

## The Making of Pancreatic $\beta$ Cells: Advances and Apprehensions

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**Abstract.** Diabetes is a dreadful disease, which in its acute stages, causes severe multiple organ failure. It is also one of the world's oldest diseases. Type 1 Diabetes is characterized by the absence of insulin and exogenous insulin dependency. Stem cell therapy is one of the promises of this era, as there are numerous studies on Rodents, Frogs, Zebra fish, Dog and Chick, elucidating the wide array of genes, transcription factors, signaling pathways and compounds, which could promote  $\beta$  cell neogenesis, regeneration, differentiation and trans-differentiation. Even though, a recent PubMed search on the keyword 'Pancreatic beta cell proliferation' revealed around 3000 reports, this review focuses on the trends attempted in recent years and infers certain critical aspects in the observations.

### 1. Introduction

Carbohydrate metabolism represents a primitive molecular adaptation and the associated pathways are conserved across numerous species [1-14]. No major organ is spared by the ireregulation of these mechanisms. Glucose homeostasis, in higher vertebrates, is accomplished predominantly by insulin, from the pancreatic  $\beta$  cells and in lesser degree by the hepatic insulin gene expression.

Diabetes is a multifactorial disease sharing its origin and aggravations from various sources such as food habits, environment, microbial infections, and family genetics. Recently, Alicia R. Timme-Laragy et al. [15] reported that zebra fish embryos, when exposed to PCB-126, which is a polychlorinated biphenyl, commonly present in electrical equipments, rubber and plastics, could develop core molecular aberrations in the formation of pancreatic  $\beta$  cells. Diabetic cases were reported as ancient as 3000 BC, in the period of Hesyra (16), but not at such an explosive proportions, this 21 century experiences [17], as the total number of diabetic individuals in 2030 is calculated to be 366 million [18].

Various other reports lament the radical change in the food habits [19, 20] and Chrono-Biological shifts [21] from the primitive hunter-gatherer groups to the present post-industrial societies to be the source of numerous complications. Circadian clocks and various clock genes are reported to be associated with various imbalances such as Lung Fibrosis [22], Oral Squamous Cell Carcinoma [23, 24], Colorectal cancer [24, 25], and also in Neurogenesis [26].

In Type-1 diabetes, the  $\beta$  cells are destroyed rapidly by autoimmunity, hence, hyperglycaemia arises, which leads to further complications. Repeated intake of exogenous insulin, on the other hand, increases the risk of recurrent hypoglycaemia.

Henceforth, one of the primary solutions for these problems could be the regeneration of pancreatic  $\beta$  cells. These artificially generated  $\beta$  cells must have the ability to withstand high endocrinal demands, despite the increased metabolic and immunological insults.

#### 1.1. Differences in the mode of diabetes progression across species

Emily J shields et al. [27] reported the extreme difference of diabetic physiology between canines and humans. In thier study, very low and dispersed islets in diabetic pancreas were found as detected by Immunofluorescence with antibodies against Synaptophysin. No compensatory mechanisms, unlike in humans, was found, during diabetic progression. This report, with its various

evidences, collectively suggests the mechanism of autoimmunity as the cause of canine diabetes, open to further research. In addition to the canine model, pigs exhibit much less sensitivity to Insulin [28], chickens need STZ injection *in ovo*, for successful diabetic induction [29] and various classifications of diabetes occurs in dogs and cats [30]. In short, these reports indicate the radical *modes operandi* differences among diabetic subjects across the species.

## 2. Molecules and Compounds

Various naturally occurring or artificially designed molecules and compounds are being reported to be directly involved in the proliferation of pancreatic beta cells. They are mentioned in Table 1.

**Table 1.** Compounds influencing  $\beta$  cell proliferation

COMPOUND	EFFECTS UPON $\beta$ CELLS	MECHANISMS	REFERENCE
Caffeine	Increases $\beta$ cell proliferation and decreases apoptosis in the presence of palmitic acid .	-Decreases the number of insulin immunoreactive cells. -Inhibits Poly ADP ribose polymerase-1(PARP-1). -Increases cyclic AMP levels which leads cell transition from G phase to S phase .	L.Chen et al [31], Siham K.Abunasef et al. [32]
Recombinant human Pdx peptide	Differentiates human biliary tree stem cells (hBTSCs) into $\beta$ cells.	Increases MafA and Pdx-1.	Vincenzo Cardinale et al. [33]
Obestatin	Reduces $\beta$ cell apoptosis and promotes $\beta$ cell generation.	Upregulates Notch 1 and downregulates FGFR2b, FGFR2c, Notch 2 and 4.	Iacopo Gesmundo 2013.[34]
Resveratrol	Optimizes the maturation of $\beta$ cell- like cells from human embryonic stem cells (hESCs).	Increases the phosphorylation of AMPK. Upregulates PI3K/AKT pathway.	Daniela Pezzolla et al.[35]
Pioglitazone	Enhances the mass of transplanted islet cells along with Alogliptin.	Maintains normoglycaemia.	Hao Yin et al. [36]
Alogliptin	Enhances the mass of transplanted islet cells along with Pioglitazone.	Maintains normoglycaemia.	Hao Yin et al. [36] Agata Jurczyk et al. [37]
Extracts of <i>Lactarius deterrimus</i> .	Increase $\beta$ cell mass.	Lowers advanced glycation end (AGE) products. Increases CXCL12 levels. Protection from oxidative stress.	Mirjana Mihailovic et al. [38]
LPS from <i>Porphyromonas Gingivalis</i> (Pg LPS) .	Compensates $\beta$ cell mass.	Stimulates insulin secretion. Increases immune response genes (Cd8a, Cd14 and Icam1) and insulin signaling pathway genes (Stat4).	Uppoor G.Bhat et al. [39]
Protein tyrosine phosphatase 1B (PTP1B)	Negatively influence $\beta$ cell mass.	Decreases phosphorylation of AKT and ERK1/2.Activation of FOX O1.	Águeda González-Rodríguez et al., 2012 [40], Siming Liu et al.,2014 [41]
Swertisin	Promotes islet neogenesis.	Activation of MEK-TKK pathway. Down regulation of Nestin, Ngn-3 and PDX-1.	Nidheesh Dadheech et al. [42]
MSDC-0160	Maintains $\beta$ cell phenotype.	Insulin sensitizer. Increases AMPK activity. Reduces mTOR activity. Phosphorylation of Akt and GSK-3.Increased expression of Pdx-1.	Nidhi Rohatgi et al. [43]
Nitric Oxide	Anti- Proliferative.	Activation of Guanylate cyclase. Increased iNOS expression.	Laura Quintana-Lopez et al. [44]

Vglycin, a soybean peptide	Promotes $\beta$ cell proliferation and prevents apoptosis and dedifferentiation.	Increases expression of IR, Akt, Erk and Ki67. FoxO1 activation. Low NGN-3 expression.	Hua Jiang et al.[45]
TLQP-21, a VGF-derived peptide	Preserves islet mass and prevents apoptosis.	Reduces cell death. Over expression of Nkx6.1. Enhances Glucose stimulated insulin secretion(GSIS).Increases cAMP levels.	Samuel B. Stephens et al. [46]
Ebselen	Increases $\beta$ cell mass, prevents apoptosis and prevents oxidative stress.	Reduces lipid peroxidation. Increases expression of Insulin transcription factors Pdx-1 and MafA.	Jana Mahadevan et al. [47]
Secreted factors by dental pulp stem cells from human exfoliated deciduous teeth (SHED)	Increase $\beta$ cell proliferation.	-	Takako Izumoto-Akita et al. [48]
Epoxy-pukalide	Induces $\beta$ cell proliferation via ERK 1/2 signalling.	Activation of ERK 1/2 pathway. Induces upregulation of cyclin D2 and cyclin E.	Jose Francisco Lopez-Acosta et al. [49]
Linoleic Acid		Upregulation of MAP-kinase and mTOR signaling pathways. Activation of ERK signaling.	In-Su Kim et al. [50]
Glucagon-Like Peptide-1 (GLP-1)	Increases $\beta$ cell proliferation.	Antiapoptotic.	B.W. Lee et al. [51]
Combination of PSN632408 and Sitagliptin.	Increase $\alpha$ and $\beta$ cell replication.	Increases GLP-1 levels. Promotes neogenesis upon pancreatic duct cells.	Ansarullah et al. [52]
Dopamine	Negatively influences $\beta$ cell proliferation. Increases apoptosis.	Increases apoptosis. Increases caspase-3. Dose-dependent activity.	Maria Jose Garcia Barrado et al. [53]
GABA	Anti-inflammatory action. Improves $\beta$ cell replication and survival.	Recovery of degranulated $\beta$ cells. Functions via GABA receptors, GABA <sub>A</sub> -Rs and GABA <sub>B</sub> -Rs.	Jide Tian et al. [54], Chandravanshi et al. [55]
Adiponectin	Promotes reconstitution of $\beta$ -cell mass	Reduction of lipotoxicity.	Risheng Ye et al. [56]
Cyclosporin	Increases proliferation of $\beta$ cells.	Possible involvement of nuclear factor of T cells (NFAT), the element of response to cAMP (CREB), and the CREB transducer (TORC2).	Ana Elena Rodríguez-Rodríguez et al. [57]
Aminopyrazine compounds such as GNF7156 and GNF4877	Stimulate $\beta$ cell proliferation.	Inhibition of dual specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A) and glycogen synthase kinase-3 beta (GSK3B).	Weijun Shen et al. [58]
Empagliflozin, a sodium glucose co-transporter 2 (SGLT2) inhibitor.	Protects $\beta$ cell mass.	Inhibition of sodium glucose cotransporter type-2 (SGLT-2).	Henrik H. Hansen et al. [59]
Vanadyl Sulfate (VOSO <sub>4</sub> ).	Normalizes blood glucose level, Increases insulin sensitivity, increases the proliferation of $\beta$ cells and $\beta$ cell number.	Possible involvement of intra-islet and extra-islet precursors.	Samira Missaoui et al. [60]
Anagliptin	Increases $\beta$ cell proliferation.	Increases Pdx-1 and GLP-1 expressions. Effects could be reduced by high fat diet.	Takanori Shinjo et al. [61]
Gastrin	Induces $\beta$ cell neogenesis and $\beta$ cell mass expansion.	Secreted by the G cells. Induces dedifferentiation of ductal cells.	Noélia Téllez et al. [62]
I-BET151, an inhibitor of bromodomain containing transcriptional factors.	Ameliorates NOD diabetes. Increases $\beta$ cell proliferation.	Activates T <sub>reg</sub> cells. Possible involvement of M2 macrophages.	Wenxian Fu et al. [63]
WS6	Induces proliferation of both $\alpha$ and $\beta$ cells.	Involvement of I $\kappa$ B kinase pathway.	Boerner et al. [64]

### 3. Signalling pathways

Many signaling pathways and their intermediates are being reported to be involved in the proliferation of beta cells. The whole organism high-content screening by Naoki Tsujiv et al. [65] suggested 20 hit-compounds involving Retinoic acid, Serotonin and Glucocorticoid signaling pathways.

#### 3.1. Adenosine signaling

Many effective drugs and compounds were screened in the study by Olov Andersson et al. [66], to be activating the Adenosine signaling in their course towards the regeneration of the  $\beta$  cells in Zebra fish. Some of them are:

1. *NECA* (5'-N-Ethylcarboxamidoadenosine)- a nonspecific adenosine agonist that activates the adenosine G Protein Coupled Receptor (GPCR) signaling, a potent  $\beta$  cell- specific enhancer acting upon A2aa receptor. Its proliferative effects were confirmed also in the studies by Ersin Akinci et al. [67]
2. *A-134974*-An adenosine kinase inhibitor that blocks the degradation of adenosine, therefore, increasing the endogenous adenosine
3. *Cilostamide*-It could affect adenosine signaling by inhibiting phosphodiesterase (PDE3), therefore, decreasing the degradation of intracellular cAMP.
4. *Zardaverine* –It affects the adenosine signaling by inhibiting PDE3 and PDE4.
5. *IB-MECA*-An adenosine agonist
6. *EHNA*-An adenosine deaminase inhibitor

#### 3.2. TGF $\beta$ signaling

TGF-  $\beta$  signaling is probably the most frequently investigated pathway, in its relation to the proliferation of pancreatic beta cells. Inhibition of this pathway is a common therapeutic strategy for treating pancreatic ductal adeno carcinoma (PDAC) and hepatocellular carcinoma (HCC) [68-69]. Inhibition of T $\beta$ R1 (ALK5) by SB431542, leads to the down-regulated nuclear expression of the cell cycle regulator p27, therefore, promotes  $\beta$  cell proliferation [70]. Nodal, a member of this pathway almost specifically promotes  $\beta$  cells without disrupting the cellular viability [71].

The TGF- $\beta$ 2 pathway also reportedly [72] promotes endothelial cell (EC) lineage, upon induced pluripotent stem cells (iPSCs), in a miR-21-dependent manner. Knockdown or disruption of this pathway could result in significant reduction of EC markers like VE-cadherin. Deletion of this pathway in CD4+ T cells could result in the progression of Autoimmune Diabetes [73] and *In vitro* redifferentiation of  $\beta$  cells [76]. Other reports [74] suggest that both TGF $\beta$ -R1 and TGF $\beta$ -R2 are not responsible for neogenesis, but for normal  $\beta$  cell proliferation. Yousef El-Gohary et al. [75] reports the involvement of the TGF- $\beta$  signaling regulators smad 7,2 and 3 in the course of  $\beta$ -cell dedifferentiation.

#### 3.3 Retinoic Acid (RA) Signaling

Another pathway which is studied in the context of pancreas development and pancreatic cancer (PC) is the Retinoic Acid Signalling (RA) pathway. Methylation of Cellular Retinoid Binding Protein 1 (CRBP1), an important regulator of this signaling, is strongly associated with exocrine acinar regeneration [77]. It should be noted that, in the work of Georgia Pennarossa et al. [78], in their attempt to generate pancreatic  $\beta$  cells from skin fibroblasts using demethylation procedures, the addition of RA along with Activin-A, resulted in increased differentiation towards pancreatic cell lineage and formation of clearly distinguished reticular patterned cell aggregates. From these two studies, it could be hypothesized that the demethylation step is the strong reason in the development of pancreatic  $\beta$  cells. In Chick and Mouse models, this pathway is positively associated with the olfactory neurogenesis [79].

#### 4. Transcription factors

The study by Chutima Talchai et al. [80] suggests dedifferentiation of  $\beta$  cells to be the cause of diabetes. This report effectively pioneered further studies (**Table 2**) involving various transcription factors (TFs) in this field.

**Table 2.** Various TFs acting upon beta cell proliferation

TRANSCRIPTION FACTOR	EFFECT UPON $\beta$ CELLS	OTHER FUNCTIONS	REFERENCES
Pdx-1	Specific to beta cells.cdk4 dependent beta cell proliferation. Maintains regional cellular identity in adult foregut in mice.	Mutation of this lead to decreased body size.	[81-89]
Ngn-3	Promotes ectopic development of beta and delta cells.Associated with beta cell neogenesis and exocrine to endocrinal conversion.		[90-92]
ZBED6	Disruption results in abnormal islet morphology.	Negatively regulates Insulin like growth factor 2 (IGF-2) mRNA expression.	[93-94].
PAX	balance between $\alpha$ cells and $\beta$ / $\delta$ cells is maintained by the antagonistic actions of the two homeodomain transcription factors Aristaless-related homeobox (ARX) and PAX4, respectively [95-97]. Development of $\beta$ and $\delta$ cells from the dorsal pancreatic bud in mouse, but not in zebra fish.	Protection from stress-induced apoptosis. In <i>Drosophila</i> , the Ey protein is the counterpart of PAX6.	[95-99]
ChREBP	Positive association with $\beta$ cell proliferation.Cyclin D2 dependent.		[100-102]

#### 5. miRNAs

miRNAs are small, non-coding RNA sequences which could affect the gene expression either post-transcriptionally or post-translationally. Overexpression and knockout studies with many invertebrates reveal that miRNAs are inevitable for many cellular and developmental regulations as many are stage-specific and tissue-specific [103]. Recently, Ksenia Tugay et al. [104] reported the association of miRNAs with age-related decline of  $\beta$  cell function. Other similar reports are mentioned in Table 3. It should be noted that gene therapy strategies using miRNA mimics could lead to non-specific gene expression changes, failed target gene suppression, interferon responses, accumulation of unnatural strands and other molecular changes, unless handled with caution [105].

**Table 3.** miRNAs on  $\beta$  cell development

miRNA	EFFECT UPON ENDOCRINE CELLS	SIGNIFICANCE	REFERENCE
miR-7	Inhibition of $\alpha$ and $\beta$ cell differentiation	Negatively regulates Pax6.	[106]
miR-375	-Adaptive $\beta$ cell expansion. -Mass and Turnover rates of both $\alpha$ and $\beta$ cells. -Phenotype-Specificity towards $\beta$ cells.	Negatively regulated by glucose.	[107-110]
miR-17	Positive association with $\beta$ cell growth and proliferation.	Associated with liver regeneration, B cell lymphomagenesis, acute ischemic stroke(AIS), and recurrence of colon cancer.	[111-115].

## 6. Stem cells

Dilli Ram Bhandari et al. [116], had come up with a simple and time saving method for the effective *In vitro* production of the insulin producing cells (IPCs) from UCB-MSCs (Umbilical Cord Blood derived Mesenchymal Stem Cells), WJ-MSCs (Wharton's Jelly derived Mesenchymal Stem Cells) and AE-SCs (Adult Embryonic Stem Cells).

This method utilizes some of the previously reported methods [117-120] with slight modifications. Growth factors such as Fibroblast growth factor (FGF), Epidermal growth factor (EGF), Keratinocyte growth factor (KGF), Vascular endothelial growth factor (VEGF) and Insulin like growth factor-1 (IGF-1) were added in the medium. Activin A along with sodium butyrate was used as key factor for the endodermal differentiation in this study. Sanna Toivonen et al. [121], reported the stimulation with Activin A and Wnt3a for the development of hepatic and pancreatic progenitors from human pluripotent stem cells (hPSCs). Georgia Pennarossa et al. [143] also used the protocol of Shi Y et al. [118] to generate pancreatic  $\beta$  cells from adult dermal fibroblasts. Expression of SOX17, PDX1 and positive response to glucose stimulation, production of Insulin and C-peptide from the differentiated cells was found to be significantly increased than the undifferentiated cells.

Rui Wei et al. [122] compared the two routes of production, the first involving nestin-positive progenitors and second involving the definitive endoderm (DE), for the successful differentiation of cells into insulin producing cells (IPCs) from human embryonic stem cells (hESCs). In spite of the similarities of the results such as islet-like cell aggregation, expression of transcription factors such as Pdx1, MafA and Nkx6.1, production of pancreatic hormones such as Insulin, C-peptide and PP, Glucose-stimulated insulin production and IPC morphology among the both methods, there were certain differences observed (Table 4).

**Table 4.** Comparison of Nestin and DE protocols for the production of IPCs

	NESTIN PROTOCOL	DEFINITIVE ENDODERM (DE) PROTOCOL
mRNA expression of Oct4	-Considered Basal-	~9 fold higher
mRNA expression of Isl1	-Considered Basal-	2.45 fold higher
mRNA expression of Pdx	-Considered Basal-	Significantly lower
miR-145, miR-7, miR-375, miR-34a, and miR-146a.	-Considered Basal-	0.61, 1.02, 4.07, 3.47, and 17.39 fold higher, respectively.
Endocrinal Cell Morphology	Multilayered and Nested.	Monolayer and epithelial.
IPCs quantity	61.7% $\pm$ 9.5%	41.6% $\pm$ 11.8%
hESC yield at Stage V	~10 <sup>8</sup> cells	5 $\times$ 10 <sup>7</sup> cells.
Duration	28 days	20 days

Altogether, this study prescribes the nestin protocol, as it involves the neuronal-trait of  $\beta$  cells and anticipates similar works with same cell lines to elucidate and modify the different obtained results.

In addition to the report by Rui Wei et al. [122], Mahmoud M. Gabr et al. [123] compared three IPS protocols involved in the human bone marrow derived mesenchymal stem cells (HBM-MSC) production. The first protocol was the one-step protocol. This method was utilized by Hisanaga et al. [124], which used Fetal bovine serum, Conophylline and Betacellulin. The second one was the two-step protocol. This protocol was utilized by Tayaramma et al. [125]. It utilized Trichostatin-A (TSA), fetal bovine serum and glucagon like peptide-1 (GLP-1). The third was the three-step protocol used by M. M. Gabr et al. [126]. It utilized  $\beta$ -mercaptoethanol, basic fibroblast growth factor, epidermal growth factor, betacellulin, activin-A, B27 supplement and nicotinamide.

They report that the cells generated using two-step (TSA-based) protocol synthesized relatively higher Insulin and Glucagon. The relative expression of pancreatic endocrine genes was also higher in this group. Other reports suggest that factors such as culture medium density [127] could be very helpful in achieving optimal differentiation.

Dong-Sik Ham et al. [128] reports an efficient formation of Insulin-producing cells from Neonatal Porcine Liver-Derived Cells (NPLCs) by the addition of PDX1/VP16, BETA2/NeuroD and v-maf musculo aponeurotic fibrosarcoma oncogene homolog A (MafA). After transplantation in STZ-mice these cells cured hyperglycaemia and Insulin secretion was achieved in 6 weeks. The Expansion and conversion of human pancreatic ductal cells into insulin-secreting endocrinal cells also was reported [129]. They have performed adenoviral induction of *MafA*, *Neurog3*, *Pdx1* and PAX6 upon the cultured ductal cells. Significant levels of Insulin expression, Insulin<sup>+</sup> cells, Proinsulin levels, and other endocrinal islet markers such as endocrine markers, including SST, GCK, PCSK1, KCNJ11, and ABCC8 were observed as results in this study.

Fazel Sahraneshin Samani et al. [130] accomplished *in-vitro* differentiated human umbilical cord blood CD133<sup>+</sup> cells into insulin producing cells. Intriguingly, the insulin levels secreted by these cells in response to glucose challenge have varied. Exendine-4 (EX-4) [131] and Laminin 411 [132] could also be used to optimally differentiate the adipose-derived mesenchymal cells (ADMSCs) and umbilical cord MSCs, respectively, into insulin producing cells. The conversion of  $\delta$  cells into  $\beta$  cells also is an age-dependent process [133].

## 7. Other factors

### 7.1. PAK1

The p21-activated kinase 1 (PAK1) is a Serine/threonine kinase, important for the whole body glucose homeostasis and also for the insulin secretion and signaling. Survivin is a multifunctional protein involved in cell cycle regulations. In pancreas, it is selectively expressed in the  $\beta$  cells and its over-expression restores the proliferation of  $\beta$  cells. Survivin is strongly positively regulated by PAK1 [134]. Activation or over expression of PAK1 is associated with inflammation and colitis-associated carcinogenesis NF- $\kappa$ B pathway [135], Gastric cancer [136,137] and Lung cancer [138]. Therapeutic targeting of PAK1 in Acute myeloid leukemia (AML) and Myelodysplastic syndrome (MDS) is highly effective in achieving tumor-specific apoptosis and differentiation of AML cells [139].

### 7.2. CCN3

Studies on knock-out mice [140], cDNA microarrays [141] and recent works by Renée Paradis et al, in 2013[142], show that the nephroblastoma overexpressed gene (Nov or Ccn3), is a novel, and, one of the transcriptional targets, up-regulated by FoxO1 in the  $\beta$  cells. The expression of Ccn3 is increased in patients with obesity. It negatively regulates  $\beta$  cell proliferation [142].

## 8. Impact of epigenetic alterations

Few evidences were reported on the impact of epigenetic modulations upon the  $\beta$  cell phenotype and proliferation. Both DNA methylation [143] and Histone methylations (H3K4me3 and H3K27me3) [144] are reported to be associated with the formation of pancreatic converted cells (PCCs) from adult dermal fibroblasts and  $\alpha$ - $\beta$  cell plasticity, respectively.

## 9. The effect of systemic microenvironment and ageing

The effect of ageing upon  $\beta$  cell survival and regeneration is contraversial [145-147]. Similar studies were conducted in various organs such as Muscle [148], Skin [149] and Neuronal cells [150]. Heterochronic and isochronic transplant studies [151] have shown the increased proliferation rates of old islet  $\beta$  cells which were transplanted to young mice and of young islet  $\beta$  cells which were transplanted to young mice. Conversely, decreased proliferation rates were observed in young islet  $\beta$  cells transplanted to old mice and old islet  $\beta$  cells transplanted to old mice.

The Ink4a (Cyclin dependent kinase inhibitor 2a) locus has certain impact upon  $\beta$  cell mass in the course of aging [152]. This locus encodes the protein p16<sup>Ink4a</sup>, which is an inhibitor of CDK4 activity. CDK4 increases  $\beta$  cell proliferation by increasing  $\beta$  cell number [153]. This locus is regulated by the Polycomb group (PcG) of protein complexes such as Polycomb repressive complex

1(PRC1) and Polycomb repressive complex 2(PRC2) [154], which contain Bmi/ubiquitin ligase-Ring1B proteins and the histone methyltransferase, named as enhancer of zeste homolog 2 (EZH2), respectively. Repression of PcG complex and expression of EZH2 enhanced  $\beta$  cell proliferation in older mice. The conversion of  $\delta$  cells into  $\beta$  cells is also an age-dependent process [133].

The inhibition of Insulin-IGF signaling (IIS) pathway by the selective ablation of median neurosecretory cells (mNSCs) could prevent ageing complications and obesity in *Drosophila* [155]. These evidences suggest the detrimental effects of excess sugar which has been forshadowed in earlier reports [19]. In *Drosophila*, the Insulin producing cells (IPCs) are regulated by the *Drosophila* insulin like peptides (DILPS), which control the metabolic and ageing processes. The receptor of *Drosophila* tachykinin-related peptides (DTKs), DTKR regulates the survival of IPCs as suggested by increased transcript levels of *Dilp2* and *Dilp3* [156]. In addition to DTKs, the *drosophila* Cbl family proteins (dCbI) also modulates the ageing processes by directly controlling DILP production via the EGFR-ERK pathway [157].

## 10. Control of cell cycle molecules upon $\beta$ cell proliferation

Based on the previous works [158,159] on  $\beta$  cell proliferation, Nathalie M. Fiaschi-Taesch et al. [160], reported the roles of key G1/S phase regulators during the course of proliferation based on adenoviral integration with both cdk6 (cyclin dependent kinase-6) and cyclin D3 (Ad.C6+D3) and its transduction into human cadaveric and rat insulinoma cells (Ins1 832/13).

Ki67 was used in this study, instead of BrdU, as it reflects the cell cycle progress right at the time of labeling. The results of labeling of cells post-proliferation with the transduction combination are shown in Table 5. These results were confirmed by live cell imaging by GFP tagged cdk6. The expression levels were verified with Rat islets, human colon cancer cells (HCT116) and human embryonic kidney cells (HEK293).

These observations suggest optimal concentrations of p21 and 27 in promoting cell cycle and a selective trafficking of molecules across nucleus during proliferation. Although this study explains some important regulations, it anticipates more details on other important molecules in different  $\beta$  cell types. The same research group [161] reported the combinatorial actions of early and late phase Cyclins and Cdks upon the proliferation of  $\beta$  cells and their *In Vitro* expansion. They also suggest that human  $\beta$  cells are more resistant to proliferation than the  $\beta$  cells of Rats. Recently, High-Throughput Screening methods (HTS) suggested p18 and p21 to be the prime candidates for the proliferation of beta cells [162].

**Table 5.** Locations and roles of G1/S molecules on  $\beta$  cell proliferation

G1/S MOLECULES	LOCATION	LEVELS DURING PROLIFERATION	PROPOSED ROLE IN PROLIFERATION
Cyclin D3,cdk6	Nuclear	Increase	Activation
p16	Nuclear	Increase and stable	Inhibitor
p27	Nuclear	Increase and stable	Inhibitor
p21(Quiescent $\beta$ cells)	Nuclear	Increase and stable	Assembly and Translocation/Inhibitor
p15	Cytoplasmic	No change	Inhibitor
p18	Cytoplasmic	No change	Inhibitor
p19	Cytoplasmic	No change	Inhibitor
pRb (Quiescent $\beta$ cells)	Nuclear	Increased phosphorylated pRb	Inhibitor
p57(Quiescent $\beta$ cells)	Nuclear	Decrease	Inhibitor

## 11. Impact of nervous system

The synthesis of insulin, in response to stimulation of the vagus nerve, is very common in normal glucose homeostasis. Blocking the parasympathetic nervous system negatively affects  $\beta$  cell proliferation [163].  $\gamma$ -aminobutyric acid (GABA), is an important neurotransmitter of the central nervous system. In the  $\beta$  cells, it is synthesized by the two isoforms of Glutamic acid decarboxylase (GAD), GAD 65 and GAD67. This signaling has significant role in the late- fetal and early post-

natal development of pancreas in mice, as GABA signaling precede Insulin expression [164]. Serotonin, another neurotransmitter and its related signaling molecules also are playing vital role in the proliferation of  $\beta$  cells [65].

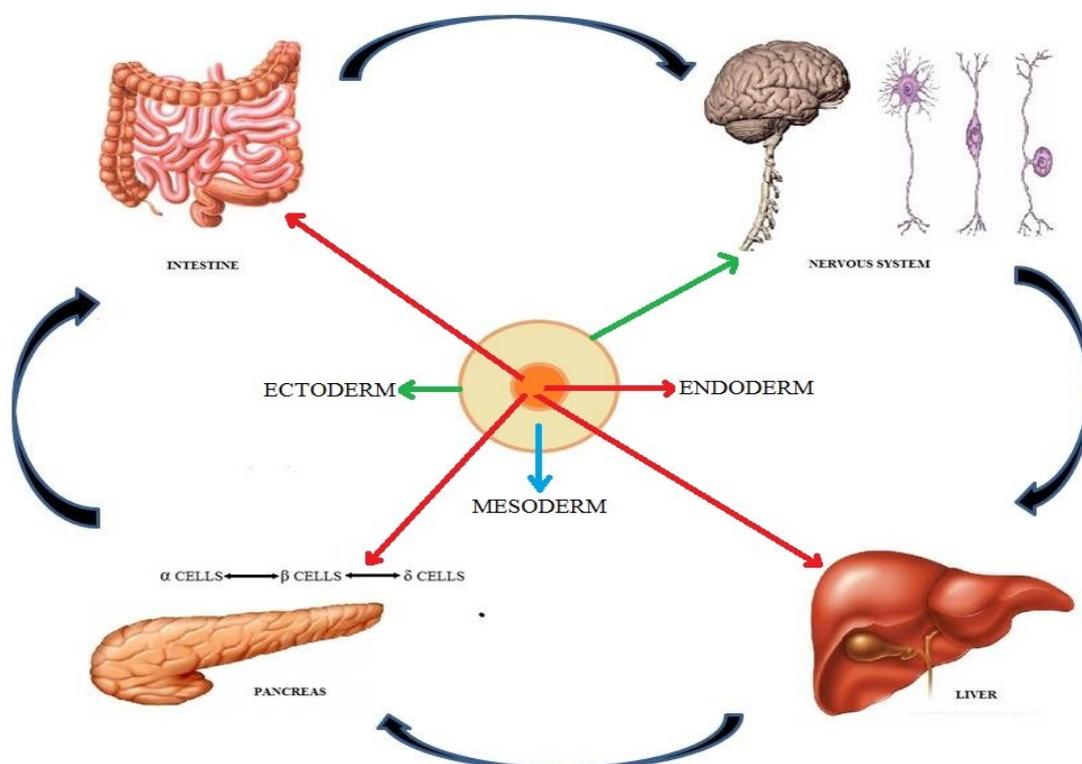
## 12. Interactions among inter and trans-dermal derivatives

The endocrinal insulin ameliorates schizophrenia; the vagus nerve and the neurotransmitter GABA stimulate  $\beta$  cell proliferation. Also

1. Andrew V. Biankin et al [165], reported with Exome sequencing and Copy number variation(CNV) methods that mutations in the genes such as ROBO1, ROBO2, SLIT2, SEMA3A, SEMA3E, SEMA5A, EPHA5 and EPHA7, which are involved in pancreatic ductal adenocarcinoma (PDAC) are also involved in axon guidance. GWAS studies suggest that ROBO2 is associated with expressive vocabulary in infants [166]. Robos are also involved in the midline crossing of axons [167] and cellular senescence [168].

3. Hussein A.N. Al-Wadei et al [169], reports the therapeutic ability of GABA along with Celecoxib, a COX-3 inhibitor, in preventing pancreatic cancer by inhibiting  $\beta$ -adrenergic effectors. Athymic nude mouse and the pancreatic lines BXPC-3 and Panc-1 were used in this study. 15nM of Epinephrin was used as stress-trigger. The inhibitory effects of Celecoxib were significantly increased by the addition of GABA.

4. By expressing the key Pdx1, MafA and Ngn3 (PMN) factors in intestinal cells, Y.J.Chen et al. [170] reports a *De Novo* formation 'Neo- $\beta$  Cell Islets'. They used double transgenic (DTG) mice generated from R26Tet $\beta$  (Rosa26 locus) and R26rtTA\*M2 mice. These neo-islets cells responded well to glucose challenge in STZ-treated mice. Islet cells could also be generated from gall bladder using the induction of *NEUROG3*, *Pdx-1* and *Maf-A* [171].



**Figure 1.** Hypothetical network among the dermal derivatives based on the recent reports

### 13. Conclusion

Altogether, these evidences suggest that there is an appreciable integration among the dermal derivatives and they could assist each other in various ways. Yet it should be noted that this 'integration' could also sabotage any therapeutic interventions upon pancreatic  $\beta$  cells, as it could affect other dermal networks. We believe that more elaborate studies on morphogenesis and pre-dermal determinants are required to confront this dilemma. Future investigations in these directions could open new doors in the area of  $\beta$  cell regeneration.

### Abbreviations

AGE: Advanced glycation end product.  
ARX: Aristaless-related homeobox  
AIS: Acute ischemic stroke  
AE-SCs : Adult Embryonic Stem Cells  
AML: Acute myeloid leukemia  
ADMSCs: Adipose- derived mesenchymal cells  
CRBP1: Cellular Retinoid Binding Protein 1  
CDK4 : Cyclin Dependent Kinase.  
DILPS: *Drosophila* insulin like peptides  
DTKs: *Drosophila* tachykinin-related peptides  
dCb: *Drosophila* Cbl family proteins  
DTG: Double Transgenic.  
DYRK1A: Dual specificity tyrosine-phosphorylationregulated kinase 1A  
EC: Endothelial cell  
EGF: Epidermal growth factor  
EX-4: Exendine-4  
EZH2: Enhancer of zeste homolog 2  
FGF : Fibroblast growth factor  
GSK3B: Glycogen synthase kinase-3 beta  
GPCR: G Protein Coupled Receptor  
GABA :  $\gamma$ -aminobutyric acid  
GAD: Glutamicacid decarboxylase  
hBTSCs: Human biliary tree stem cells  
hESCs: Human embryonic stem cells  
HCC: Hepatocellular carcinoma  
hPSCs: Human pluripotent stem cells  
hESCs : Human embryonic stem cells  
hBM-MSC: Human bone marrow derived mesenchymal stem cells.  
HTS : High-Throughput Screening methods  
iPSCs: Induced pluripotent stem cells

IPCs: Insulin producing cells  
IGF-2: Insulin like growth factor 2  
IGF-1: Insulin like growth factor-1  
IIS: Insulin-IGF signaling  
IPCs: Insulin producing cells  
KGF: Keratinocyte growth factor  
MafA: v-maf musculo aponeurotic fibrosarcoma oncogene homolog A  
MDS: Myelodysplastic syndrome  
mNSCs: Median neurosecretory cells  
NECA: 5'-N-Ethylcarboxamidoadenosine  
NPLCs: Neonatal Porcine Liver-Derived Cells  
Nov : Nephroblastoma overexpressed gene  
PDE3: Phosphodiesterase  
PDAC: Pancreatic ductal adeno carcinoma  
PCB-126: Polychlorinated biphenyl  
PARP-1: Poly ADP ribose polymerase-1  
PAK1: The p21-activated kinase 1  
PCCs: Pancreatic converted cells  
PcG : Polycomb group  
PRC1 : Polycomb repressive complex 1  
PRC2 : Polycomb repressive complex 2  
RA: Retinoic Acid Signalling  
SGLT2 : Sodium glucose co-transporter 2  
TFs: Transcription factors  
TSA: Trichostatin-A.  
UCB-MSCs: Umbilical Cord Blood derived Mesenchymal Stem Cells  
VEGF: Vascular endothelial growth factor  
WJ-MSCs : Wharton's Jelly derived Mesenchymal Stem Cells

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