Pancreatic Transcription Factors: Implications for Diabetes Therapy

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Abstract

Since both major forms of diabetes involve inadequate function of pancreatic beta cells, intensive research is ongoing to better understand how beta cells perform their complex role of secreting the hormone insulin in response to physiologic needs. Identification and characterization of pancreatic transcription factors has revealed that they play a crucial role not only in maintenance of mature beta-cell function but also at multiple stages in pancreatic development. Furthermore, recent reports have revealed their potential to convert non-beta cells into insulin-producing cells, which in some cases can function to ameliorate diabetes in experimental animals. The ability to translate these successes to the clinic will require a detailed mechanistic understanding of the molecular basis of action of these proteins. Specific gene regulation in beta cells involves the action of multiple transcription factors recruited to the promoter and functioning synergistically to activate transcription, in part through recruitment of co-activator proteins and components of the basal transcriptional machinery. In addition, the process involves modification of chromatin structure, the details of which are beginning to be elucidated. Our ability to modulate gene expression patterns may lead to developing ways to provide an unlimited supply of functional beta cells for transplantation, permitting a dramatic improvement in therapeutic options for diabetes.

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Diabetes is a serious metabolic disease whose worldwide incidence is increasing to epidemic proportions, mainly because of sedentary lifestyles and the associated obesity in the general population [1]. No cure is currently available, and current treatments are inadequate because they are associated with a relatively high incidence of long-term complications. Recently, transplantation of islet cells according to the "Edmonton protocol" has been shown to be an effective treatment for type 1 diabetes [2]. However, this approach is severely limited by a shortage of suitable donor islet tissue. There is therefore great interest in developing novel sources of functional beta cells for replacement therapy. Since normal beta cells do not proliferate efficiently *in vitro*, several experimental approaches have been initiated to achieve this goal, including engineering of beta-cell lines and use of embryonic stem cell cells and pluripotent cells derived from adult tissues [3]. In an interesting recent approach [4], pancreatic transcription factors were shown to be capable of reprogramming liver cells towards a beta-cell phenotype ("trans-differentiation"). Despite the promising nature of these results, it should be borne in mind that replacement beta cells must mimic extremely closely the function of the natural cell if long-term normoglycemia is to be achieved. This is particularly challenging given the enormous complexity of the beta cell [5]. It is therefore of great importance to understand in detail the mechanisms controlling gene-activation events, in both natural and artificial settings. The knowledge obtained will be essential for tailoring the properties of replacement beta cells to function optimally following transplantation.

Pancreatic transcription factors: role in development

The pancreas develops from dorsal and ventral budding of the foregut epithelium beginning around day E9 of mouse embryogenesis; initially, the pancreatic epithelium proliferates into the surrounding mesenchymal cells and subsequently differentiates into endocrine, acinar and duct cells [6]. Transgenic and knockout mouse models have permitted dissection of a number of key stages in this complex pathway [7]. The pioneering studies of Edlund and colleagues [8] established the paradigm that transcription factors expressed selectively in mature pancreas cells often play key roles during pancreas development; thus mice lacking a functional *Pdx1* gene show arrested pancreas development. Strikingly, this apancreatic phenotype was observed also in a human bearing mutations in both alleles of the *Pdx1* gene [9]. This result clearly identifies *Pdx1* as a crucial factor required for early development of the pancreas, though not for pancreatic specification, since buds are formed. The mechanism whereby *Pdx1* acts and the nature of its target genes during pancreas development are not yet known, though it appears that some of the effects require heterodimerization with the homeodomain protein Pbx1 [10]. The specification of endocrine cells is controlled by the Notch signaling pathway [11], which regulates expression of the key pro-endocrine transcription factor Ngn3 [12]. One important Ngn3 target gene is the bHLH transcription factor Neurod1; lack of Neurod1 leads to dramatically reduced islet cell number [7]. Several other factors (Pax4, Pax6, Nkx2.2, Nkx 6.1, Brn4) have been implicated in the switch that controls differentiation among the endocrine cell types, yet the precise mechanisms remain to be defined [13].

This approach using mouse genetics enables dissection of key steps in pancreatic organogenesis and reveals the central role of transcription factors in controlling the progressive cell fate decisions that drive the process. Furthermore, it provides a conceptual basis around which future detailed mechanistic analyses will be performed. Of particular importance will be delineation of the target genes of the various factors, and the characterization of the inter-cellular signaling molecules that control pancreas development.

Pancreatic transcription factors: role in mature beta-cell function

Cell-specific expression of pancreatic genes is controlled at the transcriptional level, in large part by combinatorial interaction among multiple transcription factors interacting with the promoter regions of the target genes [14]. Though the precise mechanisms involved have not yet been defined, a number of key principles has emerged. First, although many of the transcription factors involved are expressed in lineage-restricted fashion, expression is rarely restricted to a single cell type. Thus, Pdx1 and NeuroD1, which both play a key role in beta cell-specific gene expression, are not expressed *exclusively* in beta cells; they are also present in a restricted number of non-beta cell types: Pdx1 in intestinal cells [15] and NeuroD1 in neuronal cells and pancreatic alpha cells [16]. Combinatorial interactions clearly are an essential feature in refining the final pattern of transcription [17]. Second, the ability of transcription factors to discriminate a specific sequence is often unexpectedly low. Pdx1 and Beta2, for example, heterodimerize with ubiquitous transcription factors to increase DNA binding specificity and affinity [18,19]. Third, binding of transcription factors leads to recruitment of rate-limiting components of the transcription machinery, often mediated through binding to intermediate proteins such as transcriptional co-activators [20]. Fourth, many transcriptional activators and co-activators possess chromatin-modifying enzymatic activity, e.g., histone acetyltransferase; accordingly, altered patterns of histone modification of the insulin gene that differ among cell types have been observed [21]. The overall effect on transcription is believed to result in part from interpretation by the transcriptional machinery of the "histone code" [22], the resulting pattern of histone modification in the vicinity of the promoter.

Pancreatic transcription factors: engineering of beta cells

The essential role of Pdx1 in normal pancreatic development led Ferber et al. [23] to test whether ectopic Pdx1 expression could activate pancreatic genes in non-beta cells; indeed adenovirus-mediated activation of the insulin gene in mouse liver was observed, which was able to ameliorate streptozotocin-induced diabetes. This was shown to be associated with a persistent phenotypic shift, accompanied and perhaps controlled by activation of the endogenous liver *Pdx1* gene [4]. The ability of beta-cell transcription factors to activate insulin gene expression and mediate a "trans-differentiation" phenomenon has since been confirmed with liver and intestinal cells [4,24–27]. The efficiency of trans-differentiation varies widely with different procedures, indicating the potential for modulating the extent of the phenotype switch. In future studies, it will be important to carefully characterize the process and the properties of the engineered cells to assess their suitability for transplantation. Of considerable interest also will be their sensitivity to the autoimmune process characteristic of type 1 diabetes, and the possible ability to manipulate this characteristic experimentally.

Prospects

The study of pancreatic transcription factors has led to major advances in our understanding of the development and maintenance of cell function in this organ. It has further led to the notion that ectopic expression may lead to the generation of an important new source of beta cells to treat diabetes. The ultimate success of this idea will depend to a large extent on whether the ectopic expression of these factors, under circumstances very different from those prevailing for normal beta-cell differentiation, can nevertheless create a cell with properties that mimic beta cells sufficiently well to permit use in transplantation. Further analysis of normal and forced beta-cell differentiation will provide the answer to this key issue.

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