Upsala J Med Sci 105: 2, 7–15, 2000

Role of Tyrosine Kinase Signaling for β-Cell Replication and Survival

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ABSTRACT

Diabetes mellitus is commonly considered as a disease of a scant β-cell mass that fails to respond adequately to the functional demand. Tyrosine kinases may play a role for β-cell replication, differentiation (neoformation) and survival. Transfection of β-cells with DNA constructs coding for tyrosine kinase receptors yields a ligand-dependent increase of DNA synthesis in β-cells. A PCR-based technique was adopted to assess the repertoire of tyrosine kinases expressed in fetal islet-like structures, adult islets or RINm5F cells. Several tyrosine kinase receptors, such as the VEGFR-2 (vascular endothelial growth factor receptor 2) and c-Kit, were found to be present in pancreatic duct cells. Because ducts are thought to harbor β-cell precursor cells, these receptors may play a role for the neoformation of β-cells. The Src-like tyrosine kinase mouse Gtk (previously named Bsk/Iyk) is expressed in islet cells, and was found to inhibit cell proliferation. Furthermore, it conferred decreased viability in response to cytokine exposure. Shb is a Src homology 2 domain adaptor protein which participates in tyrosine kinase signaling. Transgenic mice overexpressing Shb in β-cells exhibit an increase in the neonatal β-cell mass, an improved glucose homeostasis, but also decreased survival in response to cytokines and streptozotocin. It is concluded that tyrosine kinase signaling may generate multiple responses in β-cells, involving proliferation, survival and differentiation.

INTRODUCTION

It is generally believed that diabetes mellitus ensues when the β-cell mass is insufficient to provide appropriate insulin secretion to maintain glucose homeostasis. The β-cell mass is a function of both β-cell regeneration and death, of which the former occurs via replication of pre-existing β-cells or β-cell neoformation from precursor cells primarily located in the ductal epithelium (3). Because the total capacity of β-cell regeneration is limited, the β-cell mass becomes inadequate to maintain normoglycemia if the destruction of β-cells is severe, as is the case when an autoimmune assault is in progress. β-cell survival may also be influenced by

other factors, such as hyperglycemia (5) and this could have a bearing on the ability of the β-cells to adequately maintain normoglycemia. Because tyrosine kinases are intimately involved in the regulation of cell replication, differentiation and survival (6), it is the aim of the present article to summarize certain attempts to characterize a role of tyrosine kinases in these processes.

TRANSFECTION OF β-CELLS WITH DNA CONSTRUCTS EXPRESSING TYROSINE KINASES

Primary β-cells were transfected with gene constructs encoding tyrosine kinases in order to establish a role of these for β-cell replication (20). Expression of v-Src in β-cells using the electroporation technique resulted in increased β-cell DNA synthesis (20). Likewise, cotransfection of constructs encoding the PDGF-BB (platelet-derived growth factor) ligand and the PDGF β-receptor by liposomal techniques also stimulated islet replication rates (21). Finally, when a DNA construct encoding a chimeric PDGF/FGFR-1 (fibroblast growth factor receptor-1) construct was transfected together with a construct encoding PDGF-BB into islets, β-cell replication was stimulated (16). In all cases, only a small fraction of cells was stimulated to synthesize DNA despite efficient transfection, suggesting that a permanent suppression of β-cell replication may exist in the adult state, thus accounting for the low replicatory activity. Such a state of growth arrest could be the consequence of active suppression via expression of antiproliferative genes, or a result of inadequate expression of signaling components required for cell cycle progression.

EXPRESSION OF TYROSINE KINASES IN β-CELLS

The expression of tyrosine kinases in insulin producing cells was assessed by a PCR- (polymerase chain reaction) based method utilizing primers recognizing conserved elements in the kinase domain (25). This was performed in order to obtain an understanding of the normal regulatory role of tyrosine kinases for β-cell function. In the insulinoma RINm5F cell line, the tyrosine kinase receptors VEGFR-2/Kdr/Flk-1 (vascular endothelial growth factor receptor-2) and c-Kit were detected, together with the cytoplasmic tyrosine kinases Tec and Hck (25). Fetal rat islets expressed VEGFR-2/Kdr/Flk-1, the IGF-1 (insulin-like growth factor) receptor, the FGFR-4, the receptor tyrosine kinase Axl/Ark/UFO and the cytoplasmic kinase Fgr (25). In adult islets of Langerhans, the IGF-1 receptor and Jak2 were detected (25). Immunohistochemical analysis revealed expression of VEGFR-2/Kdr/Flk-1 and c-Kit in pancreatic duct cells (25, 27), whereas the FGFR-4 was found to be present in both the exocrine and endocrine pancreas (27). Addition of the VEGFR-2 ligand VEGF to cultures of fetal rat islets of Langerhans, which are rich in ductal cells, increased their insulin content (25). The insulin content of fetal islets was likewise increased by addition of the c-Kit ligand stem cell factor (27). When isolated ductal structures were cultured in the presence of VEGF, DNA synthesis was stimulated

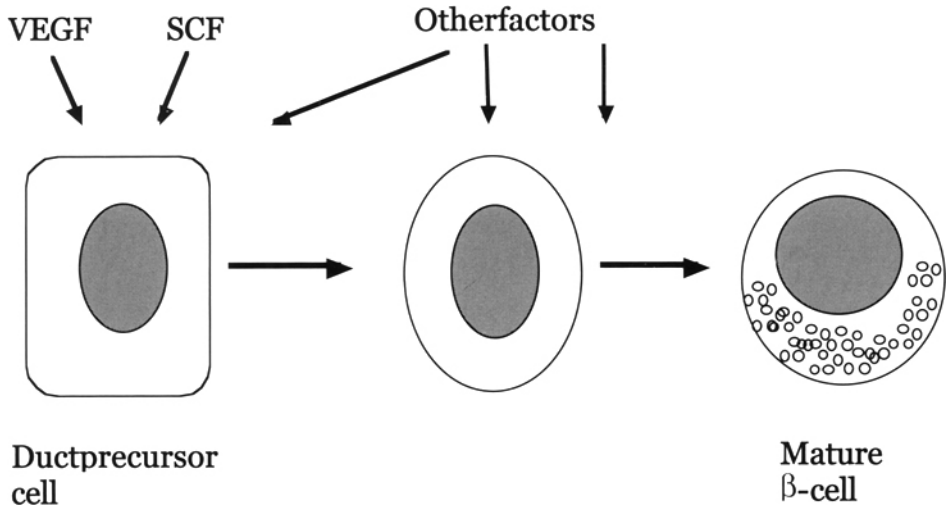

Figure 1. Model of the putative role of vascular endothelial growth factor (VEGF) and stem cell factor (SCF) for β-cell maturation from ductal precursor cells. See text for details.

(28). Furthermore, porcine islet-like cell clusters isolated from the fetal porcine pancreas increased their insulin content and lowered their insulin secretion when cultured in the presence of VEGF (28). The data suggest that VEGF stimulates ductal cells, and that this has consequences for the formation of β-cells. A model visualizing our current view on the roles of VEGFR-2 and c-Kit is shown in Fig. 1. The ligands VEGF and stem cell factor cause proliferation of precursor cells located in the ductal epithelium and this is a prerequisite for the generation of mature β-cells, which also requires the simultaneous or sequential stimulation by other factors that give rise to differentiation.

Other tyrosine kinase receptors demonstrated to be expressed in the islets of Langerhans are FGFR-1 (12), TrkA (17), c-Met (2) and the insulin receptor (14). The role of the TrkA ligand, NGF (nerve growth factor), for β-cell function remains elusive, whereas FGF-2 and HGF (the c-Met ligand) both stimulate β-cell DNA synthesis. Insulin has been reported to play a role for β-cell insulin mRNA transcription (14), but may also function as a stimulator of β-cell replication and/or survival (10).

ROLE OF MOUSE GTK FOR β-CELL PROLIFERATION

Among the tyrosine kinase sequences amplified from the RINm5F cDNA was a novel sequence which we subsequently cloned (26) and named Bsk. Bsk is identical to Iyk (19) and homologous to the rat sequence Gtk (18) and human sequence Frk/Rak (4, 13). For practical reasons, we have renamed Bsk/Iyk to mouse Gtk. Gtk has the characteristics of a Src-like tyrosine kinase with a partial myristoylation

Figure 2. Schematic display of the Gtk structure, indicating the positions of regulatory tyrosines located in the C-terminus.

signal in its N-terminus, SH2 and SH3 (Src homology- 2,- 3) domains, a tyrosine kinase domain and regulatory tyrosines in its C-terminus (26). In mouse Gtk, there are two potential regulatory tyrosines corresponding to tyrosine-527 in Src; tyrosine 497 and 504, with the latter being the more plausible candidate (Fig. 2). Mutation of these sites in order to constitutively activate mouse Gtk was performed, and the mutants were stably expressed in NIH3T3 fibroblast cells (29). Whereas the Y497F- and Y504F-mutants each alone did not affect cell proliferation, the Y497/504F-double mutant decreased cell proliferation by increasing the number of cells in the G1 phase of the cell cycle (29). This indicates regulatory roles of both the 497 and 504 tyrosines. The Y504F mutation increased kinase activity in analogy with Y527 in Src, whereas the Y497F mutation caused a translocation of mouse Gtk to the nucleus (29), and both these effects appear important for the inhibition of proliferation. When mouse Gtk (wild-type and mutants) was overexpressed in the insulinoma RINm5F cells (1), inhibitory effects on cell replication were detected. Unlike in the NIH3T3 cells, the Y504F-mutant was also effective besides the Y497/504F-mutant in inhibiting cell proliferation by increasing the number of cells in the G1-phase of the cell cycle. The expressed Y504F-Gtk mutant was partly detected in the nucleus, in contrast to what was observed in the NIH3T3 cells. The effects of mouse Gtk expression were paralleled by increased expression of the cell cycle regulatory proteins $p27^{Kip1}$ and p130 Rb2, thus providing an explanation for the inhibitory effects on cell proliferation. When mouse Gtk kinase activity was determined, complex regulatory roles of tyrosines 497 and 504 were noted. The Y497/504F-double mutant displayed a lower K_m but also a lower V_{max} compared with the wild-type, whereas the Y504F-mutant exhibited increased K_m and V_{max}. The Y497F-mutant appeared to increase both K_m and V_{max} slightly. The Y504F and Y497/504F-mutants conferred decreased viability in response to exposure to the cytokines IL-1β + IFN-γ (1). Finally, the expression of the islet hormones glucagon and insulin was assessed in the RINm5F cells expressing the mouse Gtk mutants. All clones expressing the Y504F- and the Y497/504F-mutants showed elevated glucagon mRNA contents, whereas the Y497/504F-double mutant decreased the insulin mRNA level. Thus, Gtk may play a role in the inhibition of cell cycle progression via effects of $p27^{Kip1}$ and Rb2 expression, and this may have consequences for the differentiation of cells. Furthermore, Gtk may mediate cytokine induced cytotoxicity.

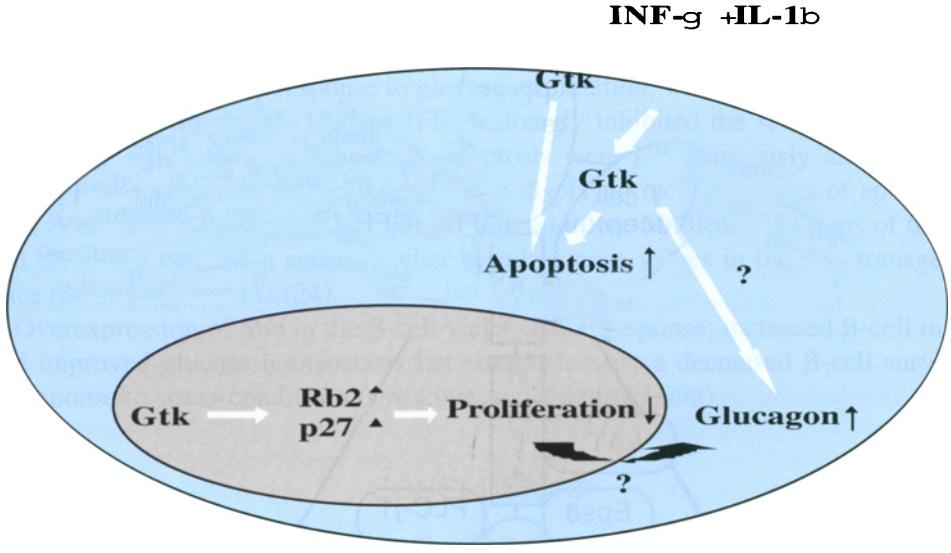

Figure 3. Possible roles of Gtk for cell function. Gtk can be found in the nucleus, in the cytoplasm and at the plasma membrane. See text for details.

Fig. 3 depicts the role of Gtk for cell function. Gtk may be found both in the nucleus, at the plasma membrane and in the cytoplasm. Nuclear Gtk increases the $p27^{Kip1}$ and p130 Rb2 levels, and this decreases cell proliferation by inhibiting the cell cycle. Gtk participates in the cytotoxicity of the cytokines IL-1β + IFN-γ, and this could reflect an action of Gtk at the plasma membrane in proximity with the cytokine receptors or a later cytoplasmic event in the signaling pathways of these cytokines. The increase in glucagon gene expression is either the consequence of decreased cell proliferation or signaling from cytoplasmic Gtk.

ROLE OF SHB FOR β-CELL SURVIVAL

The adaptor protein Shb was originally cloned as a serum-inducible gene in the insulin producing βTC-1 cell line (22). Shb has the characteristic features of proline-rich sequences in its N-terminus, a central PTB (phosphotyrosine binding) domain, several potential tyrosine phosphorylation sites and a C-terminal SH2 (Src homology 2) domain (22, 23). The proline-rich motifs interact with the SH3-domain proteins Src, Eps8, p85 PI3-kinase, Grb2 and PLCγ (phospholipase C)(7, 15, 23), whereas the PTB domain binds p36/38 LAT (15, 23). The SH2 domain has been found to associate with the PDGF receptors, the FGFR-1 (7) and the T cell receptor (23). Shb gene expression is under the control of protein kinases in NIH3T3 cells (11), βTC-1 cells (11) and in the neuronal PC12 cells (9). The phosphatase inhibitor okadaic acid and the tyrosine kinase inhibitor genistein both increased Shb mRNA contents in NIH3T3 and βTC-1 cells (11). In PC12 cells, the growth or differentiation factors NGF, FGF-2 and EGF all increased Shb gene expression (9). Shb func-

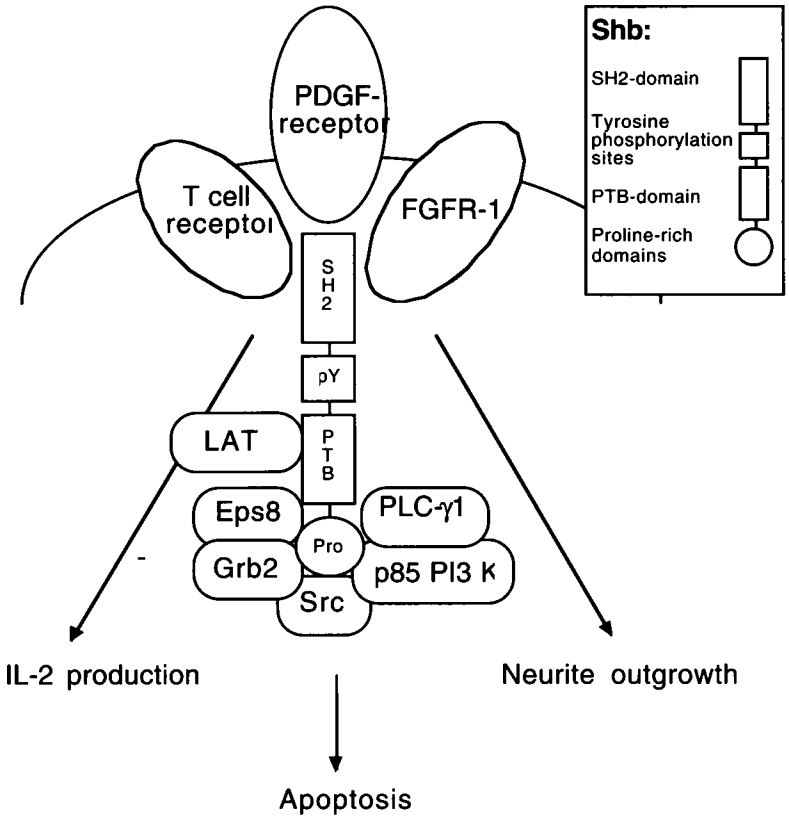

Figure 4. Schematic structure of Shb, including the different domains, and illustration of their known interactions.

tions as an adaptor protein, assembling signaling complexes in response to tyrosine kinase activation. Such complexes may mediate distinct responses in different cell types. In NIH3T3 cells, Shb overexpression resulted in increased apoptosis in response to culture at a lower serum content (8). Besides affecting cell survival, Shb overexpression has been shown to enhance NGF- and FGF-2-stimulated neurite outgrowth in the phaeochromocytoma cell line PC12 (9). In the Jurkat T lymphocyte cell line, a functional Shb molecule was required for T cell receptor-dependent stimulation of IL-2 production (15). Shb thus appears to be a versatile signaling protein that interacts with numerous receptors and transmits different signals depending on the stimulus and the responsive cell. Fig. 4 summarizes the Shb domain-interactions and the physiological responses to Shb in different systems.

To address the role of Shb for β-cell function, a transgenic mouse expressing Shb under the control of the rat insulin 2 promoter was generated (24). This construct targets expression of Shb to β-cells parallel with insulin gene expression, and thus the effects of Shb will be confined to pre-established β-cells. This transgenic mouse exhibited slightly improved glucose tolerance, an increased β-cell mass at birth

which persisted up to the age of 6 months and similar rates of β-cell DNA synthesis compared with the control mice (24). Islets isolated from the Shb-transgenic mice released more insulin in response to glucose-stimulation, whereas culture in the presence of the cytokines IL-1β plus IFN-γ strongly inhibited the secretory response from these islets. When the rates of apoptosis were simultaneously assessed, the islets isolated from the Shb-transgenic mice displayed increased rates of apoptosis, both in the absence and presence of cytokine addition. Multiple injections of the β-cell toxin streptozotocin caused higher blood glucose values in the Shb-transgenic mice than in the controls (24).

Overexpression of Shb in the β-cell yields a dual response; increased β-cell mass and improved glucose homeostasis but simultaneously a decreased β-cell survival in response to stress conditions (cytokines and streptozotocin).

CONCLUSIONS

Tyrosine kinases have the ability to affect the proliferation, differentiation/neoformation and survival of β-cells. Stimulation of tyrosine kinase receptors expressed on duct cells can promote the replication of β-cell precursor cells, which may be a prerequisite for β-cell neoformation. Expression of a Src-like tyrosine kinase, mouse Gtk, in insulin producing cells produces effects on growth and survival. Effects on β-cell growth and survival is also observed when a tyrosine kinase signaling protein, Shb, is overexpressed in these cells. It is conceivable that in several cases, tyrosine kinase signaling in β-cells may involve both growth promoting effects but also diminished capability for resisting stress-induced apoptosis. Such a dual response may be of relevance for understanding the processes that govern the development of diabetes mellitus.

ACKNOWLEDGEMENTS

This work was supported by the Juvenile Diabetes Foundation International, the Swedish Diabetes Association, the Swedish Medical Research Council (31X-10822), the Novo-Nordisk Foundation and the Family Ernfors Fund.

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