The PDGF family of tyrosine kinase receptors: a Kit to fix the beta cell?

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Abstract
Overexpression of c-Kit has recently been shown to ameliorate beta cell function by increasing the beta cell mass and insulin secretion, thus counteracting the deleterious effects of a high fat diet on glucose homeostasis. The c-Kit dependent effects are due to enhanced Akt activity that phosphorylates and inhibits the Gsk-3β kinase, thereby increasing the expression of numerous genes that promote insulin production and cell proliferation. Regulating the c–Kit/Akt/Gsk-3β pathway may provide novel means for improving beta cell function in type 2 diabetes.

Keywords diabetes, beta cell, proliferation, tyrosine kinase receptors, c-Kit, glucose tolerance

Word count 81 abstract, 1500 main text, 23 references

Abbreviations

PDGF	Platelet-derived growth factor
RTK	Receptor tyrosine kinase
Flt	Fms-like tyrosine kinase
ERK	Extracellular-signal regulated kinase
SCF	Stem cell factor
PI3K	Phosphatidyl-inositol 3' kinase
Pdx	Pancreatic and duodenal homeobox
MafA	v-Maf musculoaponeurotic fibrosarcoma oncogene family protein A
NeuroD1	Neurogenic differentiation 1
Pax	Paired box
Nkx	NK homeobox
Glut	Glucose transporter (facilitated)
Glp	Glucagon-like peptide
IR	Insulin receptor
IRS	Insulin receptor substrate
FoxO	Forkhead box O
Gsk	Glycogen synthase kinase
Tcf7L2	Transcription factor 7-like 2

The PDGF receptor family of tyrosine kinase receptors (RTK class III) consists of the PDGF receptors, c-Kit, c-Fms and Flt3 receptors. Whereas c-Fms and Flt3 primarily play a role in hematopoietic cells, c-Kit and the PDGF receptors have more diverse effects. c-Kit is of importance for the development of gametes, hematopoietic cells, melanocytes and mast cells [1], whereas the PDGF receptors are important for vascular mural cells, lung alveolar formation, intestinal villi formation, hair follicle formation and glia cells in the central nervous system [2]. These tyrosine kinase receptors have the ability to stimulate cell proliferation, increase survival, cause differentiation and cell migration by signaling through the ERK and PI3K pathways. Several oncogenic mutants of these receptors and/or their corresponding ligands have been characterized.

The group of Dr Rennian Wang, University of Western Ontario, Canada, has for several years systematically pursued a line of research investigating the role of c-Kit in beta cells and these studies have culminated in the exciting story of c-Kit dependent rescue against beta cell failure that is published in the current issue of Diabetologia [3].

The importance of PDGF for beta cells has been known for many years and early studies indicated that PDGF stimulates beta cell replication [4], that this effect is promoted by overexpression of PDGF receptors [5] and that PDGF receptors are expressed in pancreatic epithelial cells during embryonic development [6]. Recently, an age dependent decrease in PDGF receptor expression was described that diminished the proliferative potential of beta cells and beta cell specific inactivation of the PDGF receptor α-gene aggravated the susceptibility to the beta cell toxin streptozotocin [7], implicating PDGF receptors as potential targets for additional treatment regimens of diabetes. This could involve administration of PDGF ligands and/or increased expression of PDGF receptors in beta cells.

Understanding the involvement of c-Kit in beta cell function has a more recent history. Expression of c-Kit was detected in embryonic pancreatic epithelial cells or adult islets and addition of the c-Kit ligand SCF during culture of fetal islet-like structures increased their insulin content [6, 8-11]. Expression of c-Kit has been detected on both endocrine and non-endocrine epithelial cells. In addition, SCF or a c-Kit activating antibody increased the proportion insulin positive cells in human fetal islet-epithelial clusters [12, 13]. Besides playing a role for beta cell development, c-Kit also is of importance for adult beta cell function. Male mice harboring a c-Kit mutation have elevated blood glucose levels and impaired glucose tolerance [14]. They exhibit a reduced beta cell mass and insulin gene expression. Female mutant mice showed a less dramatic phenotype. Surprisingly, addition of a Gsk-3β inhibitor, 1-azakenpaullone, reversed the deleterious effect of the c-Kit mutant on glucose homeostasis suggesting the Gsk-3β pathway as responsible for the adverse effects [15].

The present investigation shows that elevated expression of c-Kit improves beta cell function particularly in male mice [3]. The authors adopted a transgenic overexpression strategy allowing the rat insulin promoter to drive c-Kit expression. As expected, isolated islets exhibited c-Kit overexpression on the RNA and protein level. The mice had improved glucose tolerance, glucose stimulated insulin secretion and islet insulin content without showing differences in peripheral insulin sensitivity.

The effects were partly due to an increased beta cell mass. Surprisingly, there was an increased mRNA content of various genes related to specific beta cell function, such as Pdx1, NeuroD1, MafA, Pax6, Nkx2-2, Glut2 and insulin. The mRNA levels of glucagon and the Glp-1 receptor were also increased. In concert, the findings point to changes in the beta cell phenotype that yield an improved insulin secretory capacity. The transgenic mice exhibited altered signaling characteristics with elevated activity of Akt and increased expression of the cell cycle protein cyclin D1. The most important effects were observed when wild type or c-Kit transgenic mice were placed on a high fat diet. The transgenic mice were resistant to high fat diet induced impairment in glucose tolerance, their weight gain was less, their fasting blood glucose levels were lower and their glucose stimulated insulin release was increased. The treatment regimen was associated with an increased transgenic beta cell mass and elevated gene expression of Pdx1, MafA, insulin, insulin receptor and IRS1. Finally, the transgene partly reversed the deleterious phenotype of the c-Kit mutant with respect to glucose metabolism, clearly implicating c-Kit in the observed beneficial effects.

The increased beta cell mass is not surprising considering that c-Kit in many systems stimulates cell replication. What is more puzzling is that the islets perform better showing increased secretory capacity and elevated expression of genes associated with the insulin secretory pathway. Is an increased beta cell mass a viable explanation for the secretory changes, allowing the cells to develop a more robust secretory phenotype over time due to less exposure to possible beta cell challenge or "exhaustion"? There are other examples of transgenic models with an increased beta cell mass that exhibit an increased insulin secretory capacity [16, 17]. However, there are also examples of experimental models with an increased beta cell mass that do not show an increased insulin secretory capacity in response to glucose in vitro [18] leaving this issue unresolved. It seems plausible that certain changes occurring in the beta cell signal-transduction signature promote both cell replication and secretory changes whereas others stimulate only one or the other of these functional aspects.

Increased Akt activity was noted and this is an anticipated finding since SCF-stimulated c-Kit is known to activate Akt [1]. On the other hand, increased expression of the insulin receptor and IRS1 was also detected and if these changes are translated into augmented signaling they would also result in stimulated Akt (Fig. 1). One potential target downstream of Akt is FoxO1 but this transcription factor was presently not investigated. Another target of Akt is Gsk-3β that becomes phosphorylated at an inhibitory site (Fig. 1). The importance of Gsk-3β in the present context is inferred from the finding that its selective beta cell specific gene ablation increases the beta cell mass, the insulin secretory response and resistance to the deleterious effects of a high fat diet [19]. In addition, Gsk-3β was also found to regulate the expression of the insulin receptor and IRS1, further tying the knot of interactions observed in the present study. As mentioned above, inhibition of the kinase by 1-azakenpaullone reversed the detrimental effects of the c-Kit mutation and Gsk-3β appears to be the common denominator for regulation of the beta cell mass and insulin secretion. Surprisingly, beta cell specific Akt overexpression did not improve the glucose stimulated insulin secretory characteristics [18], suggesting that other pathways besides Akt alone may be required for the complete phenotype. Interestingly, Gsk-3β activity potentially provides a mechanistic link to the type 2 diabetes susceptibility gene *TCF7L2* [20] since Gsk-3β regulates the levels of

β−catenin, a factor that upon entry to the nucleus forms a transcriptionally active complex with Tcf7L2.

Using a rat insulin promoter to drive transgene expression will certainly overexpress the target gene in beta cells but expression at other sites, including the hypothalamus, must be considered. There is currently no indication of possible off target expression of the transgene (whole brain expression of the transgene could not be detected) but this must not be disregarded as a possibility with respect to some metabolic effects. The failure of the transgenic mice to increase in weight upon high fat diet exposure is one such instance.

Does targeted beta cell expression of c-Kit present any potential adverse effects? Upon food deprivation, a rapid drop in blood glucose was observed and this could present a problem in conditions of scant food supply. The potential danger of an unbalanced beta cell mass was recently discussed [21] in the context of tissue-specific repression of the PDGFRα gene as well as other growth stimulating genes [22]. Perhaps restrained c-Kit-expression in beta cells is a mechanism of protection against episodes of hypoglycemia between meals due to excess insulin.

Ideally, targeted beta cell specific gene transfer of c-Kit seems an attractive strategy for treating imbalances in glucose homeostasis. However the technology as it stands at present does not allow such manipulation and therefore alternative strategies must be considered. The presently available option is using a molecular inhibitor of Gsk-3β such as 1-azakenpaullone to achieve ameliorating effects but this is an unsatisfactory strategy since Gsk-3β has multiple roles in diverse organs. Further delineation of the precise signaling cascade converging at Gsk-3β may help to identify a key component(s) more specific for the beta cell that could serve as a druggable target for treatment in type 2 diabetes. However, species differences between humans and rodents should also be taken into account since human beta cells show a much lower capacity for replication in adulthood [23] than rodent cells and thus the anticipated effect of c-Kit may in such a case become insignificant. On the other hand, if the insulinotropic effect prevails despite modest or undetectable beta cell replication in human beta cells, c-Kit activation may still be considered a relevant strategy for developing novel treatment regimens.

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Duality of interest The author declares no competing interest.

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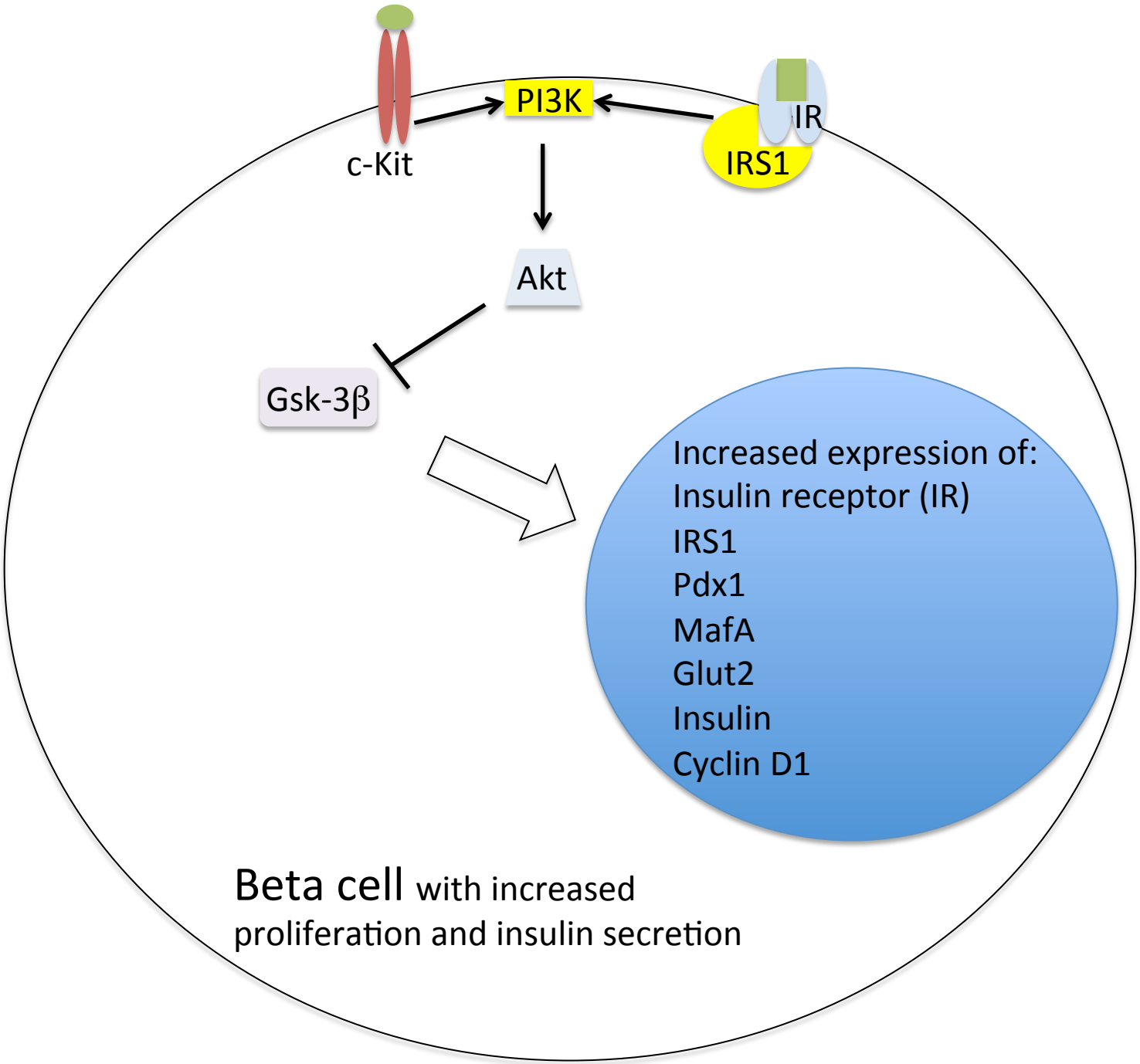
Figure legend

Fig. 1 Schematic view of c-Kit dependent effects that promote beta cell replication and insulin secretion, thereby ameliorating beta cell function. Ligand stimulated c-Kit activates Akt (via PI3K) which inhibits Gsk-3β by an inhibitory phosphorylation. The Gsk-3β kinase regulates gene expression and consequently numerous changes in beta cell gene transcription follow due to its inhibition, including those of the insulin receptor (IR) and the IR docking protein IRS1. Increased expression and activity of these signaling components will cause further activation of Akt. In addition, increased expression of genes promoting glucose stimulated insulin secretion, *e.g.* Pdx1, MafA, Glut2 and insulin, and those increasing the beta cell mass (cyclin D1) are observed under these conditions.


c-Kit
PI3K
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Increased expression of:
Insulin receptor (IR)
IRS1
Pdx1
MafA
Glut2
Insulin
Cyclin D1
Beta cell with increased
proliferation and insulin secretion