTCH signaling pathway can play either an oncogene or suppressor role [45].

6) Expression of the human epidermal growth factor 2 receptor (HER2) is upregulated in 20–30% of breast cancer cases, causing faster growth and more aggressive tumors. Alternative splicing generates a functionally different variant of HER2 (termed Herstatin), which is formed by retention of the eighth intron in the mRNA. Herstatin acts as a tumor suppressor effectively blocking HER2 activity and cell proliferation, which facilitates apoptosis [46].

7) SOCS1 is known as a suppressor in many types of cancer, but it also can function as an oncogene [47].

8) The AMP-activated protein kinase (AMPK) is historically considered a component of a tumor-sup-
pressing complex. Nevertheless, recent data indicate its oncogenic role [48].

9) The Kruppel-like transcription factor 4 (KLF4) functions as a tumor suppressor in many types of cancer. However, it was reported that KLF4 has an oncogenic function in breast cancer and other types of cancer. KLF4 changes its function in PDAC during tumor development. It has an oncogenic function in PDAC initiation and a suppressor function in tumor progression [34]. Another member of this family, KLF5, displays the same dualism [49]. In this regard, KLF4 and KLF5 are similar to PDX1, which is the subject of this review.

The mechanisms of multifunctionality can be different – from alternative splicing to a change of partners interacting with the gene or its product at different stages of embryonic development, tumor progression, or in different types of cells.

Interactions is a widely used mechanism of multifunctionality [50, 51]. Transcription factors regulate gene expression by interacting with chromatin DNA. Recruiting of coregulatory proteins is a key aspect of transcription factor functioning, and each of the factors can interact with a specific set of coregulators different from other factors. This set is also different in different cells and in different stages of such processes as development and progression of cancer. Currently, we have a wealth of data, although fragmentary, on interactions of various transcription factors. Sometimes interaction networks include a lot of partners. For example, it was shown that over four hundred proteins bind to a histone acetyltransferase and the transcriptional coactivators p300 and CBP, and among them tens of transcription factors. While this paradigm is well known, we are still far from complete understanding of the mechanisms of functioning of these interactomes, and, moreover, we do not fully understand even functions of individual transcription factors. Sometimes interaction networks include a lot of partners. For example, it was shown that over four hundred proteins bind to a histone acetyltransferase and the transcriptional coactivators p300 and CBP, and among them tens of transcription factors. While this paradigm is well known, we are still far from complete understanding of the mechanisms of functioning of these interactomes, and, moreover, we do not fully understand even functions of individual transcription factors on a single promoter [50]. This is fully true for key factors in pancreas development, and for PDX1.

Acknowledgments

The authors are thankful to Dr. B. O. Glazov for critical reading of the manuscript.

This work was supported by the Russian Science Foundation (project No. 14-50-00131).

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