The transcription coactivator Yes-associated protein (YAP) influences β-cell proliferation and diabetes

Dissertation

zur Erlangung des Grades eines Doktors der Naturwissenschaften
im Fachbereich Biologie/Chemie der Universität Bremen

vorgelegt von
Sahar Mohammadrafiezadeh, M.Sc.
Bremen, 17.12.2019

1. Gutachter: Prof. Dr. Kathrin Maedler
2. Gutachter: Prof. Dr. Andreas Birkenfeld
Date of defense: 10.02.2020

Versicherung an Eides Statt

Ich, Sahar, Mohammadrafiezadeh (Vorname, Name)

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.
Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe.
Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung.

Bremen, 17.12.2019, Sahar Mohammadrafiezadeh

_____

Ort, Datum Unterschrift

Contents

Summary

Beta cell failure is a hallmark of both type 1 and type 2 diabetes. The mechanisms of the initiation of beta cell dysfunction and beta cell death are not completely understood. Investigating the mechanism of action of various signal molecules involved in beta cell apoptosis and proliferation can result in novel targets for diabetes treatment. The Hippo pathway is a vital cascade that plays a fundamental role during cell and organ development. It also regulates beta cell proliferation, apoptosis, and differentiation through its main components including NF2, MST1/2, LATS1/2 and YAP. Yes associated protein (YAP) is a main downstream target of Large Tumor Suppressor (LATS) 1/2 and transcriptional co-activator that enhances expression of several genes by interaction with TEAD transcription factor.

YAP is highly expressed during pancreas development. As soon as endocrine islet cells origin, YAP is limited to exocrine and duct cells and excluded from the endocrine part. Also later in mature beta cells, I found that YAP is not expressed.

In my doctoral thesis I asked the question whether YAP re-expression can restore the almost absent proliferative capacity in mature beta cells. We also clarified the effect of YAP on pancreatic beta cells in both physiological and diabetic states.

Therefore, I re-expressed the active form of YAP specifically in beta cells and in human islets. Indeed, I found that YAP re-expression enhances beta cell proliferation without changing beta cell function and identity. The Forkhead Box M1 (FOXM1)-YAP crosstalk plays a crucial role in switching on beta cell proliferation, regeneration and cell cycle progression. In parallel, YAP re-expression has an anti-apoptotic effect on beta cells under diabetic conditions.

In a second part of this study, I analyzed the differential expression of mechanistic target of rapamycin complexes (mTORC), master regulators of nutritional status at both cellular and organismic levels, in human and mouse diabetic islets under diabetogenic conditions. Our results revealed a hyperactivity of mTORC1 in human islets from patients with type 2 diabetes. Moreover, specific mTORC1 inhibition can restore beta cell function in diabetes.

Altogether, my data suggest that high metabolic overload leads to mTORC1 hyperactivity; such beta cell stress impairs beta cell function and survival during the progression of diabetes. As beta cells have lost important pro-proliferative factors during maturation and identity, such as YAP, they are unable to compensate for a chronic high metabolic demand. The results of my work propose that a transient overexpression of YAP restores β-cell proliferation during stress and could stand as future β-cell regeneration therapy for functional beta cell mass expansion. It could further be used as tool for cell replacement therapy to restore β-cell survival during islet transplantation.

Zusammenfassung

Sowohl Typ-1 als auch Typ-2-Diabetes sind gekennzeichnet durch das Versagen der Betazellen im Pankreas. Die Mechanismen, wie es tatsächlich initial zur Dysfunktion und dem Tod der Betazelle kommt, sind noch weitgehend unverstanden. Die Untersuchung des Wirkungsmechanismus verschiedener Signalmoleküle, die an der Apoptose und Proliferation von Betazellen beteiligt sind, kann neue Strategien für die Diabetesbehandlung identifizieren. Der HIPPO Signalweg ist eine lebenswichtige Kaskade, die bei der Zell- und Organentwicklung eine grundlegende Rolle spielt und auch Proliferation, Apoptose und Differenzierung der Betazellen über seine Hauptkomponenten NF2, MST1/2, LATS1/2 und YAP kontrolliert.
YES assoziiertes Protein (YAP) ist ein Haupttarget des Large Tumor Suppressor (LATS) 1/2 Proteins und transkriptioneller Co-Aktivator, das die Expression mehrerer Gene durch Interaktion mit dem TEAD-Transkriptionsfaktor aktiviert.
Während der Pankreasentwicklung ist YAP in allen Zellen stark exprimiert. Sobald jedoch endokrine Inselzellen entstehen, ist YAP auf exokrine und duktale Zellen beschränkt und vom endokrinen Teil ausgeschlossen. Auch später in adulten Betazellen fand ich heraus, dass YAP nicht exprimiert wird.
In meiner Doktorarbeit stellte ich die Frage, ob eine Wiederherstellung von YAP in Betazellen deren stark limitierte Kapazität zur Proliferation erneuern kann. Dabei habe ich die Wirkung von YAP auf die Beta-Zellen der Bauchspeicheldrüse sowohl im physiologischen als auch im diabetischen Zustand untersucht.
Die aktive Form von YAP wurde in Betazellen und in humanen Inselzellen überexprimiert. Ich konnte feststellen, dass die YAP-Reexpression die Proliferation von Betazellen fördert, ohne die Funktion und Identität der Beta-Zellen zu verändern. Das Zusammenspiel von Forkhead Box M1 (FOXM1) mit YAP spielt eine entscheidende Rolle bei der Einschaltung der Betazellproliferation, Regeneration und Zellzyklusprogression. Parallel dazu hat die YAP-Reexpression eine antiapoptotische Wirkung auf Betazellen unter diabetischen Bedingungen.
Im zweiten Teil dieser Studie analysierte ich die differentielle Expression der mechanistischen Targets von Rapamycin (mTORC), Master-Regulatoren des Ernährungsstatus auf zellulärer und organismischer Ebene, in diabetischen Inseln unter diabetogenen Bedingungen. Unsere Ergebnisse zeigen eine Hyperaktivität von mTORC1 in menschlichen Inseln von Patienten mit Typ-2-Diabetes. Darüber hinaus kann die spezifische mTORC1-Hemmung die Beta-Zellfunktion im Diabetes wiederherstellen.
Insgesamt deuten meine Daten darauf hin, dass eine hohe metabolische Überlastung zur mTORC1-Hyperaktivität führt, und dass ein solcher Betazellstress die Funktion und das Überleben der Betazellen während des Fortschreitens des Diabetes beeinträchtigt. Da Betazellen während der Reifung und Identität wichtige proproliferative Faktoren wie YAP verlieren, können sie einen chronisch erhöhten Stoffwechselbedarf nicht kompensieren. Die Ergebnisse meiner Arbeit deuten darauf hin, dass eine vorübergehende Überexpression von YAP die Betazellproliferation unter Stress wiederherstellt und als zukünftige Betazellregenerationstherapie zur Wiederherstellung physiologisch-funktioneller Betazellen stehen könnte. Es könnte auch als Werkzeug in der Zellersatztherapie fungieren; zur Wiederherstellung des β-Zellüberlebens während der Inseltransplantation.

Abbreviations

AA	Amino acids
APCs	Antigen presenting cells
BCL-2	B-cell lymphoma
BCL-XL	B-cell lymphoma-extra large
CTGF	Connective tissue growth factor
DACH1/2	Dachshund Homologs 1/2
DAG	Diacylglycerol
4E-BP1	4E-binding protein
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial–mesenchymal transition
ER	Endoplasmic reticulum
Ex	Expanded
FAT 1–4	FAT atypical cadherins 1–4
FFA	Free fatty acids
FKBP12	FK506-binding protein 12
FOXOs	forkhead family of transcription factors
FRB	FKBP12-rapamycin binding
FRMD1/6	FE1-4RM Domain-containing Proteins 1/6
GABP	GA-binding protein
GCG	Glucagon
GCK	Germinal center kinases
GHRL	Ghrelin
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
GPCRs	G-protein-coupled receptors
GSK3	Glycogen synthase kinase-3
HbA1c	Higher glycated hemoglobin A1c
HFD	High-fat diet
hPSCs	human pluripotent stem cells
Hpo	Hippo
IAPP	Islet amyloid polypeptide
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1β	Interleukin 1 beta
INF-γ	Interferon gamma
INS	Insulin
I/R	Ischemia/reperfusion
IR	Insulin receptor
KD-mTOR	Kinase-dead mutant of mTOR
LATS1/2	Large tumor suppressor homolog 1/2
LPA	Lysophosphatidic acid
LPAR	LPA receptor
mSIN1	Mammalian stress-activated protein kinase interacting protein 1
MST1/2	Mammalian sterile 20-like 1/2
MOB1a/b	MOB kinase activator 1A/B
mTORC1/2	Mammalian target of rapamycin complex 1/2
mTORC2	PDK2
NDR	Nuclear Dbf2-related
NF2	Neurofibromatosis type-2
NF-kB	Nuclear factor kappa B

NO	Nitric oxide
p53BP-2	p53-binding protein-2
PACAP	Pituitary adenylate cyclase-activating polypeptide
PDK1	Protein kinase 3-phosphoinositide-dependent protein kinase-1
PI3Ks	Phosphatidylinositol 3-kinases
PIP2	Phosphorylate phosphatidylinositol (4,5) bisphosphate
PIP3	Phosphatidylinositol (3,4,5) bisphosphate
PKA	Protein kinase A
PKB/AKT	Protein kinase B
PRAS40	Proline-rich AKT substrate 40 kDa
RA	Ras association domain
Rictor	Rapamycin insensitive companion of mTOR
RTK	Receptor tyrosine kinase
S1P	Sphingosine-1-phosphate
S1PR	S1P receptor
S6K1	S6 kinase
Sav	Salvador
SC-β	Stem cell-derived insulin-producing beta
SOD	Superoxide dismutase
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TAO-1	Thousand-and-one amino acids kinase-1
TNF-α	Tumour Necrosis Factor alpha
TRXs	Thioredoxins
TRXR	Thioredoxin reductase
TXNIP	Thioredoxin interacting protein
ULK1	Unc-51-like autophagy-activating kinase 1
UTR	Untranslated region
Wts	Warts
WWC1/2	WW, C2 Domain Containing 1/2
YAP	Yes-associated protein
Yki	Yorki
ZO1/2	Zonula Occludens 1/2

1. Introduction

1.1. The pancreas

1.1.1. Pancreas: anatomy and structure

The pancreas is an essential organ made of two exocrine and endocrine parts, placed across the back of the abdomen behind the stomach. The term “pancreas” is rooted from the Greek, ‘Pan’ pointing ‘all’ and ‘creas’ defining ‘flesh’ which is in the upper abdomen behind the stomach. From proximal to distal, the regions of the pancreas are defined as head, neck, body, and tail, in human (Slack 1995). The head lies close to the duodenum while the tail expands to the hilum of the spleen. The body of the pancreas is located posterior to the distal portion of the stomach between the tail and the neck. The size of a human pancreas is 14-20 cm, and weighs about 100 grams (Longnecker 2014) (Fig.1). The exocrine and endocrine parts of pancreas play important roles in digestion and blood sugar regulation, respectively.

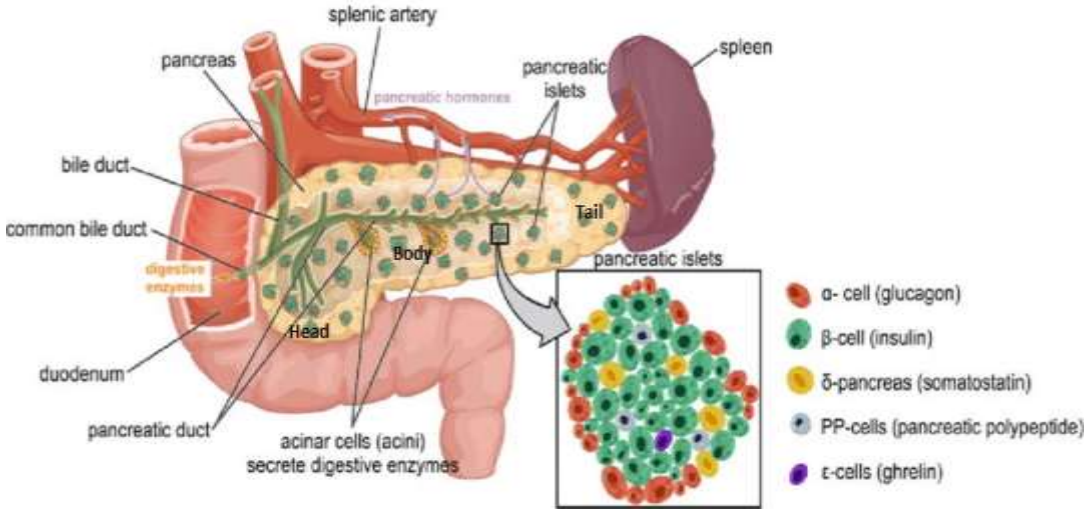

Figure 1. Overview of pancreas localization and anatomy. The pancreas including an endo- and an exocrine part settled in the upper abdomen. The exocrine cells secrete bicarbonate, mucins and digestive enzymes through the pancreatic duct system into the small intestine, while the endocrine islet cells have a fundamental role for secretion of specific hormones into the bloodstream. Image from (College 2016).

During the development, human pluripotent stem cells (hPSCs) differentiate to various mature pancreatic cells under activation or inhibition of several cell signaling pathways (Jacobson and Tzanakakis 2017). The exocrine cells are responsible for secretion of digestive enzymes, bicarbonate, and mucins into the intestine (Slack 1995). The exocrine part is made of ductal and acinar cells. Ductal cells are responsible for bicarbonate and mucins production and acinar cells release digestive enzymes such as amylase, lipase, trypsin, and chymotrypsin into duodenum during food digestion process (Jacobson and Tzanakakis 2017). These digestive enzymes play

an important role in carbohydrate, fat, and protein decomposition to glucose (Glu), free fatty acids (FFA), and amino acids (AA), respectively (Henderson 1969). On the other hand, the endocrine part, consisting of the islets of Langerhans, emerges through the aggregation of five different cell types including beta (β), alpha (α), delta (δ), pancreatic polypeptide (PP), and epsilon (ε) cells which secrete insulin (INS), glucagon (GCG), somatostatin (SST), pancreatic polypeptide (PPY), and ghrelin (GHRL) into the blood stream in the adult organism, respectively (Slack 1995, Jacobson and Tzanakakis 2017, Da Silva Xavier 2018) (Fig.1).

1.1.3. Pancreas: Islet structure and function

The Islets of Langerhans are distributed all over the pancreas and secrete different hormones important for regulation of glucose metabolism and energy homeostasis (Da Silva Xavier 2018). The total number of islets in a human pancreas is between 3.2 and 14.8 million, and the size of islets differs between 0.5 to 2.0 cm^3 (Hellman 1959, Hellman 1959, Saito, Iwama et al. 1978, Da Silva Xavier 2018). The distribution of islets is equal throughout the head and body regions but is more than two times higher in the tail region of the pancreas (Wang, Misawa et al. 2013).

1.2. The pancreatic beta cells

1.2.1. Beta cell function

β-cells play a critical role in response to high blood glucose levels and the maintenance of glucose homeostasis by secreting insulin into the blood stream. In 1921, a key time point in diabetes treatment, Frederick Banting and Charles Best figured out that insulin can reduce blood glucose in dogs indicating that loss of the hormone insulin is a fundamental reason of developing diabetes. In collaboration with James Collip, this investigation subsequently expanded to clinical use of insulin leading to the Nobel Prize in Physiology and Medicine to Banting and Macleod, most controversially, the latter received the award in his role as department chief, while Best, the student, who had done the actual work together with Banting, came away empty-handed (Da Silva Xavier 2018). Insulin is an anabolic hormone with important metabolic roles in regulation of blood glucose level and systemic energy homeostasis via stimulation of glucose uptake from the blood circulation in the peripheral tissues such as muscle and fat, suppressing gluconeogenesis in the hepatic cells, and lipids biogenesis in the adipose cells (Muoio and Newgard 2008, Weiss, Steiner et al. 2014).

1.2.2. Insulin structure and function

The insulin gene is expressed in β-cells and is located on chromosome 11 (Owerbach, Bell et al. 1981). Its polypeptide is made of two chains including A and B chains. Chain A with 21 amino acids is bound to chain B with 30 amino acids via two disulfide bonds derived from cysteine residues. The chain A has also an intra-chain disulfide bond (Fig. 2). Insulin is translated initially as a preproinsulin single-chain polypeptide, which has 12 kD molecular weight and changes to proinsulin during post translational process in the endoplasmic reticulum (ER). Proinsulin, an intermediate structure of insulin, is made by cleavage of preproinsulin while the latter is translocated to ER. Therefore, the primary structure of proinsulin is converted to the secondary and tertiary structures of insulin by interfering correct disulfide pairing and formation of three-dimensional protein structure. Proinsulin protein is a 9 kD polypeptide including both A and B chains. Insulin molecular weight changes to 6 kD following post translational modifications (Weiss, Steiner et al. 2014)(Fig. 2). Insulin expression is regulated by different transcription factors such as PDX1, MafA, and neuroD1, which play vital roles in β-cell differentiation during pancreatic development (Artner and Stein 2008).

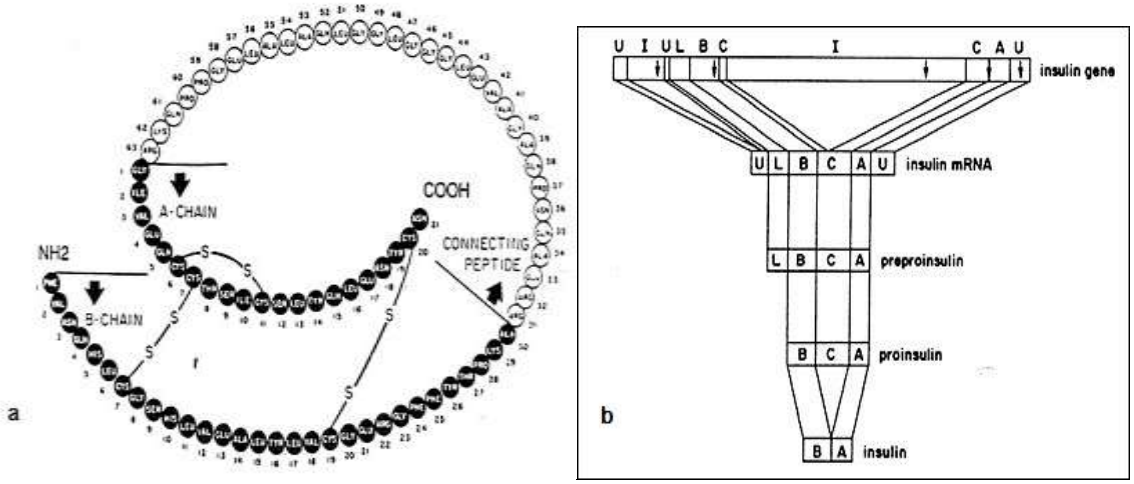

Figure 2. A, Primary structures of porcine insulin and porcine proinsulin. The sequence of human insulin is identical to that of porcine insulin except for the change of Ala ^{B30} to Thr ^{B30} in human insulin**. B, Diagrammatic illustration of the processing of insulin.** The transcription and translation of the human insulin gene, as well as processing of preproinsulin to insulin is illustrated (images and legend from (Weiss, Steiner et al. 2014)).

1.2.3. Mechanism of Insulin secretion

Glucose concentrations higher than 8-10 mM lead to glucose transportation via Glut 2 transporter into the β-cell and consequent depolarization of the β-cell. Glucokinase immediately phosphorylates the transported glucose in carbon six position and converts it to glucose-6-phosphate, which can be a target for glycolysis. Glycolysis is a cellular pathway performed in cytoplasm as the beginning of cellular respiration and leading to glucose transformation into two pyruvates, two ATPs, and the reduction of two NADs+ to NADHs, which are going to be used in mitochondria for production of more ATPs through electron transport chain (Wilcox 2005). ATPs produced by oxidative phosphorylation in the mitochondria binds to ATP-dependent potassium channels leading to closure of K channels and consequent depolarization of the cell membrane. At the rest time, potassium channels allow potassium to leave the β-cell through facilitated diffusion preventing the β-cell from being depolarized or getting more positive charge within the cell. Therefore, there will be a lot of potassium ions outside the β-cell compared to the inside. The potassium channel has a receptor on it, which can bind to ATP and results in potassium channel blocking. Subsequently, the channels are opened and calcium ions into the cell. This triggers the release of the secretory insulin vesicles via granules exocytosis outside of the cell (Soria, Quesada et al. 2004). In addition to the voltage-sensitive pathways, there are other amplification pathways contributed in insulin secretion including various hormones and neurotransmitters such as epinephrine, galanin, somatostatin, acetylcholine, and glucagon-like peptide 1 (GLP-1) as well as several cellular metabolic intermediates (Bratanova-Tochkova, Cheng et al. 2002, Wilcox 2005)(Fig. 3).

Insulin secretion may be influenced by various factors such as the level of insulin gene transcription, translation, post-translational modification in the Golgi, and also β-cell mass turnover (Nielsen, Galsgaard et al. 2001). Insulin influences glucose metabolism and utilization while glucose also impacts insulin production and secretion. Moreover, various factors can stimulate the pancreatic islet insulin secretion such as fatty acids, amino acids, acetylcholine, pituitary adenylate cyclase-activating polypeptide (PACAP), glucose-dependent insulinotropic polypeptide (GIP), and GLP-1 (Wilcox 2005). For example, the transportation of arginine, a positively charged amino acid, by CAT2A leads to β-cell depolarization and insulin secretion (Bratanova-Tochkova, Cheng et al. 2002). Some hormones like acetylcholine elevates diacylglycerol (DAG) production, which influences PKC activation and subsequently insulin secretion. Moreover, various hormones such as vasoactive intestinal peptide, PACAP, GLP-1, and GIP lead to adenylyl cyclase activity resulting in cAMP elevation and PKA activity, which triggers insulin secretion (Sharp 1979, Bratanova-Tochkova, Cheng et al. 2002, Wilcox 2005).

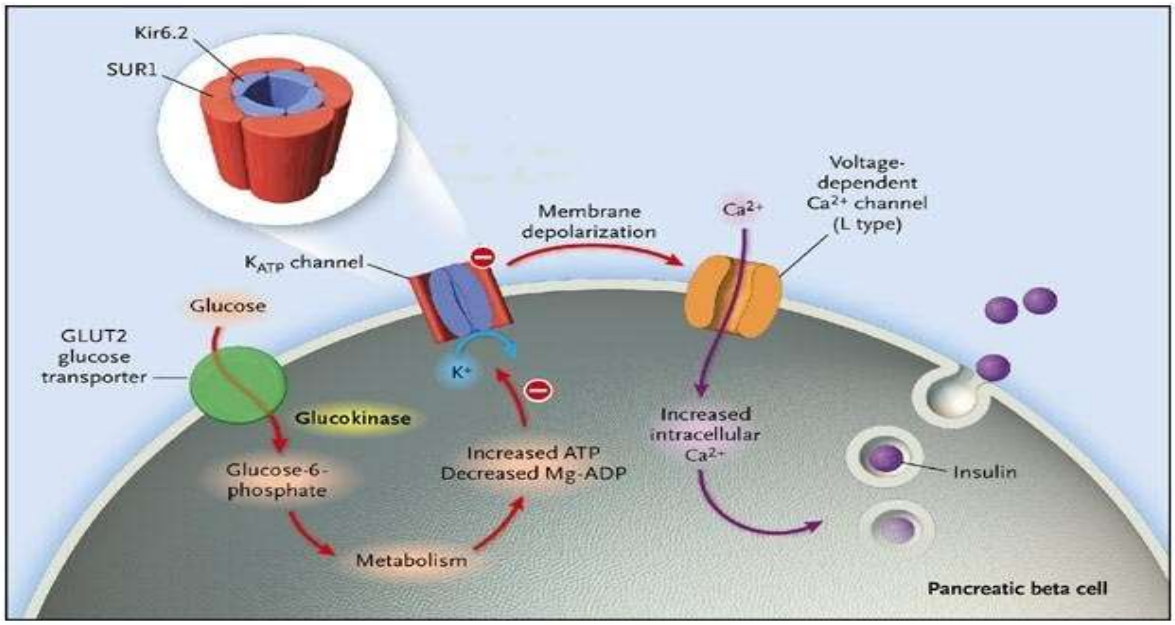

Figure 3. Schematic Representation of the Pancreatic Beta Cell, Illustrating the Role of the ATP-Sensitive Potassium (K_{ATP}) Channel in Insulin Secretion. Glucose enters the beta cell by way of the GLUT2 glucose transporter. Once inside the cell, glucose is metabolized, leading to changes in the intracellular concentration of adenine nucleotides that inhibit the K_{ATP} channel and thus cause channel closure. The K_{ATP} channel consists of four sulfonylurea-receptor (SUR1) subunits and four Kir6.2 subunits in an octomeric structure. Channel closure leads to membrane depolarization, which subsequently activates voltage-dependent calcium (Ca^{2+}) channels, leading in turn to an increase in intracellular Ca^{2+}, which triggers insulin exocytosis. Sulfonylureas initiate secretion by directly binding to the SUR1 subunits of K_{ATP} channels and causing channel closure. Mg-ADP denotes magnesium ADP. Image and legend from (Gloyn, Pearson et al. 2004)).

1.2.4. Insulin action

Secreted insulin binds to insulin receptor (IR) located on the cell membrane of target cells and influences glucose homeostasis, cellular growth, and proliferation in different organs, although the major targets of insulin are liver, fat, and muscles tissues (Watanabe, Hayasaki et al. 1998). Insulin action is categorized in different types, the first one is an immediate effect, which refers to early response in several seconds after insulin secretion such as glucose transporting and its phosphorylation. The second one is named mid-term, which happens within 5-60 minutes after insulin is administrated consisting of insulin effect on gene expression and protein production. The third one is long-term effect, which can lead to DNA replication, cell division, and cell differentiation prolonged from several hours to several days after insulin administration. Furthermore, insulin effect is also dose dependent, for example glycogenolysis can be inhibited by lower insulin levels compared to gluconeogenesis (Watanabe, Hayasaki et al. 1998).
Insulin fate starts by conflicting with liver, which is named insulin clearance to regulate the amount of insulin reaching peripheral tissues. Insulin clearance is a liver mechanism to reduce insulin

concentration in the peripheral circulation through insulin degradation. The remaining insulin, which escaped from liver insulin clearance transfers to the heart through the hepatic vein and is pumped to arterial circulation to influence target tissues such as fat, liver, skeletal muscles and brain. Insulin prevents glycogenolysis and gluconeogenesis in liver cells. In muscles and adipose tissues, insulin leads to enhancement of exocytosis of Glut-4 vesicles to the cell membrane, which result in increased glucose uptake. In adipose tissue, insulin increases glucose uptake through activating lipogenesis (Tokarz, MacDonald et al. 2018, Vargas and Sepulveda 2018). Moreover, insulin activates hexokinase and 6-phosphofructokinase enzymes leading to elevation of glycolysis and also increases glycogen synthesis and uptake of triglycerides in both adipocyte and myocyte. Therefore, insulin decreases plasma FFA levels through suppressing the rate of lipolysis and increasing triglycerides uptake from the blood into adipocytes. In hepatocyte and myocyte, insulin suppresses the rate of glycogen breakdown resulting in lower blood glucose level. Furthermore, FFA oxidation is suppressed in hepatocyte and myocyte through insulin action. Insulin action leads to increasing AA transportation into hepato, myo, and adipocytes and consequent elevation of protein synthesis (Dimitriadis, Mitrou et al. 2011). The final destination of insulin is kidney to be degraded and cleared from blood circulation (Tokarz, MacDonald et al. 2018).

1.2.5. Insulin receptor isoforms and signaling pathway

When insulin is secreted by β-cells into the blood stream, it binds to its receptor located on the surface of target cells and starts insulin mediated signal transduction. Each cell has a specific range of insulin receptors, for instance hepatocytes and adipocytes have more insulin receptors compared to erythrocytes, which leads to different responses in various cells. Insulin receptor is made of two α and two β subunits, which are connected via disulfide bond. The α subunits are responsible for binding to insulin while the β subunits, consisting of tyrosine kinase domains, can phosphorylate tyrosine residues in the target proteins located in the cytoplasm and start different signaling cascades. There are two isoforms of insulin receptors made during post-translational process by various exon 11 splicing. The insulin isoform with exon 11, has higher affinity to insulin, is abundant in hepatocyte compared to muscle. The fat cells produce both insulin receptor isoforms. Therefore, this fact can explain the higher response of liver to insulin compared to other organs (Watanabe, Hayasaki et al. 1998).
Insulin receptor belongs to a receptor tyrosine kinase (RTK) family and is activated by insulin leading to conformational change of the RTK and its self-phosphorylation at tyrosine residues. Phosphatidylinositol 3-kinases (PI3Ks) are a superfamily including three classes such as the class IA enzymes activated directly by cell surface receptors like RTKs, GPCRs, and certain oncogenes. The class IA PI3Ks consist of a p110 and a p85 as catalytic and regulatory subunits, respectively. The p85 subunit of PI3K mediates an interaction to tyrosine phosphate motif of RTKs. This interaction activates p110 catalytic domain which catalyzes conversion of phosphorylate phosphatidylinositol (4,5) bisphosphate (PIP2) into phosphatidylinositol (3,4,5) bisphosphate (PIP3) (Liu, Cheng et al. 2009). The PIP3 is a key second messenger in the insulin signaling cascade, which is increased very fast in the first minute of insulin engagement to its receptor. Then, PIP3 mediates a docking of PH domain in N terminus of its effector protein kinase

B (PKB/AKT) leading to phosphorylation of AKT at Thr308 and Ser473 by protein kinase 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2 (known as mTORC2), respectively, resulting in full activation of AKT. Activated AKT phosphorylates and regulates activity of its downstream targets such as glycogen synthase kinase-3 (GSK3), mammalian target of rapamycin complex 1 (mTORC1), and the forkhead family of transcription factors (FOXOs), which modulate glucose metabolism, cell growth, protein synthesis, cell survival, and cell death (Lizcano and Alessi 2002, Liu, Cheng et al. 2009). AKT activation induces glycolysis in heart cells by phosphorylation and activating cardiac-specific form of 6-phosphofructo-2-kinase, which stimulates ATP production. Moreover, AKT inhibits FOXOs transcription factors via phosphorylation at three residues leading to their direct engagement to phospho-binding proteins 14-3-3 and subsequent exclusion of FOXOs from nucleus to cytoplasm and their ultimate degradation via ubiquitin proteasome pathway. The major role of insulin is increasing glucose transportation by activating insulin receptors, which leads to phosphorylation of the proto-oncogene Cbl resulting in activation of the TC10 family of Rho GTP-binding proteins and its consequent interaction with unknown effector proteins to allow insulin-stimulated GLUT4 translocation from its intracellular stores to the plasma membrane (Fig. 4) (Lizcano and Alessi 2002). Moreover, Insulin signaling plays an important role in regulation of hepatic glucose output through glucagon secretion. Activated AKT enhances gamma-aminobutyric acid receptor ($GABA_AR$) exocytosis to α-cells membrane. Then, GABA ,co-secreted by insulin from β-cells, binds to its receptor, $GABA_AR$, on α-cells and modulates glucagon secretion and ultimately reduces hepatic glucose output and gluconeogenesis (Rorsman, Berggren et al. 1989, Xu, Kumar et al. 2006, Cherrington, Moore et al. 2007, Kawamori, Kurpad et al. 2009).

In conclusion, insulin affects metabolic processes in three major target organs; skeletal muscles, liver and adipose tissue through stimulation of nutrition transmission (i.e. glucose, amino acids, and fatty acids), induction of glycogen, protein and lipid synthesis mediated by the insulin receptor signaling and its downstream hubs, such as AKT/mTOR, S6 and GSK3. Insulin signaling has different impacts on intracellular pathways such as glucose and FFA metabolisms (e.g. increasing glycolysis, glycogenesis, lipogenesis and inhibition of glycogenolysis, gluconeogenesis, lipolysis). In muscle and adipose tissues, insulin signaling pathway triggers glucose uptake mediated by an enhancement of Glut 4 vesicles exocytosis to the cell membrane resulting in glucose absorption (Lizcano and Alessi 2002, Dimitriadis, Mitrou et al. 2011).

Loss of insulin secretion or insulin resistance triggers glucose metabolism disorder named diabetes. Impairment of insulin signaling pathway is a fundamental cause that constructs insulin resistance and consequently pathology of diabetes.

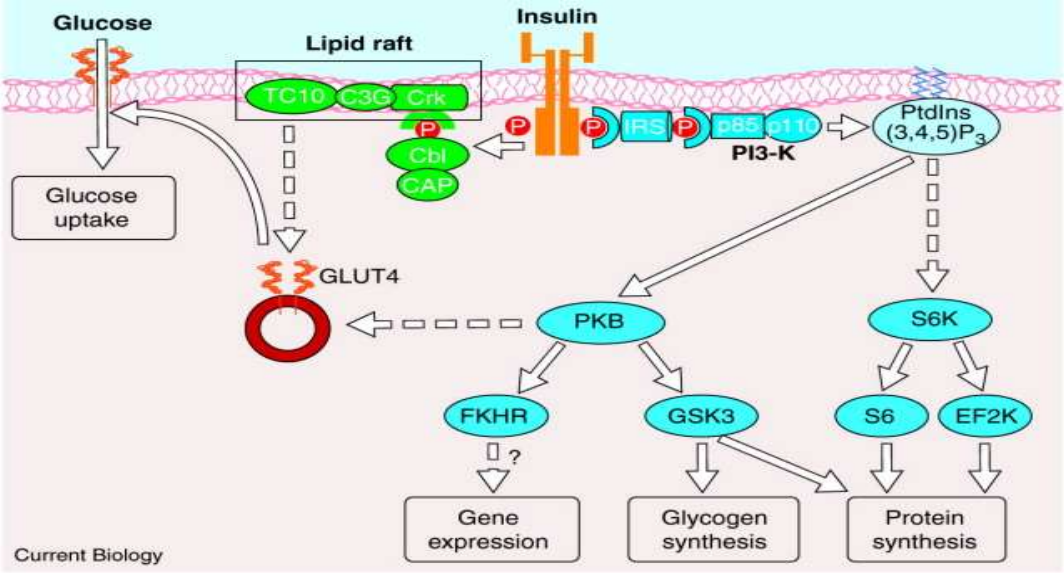

Figure 4. Overview of the regulation of major metabolic responses in cells by insulin. Insulin binds to its receptor inducing auto phosphorylation at a number of tyrosine residues recognized by the phosphor tyrosine-binding (PTB) domain of adaptor proteins termed insulin receptor substrates (IRS). IRSs are recruited to the receptor and phosphorylated at tyrosine residues by the insulin receptor, some of which are recognized by the Src homology 2 (SH2) domain of the p85 regulatory subunit of a lipid kinase, PI 3-kinase. The catalytic subunit of PI 3-kinase, p110, then phosphorylates PtdIns(4,5)P_2 at the plasma membranes of cells to generate the second messenger PtdIns(3,4,5)P_3, which stimulates insulin-dependent processes. Activated insulin receptor also phosphorylates the protein Cbl, which is in a complex with the adaptor protein CAP. This results in the Cbl–CAP complex being recruited to a region of the plasma membrane termed a lipid raft. At this location, Cbl interacts with the adaptor protein Crk, which is constitutively associated with the Rho-family guanine nucleotide exchange factor, C3G. C3G then activates members of the GTP-binding protein family, TC10, which themselves activate unknown effector molecules to promote GLUT4 translocation. Dotted arrows are steps of the pathway that have not yet been fully elucidated. Image and legend from (Lizcano and Alessi 2002)).

1.3. Diabetes

Diabetes mellitus is a chronic metabolic disease with worldwide prevalence. The number of people diagnosed with diabetes increases every minute. WHO statistics report declared that prevalence rate of diabetes increased with quadrupled ratio since 1980 to 422 million adults in 2014, which means a dramatic increase in the number of diabetic patients (WHO 2018). Diabetes is a metabolic disorder, which relates to glucose blood level dysregulation associated with several short-term and long-term complications such as cardiovascular disease, stroke, and kidney disease. Diabetes mellitus is categorized in two major types: Type 1 diabetes mellitus (T1D) and Type 2 diabetes mellitus (T2D) (endocrineweb 2018, September 27). Type 1 diabetes consisting only 10% and the rest 90% is related to type 2 diabetes of diabetics (Lizcano and Alessi 2002).

1.3.1. Type 1 diabetes mellitus

T1D is an autoimmune disease caused by autoimmune-mediated reactions selectively targeting insulin producing pancreatic β-cells (Mathis, Vence et al. 2001). Genetic predisposition as well as environmental factors such as viral infection are key important determinants for development of T1D (Wållberg and Cooke 2013). During the progression of T1D, increased production and secretion of pro-inflammatory cytokines (e.g. IL-1β, TNF-α, INF-γ and chemokines (e.g. CXCL10) by infiltrated T-cells and other immune cells such as macrophages trigger β-cell death and destruction which leads to insulin deficiency and development of hyperglycemia (Christen and von Herrath 2004, Wållberg and Cooke 2013). Also, deleterious interaction between antigen presenting cells (APCs) and T-cells promotes high local production of ROS and other apoptotic triggers like the perforin/granzyme B and Fas/FasL systems that activate pro-apoptotic molecules such mitochondrial-dependent apoptotic players such as Bid which further contribute in a process of β-cell destruction in T1D. Elevated expression of innate immune receptors by stressed β-cells further accelerate their own destruction through deadly cross-communication between infiltrating immune cells and the targeted β-cells (Thomas, McKenzie et al. 2009, Wållberg and Cooke 2013). Of note, activated transcription factors NFκB and STAT1 induced by pro-inflammatory cytokines Interleukin 1 beta (IL-1β), Tumour Necrosis Factor alpha *(*TNFα*)* and Interferon gamma *(*IFNγ*)* induce expression of inducible nitric oxide (NO) synthase (iNOS) leading to production of the highly toxic free radical NO and subsequent oxidative damage and ultimate β-cell apoptosis (Thomas, McKenzie et al. 2009). Altogether, the invasion of T-cells in cooperation with other immune cells trigger loss of β-cells leading to lower insulin production and higher blood glucose level in T1D placing the loss of islet β-cells as critical pathogenic hallmark of T1D.

1.3.2. Type 2 diabetes mellitus

T2D is the most common type of diabetes (Wilcox 2005). T2D origins from genetic predisposition, obesity, and life style such as physical inactivity, sedentary lifestyle, cigarette smoking, hypertension, and generous consumption of alcohol. T2D seems to start with insulin resistance in insulin-dependent tissues such as muscles and fat; in this stage, β-cells try to compensate for the higher insulin demand by producing more insulin, but as insulin resistance gets chronic in the body, in genetically-predisposed individuals, β-cells are overworked, exhausted and ultimately cannot produce enough insulin leading to impaired insulin secretion and development of hyperglycemia (Muoio and Newgard 2008, DeFronzo, Ferrannini et al. 2015, Cersosimo, Triplitt et al. 2018, endocrineweb 2018, September 27). In addition, it is revealed that pancreatic α-cells also play an important role in the T2D pathogenesis by over-secretion of glucagon (Cersosimo, Triplitt et al. 2018, endocrineweb 2018, September 27). The individuals suffered from prediabetes can be detected by tests for impaired fasting glucose (IFG) levels, impaired glucose tolerance (IGT), or higher glycated hemoglobin A1c (HbA1c) (DeFronzo, Ferrannini et al. 2015). Determining the prediabetes stage may lead to prevention of diabetes by changing lifestyle (weight loss and exercise) as well as using antidiabetic and anti-obesity medications (DeFronzo, Ferrannini et al. 2015). β-cell resistance to GLP-1 and consequent reduced secretion of insulin and increased secretion of glucagon, may also lead to hyperglycemia through elevation of hepatic glucose production. Concomitantly, insulin resistance in adipose tissue has an influence on

lipolysis and elevates plasma FFA level that accelerates insulin resistance in muscles and liver and when through a vicious cycle cam further contribute to β-cell failure (DeFronzo, Ferrannini et al. 2015). Long term exposure of islets to high levels of FFA such as palmitate or glucose lead to lower insulin secretion from β-cells in response to glucose stimulation as well as β-cell apoptosis, while monounsaturated fatty acids such as oleate intercepts β-cells death induced by palmitate- and glucose (Dyntar, Eppenberger-Eberhardt et al. 2001, Sivitz 2001, Maedler, Oberholzer et al. 2003, Kato, Shimano et al. 2008, Oh, Bae et al. 2018). As described above, first of all insulin resistance appears, thenT2D occurs upon β-cell failure. Different factors including, genetic abnormalities (Morris, Voight et al. 2012), incretin hormone, GLP-1, and GIP resistance and/or deficiency (Nauck, Vardarli et al. 2011, Madsbad 2014), lipotoxicity (Kato, Shimano et al. 2008, DeFronzo 2010), glucotoxicity (Bensellam, Laybutt et al. 2012), hypersecretion of islet amyloid polypeptide (IAPP) (Ritzel, Meier et al. 2007), reactive oxygen stress (Collins, Pi et al. 2012), and activation of inflammatory pathways (DeFronzo 2010) lead to impaired insulin secretion and β-cell apoptosis (Marchetti, Bugliani et al. 2007) resulting in β-cell mass decreasing by 30–40% in T2D (Rahier, Guiot et al. 2008).

1.4. β-cell Apoptosis in diabetes

Apoptosis or programmed cell death occurs during all stages of development, aging, maintaining cell population in tissues and immune defense response. Any disturbance of apoptosis may lead to disease, e.g. excessive apoptosis leads to tissue dysfunction such as diabetes, autoimmune lymphoproliferative syndrome and cardiomyopathy, and an uncontrolled reduction of cell death causes cancer (Elmore 2007, Favaloro, Allocati et al. 2012). Apoptosis executes via two different major pathways named extrinsic (receptor-mediated, e.g. Fas/TNFR) and intrinsic (mitochondria-driven) pathways. The prototype death receptor, Fas/TNFR is a signaling cascade leading to caspases 8 and 3 activation and finally cell death. The intrinsic pathway is a non-receptor-mediated pathway, which starts by cellular stress and regulated by the balance between pro-apoptotic (e.g. Bid and Bax) and anti-apoptotic (e.g. BCL-2 and BCL-XL) members of the Bcl-2 protein family (Elmore 2007, Thomas, McKenzie et al. 2009)(Fig.5). In T1D and T2D, apoptosis is a major contributing factor in the process of β-cell loss (Thomas, McKenzie et al. 2009, Tomita 2016).

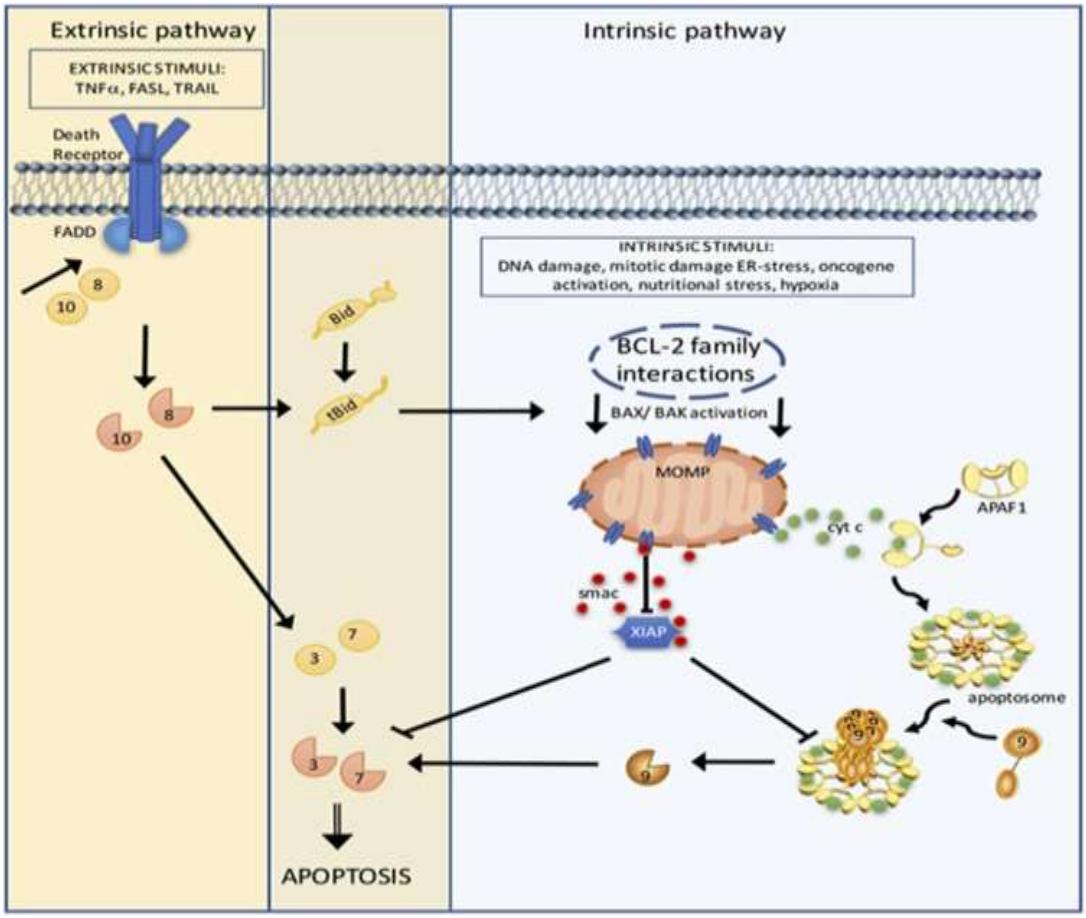

Figure 5. The extrinsic and intrinsic apoptotic pathways. The extrinsic pathway is initiated by engagement of DRs via their respective ligands TNF, FASL/CD95L, or TRAIL. Together with the adaptor FAS-associated death-domain (FADD) protein and the initiator procaspase-8 (or -10) they form the death-inducing signaling complex (DISC). This assembly enables the dimerization and autoactivation of the initiator caspases, which in turn cleave and activate the executioner caspase-3 and -7, ultimately leading to apoptosis unless they are inhibited by XIAP. The intrinsic pathway can be engaged by diverse intracellular stresses that modulate BCL-2 family protein interactions that control the activation of the BCL-2 effector proteins BAX and BAK. Once activated, BAX and BAK cause MOMP, leading to the release of proapoptotic IMS proteins. Cytochrome *c* (Cyt *c*) engages APAF1 and induces its oligomerization, leading to apoptosome formation that recruits and activates the initiator procaspase-9. Active caspase-9 cleaves and activates the executioner caspase-3 and -7. Simultaneously with Cyt *c*, Smac is released from the IMS and inhibits XIAP. The extrinsic and intrinsic pathways are linked; caspase-8 can cleave the BH3-only protein BH3-interacting domain death agonist (Bid), leading to its active, truncated form tBid, which in turn activates BAX/BAK. Numbers in circles indicate the respective pro- and active caspase; interrupted circles represent active caspases. Image and legend from (Lee and Pervaiz 2007, Kalkavan and Green 2018).

As caspase-3 is a main effector caspase of the apoptotic pathways and TUNEL is a detector of DNA fragmentation, *in situ* TUNEL and caspase-3 staining are favorite techniques for detecting apoptosis in islets (Thomas, McKenzie et al. 2009, Tomita 2010). Numerous studies show that glucose increases IL-1 β expression (an inflammatory cytokine) in β-cells and intra islet

macrophages leading to NF-kβ activation and Fas induction resulting in β-cell death in human islets (Maedler, Spinas et al. 2001, Maedler, Sergeev et al. 2002, Ehses, Perren et al. 2007). High glucose level upregulates Fas receptor resulting in Fas-FasL interaction and consequent activation of caspase-8 and caspase-3, DNA fragmentation, and β-cell apoptosis (Maedler, Spinas et al. 2001, Maedler, Fontana et al. 2002, Maedler, Sergeev et al. 2002) (Fig.6).

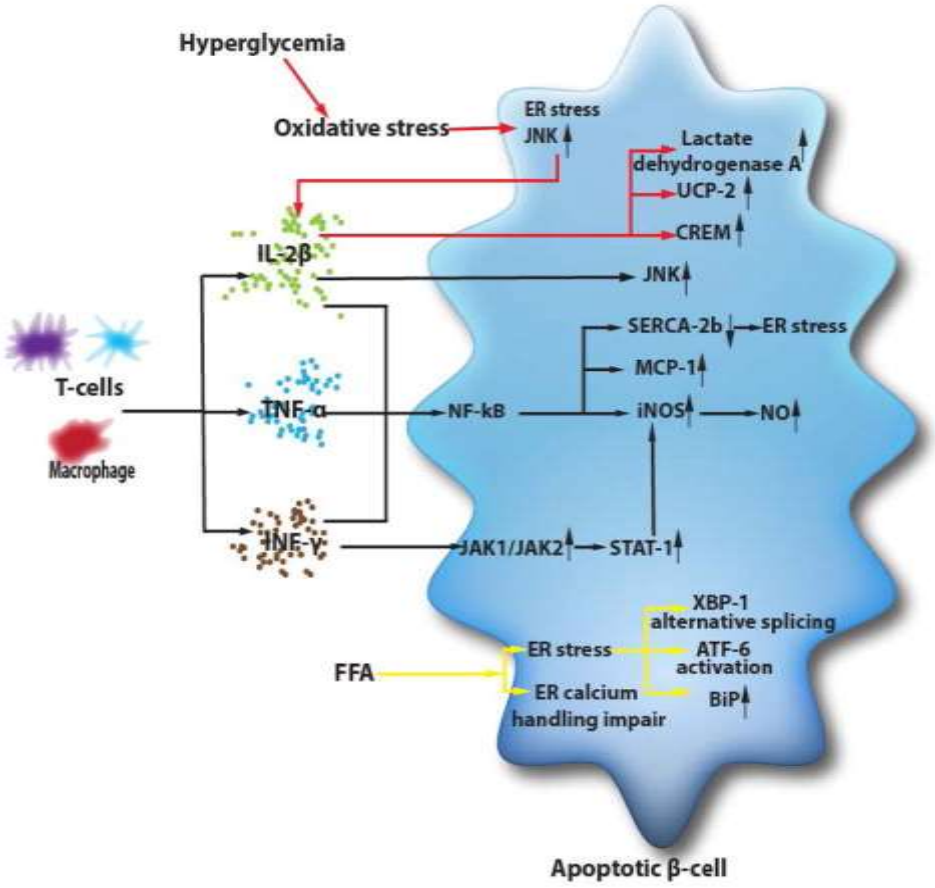

Figure 6. Schematic illustration of pancreatic beta-cell apoptosis and dysfunction. An increased pro-inflammatory cytokines production/secretion (e.g. IL-1β, TNF-α, INF-γ) as well as evaluated ROS mediated by hyperglycemia lead to beta-cell apoptosis resulting in insufficient insulin production and secretion. FFAs also trigger ER stress and apoptotic processes. Image from (Fu, R Gilbert et al. 2013).

In T2D, ROS and ER stress mediated by chronically elevated glucose, saturated fatty acids, as well as IAPP, and inflammation result in β-cell apoptosis (Butler, Janson et al. 2003). Chronic hyperglycemia has been observed to induce β-cell apoptosis and T2D in different animal models such as *Psammomys obesus* (Prentki and Nolan 2006) (Donath, Gross et al. 1999), the Zucker diabetic fatty rat (Pick, Clark et al. 1998), and the domestic cat (Zini, Osto et al. 2009). High glucose (16.5 mM for 5 days) results in upregulation of the BH3 only genes (Bid and Bad) and downregulation of anti-apoptotic BCL-xL (Federici, Hribal et al. 2001). Glucose induces thioredoxin interacting protein (TXNIP) leading to apoptosis through upregulation of caspase-3

and inhibition of thioredoxin enzyme activity (Chen, Saxena et al. 2008). Hyperglycemia leads to β-cell apoptosis via increasing ROS contributing to tissue dysfunction in two ways: dysregulation of redox-sensitive signaling pathways, and oxidative damage to biological structures such as DNA, proteins, and lipids (Guichard, Moreau et al. 2008). High plasma level of saturated FFA like palmitate triggers β-cell death through activation of ER stress, nuclear factor kappa B (NF-kB) and induction of other inflammatory factors (Huang, Lin et al. 2007, Zraika, Hull et al. 2009) (Donath, Böni-Schnetzler et al. 2009) (Lee, Sohn et al. 2001). Furthermore, chronic inflammation in response to the stress induced by excess nutrition leads to β-cell apoptosis through enhancing infiltration of islet area with monocytes/macrophages and lymphocytes (Eguchi and Nagai 2017). However, the initial trigger for the onset of β-cell death in T2D is still under researching. Therefore, designing new medicine for inhibition of β-cell apoptosis is pretty difficult and for catching this valuable goal we should know more about different signaling pathways which are involved in β-cell apoptosis.

1.5. Signaling pathways related to diabetes

1.5.1.1. Mammalian target of rapamycin complex (mTORC)

As said above, β-cell failure is a fundamental cause of both T1D and T2D. There are different pathways involved in regulation β-cell proliferation, apoptosis, and function such as mammalian target of rapamycin complex (mTORC) and Hippo pathways. mTORC was identified for the first time in *Saccharomyces Cerevisiae* (Hay and Sonenberg 2004). mTOR , a central regulator of cell metabolism and growth, is a conserved 289-kDa Ser/Thr kinase existing as two complexes of mTORC1 and mTORC2. mTOR regulates the protein synthesis, which is necessary for anabolic growth and proliferation through the availability of nutrients, cellular energy levels, oxygen levels, and mitogenic signals (Ardestani, Lupse et al. 2018). Nutrients (amino acids and glucose), growth factors (for example, insulin and IGF-1), and the cellular energy level (high ATP:AMP) lead to mTORC1 activation resulting in shifting intracellular catabolism to growth promoting anabolism, and increasing the synthesis of proteins, lipids, and nucleotides. However; activation of mTORC2 is under regulation of extracellular stimuli such as growth factors, RTK, and insulin, which lead to cell proliferation and survival, cytoskeletal dynamics, ion transport, and growth regulation through phosphorylation of a conserved serine residue of AKT, PKCα, and SGK1 (Cybulski and Hall 2009, Dalle Pezze, Sonntag et al. 2012, Ardestani, Lupse et al. 2018)(Fig.7).

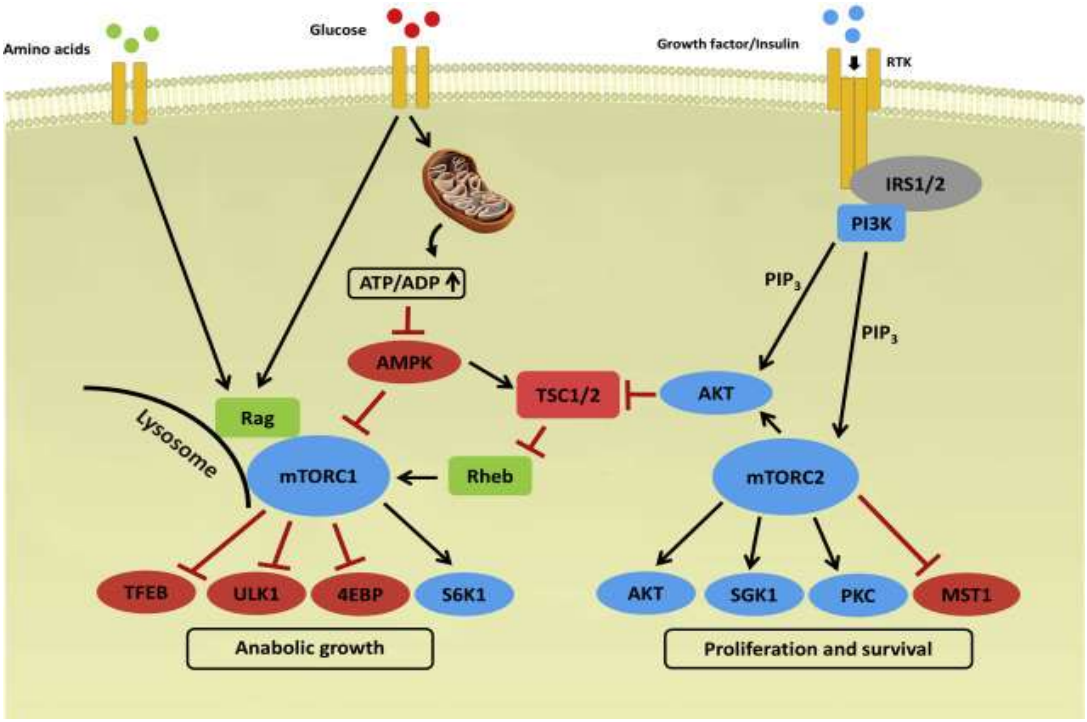

Figure 7. A Simplified Presentation of mTOR signaling under Physiological Conditions. In response to cellular stimulation, PI3K generates PIP3, which promotes the activation of mTORC2 and AKT. AKT phosphorylates and inhibits the upstream inhibitor of mTORC1, known as TSC1/2, leading to GTPase Rheb stimulation and mTORC1 activation. Also, nutrients such as AA and Glu function through the Rag family of GTPases to activate mTORC1 by localizing mTORC1 to the lysosome. Under high ATP: ADP ratio, AMPK is inhibited, and thus mTORC1 is activated through loss of both phosphorylation-dependent activation of TSC2 and direct inactivation of mTORC1. Phosphorylation of AKT by mTORC2 enhances its activity, and mTORC2 activation is at least partially mediated by PI3K-dependent PIP3 production. Image from (Ardestani, Lupse et al. 2018).

The mTORC1 complex includes mTOR catalytic subunit, regulatory associated protein of mTOR (Raptor), proline-rich AKT substrate 40 kDa (PRAS40), and the protein mLST8/GβL, and promotes cellular growth and suppresses catabolic processes during stress. Raptor plays a role as an adaptor and its interaction with S6K1 and 4E-BP1 is crucial for phosphorylation of them by mTORC1. Rapamycin inhibits mTORC1 activity that restrains phosphorylation of downstream targets related to disruption of mTOR-raptor interaction resulting in cell apoptosis (Laplante and Sabatini 2012, Saxton and Sabatini 2017). Chen, Zheng *et al.,* had shown that rapamycin association with FK506-binding protein 12 (FKBP12) leads to its interaction with FKBP12-rapamycin binding (FRB) domain in C terminus of mTORC1 resulting in mTORC1 deactivation and inhibition (Chen, Zheng et al. 1995). The mTORC1 leads to translation and higher protein synthesis through phosphorylating and deactivating of eukaryotic initiation factor 4E-binding protein (4E-BP1), the repressor of mRNA translation, and phosphorylation and activation of S6 kinase (S6K1), the regulator of cell growth, protein translation, and proliferation (Hay and Sonenberg 2004). The mTORC2 consists of mTOR catalytic subunit, rapamycin insensitive

companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), and mLST8/GbL (Liu, Cheng et al. 2009, Zarogoulidis, Lampaki et al. 2014). In the beginning, it had shown that the mTORC2 does not associate with FKBP12-rapamycin during short term treatment (Jacinto, Loewith et al. 2004). Later on, Sarbassov, A *et al.,* had shown that rapamycin can also inhibit mTORC2 complex during long term incubation (Sarbassov, Ali et al. 2006).

Activation of the insulin signaling pathway leads to full activity of AKT resulting in TSC2 phosphorylation and inhibition, which is a negative regulator of mTORC1 (Hay and Sonenberg 2004, Saxton and Sabatini 2017). Therefore, activated AKT leads to activation of mTORC1 through disturbing TSC2 binding to mTORC1 resulting in promotion of phosphorylation of two important downstream targets of mTOR: S6K and 4EBP1 (Ardestani, Lupse et al. 2018). The mTORC1 regulates protein synthesis via phosphorylation of 4EBP, which is a repressor of eIF4E through suppression of eIF4E interaction with eIF4G, thereby abrogating ribosome binding (Hay and Sonenberg 2004). In addition, mTORC1 also can phosphorylate and negatively regulate unc-51-like autophagy-activating kinase 1 (ULK1), the initiator of the autophagic cascade (Kim and Guan 2015).

mTOR hyperactivity is associated with cancer through regulating translation of downstream targets including increasing translation of mRNAs encoding positive regulators of cell cycle progression, such as cyclin D1 and c-Myc, or decreasing translation of negative regulators, such as the cyclin-dependent kinase inhibitor, p27 (Gera, Mellinghoff et al. 2004). Since mTOR plays a fundamental role in cellular metabolism and function of both pancreatic islet β-cell and immune cell, it can make both anti and prodiabetic effects through induction of growth or proliferation in β-cell and modifying immune cell fuel metabolism resulting in opposing impairments of insulin secretion in diabetes and amplifying immune cell contributions to β-cell dysfunction and the development of diabetes, respectively (Tuo and Xiang 2018).

In rat and human β-cells, chronic high glucose and lipid levels lead to activity and cytotoxic lipid droplet accumulation in an mTORC1-dependent manner while mTORC1 inhibition by rapamycin results in an increasing insulin secretion and also reduces β-cell apoptosis under nutrient overload condition (Vernier, Chiu et al. 2012, Ardestani, Lupse et al. 2018). mTORC1 regulates cell cycle progression by modulation of cyclins D2 and D3 and Cdk4 activities. Besides, rapamycin treatment can exert negative effects on islet transplantation and β-cell adaptation to insulin resistance (Balcazar, Sathyamurthy et al. 2009). In two different transgenic mouse models, β-cell-specific deletion of TSC1/TSC2, repressors of Rheb, and Rheb overexpression in β-cells, a positive upstream regulator of mTORC1, lead to mTORC1 activity/upregulation subsequently resulting in elevated β-cell size, expansion of β-cell mass, hyperinsulinemia, and an improvement in glucose tolerance (Mühlemann , Hamada, Hara et al. 2009, Ardestani, Lupse et al. 2018). In mice, β-cell-specific ablation of Raptor, an essential component of mTORC1, displays mTORC1 inactivity leading to β-cell growth and mass reduction, loss of functional maturity, elevating β-cell apoptosis and GSIS impairment (Ni, Gu et al. 2017). However; Alejandro, E *et al.,* had shown that β-cell-specific overexpression of a kinase-dead mutant of mTOR (KD-mTOR) in mice leads to development of glucose intolerance on a high-fat diet (HFD) mediated by PDX1 deficiency and insulin secretion defect without alterations in β-cell mass that suggest mTOR kinase activity is crucial for β-cell function (Alejandro, Bozadjieva et al. 2017). Impaired fetal nutrition promotes the risk of T2D through reducing embryonic β-cell growth and poor function related to insufficient

amino acid (specially leucine), which modulates endocrine and pancreatic progenitor growth in an mTOR-dependent manner (Elghazi, Blandino-Rosano et al. 2017). In mice, S6K1 deficiency results in hypoinsulinaemic, glucose intolerant, impaired glucose-induced insulin secretion and pancreatic insulin content due to the β cell size and mass reduction (Pende, Kozma et al. 2000). In conclusion, mTORC1, a central regulator of metabolic and nutrient cues acts as a double edge sword in the regulation of β-cell mass and function and plays critical roles in both β-cell proliferation under physiological condition and apoptosis related to its long term activation. Different studies including our recent work within this thesis (Yuan, Rafizadeh et al. 2017)identified that chronic activation of mTORC1 leads to β-cell failure in T2D.

1.6. The Hippo Pathway

1.6.1. The Hippo pathway: function and components

Hippo pathway is an evolutionarily conserved regulator of organ size, identified for the first time from genetic screening in fruit fly *Drosophila* (Chan, Lim et al. 2011). Hippo pathway plays a very crucial role in regulating organ size during development process via controlling proliferation and apoptosis (Halder and Johnson 2011). Therefore, because of its fundamental effect, any mutation or dysregulation of Hippo pathway components may lead to pathological disorders such as cancer, diabetes, cardiovascular and neurodegenerative diseases (Johnson and Halder 2014, Plouffe, Hong et al. 2015, Yu, Zhao et al. 2015, Ardestani and Maedler 2017). In mice, deletion of Hippo components such as NF2, MST1/2, SAV1, MOB1A/B, and LATS1/2 or overexpression of YAP all lead to overexpression of TEAD downstream target genes, which elevate expansion of progenitor cells and tissue overgrowth which will be addressed later (Camargo, Gokhale et al. 2007, Dong, Feldmann et al. 2007, Zhou, Conrad et al. 2009, Meng, Moroishi et al. 2016).
Hippo pathway's various compounds are described as Hippo (Hpo), Salvador (Zhang, Zhang et al.), Warts (Wts), mats, and Yorki (Yki) in drosophila and Mammalian sterile 20-like 1/2 (MST1/2, also called STK4/3), Salvador (SAV1), Large tumor suppressor homolog 1/2 (LATS1/2), MOB kinase activator 1A/B (MOB1a/b), and Yes-associated protein (YAP) transcriptional homologs in mammals (Yu, Zhao et al. 2015)(Fig.8). There are several associated regulatory proteins targeted by Hippo pathway, which play roles in cell survival, cell cycle progression, and tissue regeneration (Johnson and Halder 2014, Plouffe, Hong et al. 2015, Yu, Zhao et al. 2015). It had been shown that Hippo pathway inhibition related to Warts deletion triggers excessive cell proliferation and overgrowth of tissue (Justice, Zilian et al. 1995). Different studies proved that mutation of each core component: Salvador (Zhang, Zhang et al.), Hippo (hpo), or Mob (Matsui, Nakano et al.) as tumor suppressor result in organ size changes via overgrowth (Kango-Singh, Nolo et al. 2002, Harvey, Pfleger et al. 2003, Lai, Wei et al. 2005). Deletion of Yki in drosophila or YAP in mice, diminishes the overgrowth phenotypes caused by mutation of upstream component of Hippo pathway (Huang, Wu et al. 2005, Zhang, Bai et al. 2010, Zhou, Zhang et al. 2011).

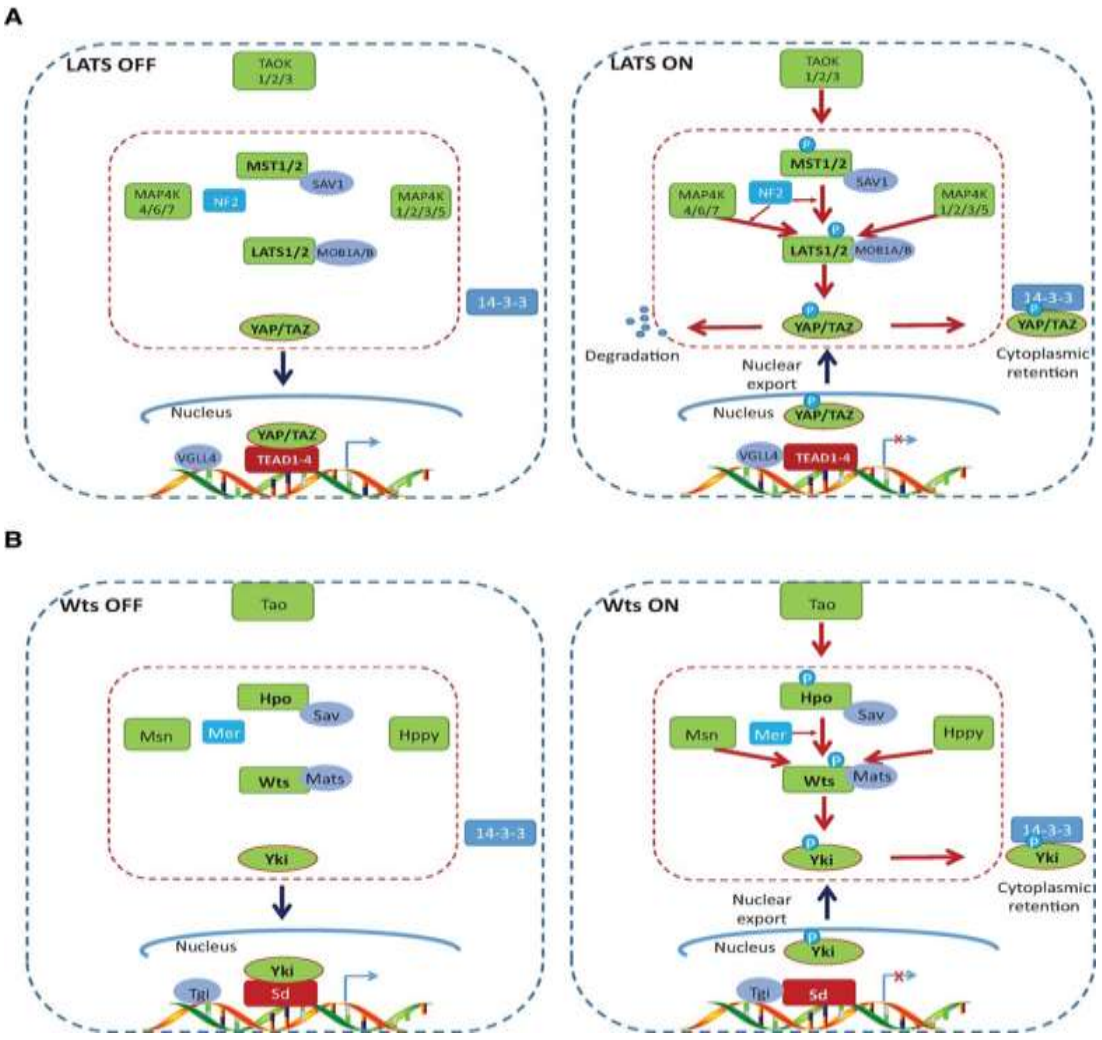

Figure 8. The components of Hippo pathway in mammals and drosophila A, The mammalian Hippo pathway core component. When the Hippo pathway is off, YAP and TAZ are active and compete with VGLL4 for TEAD binding and activation of gene transcription in the nucleus. TAO kinases can activate the Hippo pathway by MST1/2 phosphorylation at its activation loop. MST1/2 in turn phosphorylate LATS1/2, facilitated by scaffold proteins SAV1, MOB1A/B, and NF2. MAP4K4/6/7 and MAP4K1/2/3/5 also phosphorylate and activate LATS1/2. Phosphorylation of LATS1/2 by MAP4K4/6/7 requires NF2 (also known as Mer). Activated LATS1/2 phosphorylate YAP and TAZ, leading to 14-3-3-mediated YAP and TAZ cytoplasmic retention and SCF-mediated YAP and TAZ degradation. B, The *Drosophila* Hippo pathway. Active Yki competes Tgi to interact with Sd in the nucleus and activates the transcription of Sd target genes. When Hpo is activated by Tao kinase or dimerization, it phosphorylates and activates Wts with the assistance of the scaffold proteins Sav and Mats as well as Mer. It is unclear whether Msn and Hppy require Mer and Sav to phosphorylate and activate Wts. Active Wts phosphorylates and inactivates Yki, leading to 14-3-3-mediated Yki cytoplasmic retention. Image and legend from (Meng, Moroishi et al. 2016)).

In humans, the *Neurofibromatosis type-2* (NF2) encoded protein, Merlin (Mer), and its mutated variant had shown the development of tumors in the central nervous system. Mer acts as a tumor-suppressor gene due to *NF2*-deficiency and shows a failure of contact dependent growth arrest

(Hamaratoglu, Willecke et al. 2006). Mer is an adaptor protein transducing a signal from membrane receptors to intracellular downstream components such as PAK1 and CD44 whose overexpression restrains cell proliferation. Mer, as a conserved growth-suppressor, acts with Expanded (Ex) in *Drosophila* that leads to a growth-suppressing signal transduction from an unknown receptor (Hamaratoglu, Willecke et al. 2006).

Ex and Mer, and Kibra complex , act upstream of ,Hpo and, antagonize tissue growth through activation of thousand-and-one amino acids kinase-1 (TAO-1) (mammalian homolog TAOK-3), which finally triggers phosphorylation and inactivation of Yki resulting in downregulation of Yki target genes (Polesello, Huelsmann et al. 2006, Thompson and Cohen 2006, Yu and Guan 2013) (Boggiano, Vanderzalm et al. 2011)(Fig.9).

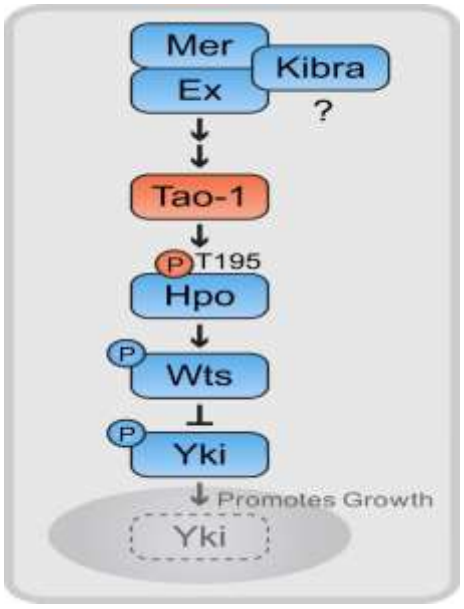

Figure 9. A Model for the function of Tao-1 in the HSW Pathway. Tao-1 directly phosphorylates Hpo at T195 in the kinase activation loop, leading to the activation of Wts and inhibition of Yki, which remains cytoplasmic when phosphorylated. Genetic and biochemical experiments position Tao-1 upstream of Hpo and suggest that Mer and Ex function through Tao-1 to activate HSW signaling. Kibra, Mer, and Ex can form a protein complex and promote HSW activation, but it remains unclear if Kibra functions upstream or in parallel to Tao-1. Image and legend from (Boggiano, Vanderzalm et al. 2011).

Another gene which acts as tumor suppressor is *RASSF* that contains a Ras association domain (RA), an N-terminal C1-type zinc finger, and a C-terminal Sav RASSF Hippo (Dong, Feldmann et al.) domain. In human, RASSF bound to MST1 and RASSF1-MST1 complex may function as a proapoptotic effector and modulate MST1 activity (Khokhlatchev, Rabizadeh et al. 2002). In drosophila, it had been shown that RASSF inhibits Hpo through competition with Sav for Hpo and enrolls a PP2A complex (dSTRIPAK) to dephosphorylate and deactivate Hpo (Polesello, Huelsmann et al. 2006).

1.6.2. Mammalian Sterile-20-like kinase 1/2 (MST1/2): structure and function

The MST1/2 is a Ser/Thr kinase that belongs to the Class II germinal center kinases (GCK) family of kinases consisting of an N-terminal catalytic domain in the Ste20 class and a non-catalytic tail including, an auto-inhibitory segment and a coiled-coil domain that mediates dimerization, which can be activated by TAO kinase (Praskova, Khoklatchev et al. 2004, Meng, Moroishi et al. 2016). MST1/2 plays a role in the regulation of the cytoskeleton and apoptosis (Glantschnig, Rodan et al. 2002). In mammals, TAO kinases can initiate the hippo pathway by phosphorylation of a single site MST1 (Thr183)/MST2 (Thr180) named the activation loop resulting in MST1/2 activation (Boggiano, Vanderzalm et al. 2011). Moreover, MST1/2 dimerization results in auto-phosphorylation of activation loop of MST1/2 leading to full activation of MST1/2 (Glantschnig, Rodan et al. 2002, Praskova, Khoklatchev et al. 2004). Previously our lab identified that MST1/2, a core component of Hippo, as a fundamental regulator of apoptotic β-cell death and dysfunction (Ardestani, Paroni et al. 2014).

1.6.3. Large Tumor Suppressor (LATS1/2): structure and function

The LATS1/2 belongs to the nuclear Dbf2-related (NDR) family of kinases (Pearce, Komander et al. 2010) made of the activation loop and the hydrophobic motif. It is involved in tumorigenesis via modulating the regulation of apoptosis and proliferation (Visser and Yang 2010). MST2 can phosphorylate the hydrophobic motif of LATS1 (T1079) and MOB1 at its N-terminal tail (T35 and T12), which contributes to LATS1 activation and LATS1-MOB1 complex separation from MST2 (Meng, Moroishi et al. 2016). It has been shown that the hydrophobic motifs of LATS1/2 can also be phosphorylated via two groups of MAP4Ks (mitogen-activated protein kinase kinase kinase kinase), MAP4K1/2/3/5 (homologs of Drosophila Happyhour [Hppy]) and MAP4K4/6/7 (homologs of Drosophila Misshapen [Msn]) resulting in LATS1/2 activation (Meng, Moroishi et al. 2015, Zheng, Wang et al. 2015). Activated LATS1/2 phosphorylates YAP/TAZ resulting in sequential YAP/TAZ deactivation (Zhao, Wei et al. 2007, Meng, Moroishi et al. 2015).

1.6.4. Mechanism of Hippo regulation

1.6.4.1 Cell contact and mechanical signal cross talk Hippo pathway

Cell-cell contact, extracellular matrix (ECM) stiffness, and mechanical signals are important stimuli which regulate Hippo pathway output. For example, adherens and tight junctions can regulate Hippo pathway through activating LATS1/2 (Zhao, Wei et al. 2007, Silvis, Kreger et al. 2011). Moreover, interaction with the ECM which is required for cell survival and growth leads to YAP nuclear localization through Rho-GTPases or the FAK–Src–PI3K pathway activation (Zhao, Li et al. 2012, Kim and Gumbiner 2015).

1.6.4.2 Hippo pathway and G-protein-coupled receptors (GPCRs)

There are 2 crucial factors: hormonal signals (autocrine, paracrine, and endocrine) and nutrients that have a role in regulation of tissue growth. Hormones or growth factor stimulating signals regulate the Hippo pathway through several molecules, such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), activating and stabilizing YAP/TAZ through their GPCRs, LPA receptor (LPAR), and S1P receptor (S1PR) in order to control tissue growth and homeostasis (Yu, Zhao et al. 2012). Different studies had shown that GPCRs can either stimulate or suppress the LATS1/2 kinase through the nature of downstream G proteins targets. For example, activated Gα12/13- and Gαq/11 mediated by epinephrine or glucagon induction, activate Rho-GTPases, which in turn inhibit LATS1/2 (Yu, Zhao et al. 2012). Activated GαS-coupled receptors mediated by LPA, S1P and thrombin stimulation activate protein kinase A (PKA) and Rho GTPases that increase LATS1/2 kinase activity resulting in YAP and TAZ inhibition in a protein kinase A (PKA) dependent manner (Yu, Zhang et al. 2013). For example, elevated GPCR expression mediated by estrogen suppresses LATS1/2, designating a role of YAP/TAZ activation by estrogen in breast cancer (Zhou, Wang et al. 2015).

1.6.4.3 Hippo and Stress signals

Several stress signals such as energy stress, endoplasmic reticulum stress, and hypoxia, can regulate Hippo pathway components such as hydrogen peroxide, which stimulates MST1/2 during cellular oxidative stress (Taylor, Wang et al. 1996, Lehtinen, Yuan et al. 2006, Geng, Sun et al. 2015). In response to oxidative stress in cardiomyocytes, FOXO1 interacts with YAP and mediates transcription of catalase and MnSOD genes resulting in reduction of oxidative stress and restoration of YAP activity protecting heart cell against ischemia/reperfusion (I/R) injury (Shao, Zhai et al. 2014)(Fig.10).

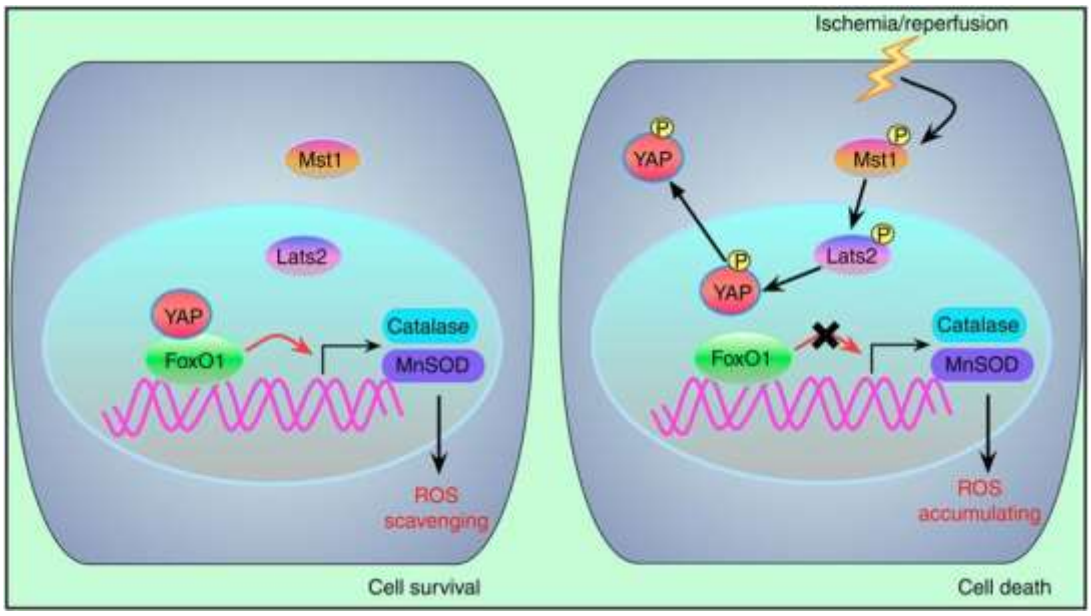

Figure 10. A scheme of the cross talk between Hippo signaling and FoxO1. In cardiomyocytes, YAP and FoxO1 form a functional complex that mediates catalase and MnSOD expression. In response to I/R, activation of the Hippo signaling cascade induces YAP inactivation, which leads to antioxidant gene suppression, ROS accumulation and cell death. Image and legend from (Shao, Zhai et al. 2014).

In HEK293T, glucose deprivation triggers energy stress activating AMPK that phosphorylates and stabilizes tight-junction protein AMOTL1 at S793 residue leading to LATS1/2 activation (DeRan, Yang et al. 2014)(Fig.11). Activated AMPK mediated by energy stress, also phosphorylates YAP at multiple sites, and inhibit YAP and TEAD binding (Mo, Meng et al. 2015, Wang, Xiao et al. 2015). In Hela cells, treatment by metformin leads to LKB1 activation resulting in YAP inhibition (Nguyen, Babcock et al. 2013).

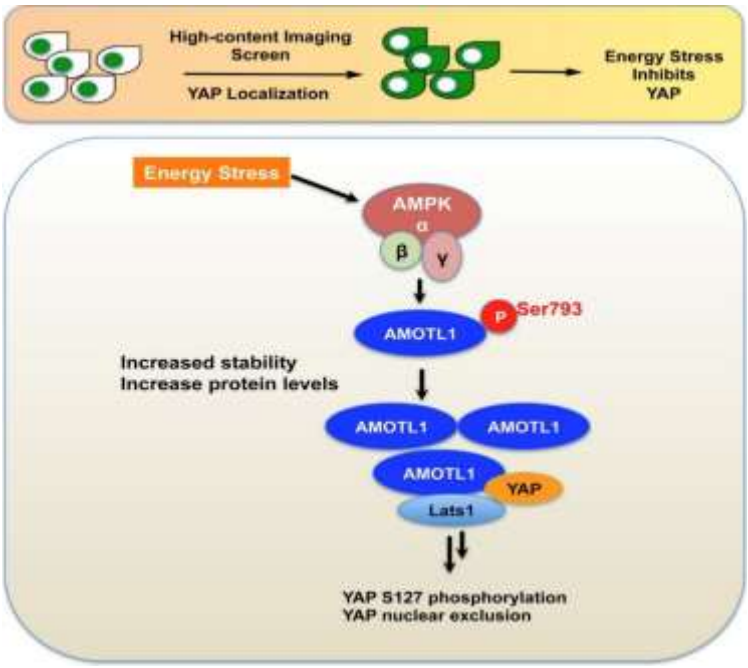

Figure 11. Energy stress inhibits YAP. AMPK directly phosphorylates AMOTL1, an upstream inhibitor of YAP, at S793 resulting in YAP deactivation. Image and legend from (DeRan, Yang et al. 2014)).

1.6.5. Yes-associated protein (YAP)

1.6.5.1. YAP: Structure and function

In 1994, YAP was identified as a protein that associates with the Src family non-receptor tyrosine kinase and plays critical roles (Sudol 1994) in regulating early embryonic development, growth of several tissue types, adult organs, particularly during tissue repair and regeneration mediated by driving the transcription of genes that enhance cell proliferation, survival, and stem cell maintenance (Low, Pan et al. 2014, Piccolo, Dupont et al. 2014, Hansen, Moroishi et al. 2015, Yu, Zhao et al. 2015). YAP (a homolog of Yki in drosophila), a major effector of the mammalian Hippo tumor suppressor pathway, is a conserved oncoprotein encoded by the *YAP* gene settled in the human chromosome 11q22, which only bind to DNA by cooperation with transcriptional factors (Lorenzetto, Brenca et al. 2014). *YAP* gene encodes 2 isoforms of YAP that are different in WW binding domain including YAP1 and YAP2 consisting of 1 and 2 WW domains, respectively (Sudol 1994, Komuro, Nagai et al. 2003). YAP polypeptide is a 65 kDa protein consisting of 488 amino acid residue, which divides in different domains and motifs including a TEA DNA-binding, a coiled-coil, and WW domains as well as a PDZ interaction and an SH3 binding motifs (Shen and Stanger 2015, Abylkassov and Xie 2016)(Fig.12). YAP binds to TEAD family of transcription

factors through TEA DNA-binding and WW domains involved in transcriptional coactivator binding, which in turn, binds to the PPxY motif present on transcription factors (Kanai, Marignani et al. 2000). The PDZ binding motif of YAP is responsible for nuclear localization and critical for YAP-mediated oncogenesis transformation (Shimomura, Miyamura et al. 2014). One of the interacting proteins with WW1 motif of YAP is p53-binding protein-2 (p53BP-2), which interface through its SH3 domains. The WW1 domain of YAP bind to the rich proline (YPPPPY) motif of p53BP-2, and the SH3 domain of p53BP-2 interface with the VPMRLR sequence of YAP and regulate the apoptotic activity of p53 (Espanel and Sudol 2001).

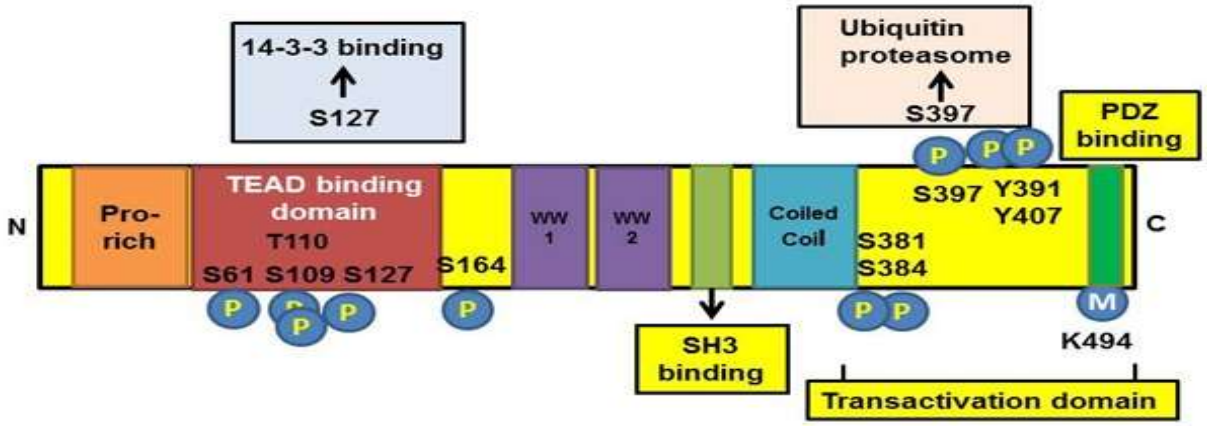

Figure 12. YAP protein various domains. YAP domains are with different color from the N-terminus to the C-terminus. YAP, yes-associated protein; TEAD, TEA domain; SH3, SRC homology 3; P, phosphorylation; M, methylation. Image and legend from (Abylkassov and Xie 2016)).

YAP is negatively regulated by the Hippo pathway playing crucial roles in development, regulation of organ size, and apoptosis. Therefore, any dysregulation in the Hippo-YAP/TAZ may contribute in developmental defects, tissue atrophy, defective tissue repair, tissue overgrowth, and tumor formation indicating a powerful impact of Hippo/YAP signaling in tissues homeostasis and growth (Warren, Xiao et al. 2018).

1.6.5.2. Mechanisms of YAP Regulation

YAP activity is regulated by Hippo-dependent or independent pathways. Upstream regulators of YAP in context of Hippo signaling are TAO kinase, FAT atypical cadherins 1–4 (FAT 1–4), WW, C2 Domain Containing 1 (WWC1) and 2 (WWC2), FE1-4RM Domain-containing Proteins 1 and 6 (FRMD1 and FRMD6), Dachshund Homologs 1 and 2 (DACH1 and DACH2), Dachsous, NF2/Merlin, and Zonula Occludens 1 and 2 (ZO1 and ZO2) (Pan 2010, Genevet and Tapon 2011, Meng, Moroishi et al. 2016, Ye and Eisinger-Mathason 2016, Bae, Kim et al. 2017).

Various signals such as contact inhibition, energy stress, serum deprivation, and F-actin disassembly make the Hippo pathway active (ON) resulting in LATS1/2 activation (Meng, Moroishi et al. 2015) that leads to YAP phosphorylation at Ser127, among other sites, resulting in their binding to 14-3-3 protein. This leads to YAP subsequent translocation from the nucleus to the

cytoplasm (Zhao, Wei et al. 2007). Moreover, phosphorylation of YAP at Ser381 primes further phosphorylation by Casein kinase 1δ/ε and subsequent recruitment of the SCF E3 ubiquitin ligase ultimately leading to YAP/TAZ ubiquitination and proteasome degradation (Liu, Zha et al. 2010, Zhao, Li et al. 2010).

GPCR, including three subunits, α, β, and γ, regulates YAP through two types of ligands-agonists: 1) LPA, S1P and thrombin, and 2) glucagon and epinephrine, which lead to YAP inhibition and activation, respectively (Zhou, Wang et al. 2014, Abylkassov and Xie 2016)(Fig.14). LPA, S1P, and thrombin stimulate Gαs that activates cAMP-PKA leading to LATS1/2 activation, while glucagon and epinephrine stimulate Gα11, Gα12, Gα13, Gαi, Gαo and Gαq activating Rho GTPase inducing F-actin polymerization resulting in LATS1/2 inhibition and subsequently YAP activation (Moroishi, Hansen et al. 2015). Furthermore, it is revealed that Wnt signaling triggers separation of YAP from the β-catenin destruction complex, and thus causing accumulation of nuclear YAP (Fig.12) (Azzolin, Panciera et al. 2014, Abylkassov and Xie 2016). In addition, YAP can be activated through Leukemia inhibitory factor receptor and epidermal growth factor via separating it from Hippo kinase (Moroishi, Hansen et al. 2015). YAP activity may be regulated by miRNA 31, which inhibits LATS2 3' untranslated region (UTR) leading to elevated level of YAP in the nucleus and this, in turn, promotes transcription of oncogenes such as cyclin D1 (Mitamura, Watari et al. 2014). Moreover, AKT can promote cellular survival in the face of apoptotic stimuli through phosphorylation of YAP at Ser127 and suppression of its ability to enhance p73-mediated transcription of proapoptotic genes in response to DNA damaging agents leading to apoptosis (Basu, Totty et al. 2003). Another YAP regulator is Protein kinase Cζ (PKCζ) demonstrated to be a negative regulator of intestinal tumorigenesis. PKCζ phosphorylates YAP at Ser109 and Thr110 resulting in the retention of YAP in the cytoplasm (Llado, Nakanishi et al. 2015). In addition to regulation of YAP phosphorylation, there is another mechanism through methylation mediated by Set7 (Setd7), a SET-domain-containing lysine methyltransferase that methylates Lys494 of YAP resulting in YAP translocation (Llado, Nakanishi et al. 2015).

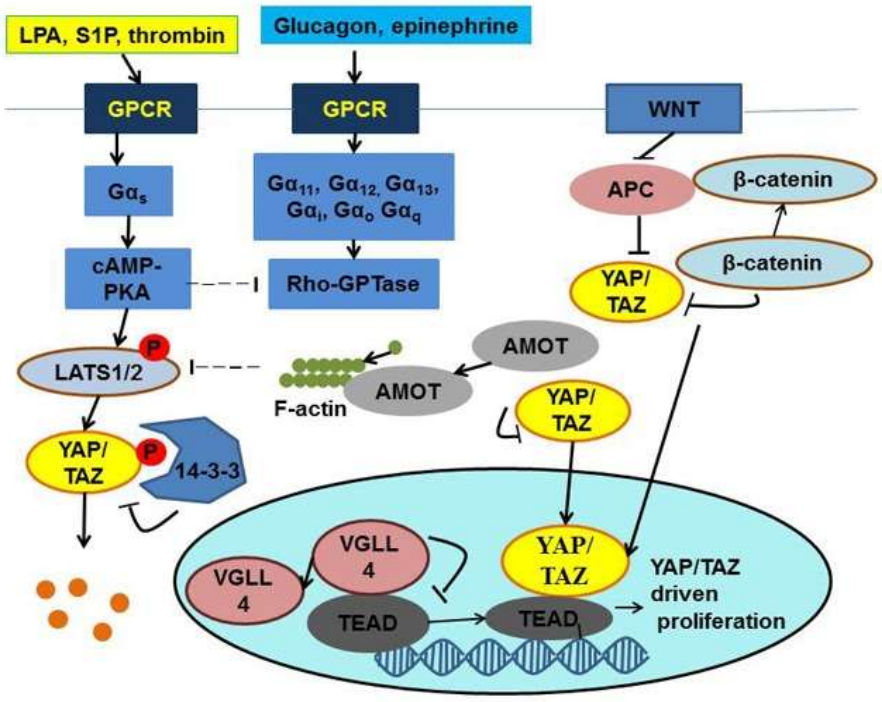

Figure 13. Regulation of the Yap/TAZ by GPCR and Wnt. Image from (Abylkassov and Xie 2016).

1.6.5.3. YAP downstream targets

In Drosophila, Yki increases expression of cyclin E and Diap1 genes, which leads to induction of proliferation and suppression of apoptosis (Udan, Kango-Singh et al. 2003, Huang, Wu et al. 2005). In MCF10A cells, upregulation of YAP induces epithelial–mesenchymal transition (EMT), a hallmark of tumorigenic transformation (Overholtzer, Zhang et al. 2006). Moreover, It has been shown that YAP expression and nuclear localization increased in multiple human cancer tissues (Zhao, Wei et al. 2007). For example, Camargo *et al.,* identified overexpression of YAP leads to liver oversize and tumor growth (Camargo, Gokhale et al. 2007). In addition, different studies have observed that various transcription factors, such as ErbB4, Runx2, TEAD, and p73 interact with YAP (Yagi, Chen et al. 1999, Vassilev, Kaneko et al. 2001, Basu, Totty et al. 2003, Komuro, Nagai et al. 2003). From a transcription activity-based screen, TEAD is identified as the most potent YAP target and is required by YAP to induce gene expression, cell growth, anchorage-independent growth, and EMT (Zhao, Ye et al. 2008). Furthermore, Zhao *et al.,* demonstrated that the connective tissue growth factor (CTGF) is a direct target gene of YAP and also showed that TEAD regulates cell growth and colony formation in soft agar in a YAP-mediated manner (Zhao, Ye et al. 2008). In *Drosophila*, it has been shown that the interaction between Sd and Yki promotes tissue growth and organ size (Zhao, Ye et al. 2008). The transcription factor FOXO1 consisting of the Forkhead box a conserved DNA-binding domain, plays a role in regulation of cell cycle, apoptosis, atrophy, autophagy, and energy homeostasis (Huang and Tindall 2007, Calnan

and Brunet 2008). During oxidative stress, FOXO1 also has a fundamental role to protect cells via regulating expression of antioxidant genes such as catalase and MnSOD (Kops, Dansen et al. 2002, Nemoto and Finkel 2002). In the heart, deletion of FOXO1 leads to an increase in apoptosis and a decrease in cardiac function in response to myocardial infarction, concluding FOXO1 plays a vital role in cardiomyocyte survival when oxidative stimuli are present (Sengupta, Molkentin et al. 2011). However, the details of the signaling cascade triggering regulation of FOXO1 activity during I/R is yet unknown. But it had been shown that FOXO1 is a downstream effector of YAP and YAP-FOXO1 complex regulates the expression of antioxidant genes in response to I/R through cardiomyocyte survival, which is negatively regulated via activation of Hippo signaling (Shao, Zhai et al. 2014)(Fig.10).

1.6.5.4. The role of YAP in pancreas development

As said above, Hippo pathway plays a critical role during organ development. In the course of pancreas development, Hippo provides a balance between proliferation and differentiation of stem/progenitor cells, which are a common pool for generation of all endocrine and exocrine cells (Gu, Dubauskaite et al. 2002, George, Day et al. 2012). Hippo pathway is very important to form a pancreas of correct size, cell type composition, and physiologic function. Development of pancreas includes two steps: the “primary transition” (~E10), in which there is a modest increase in the production of pancreatic hormones, and the “secondary transition” (~E14–16), during which there is a dramatic increase in the production of digestive enzymes and hormones (Pan and Wright 2011). Deletion of MST1/2 results in higher YAP activity throughout the exocrine compartment correlated with higher levels of cell proliferation resulting in pancreas oversize in mouse (George, Day et al. 2012). Activated YAP directly upregulates the genes necessary for progression of cell proliferation, such as CTGF and the epidermal growth factor (EGF) family member amphiregulin in mammalian cells (Zhao, Ye et al. 2008). George, Day et al. demonstrated that YAP is highly expressed throughout the positive-PDX1 embryonic day 12.5 (E12.5) in mouse pancreas via immunohistochemical staining for PDX1, as shown in Fig.14 A and B. They showed that YAP localizes in the nuclear cells within the acinus-fated “tip” region, while cells within the duct- and endocrine-destined “trunk” (green arrow) showed either strongly YAP positive or, alternatively, deprived of YAP expression. YAP expression is limited to prospective ductal and acinar regions during the secondary transition (~E16.5) characterized by massive cell proliferation and differentiation (Fig.14C). The number of YAP-positive cells decreased in the adult mouse pancreas after development (Fig.14D) and increased in ductal and terminal-duct centro acinar cells (George, Day et al. 2012). Furthermore, they approved that YAP is not expressed in islets while it has a weak cytoplasmic staining pattern in acinar cells in both mouse and human pancreas shown in Fig.14E and F. In contrast, P-MST1/2 is obviously detected in islets area whereas its expression in ductal and acinar cells is very weak (Fig.14G and H) (George, Day et al. 2012).

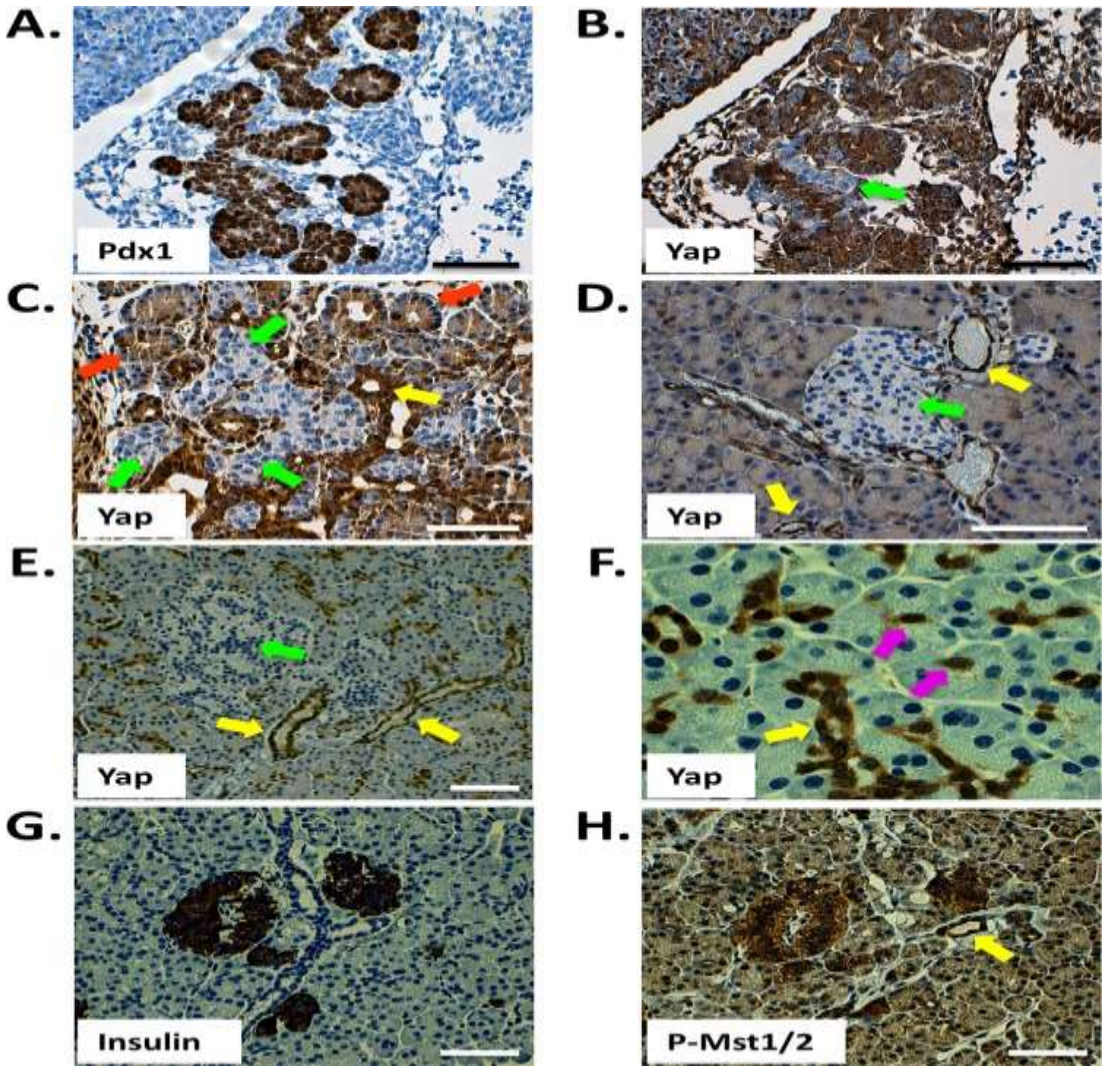

Figure 1. Immunohistochemical detection of Yap and active Mst1/2 in the mammalian pancreas A and B, Yap is broadly expressed throughout the Pdx1-positive E12.5 mouse pancreas. C, Yap expression in the E16.5 pancreas. D, Yap expression within the pancreas of the adult mouse (at 6 weeks) is largely confined to the ductal network, including terminal duct centroacinar cells, and is undetectable within islets. E and F, Yap expression in the human pancreas (at 35 years) mirrors that of the mouse, with reactivity observable throughout the ductal network and absent from islets. G and H, active Hippo signaling, as determined by phosphorylation-specific Mst1/2 antibody, is present throughout the adult human pancreas. Highest levels of active Hippo signaling are found within islets. Arrows denote prospective or mature cell types, as follows: green, endocrine; orange, acinar; yellow, ducts; violet, centroacinar. Image and legend from (George, Day et al. 2012)).

Another study observed that specific deletion of MST1/2 in the developing pancreatic epithelium results in an increase of total YAP and a decrease of phosphorylated YAP leading to loss of acinar cell identity and causing a decrease of pancreas mass (Gao, Zhou et al. 2013). However, it had been shown that specific deletion of MST1/2 in hepatocyte results in overgrowth of liver (Zhou, Conrad et al. 2009, Lu, Li et al. 2010, Song, Mak et al. 2010). Recently, Rosado *et al.,* have

reported that inhibition of YAP stimulates differentiation and constant activity of YAP impairs β-cell differentiation (Rosado-Olivieri, Anderson et al. 2019). YAP has an impact on differentiation of endocrine progenitor by limiting differentiation and impairing glucose-stimulated insulin secretion in stem cell-derived insulin-producing beta (SC-β) cells (Rosado-Olivieri, Anderson et al. 2019). They also found that reduced YAP activity via either chemical or genetic suppression leads to an increased generation of endocrine cells and SC-β cells (Rosado-Olivieri, Anderson et al. 2019). Newly specified acinar cells endure maturation and expansion during the secondary transition (Pan and Wright 2011). During the secondary transition, MST1 and YAP, but not MST2 are expressed while all of them are silenced at the time of birth (Gao, Zhou et al. 2013). Since improper YAP expression causes interruption of pancreatic development through interfering with both acinar and endocrine differentiation during the secondary transition (E13.5-E17.5). Therefore, YAP expression should be limited to mid-gestation and suppressed during or immediately after the second transition for pancreatic development (Gao, Zhou et al. 2013).
In conclusion, the hippo pathway regulates pancreas size through regulation of YAP, which control pancreas development and proliferation of progenitor cells during development of mammalian pancreas.

1.6.5.5. The effect of YAP in regeneration

Presence of stem cells, for example in the intestine or skin, and reactivation of cell proliferation in fully differentiated cells, for example in hepatocytes in the liver, endow the regenerative potential in a tissue (Baddour, Sousounis et al. 2012, Mao and Mooney 2015). Regenerative medicine tries to mimic the repair mechanisms in organs with inadequate regeneration, for example by transplanting progenitor or stem cells or by triggering endogenous repair mechanisms. Although just stimulating cell proliferation in differentiated cells is not enough to present a detectable regenerative potential in a tissue like heart (Pasumarthi, Nakajima et al. 2005, Engel, Hsieh et al. 2006, Hassink, Pasumarthi et al. 2007, Kühn, Del Monte et al. 2007). In parallel, clinical trials of stem cell transplantation indicated a low efficiency (Trounson and McDonald 2015). As said above Hippo has a canonical role in the maintenance of organ size and regulation of development; there are several studies, which showed the effect of Hippo in regeneration of different organs. Upregulated Hippo components inhibit proliferation and regeneration in cardiomyocyte in heart failure (Halder and Johnson 2011, Leach, Heallen et al. 2017). Heart specific Salvador-deficiency improves regeneration of ischaemic heart failure due to myocardial infarction through enhanced scar border vascularity, declined fibrosis, and restoration of pumping function in mouse models. Deletion of Salvador in cardiomyocyte triggers the expression of proliferative and stress response genes such as *Park2* necessary for heart repair in mouse (Leach, Heallen et al. 2017). Cardiomyocyte-specific ablation of Salvador also upregulates some genes involved in cell cycle, heart contraction, heart growth, and cellular response to stress, declaring that *SalvCKO* cardiomyocytes were restoring the maturity of cardiomyocyte with an efficient stress response. Moreover, Salvador deficiency in cardiomyocyte diminishes expression of some genes involved in protein translation and metabolism including the ubiquitin proteasome pathway and inflammation, suggesting that inflammation and misfolded protein response were more efficiently addressed in *SalvCKO* myocardial infarction (Leach, Heallen et al. 2017). Furthermore, another study also indicated that deletion of Hippo components such as LATS1/2 or Salvador in

cardiomyocyte of mice with myocardial infract ameliorates heart function through upregulation of cardiomyocyte renewal and regeneration genes downstream targets of YAP resulting in cell cycle progression, enhancement of cytoskeletal remodeling and protection of cell membrane from contractile stress (Heallen, Morikawa et al. 2013, Morikawa, Zhang et al. 2015, Tao, Kahr et al. 2016). In hepatocyte, it had been shown that age-related hepatocyte regeneration defects are mediated by impaired YAP expression and hepatocyte proliferation in old mice. Temporary downregulated Hippo core kinases, MST1 and MST2, using siRNA stimulates proliferation in quiescent liver cells resulting in hepatocyte regeneration in aged mice after partial hepatectomy through upregulation of proliferation genes such as FOXM1B induced by YAP (Loforese, Malinka et al. 2017). Recent study indicated that Hippo pathway has an impact on proliferation and specification of pancreatic progenitor cells. Constitutive activation of YAP increases proliferation and the self-renewal of pancreatic progenitors while decreases endocrine differentiation *in vitro* (Rosado-Olivieri, Anderson et al. 2019).
In conclusion, according to Hippo's role in the regulation of organ size and development, I suggest that switching Hippo "OFF" or temporarily activate YAP may result in improvement of proliferation and regeneration. To prove this hypothesis is the aim of my doctoral thesis.

1.7. Aim of thesis

The identification of cellular pathway(s) and molecular players responsible for β-cell failure is urgently needed in order to fully understand disease mechanisms and for optimal and especially so far non existing therapeutic interventions, which target to the core problem in diabetes: the loss of functional β-cells.
There are several signaling pathways, such as mTOR, Hippo, JNK, and AKT (Bernal-Mizrachi, Wen et al. 2001, Tuttle, Gill et al. 2001, Ardestani, Paroni et al. 2014), which are involved in β-cells failure in diabetes, but the in-depth mechanism of actions are not known yet. Hippo plays a critical role in controlling organ size, via affecting cell size and numbers through regulation of YAP. Therefore, the first aim of my thesis was to investigate the Hippo pathway as a possible regulator of β-cell turnover.
I identified that exogenously introduced YAP is able to foster human β-cell proliferation and confers an intrinsic resistance to β-cell death under a diabetogenic environment.

The second aim of my thesis was to elucidate the potential regulation of mTOR signaling complexes in metabolically stressed islets
I found that mTORC1 is highly upregulated in stressed human islets.

The knowledge of both parts of my thesis are important for understanding the molecular mechanism of the diabetes pathology and open new insights for designing a new treatment for diabetes with a major focus on the restoration of survival and function of insulin-producing β-cells.

References

Abylkassov, R. and Y. Xie (2016). "Role of Yes-associated protein in cancer: An update." Oncology letters **12**(4): 2277-2282.

Alejandro, E. U., et al. (2017). "Overexpression of kinase-dead mTOR impairs glucose homeostasis by regulating insulin secretion and not β-cell mass." Diabetes **66**(8): 2150-2162.

Ardestani, A., et al. (2018). "mTORC1 signaling: a double-edged sword in diabetic β cells." Cell metabolism **27**(2): 314-331.

Ardestani, A. and K. Maedler (2017). "The Hippo signaling pathway in pancreatic β-cells: functions and regulations." Endocrine reviews **39**(1): 21-35.

Ardestani, A., et al. (2014). "MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes." Nature medicine **20**(4): 385.

Ardestani, A., et al. (2014). "MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes." Nat Med **20**(4): 385-397.

Artner, I. and R. Stein (2008). Transcriptional regulation of insulin gene expression. Pancreatic Beta Cell in Health and Disease, Springer**:** 13-30.

Azzolin, L., et al. (2014). "YAP/TAZ incorporation in the β-catenin destruction complex orchestrates the Wnt response." Cell **158**(1): 157-170.

Baddour, J. A., et al. (2012). "Organ repair and regeneration: an overview." Birth Defects Research Part C: Embryo Today: Reviews **96**(1): 1-29.

Bae, J. S., et al. (2017). "The Hippo signaling pathway provides novel anti-cancer drug targets." Oncotarget **8**(9): 16084.

Balcazar, N., et al. (2009). "mTORC1 activation regulates β-cell mass and proliferation by modulation of cyclin D2 synthesis and stability." Journal of Biological Chemistry **284**(12): 7832-7842.

Basu, S., et al. (2003). "Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis." Molecular cell **11**(1): 11-23.

Bensellam, M., et al. (2012). "The molecular mechanisms of pancreatic β-cell glucotoxicity: recent findings and future research directions." Molecular and cellular endocrinology **364**(1-2): 1-27.

Bernal-Mizrachi, E., et al. (2001). "Islet beta cell expression of constitutively active Akt1/PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia." J Clin Invest **108**(11): 1631-1638.

Boggiano, J. C., et al. (2011). "Tao-1 phosphorylates Hippo/MST kinases to regulate the Hippo-Salvador-Warts tumor suppressor pathway." Developmental cell **21**(5): 888-895.

Bratanova-Tochkova, T. K., et al. (2002). "Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion." Diabetes **51**(suppl 1): S83-S90.

Butler, A. E., et al. (2003). "β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes." Diabetes **52**(1): 102-110.

Calnan, D. and A. Brunet (2008). "The foxo code." Oncogene **27**(16): 2276.

Camargo, F. D., et al. (2007). "YAP1 increases organ size and expands undifferentiated progenitor cells." Current Biology **17**(23): 2054-2060.

Cersosimo, E., et al. (2018). Endotext: Pathogenesis of Type 2 Diabetes Mellitus.

Chan, S. W., et al. (2011). "The Hippo pathway in biological control and cancer development." Journal of cellular physiology **226**(4): 928-939.

Chen, J., et al. (2008). "Thioredoxin-interacting protein: a critical link between glucose toxicity and β-cell apoptosis." Diabetes **57**(4): 938-944.

Chen, J., et al. (1995). "Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue." Proceedings of the National Academy of Sciences **92**(11): 4947-4951.

Cherrington, A., et al. (2007). Insulin action on the liver in vivo, Portland Press Ltd.

Christen, U. and M. G. von Herrath (2004). "IP-10 and type 1 diabetes: a question of time and location." Autoimmunity **37**(4): 273-282.

College, O. (2016). Chapter 17.9 The Endocrine Pancreas, Figure 1. Anatomy & Physiology.

Collins, S., et al. (2012). "Uncoupling and reactive oxygen species (ROS)–A double-edged sword for β-cell function?“Moderation in all things”." Best practice & research Clinical endocrinology & metabolism **26**(6): 753-758.

Cybulski, N. and M. N. Hall (2009). "TOR complex 2: a signaling pathway of its own." Trends in biochemical sciences **34**(12): 620-627.

Da Silva Xavier, G. (2018). "The cells of the islets of langerhans." Journal of clinical medicine **7**(3): 54.

Dalle Pezze, P., et al. (2012). "A dynamic network model of mTOR signaling reveals TSC-independent mTORC2 regulation." Sci. Signal. **5**(217): ra25-ra25.

DeFronzo, R. (2010). "Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009." Diabetologia **53**(7): 1270-1287.

DeFronzo, R. A., et al. (2015). "Type 2 diabetes mellitus." Nature reviews Disease primers **1**: 15019.

DeRan, M., et al. (2014). "Energy stress regulates hippo-YAP signaling involving AMPK-mediated regulation of angiomotin-like 1 protein." Cell reports **9**(2): 495-503.

Dimitriadis, G., et al. (2011). "Insulin effects in muscle and adipose tissue." Diabetes research and clinical practice **93**: S52-S59.

Donath, M. Y., et al. (2009). "Islet inflammation impairs the pancreatic β-cell in type 2 diabetes." Physiology **24**(6): 325-331.

Donath, M. Y., et al. (1999). "Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes." Diabetes **48**(4): 738-744.

Dong, J., et al. (2007). "Elucidation of a universal size-control mechanism in Drosophila and mammals." Cell **130**(6): 1120-1133.

Dyntar, D., et al. (2001). "Glucose and palmitic acid induce degeneration of myofibrils and modulate apoptosis in rat adult cardiomyocytes." Diabetes **50**(9): 2105-2113.

Eguchi, K. and R. Nagai (2017). "Islet inflammation in type 2 diabetes and physiology." The Journal of clinical investigation **127**(1): 14-23.

Ehses, J. A., et al. (2007). "Increased number of islet-associated macrophages in type 2 diabetes." Diabetes **56**(9): 2356-2370.

Elghazi, L., et al. (2017). "Role of nutrients and mTOR signaling in the regulation of pancreatic progenitors development." Molecular metabolism **6**(6): 560-573.

Elmore, S. (2007). "Apoptosis: a review of programmed cell death." Toxicologic pathology **35**(4): 495-516.

endocrineweb (2018, September 27). "Type 2 Diabetes Complications." from https://www.endocrineweb.com/conditions/type-2-diabetes/type-2-diabetes-complications.

endocrineweb (2018, September 27). "Type 2 Diabetes Complications." from https://www.endocrineweb.com/conditions/type-2-diabetes/type-2-diabetes-complications.

Engel, F. B., et al. (2006). "FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction." Proceedings of the National Academy of Sciences **103**(42): 15546-15551.

Espanel, X. and M. Sudol (2001). "Yes-associated protein and p53-binding protein-2 interact through their WW and SH3 domains." Journal of Biological Chemistry **276**(17): 14514-14523.

Favaloro, B., et al. (2012). "Role of apoptosis in disease." Aging (Albany NY) **4**(5): 330.

Federici, M., et al. (2001). "High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program." Diabetes **50**(6): 1290-1301.

Fu, Z., et al. (2013). "Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes." Current diabetes reviews **9**(1): 25-53.

Gao, T., et al. (2013). "Hippo signaling regulates differentiation and maintenance in the exocrine pancreas." Gastroenterology **144**(7): 1543-1553. e1541.

Genevet, A. and N. Tapon (2011). "The Hippo pathway and apico–basal cell polarity." Biochemical Journal **436**(2): 213-224.

Geng, J., et al. (2015). "Kinases Mst1 and Mst2 positively regulate phagocytic induction of reactive oxygen species and bactericidal activity." Nature immunology **16**(11): 1142.

George, N. M., et al. (2012). "Hippo signaling regulates pancreas development through inactivation of Yap." Molecular and cellular biology **32**(24): 5116-5128.

Gera, J. F., et al. (2004). "AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression." Journal of Biological Chemistry **279**(4): 2737-2746.

Glantschnig, H., et al. (2002). "Mapping of MST1 kinase sites of phosphorylation activation and autophosphorylation." Journal of Biological Chemistry **277**(45): 42987-42996.

Gloyn, A. L., et al. (2004). "Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6. 2 and permanent neonatal diabetes." New England Journal of Medicine **350**(18): 1838-1849.

Gu, G., et al. (2002). "Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors." Development **129**(10): 2447-2457.

Guichard, C., et al. (2008). NOX family NADPH oxidases in liver and in pancreatic islets: a role in the metabolic syndrome and diabetes?, Portland Press Limited.

Halder, G. and R. L. Johnson (2011). "Hippo signaling: growth control and beyond." Development **138**(1): 9-22.

Hamada, S., et al. (2009). "Upregulation of the mammalian target of rapamycin complex 1 pathway by Ras homolog enriched in brain in pancreatic β-cells leads to increased β-cell mass and prevention of hyperglycemia." Diabetes **58**(6): 1321-1332.

Hamaratoglu, F., et al. (2006). "The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis." Nature cell biology **8**(1): 27.

Hansen, C. G., et al. (2015). "YAP and TAZ: a nexus for Hippo signaling and beyond." Trends in cell biology **25**(9): 499-513.

Harvey, K. F., et al. (2003). "The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis." Cell **114**(4): 457-467.

Hassink, R. J., et al. (2007). "Cardiomyocyte cell cycle activation improves cardiac function after myocardial infarction." Cardiovascular research **78**(1): 18-25.

Hay, N. and N. Sonenberg (2004). "Upstream and downstream of mTOR." Genes & development **18**(16): 1926-1945.

Heallen, T., et al. (2013). "Hippo signaling impedes adult heart regeneration." Development **140**(23): 4683-4690.

Hellman, B. (1959). "Actual distribution of the number and volume of the islets of Langerhans in different size classes in non-diabetic humans of varying ages." Nature **184**(4697): 1498-1499.

Hellman, B. (1959). "The frequency distribution of the number and volume of the islets Langerhans in man. I. Studies on non-diabetic adults." Acta Societatis Medicorum Upsaliensis **64**: 432.

Henderson, J. (1969). "Why are the islets of Langerhans?" The Lancet **294**(7618): 469-470.

Huang, C.-j., et al. (2007). "High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress–mediated β-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes." Diabetes **56**(8): 2016-2027.

Huang, H. and D. J. Tindall (2007). "Dynamic FoxO transcription factors." J Cell Sci **120**(15): 2479-2487.

Huang, J., et al. (2005). "The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP." Cell **122**(3): 421-434.

Jacinto, E., et al. (2004). "Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive." Nature cell biology **6**(11): 1122.

Jacobson, E. F. and E. S. Tzanakakis (2017). "Human pluripotent stem cell differentiation to functional pancreatic cells for diabetes therapies: Innovations, challenges and future directions." Journal of biological engineering **11**(1): 21.

Johnson, R. and G. Halder (2014). "The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment." Nature reviews Drug discovery **13**(1): 63.

Justice, R. W., et al. (1995). "The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation." Genes & development **9**(5): 534-546.

Kalkavan, H. and D. R. Green (2018). "MOMP, cell suicide as a BCL-2 family business." Cell death and differentiation **25**(1): 46.

Kanai, F., et al. (2000). "TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins." The EMBO journal **19**(24): 6778-6791.

Kango-Singh, M., et al. (2002). "Shar-pei mediates cell proliferation arrest during imaginal disc growth in Drosophila." Development **129**(24): 5719-5730.

Kato, T., et al. (2008). "Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets." Diabetes **57**(9): 2382-2392.

Kawamori, D., et al. (2009). "Insulin signaling in α cells modulates glucagon secretion in vivo." Cell metabolism **9**(4): 350-361.

Khokhlatchev, A., et al. (2002). "Identification of a novel Ras-regulated proapoptotic pathway." Current biology **12**(4): 253-265.

Kim, N.-G. and B. M. Gumbiner (2015). "Adhesion to fibronectin regulates Hippo signaling via the FAK–Src–PI3K pathway." J Cell Biol **210**(3): 503-515.

Kim, Y. C. and K.-L. Guan (2015). "mTOR: a pharmacologic target for autophagy regulation." The Journal of clinical investigation **125**(1): 25-32.

Komuro, A., et al. (2003). "WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus." Journal of Biological Chemistry **278**(35): 33334-33341.

Kops, G. J., et al. (2002). "Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress." Nature **419**(6904): 316.

Kühn, B., et al. (2007). "Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair." Nature medicine **13**(8): 962.

Lai, Z.-C., et al. (2005). "Control of cell proliferation and apoptosis by mob as tumor suppressor, mats." Cell **120**(5): 675-685.

Laplante, M. and D. M. Sabatini (2012). "mTOR signaling in growth control and disease." Cell **149**(2): 274-293.

Leach, J. P., et al. (2017). "Hippo pathway deficiency reverses systolic heart failure after infarction." Nature **550**(7675): 260.

Lee, J. Y., et al. (2001). "Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4." Journal of Biological Chemistry **276**(20): 16683-16689.

Lee, S. C. and S. Pervaiz (2007). "Apoptosis in the pathophysiology of diabetes mellitus." The international journal of biochemistry & cell biology **39**(3): 497-504.

Lehtinen, M. K., et al. (2006). "A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span." Cell **125**(5): 987-1001.

Liu, C.-Y., et al. (2010). "The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCFβ-TrCP E3 ligase." Journal of Biological Chemistry **285**(48): 37159-37169.

Liu, P., et al. (2009). "Targeting the phosphoinositide 3-kinase pathway in cancer." Nature reviews Drug discovery **8**(8): 627.

Lizcano, J. M. and D. R. Alessi (2002). "The insulin signalling pathway." Current biology **12**(7): R236-R238.

Llado, V., et al. (2015). "Repression of intestinal stem cell function and tumorigenesis through direct phosphorylation of β-catenin and Yap by PKCζ." Cell reports **10**(5): 740-754.

Loforese, G., et al. (2017). "Impaired liver regeneration in aged mice can be rescued by silencing Hippo core kinases MST1 and MST2." EMBO molecular medicine **9**(1): 46-60.

Longnecker, D. S. (2014). "Anatomy and Histology of the Pancreas." Pancreapedia: The Exocrine Pancreas Knowledge Base.

Lorenzetto, E., et al. (2014). "YAP1 acts as oncogenic target of 11q22 amplification in multiple cancer subtypes." Oncotarget **5**(9): 2608.

Low, B. C., et al. (2014). "YAP/TAZ as mechanosensors and mechanotransducers in regulating organ size and tumor growth." FEBS letters **588**(16): 2663-2670.

Lu, L., et al. (2010). "Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver." Proceedings of the National Academy of Sciences **107**(4): 1437-1442.

Madsbad, S. (2014). "The role of glucagon-like peptide-1 impairment in obesity and potential therapeutic implications." Diabetes, Obesity and Metabolism **16**(1): 9-21.

Maedler, K., et al. (2002). "FLIP switches Fas-mediated glucose signaling in human pancreatic β cells from apoptosis to cell replication." Proceedings of the National Academy of Sciences **99**(12): 8236-8241.

Maedler, K., et al. (2003). "Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic β-cell turnover and function." Diabetes **52**(3): 726-733.

Maedler, K., et al. (2002). "Glucose-induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets." The Journal of clinical investigation **110**(6): 851-860.

Maedler, K., et al. (2001). "Glucose induces β-cell apoptosis via upregulation of the Fas receptor in human islets." Diabetes **50**(8): 1683-1690.

Mao, A. S. and D. J. Mooney (2015). "Regenerative medicine: current therapies and future directions." Proceedings of the National Academy of Sciences **112**(47): 14452-14459.

Marchetti, P., et al. (2007). "The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients." Diabetologia **50**(12): 2486-2494.

Mathis, D., et al. (2001). "β-Cell death during progression to diabetes." Nature **414**(6865): 792.

Matsui, Y., et al. (2008). "Lats2 is a negative regulator of myocyte size in the heart." Circulation research **103**(11): 1309-1318.

Meng, Z., et al. (2016). "Mechanisms of Hippo pathway regulation." Genes & development **30**(1): 1-17.

Meng, Z., et al. (2015). "MAP4K family kinases act in parallel to MST1/2 to activate LATS1/2 in the Hippo pathway." Nature communications **6**: 8357.

Mitamura, T., et al. (2014). "microRNA 31 functions as an endometrial cancer oncogene by suppressing Hippo tumor suppressor pathway." Molecular cancer **13**(1): 97.

Mo, J.-S., et al. (2015). "Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway." Nature cell biology **17**(4): 500.

Morikawa, Y., et al. (2015). "Actin cytoskeletal remodeling with protrusion formation is essential for heart regeneration in Hippo-deficient mice." Sci. Signal. **8**(375): ra41-ra41.

Moroishi, T., et al. (2015). "The emerging roles of YAP and TAZ in cancer." Nature Reviews Cancer **15**(2): 73.

Morris, A. P., et al. (2012). "Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes." Nature genetics **44**(9): 981.

Mühlemann, M. "Intestinal stem cells and the Na\(^+\)-D-Glucose Transporter SGLT1: potential targets regarding future therapeutic strategies for diabetes."

Muoio, D. M. and C. B. Newgard (2008). "Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes." Nature reviews Molecular cell biology **9**(3): 193.

Nauck, M., et al. (2011). "Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down?" Diabetologia **54**(1): 10-18.

Nemoto, S. and T. Finkel (2002). "Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway." Science **295**(5564): 2450-2452.

Nguyen, H. B., et al. (2013). "LKB1 tumor suppressor regulates AMP kinase/mTOR-independent cell growth and proliferation via the phosphorylation of Yap." Oncogene **32**(35): 4100.

Ni, Q., et al. (2017). "Raptor regulates functional maturation of murine beta cells." Nature communications **8**: 15755.

Nielsen, J. H., et al. (2001). "Regulation of beta-cell mass by hormones and growth factors." Diabetes **50**(suppl 1): S25.

Oh, Y. S., et al. (2018). "Fatty acid-induced lipotoxicity in pancreatic beta-cells during development of type 2 diabetes." Frontiers in endocrinology **9**: 384.

Overholtzer, M., et al. (2006). "Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon." Proceedings of the National Academy of Sciences **103**(33): 12405-12410.

Owerbach, D., et al. (1981). "The insulin gene is located on the short arm of chromosome 11 in humans." Diabetes **30**(3): 267-270.

Pan, D. (2010). "The hippo signaling pathway in development and cancer." Developmental cell **19**(4): 491-505.

Pan, F. C. and C. Wright (2011). "Pancreas organogenesis: from bud to plexus to gland." Developmental dynamics **240**(3): 530-565.

Pasumarthi, K. B., et al. (2005). "Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice." Circulation research **96**(1): 110-118.

Pearce, L. R., et al. (2010). "The nuts and bolts of AGC protein kinases." Nature reviews Molecular cell biology **11**(1): 9.

Pende, M., et al. (2000). "Hypoinsulinaemia, glucose intolerance and diminished β-cell size in S6K1-deficient mice." Nature **408**(6815): 994.

Piccolo, S., et al. (2014). "The biology of YAP/TAZ: hippo signaling and beyond." Physiological reviews **94**(4): 1287-1312.

Pick, A., et al. (1998). "Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat." Diabetes **47**(3): 358-364.

Plouffe, S. W., et al. (2015). "Disease implications of the Hippo/YAP pathway." Trends in molecular medicine **21**(4): 212-222.

Polesello, C., et al. (2006). "The Drosophila RASSF homolog antagonizes the hippo pathway." Current biology **16**(24): 2459-2465.

Praskova, M., et al. (2004). "Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras." Biochemical Journal **381**(2): 453-462.

Prentki, M. and C. J. Nolan (2006). "Islet β cell failure in type 2 diabetes." The Journal of clinical investigation **116**(7): 1802-1812.

Rahier, J., et al. (2008). "Pancreatic β-cell mass in European subjects with type 2 diabetes." Diabetes, Obesity and Metabolism **10**: 32-42.

Ritzel, R. A., et al. (2007). "Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islets." Diabetes **56**(1): 65-71.

Rorsman, P., et al. (1989). "Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels." Nature **341**(6239): 233.

Rosado-Olivieri, E. A., et al. (2019). "YAP inhibition enhances the differentiation of functional stem cell-derived insulin-producing β cells." Nature communications **10**(1): 1464.

Saito, K., et al. (1978). "Morphometrical analysis on topographical difference in size distribution, number and volume of islets in the human pancreas." The Tohoku journal of experimental medicine **124**(2): 177-186.

Sarbassov, D. D., et al. (2006). "Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB." Molecular cell **22**(2): 159-168.

Saxton, R. A. and D. M. Sabatini (2017). "mTOR signaling in growth, metabolism, and disease." Cell **168**(6): 960-976.

Sengupta, A., et al. (2011). "FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress." Journal of Biological Chemistry **286**(9): 7468-7478.

Shao, D., et al. (2014). "A functional interaction between Hippo-YAP signalling and FoxO1 mediates the oxidative stress response." Nature communications **5**: 3315.

Sharp, G. (1979). "The adenylate cyclase-cyclic AMP system in islets of Langerhans and its role in the control of insulin release." Diabetologia **16**(5): 287-296.

Shen, Z. and B. Z. Stanger (2015). "YAP regulates S-phase entry in endothelial cells." PloS one **10**(1): e0117522.

Shimomura, T., et al. (2014). "The PDZ-binding motif of Yes-associated protein is required for its co-activation of TEAD-mediated CTGF transcription and oncogenic cell transforming activity." Biochemical and biophysical research communications **443**(3): 917-923.

Silvis, M. R., et al. (2011). "α-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1." Sci. Signal. **4**(174): ra33-ra33.

Sivitz, W. I. (2001). "Lipotoxicity and glucotoxicity in type 2 diabetes: effects on development and progression." Postgraduate medicine **109**(4): 55-64.

Slack, J. (1995). "Developmental biology of the pancreas." Development **121**(6): 1569-1580.

Song, H., et al. (2010). "Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression." Proceedings of the National Academy of Sciences **107**(4): 1431-1436.

Soria, B., et al. (2004). "Novel players in pancreatic islet signaling: from membrane receptors to nuclear channels." Diabetes **53**(suppl 1): S86-S91.

Sudol, M. (1994). "Yes-associated protein (YAP65) is a proline-rich phosphoprotein that binds to the SH3 domain of the Yes proto-oncogene product." Oncogene **9**(8): 2145-2152.

Tao, G., et al. (2016). "Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury." Nature **534**(7605): 119.

Taylor, L. K., et al. (1996). "Newly identified stress-responsive protein kinases, Krs-1 and Krs-2." Proceedings of the National Academy of Sciences **93**(19): 10099-10104.

Thomas, H. E., et al. (2009). "Beta cell apoptosis in diabetes." Apoptosis **14**(12): 1389.

Thompson, B. J. and S. M. Cohen (2006). "The Hippo pathway regulates the bantam microRNA to control cell proliferation and apoptosis in Drosophila." Cell **126**(4): 767-774.

Tokarz, V. L., et al. (2018). "The cell biology of systemic insulin function." J Cell Biol **217**(7): 2273-2289.

Tomita, T. (2010). "Immunocytochemical localisation of caspase-3 in pancreatic islets from type 2 diabetic subjects." Pathology **42**(5): 432-437.

Tomita, T. (2016). "Apoptosis in pancreatic β-islet cells in Type 2 diabetes." Bosnian journal of basic medical sciences **16**(3): 162.

Trounson, A. and C. McDonald (2015). "Stem cell therapies in clinical trials: progress and challenges." Cell stem cell **17**(1): 11-22.

Tuo, Y. and M. Xiang (2018). "mTOR: A double-edged sword for diabetes." Journal of leukocyte biology.

Tuttle, R. L., et al. (2001). "Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha." Nat Med **7**(10): 1133-1137.

Udan, R. S., et al. (2003). "Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway." Nature cell biology **5**(10): 914.

Vargas, E. and M. A. C. Sepulveda (2018). Biochemistry, Insulin, Metabolic Effects. StatPearls [Internet], StatPearls Publishing.

Vassilev, A., et al. (2001). "TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm." Genes & development **15**(10): 1229-1241.

Vernier, S., et al. (2012). "β-cell metabolic alterations under chronic nutrient overload in rat and human islets." Islets **4**(6): 379-392.

Visser, S. and X. Yang (2010). "LATS tumor suppressor: a new governor of cellular homeostasis." Cell cycle **9**(19): 3892-3903.

Wållberg, M. and A. Cooke (2013). "Immune mechanisms in type 1 diabetes." Trends in immunology **34**(12): 583-591.

Wang, W., et al. (2015). "AMPK modulates Hippo pathway activity to regulate energy homeostasis." Nature cell biology **17**(4): 490.

Wang, X., et al. (2013). "Regional differences in islet distribution in the human pancreas-preferential beta-cell loss in the head region in patients with type 2 diabetes." PloS one **8**(6): e67454.

Warren, J., et al. (2018). "YAP/TAZ activation as a target for treating metastatic cancer." Cancers **10**(4): 115.

Watanabe, M., et al. (1998). "Histologic distribution of insulin and glucagon receptors." Brazilian journal of medical and biological research **31**(2): 243-256.

Weiss, M., et al. (2014). Insulin biosynthesis, secretion, structure, and structure-activity relationships. Endotext [Internet], MDText. com, Inc.

WHO (2018). "Diabetes." from https://www.who.int/news-room/fact-sheets/detail/diabetes.

Wilcox, G. (2005). "Insulin and insulin resistance." Clinical biochemist reviews **26**(2): 19.

Xu, E., et al. (2006). "Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system." Cell metabolism **3**(1): 47-58.

Yagi, R., et al. (1999). "A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator." The EMBO journal **18**(9): 2551-2562.

Ye, S. and T. K. Eisinger-Mathason (2016). "Targeting the Hippo pathway: Clinical implications and therapeutics." Pharmacological research **103**: 270-278.

Yu, F.-X. and K.-L. Guan (2013). "The Hippo pathway: regulators and regulations." Genes & development **27**(4): 355-371.

Yu, F.-X., et al. (2013). "Protein kinase A activates the Hippo pathway to modulate cell proliferation and differentiation." Genes & development **27**(11): 1223-1232.

Yu, F.-X., et al. (2015). "Hippo pathway in organ size control, tissue homeostasis, and cancer." Cell **163**(4): 811-828.

Yu, F.-X., et al. (2012). "Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling." Cell **150**(4): 780-791.

Yuan, T., et al. (2017). "Reciprocal regulation of mTOR complexes in pancreatic islets from humans with type 2 diabetes." Diabetologia **60**(4): 668-678.

Zarogoulidis, P., et al. (2014). "mTOR pathway: A current, up-to-date mini-review." Oncology letters **8**(6): 2367-2370.

Zhang, H., et al. (2010). "Gestational diabetes mellitus resulting from impaired β-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen." Diabetes **59**(1): 143-152.

Zhang, N., et al. (2010). "The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals." Developmental cell **19**(1): 27-38.

Zhao, B., et al. (2010). "A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCFβ-TRCP." Genes & development **24**(1): 72-85.

Zhao, B., et al. (2012). "Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis." Genes & development **26**(1): 54-68.

Zhao, B., et al. (2007). "Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control." Genes & development **21**(21): 2747-2761.

Zhao, B., et al. (2008). "TEAD mediates YAP-dependent gene induction and growth control." Genes & development **22**(14): 1962-1971.

Zheng, Y., et al. (2015). "Identification of Happyhour/MAP4K as alternative Hpo/Mst-like kinases in the Hippo kinase cascade." Developmental cell **34**(6): 642-655.

Zhou, D., et al. (2009). "Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene." Cancer Cell **16**(5): 425-438.

Zhou, D., et al. (2011). "Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance." Proceedings of the National Academy of Sciences **108**(49): E1312-E1320.

Zhou, X., et al. (2015). "Estrogen regulates Hippo signaling via GPER in breast cancer." The Journal of clinical investigation **125**(5): 2123-2135.

Zhou, X., et al. (2014). "G protein-coupled receptors: bridging the gap from the extracellular signals to the Hippo pathway." Acta Biochim Biophys Sin **47**(1): 10-15.

Zini, E., et al. (2009). "Hyperglycaemia but not hyperlipidaemia causes beta cell dysfunction and beta cell loss in the domestic cat." Diabetologia **52**(2): 336.

Zraika, S., et al. (2009). "Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced beta cell apoptosis." Diabetologia **52**(4): 626-635.

2. Results

Manuscript I

2.1. Pro-proliferative and anti-apoptotic action of exogenously introduced YAP in pancreatic β-cells

Ting Yuan[1]*, **Sahar Rafizadeh**[1]*, Zahra Azizi[1]*, Blaz Lupse[1], KanakaDurga Devi Gorrepati[1], Sushil Awal[1], Jose Oberholzer[2], Kathrin Maedler[1,3] & Amin Ardestani[1]

[1]Centre for Biomolecular Interactions Bremen, University of Bremen, Germany

[2]Division of Transplantation, University of Illinois at Chicago, Chicago, Illinois, USA

[3]German Center for Diabetes Research (DZD) project partner, University of Bremen

*shared first authors

Published in JCI Insight. 2016 Nov 3; 1(18): e86326.

DOI:10.1172/jci.insight.86326

https://doi.org/10.1172/jci.insight.86326

My Contribution:

Designed and performed experiments and analyzed data (partially for Figures 4 and 5, completely for Figure 6).

Manuscript II

2.2. Reciprocal regulation of mTOR complexes in human type 2 diabetic pancreatic islets

Ting Yuan[1]*, **Sahar Rafizadeh[1]***, Kanaka Durga Devi Gorrepati[1]*, Blaz Lupse[1],Jose Oberholzer[2], Kathrin Maedler[1,3] & Amin Ardestani[1]

[1]Centre for Biomolecular Interactions Bremen, University of Bremen, Germany

[2]Division of Transplantation, University of Illinois at Chicago, Chicago, Illinois, USA

[3]German Center for Diabetes Research (DZD) project partner, University of Bremen

*shared first authors

Published in Diabetologia, 2017 Apr;60(4):668-678.

DOI: 10.1007/s00125-016-4188-9

https://doi.org/10.1007/s00125-016-4188-9

Contribution:

Designed and performed experiments and analyzed data for Figure 2a-c.

3-Discussion

3.1. YAP regulates β-cell turnover

Diabetes is caused by a lack of insulin due to either aberration in pancreatic development, reduced β-cell mass, or β-cell dysfunction. Therefore, strategies to foster β-cell regeneration as well as enhance β-cell stress response and viability represent an important step toward potential diabetes therapies. The Hippo pathway plays a crucial role in pancreatic development through regulating cell cycle and organ size via YAP (Ardestani and Maedler 2017). Numerous studies show the YAP regenerative potential in several organs (e.g. liver, intestine and heart) (Plouffe, Hong et al. 2015). YAP induces regeneration of embryonic cardiomyocytes (Xin, Kim et al. 2013) and through binding to DNA in cooperation with TEAD it regulates different genes which foster the cell to in enter the cell cycle (Kanai, Marignani et al. 2000). Camargo *et al.* had indicated that YAP overexpression increases liver size more than four times (Camargo, Gokhale et al. 2007). In a study led by Riley et al., it was revealed that overexpression of CTGF, a vital regulator of embryonic β-cell proliferation and a downstream target of YAP, elicits higher levels of proliferation and mass expansion of immature β-cells (Riley, Pasek et al. 2015). My results suggest that YAP re-expression induces proliferation and mass expansion of β-cells that normally do not undergo proliferation. We showed that β-cell-specific-YAP re-expression induces anti-apoptotic and pro-proliferative responses in β-cells and primary human islets.

YAP expression is limited to the exocrine part during pancreatic development (George, Day et al. 2012) and silenced at the mRNA level in the neurogenin-3-dependent specification of the pancreas endocrine lineage but not in exocrine and duct cells (George, Boerner et al. 2015). I have also shown that YAP is not expressed in the β-cell line INS1-E as well as in human islets. Therefore, re-expression of YAP may elevate the entry of non-dividing or hardly dividing cells such as β-cells into the cell cycle. Aligned with this idea, one of the best examples of Hippo cell cycle involvement occurs during liver development. In hepatocytes, specific loss of MST1/2 results in YAP activity, which finally leads to upregulation of TEAD downstream target genes and entrance into the cell cycle and consequent liver enlargement (Lu, Li et al. 2010). Likewise, George *et al.*, also had shown that overexpression of the active form of YAP in human cadaveric islets promotes β-cell proliferation and did not affect β-cell function and insulin secretion (George, Boerner et al. 2015). My data revealed that β-cell specific re-expression of the active form of YAP induces proliferation and rescues the β-cells from apoptosis without compromising their function *in vitro*.

Furthermore, our results indicated that β-cell specific overexpression of the active form of YAP upregulates FOXM1 and also increases the number of Ki67/Brdu positive β-cells, which confirms the pro-proliferative role of YAP in primary human islets. Therefore, YAP may be a good target to enhance β-cell mass. In line with our results, different studies had shown that YAP increases the activity of TEAD transcription factor, which promotes the expression of different downstream target genes related to cell proliferation or migration such as CTGF, CYR61 (Plouffe, Lin et al.

2018), and FOXM1 (Fan, Cai et al. 2015) and represses some other genes such as LGR5 (Plouffe, Lin et al. 2018). FOXM1 is a proliferation-specific member of the Fox family of transcription factors expressed in all proliferating tissues in the mouse embryo and downregulated after differentiation (Westendorf, Rao et al. 1994, Korver, Roose et al. 1997, Yao, Sha et al. 1997, Ye, Kelly et al. 1997). FOXM1 has been established as a key cell cycle regulator of the G1/S progression and G2/M transition. It regulates cell proliferation through affecting expression of some genes including cyclin B1 and Cdc25B phosphatase (Wang, Kiyokawa et al. 2002, Krupczak-Hollis, Wang et al. 2004, Costa, Kalinichenko et al. 2005). Several studies in cardiomyocytes, embryo and hepatocytes had shown that FOXM1 ablation interferes with proliferation resulting in lethality or impressive reduction of tissue specific cell number mediated by inhibition of mitosis (Korver, Schilham et al. 1998, Wang, Kiyokawa et al. 2002, Krupczak-Hollis, Wang et al. 2004). The aforementioned findings and the fact that the liver and pancreas come from a common embryological origin, we hypothesized that FOXM1 would be a YAP-dependent molecular signal involved in pancreatic β-cell proliferation. Likewise, previous studies including FOXM1 deficiency in the entire pancreas revealed that FOXM1, which is highly expressed in embryonic and neonatal endocrine cells, plays a crucial role in β-cell proliferation and mass expansion with age (Zhang, Ackermann et al. 2006), after partial pancreatectomy (Misfeldt, Costa et al. 2008), and during pregnancy (Zhang, Zhang et al. 2010). FOXM1 is upregulated in response to obesity in nondiabetic C57BL/6 (B6) *Leptin ob/ob* mice (Davis, Lavine et al. 2010). During obesity development, different neurotransmitters released from vagal nerves are involved in compensatory β-cell proliferation in various animal models and may promote β-cells proliferation and maintain glucose homeostasis through a FOXM1-dependent mechanism (KANETO, KOSAKA et al. 1967, Niijima 1989, Kiba, Tanaka et al. 1996, Edvell and Lindström 1998, Lausier, Diaz et al. 2010, Yamamoto, Imai et al. 2017). Based on all previous studies together with my results, we described FOXM1 as a YAP-dependent molecular signal to switch on β-cell proliferation signaling networks and cell growth.

My results indicate that induction of apoptosis as represented by elevated level of cleaved-caspase 3 and cleaved-PARP triggered by a complex diabetogenic milieu including gluco- and lipo-toxicity, pro-inflammatory cytokines, and oxidative stress are antagonized by overexpression of YAP. Therefore, I suggest that transiently upregulated YAP is a pro-survival signal that efficiently blocks apoptosis in stressed β-cells and human islets. Activated caspase 8 mediated by FAS or oxidative stress cleaves caspases 1, 4, and 5 leading to activation of caspases 3, 6, and 7 (Graves, Gotoh et al. 1998). Since my results revealed that YAP downregulates cleaved-caspase 3 and cleaved-PARP, we can mention that YAP ameliorates the pro-apoptotic effect of various diabetic conditions (glucotoxicity, lipotoxicity, etc) in the β-cells.

Conversion of harmful ROS including hydrogen peroxide (H_2O_2) and superoxide to H_2O is regulated through thioredoxins (TRXs), a redox-sensitive signaling complex which consists of TRXs, thioredoxin reductase (TRXR), and NADPH. Several studies have shown that dysregulated reduction-oxidation (redox) responses initiate and lead to the progression of multiple diseases such as diabetes and cancer (Watanabe, Nakamura et al. 2010, Yoshihara, Chen et al. 2010, Masutani, Yoshihara et al. 2011). In both types of diabetes, oxidative stress is a common mechanism triggering β-cell exhaustion, impaired insulin secretion, and β-cell apoptosis (Yoshihara, Masaki et al. 2014). In this line, previous studies have shown that overexpression of TRX1 protects β-cells from destruction and ameliorates the development of diabetes in mouse

models of T1D (NOD) and T2D (db/db) (Hotta, Tashiro et al. 1998, Yamamoto, Yamato et al. 2008). In my study, I identified a signaling link between YAP and TRX system components that mediated responses to oxidative stress in stressed β-cells induced by different diabetic stimuli. My results did not show any influence on β-cell function after exogenous re-expression of YAP in human islets under normal conditions, indicating that YAP may only act during metabolic stress and that YAP may not have a risk of developing hypoglycemia under basal conditions.

Oxidative stress induced by chronically elevated glucose levels or an excessive amount of free fatty acid trigger β-cell failure due to high endogenous production of ROS and low expression of anti-oxidative enzymes such as *Superoxide dismutase* (SOD), catalase, and glutathione peroxidase (GPx) (Wang and Wang 2017). By gene expression analysis of an array of 25 ROS-related genes, I identified TRX1 (encoded by *TXN1*) and TRX2 (encoded by *TXN2*) as YAP-induced antioxidant proteins whose upregulation is indispensable for YAP-mediated β-cell protection under diabetic conditions. Aligned with my data, Wu et al., had shown that inhibition of Ets family transcription factor called GA-binding protein (GABP) leads to YAP depletion resulting in downregulation of mitochondrial and antioxidant genes. Restoration of GABP by MST1/2 deletion induces YAP mRNA transcription and upregulation of antioxidant genes including TXN1 and TXN2 in human HCCs (Wu, Xiao et al. 2013). Yki (homolog of YAP in Drosophila) activity upregulates antioxidant enzymes such as TRXR-1, subunits of the electron transport chain complex I, and a dramatic reduction in intracellular ROS (Nagaraj, Gururaja-Rao et al. 2012). Therefore, I suggest that YAP re-expression may trigger survival through upregulation of TRX, which is a key molecular signal in the oxidative response pathway. Shao et al, had shown that activated MST1/2 mediated by hydrogen peroxide leads to YAP downregulation resulting in FOXO1 inhibition in the mouse liver. The FOXO1 transcription factor interacts with YAP and reduces cellular oxidative stress through expression of MnSOD and catalase genes (Shao, Zhai et al. 2014). However, my data did not reveal that YAP has any influence on SOD expression in INS1-E β-cells.

TRX1 is a negative regulator of MST1. It inhibits MST1 through interacting with the MST1' regulatory domain called SARAH, which subsequently suppresses MST1 homodimerization, and autophosphorylation (Chae, Hwang et al. 2012). Previously, our lab identified MST1 as pro-apoptotic kinase in the β-cells whose aberrant upregulation induces β-cell death as well as impaired insulin secretion (Ardestani, Paroni et al. 2014). It has been shown in other cell types that oxidative stress disrupts the TRX-MST1 association which leads to MST1-driven apoptosis under oxidative environment (Radu and Chernoff 2009, Chae, Hwang et al. 2012). All together YAP-induced TRX upregulation may function as negative feedback loop where TRX directly interacts and inhibits MST1 thereby restoring β-cell viability (Ardestani, Paroni et al. 2014, Rojas, Bermudez et al. 2018). The aforementioned findings and my data revealed a novel signaling axis (YAP-TRX) whose functional operation improves β-cell viability in response to diabetogenic conditions.

Also, my results indicate that genetic or chemical inhibition of the stress-response TRX–system results in the downregulation of exogenously expressed YAP protein level suggesting an existence of bidirectional regulation between TRXs and YAP in which TRXs target YAP protein stability in addition to their own transcriptional regulation directed by YAP.

3-2- mTORC1 is hyper-activitated in the β-cell

In T2D, nutrient overload including chronically elevated glucose, free fatty acids and amino acids trigger compensatory mechanisms resulting in an increased β-cells mass and function in order to cope with systemic insulin resistance. However, with progression of disease, such compensatory responses fails in genetically-susceptible individuals due to β-cell exhaustion, decompensation and ultimate death (Um, Frigerio et al. 2004, Tremblay, Brûlé et al. 2007, Shigeyama, Kobayashi et al. 2008). In β-cells, mTORC1 plays an important role in the adaptive responses through increasing β-cell mass and compensating for chronically elevated glucose by further insulin secretion (Bartolomé, Guillén et al. 2010). Likewise, chronic mTORC1 hyperactivity mediated by β-cell specific ablation of TSC2 triggers an early increased β-cell mass improving glucose homeostasis in young mice (Shigeyama, Kobayashi et al. 2008). However, constitutive mTORC1 activity diminishes β-cell mass, impairs autophagy response resulting in β-cell failure and hyperglycemia, in older TSC2 mice with specific β-cell deletion (Bartolomé, Kimura-Koyanagi et al. 2014). Previously and in the same line, Shigeyama *et al.*, had shown that the constitutive mTORC1 activity correlates with the loss of β-cell number, β-cell death recapitulating and β-cell phenotypical abnormalities observed in rodent and human T2D islets (Shigeyama, Kobayashi et al. 2008). Therefore, we aimed to study whether mTORC1 is hyper activated in metabolically stressed human T2D islets. And we further asked ourselves what are the consequences of mTORC1 signaling inhibition on β-cell function.

We detected mTORC1 hyperactivity in human islets of individuals with T2D, which is in same line with previous observations in animal models of T2D. The *multicellular* islet of Langerhans comprises multiple cell types including major fractions of insulin-producing β-cells as well as glucagon-producing α-cells. In order to locate a potential source of activated mTORC1 in human islets, I performed an in situ immunofluorescence analysis of pS6 (activated mTORC1 marker) together with insulin or glucagon. My data indicated that (1) major pS6-expressing cells are β-cells and (2) such cells are highly elevated in islets isolated from individuals with T2D suggesting β-cells as principal sources of activated mTORC1 in human T2D islets.

Our further investigation found an opposite regulation of the two distinct mTOR complexes in human T2D islets with contaminant upregulation of mTORC1 and downregulation of mTORC2. Moreover, our results revealed that pharmacological inhibition of mTORC1 signaling (by selective inhibitor of mTORC1 downstream kinase S6K1) as well as its genetic inhibition (by silencing of mTORC1 integral component Raptor) improves GSIS in human T2D islets as well as in mouse diabetic islets. Putting altogether, mTORC1 seems to be required for β-cell adaptation and compensation to insulin resistance by promoting β-cell proliferation and hypertrophy (Blandino-Rosano, Chen et al. 2012). However, later on, prolonged nutrient excess induces sustained hyperactivity of mTORC1 resulting in β-cell dysfunction and apoptosis (Shigeyama, Kobayashi et al. 2008, Bartolomé, Kimura-Koyanagi et al. 2014).

3-3- mTOR and Hippo crosstalk

Considering the importance of organ development, there are several cell signaling pathways involved in regulation of proliferation and organ size. During the last decade, the Hippo pathway has been established as key regulator of cell growth and organ size. Hippo disturbance influences tumorigenesis through hyperactivity of YAP (Dong, Feldmann et al. 2007, Zhao, Wei et al. 2007, Lee, Lee et al. 2010, Lu, Li et al. 2010). Tumaneng *et al.*, have shown that there is a cross talk between YAP/Hippo and mTOR regulating cell size, tissue growth, and hyperplasia. YAP enhances mTOR activation via miRNA-mediated inhibition of PTEN, a negative upstream regulator of mTOR (Tumaneng, Schlegelmilch et al. 2012). Another example in glioma cell, hippo kinase MST1 regulates cell cycle and proliferation by inhibition of AKT/mTOR pathway (Chao, Wang et al. 2015). Oppositely, AKT improves cell survival through direct phosphorylation of MST1 at Thr120 and Thr387 residues and its subsequent inhibition (Cinar, Fang et al. 2007, Jang, Yang et al. 2007, Yuan, Kim et al. 2010). In the same line, the other mTOR complex, mTORC2 has a negative impact on Hippo pathway in cardiomyocytes by phosphorylating MST1 at Ser438 residue in the SARAH domain, thereby restraining homodimerization and activity of MST1 which leads to restoration of cardiac function. Cardiomyocyte-specific ablation of the Rictor, a principal component of mTORC2, induces cardiac dysfunction and dilation, damaging cardiac growth and adaptation in the presence of pressure overload mediated by MST1 hyperactivity (Sciarretta, Zhai et al. 2015). Aligned with the aforementioned studies and my data from both papers, I propose that there is a connection between YAP and mTORC1. I speculate that YAP over-expression may trigger mTORC1 upregulation by 2 mechanisms. One mechanism is that YAP promotes TRX1/2 upregulation that restrains MST1/2 activity, thus results in higher AKT and subsequent mTORC1 activation. Second one is that overexpression of YAP inhibits PTEN via its miRNA inhibitor miR-29, which subsequently results in AKT and mTORC1 hyper-activation. Further mechanistic studies are required to carefully test such hypotheses in the β-cells.

3-3- Conclusion

The role of YAP as well as mTOR in pancreatic β-cells in human and rodent islets under physiological and diabetes-relevant conditions was systematically addressed in this study. Our results indicat that:

1) YAP plays a crucial role in induction of proliferation and survival through upregulating transcription factor FOXM1 as well as stress-response proteins TRX1/2. The identification of YAP as a pro-proliferative and pro-survival signal provides a novel area for potential therapeutic strategies aiming at enhancing β-cell mass in diabetes. The role of YAP to elicit β-cell proliferation and survival was reported for the first time in our study. We have also distinguished the potential proteins in which YAP elicits β-cell proliferation and survival with attributing FOXM1 and TRX1/2 as molecules mediating YAP responses to promote proliferation and viability respectively.
2) We reported mTORC1 hyperactivity as well as mTORC2 deficiency in human islets from patients with T2D as well as under hyperglycermic/diabertogenic conditions. We detected β-cells as potential source of mTORC1 hyperactivity in T2D islets. Targeting mTORC1 signaling improved insulin secretion in human and mouse diabetic islets.

3-4- Outlook

Despite important roles of Hippo/YAP and mTOR signals in β-cell biology described in my thesis, some critical questions still exist, which would be of great interest for future studies:

a) We identified the pro-proliferative and anti-apoptotic effect of re-expressed YAP in β-cells in *in vitro* conditions. What would be the impact of YAP overexpression in pancreatic β-cell in terms of proliferation, regeneration, function and survival *in vivo*? I have been part of the initiative to generate β-cell-specific YAP overexpressing mice in our lab. In depth characterization of these mice will provide further insights into the cell-intrinsic as well as paracrine roles of YAP expression in β-cells *in vivo*.
   This includes but is not limited to:
   1) Whether YAP re-expression facilitates β-cell regeneration and repair in STZ- and HFD-treated diabetic mice?
   2) What would be the impact of YAP re-expression in T2D human and mouse islets?
b) Would YAP overexpression have any influence on mTORC1 and/or mTORC2 activities?
c) As mTORC2 declined in diabetic islets, what would be the impact of increased mTORC2 activity on β-cell survival and function under diabetic conditions?

Reference

Ardestani, A. and K. Maedler (2017). "The hippo signaling pathway in pancreatic β-cells: functions and regulations." Endocrine reviews **39**(1): 21-35.

Ardestani, A., et al. (2014). "MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes." Nature medicine **20**(4): 385.

Bartolomé, A., et al. (2010). "Role of the TSC1-TSC2 complex in the integration of insulin and glucose signaling involved in pancreatic β-cell proliferation." Endocrinology **151**(7): 3084-3094.

Bartolomé, A., et al. (2014). "Pancreatic β-cell failure mediated by mTORC1 hyperactivity and autophagic impairment." Diabetes **63**(9): 2996-3008.

Blandino-Rosano, M., et al. (2012). "mTORC1 signaling and regulation of pancreatic β-cell mass." Cell Cycle **11**(10): 1892-1902.

Camargo, F. D., et al. (2007). "YAP1 increases organ size and expands undifferentiated progenitor cells." Current Biology **17**(23): 2054-2060.

Chae, J. S., et al. (2012). "Thioredoxin-1 functions as a molecular switch regulating the oxidative stress-induced activation of MST1." Free Radical Biology and Medicine **53**(12): 2335-2343.

Chao, Y., et al. (2015). "Mst1 regulates glioma cell proliferation via the AKT/mTOR signaling pathway." Journal of neuro-oncology **121**(2): 279-288.

Cinar, B., et al. (2007). "The pro-apoptotic kinase Mst1 and its caspase cleavage products are direct inhibitors of Akt1." The EMBO journal **26**(21): 4523-4534.

Costa, R. H., et al. (2005). "New and unexpected: forkhead meets ARF." Current opinion in genetics & development **15**(1): 42-48.

Davis, D. B., et al. (2010). "FoxM1 is up-regulated by obesity and stimulates β-cell proliferation." Molecular Endocrinology **24**(9): 1822-1834.

Dong, J., et al. (2007). "Elucidation of a universal size-control mechanism in Drosophila and mammals." Cell **130**(6): 1120-1133.

Edvell, A. and P. Lindström (1998). "Vagotomy in young obese hyperglycemic mice: effects on syndrome development and islet proliferation." American Journal of Physiology-Endocrinology and Metabolism **274**(6): E1034-E1039.

Fan, Q., et al. (2015). "FOXM1 is a downstream target of LPA and YAP oncogenic signaling pathways in high grade serous ovarian cancer." Oncotarget **6**(29): 27688.

George, N. M., et al. (2015). "Exploiting expression of Hippo effector, Yap, for expansion of functional islet mass." Molecular Endocrinology **29**(11): 1594-1607.

George, N. M., et al. (2012). "Hippo signaling regulates pancreas development through inactivation of Yap." Molecular and cellular biology **32**(24): 5116-5128.

Graves, J. D., et al. (1998). "Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1." The EMBO journal **17**(8): 2224-2234.

Hotta, M., et al. (1998). "Pancreatic β cell–specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes." Journal of Experimental Medicine **188**(8): 1445-1451.

Jang, S.-W., et al. (2007). "Akt phosphorylates MstI and prevents its proteolytic activation, blocking FOXO3 phosphorylation and nuclear translocation." Journal of Biological Chemistry **282**(42): 30836-30844.

Kanai, F., et al. (2000). "TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins." The EMBO journal **19**(24): 6778-6791.

KANETO, A., et al. (1967). "Effects of stimulation of the vagus nerve on insulin secretion." Endocrinology **80**(3): 530-536.

Kiba, T., et al. (1996). "Ventromedial hypothalamic lesion-induced vagal hyperactivity stimulates rat pancreatic cell proliferation." Gastroenterology **110**(3): 885-893.

Korver, W., et al. (1997). "The winged-helix transcription factor Trident is expressed in actively dividing lymphocytes." Immunobiology **198**(1-3): 157-161.

Korver, W., et al. (1998). "Uncoupling of S phase and mitosis in cardiomyocytes and hepatocytes lacking the winged-helix transcription factor Trident." Current Biology **8**(24): 1327-S1321.

Krupczak-Hollis, K., et al. (2004). "The mouse Forkhead Box m1 transcription factor is essential for hepatoblast mitosis and development of intrahepatic bile ducts and vessels during liver morphogenesis." Developmental biology **276**(1): 74-88.

Lausier, J., et al. (2010). "Vagal control of pancreatic β-cell proliferation." American Journal of Physiology-Endocrinology and Metabolism **299**(5): E786-E793.

Lee, K.-P., et al. (2010). "The Hippo–Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis." Proceedings of the National Academy of Sciences **107**(18): 8248-8253.

Lu, L., et al. (2010). "Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver." Proceedings of the National Academy of Sciences **107**(4): 1437-1442.

Masutani, H., et al. (2011). "Thioredoxin binding protein (TBP)-2/Txnip and α-arrestin proteins in cancer and diabetes mellitus." Journal of clinical biochemistry and nutrition: 1110240115-1110240115.

Misfeldt, A. A., et al. (2008). "β-Cell proliferation, but not neogenesis, following 60% partial pancreatectomy is impaired in the absence of FoxM1." Diabetes **57**(11): 3069-3077.

Nagaraj, R., et al. (2012). "Control of mitochondrial structure and function by the Yorkie/YAP oncogenic pathway." Genes & development **26**(18): 2027-2037.

Niijima, A. (1989). "Nervous regulation of metabolism." Progress in neurobiology **33**(2): 135-147.

Plouffe, S. W., et al. (2015). "Disease implications of the Hippo/YAP pathway." Trends in molecular medicine **21**(4): 212-222.

Plouffe, S. W., et al. (2018). "The Hippo pathway effector proteins YAP and TAZ have both distinct and overlapping functions in the cell." Journal of Biological Chemistry **293**(28): 11230-11240.

Radu, M. and J. Chernoff (2009). "The DeMSTification of mammalian Ste20 kinases." Current Biology **19**(10): R421-R425.

Riley, K. G., et al. (2015). "Connective tissue growth factor modulates adult β-cell maturity and proliferation to promote β-cell regeneration in mice." Diabetes **64**(4): 1284-1298.

Rojas, J., et al. (2018). "Pancreatic beta cell death: novel potential mechanisms in diabetes therapy." Journal of diabetes research **2018**.

Sciarretta, S., et al. (2015). "mTORC2 regulates cardiac response to stress by inhibiting MST1." Cell reports **11**(1): 125-136.

Shao, D., et al. (2014). "A functional interaction between Hippo-YAP signalling and FoxO1 mediates the oxidative stress response." Nature communications **5**: 3315.

Shigeyama, Y., et al. (2008). "Biphasic response of pancreatic β-cell mass to ablation of tuberous sclerosis complex 2 in mice." Molecular and cellular biology **28**(9): 2971-2979.

Tremblay, F., et al. (2007). "Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient-and obesity-induced insulin resistance." Proceedings of the National Academy of Sciences **104**(35): 14056-14061.

Tumaneng, K., et al. (2012). "YAP mediates crosstalk between the Hippo and PI (3) K–TOR pathways by suppressing PTEN via miR-29." Nature cell biology **14**(12): 1322.

Um, S. H., et al. (2004). "Absence of S6K1 protects against age-and diet-induced obesity while enhancing insulin sensitivity." Nature **431**(7005): 200.

Wang, J. and H. Wang (2017). "Oxidative stress in pancreatic beta cell regeneration." Oxidative medicine and cellular longevity **2017**.

Wang, X., et al. (2002). "The Forkhead Box m1b transcription factor is essential for hepatocyte DNA replication and mitosis during mouse liver regeneration." Proceedings of the National Academy of Sciences **99**(26): 16881-16886.

Watanabe, R., et al. (2010). "Anti-oxidative, anti-cancer and anti-inflammatory actions by thioredoxin 1 and thioredoxin-binding protein-2." Pharmacology & therapeutics **127**(3): 261-270.

Westendorf, J. M., et al. (1994). "Cloning of cDNAs for M-phase phosphoproteins recognized by the MPM2 monoclonal antibody and determination of the phosphorylated epitope." Proceedings of the National Academy of Sciences **91**(2): 714-718.

Wu, H., et al. (2013). "The Ets transcription factor GABP is a component of the hippo pathway essential for growth and antioxidant defense." Cell reports **3**(5): 1663-1677.

Xin, M., et al. (2013). "Hippo pathway effector Yap promotes cardiac regeneration." Proceedings of the National Academy of Sciences **110**(34): 13839-13844.

Yamamoto, J., et al. (2017). "Neuronal signals regulate obesity induced β-cell proliferation by FoxM1 dependent mechanism." Nature communications **8**(1): 1930.

Yamamoto, M., et al. (2008). "Transgenic expression of antioxidant protein thioredoxin in pancreatic β cells prevents progression of type 2 diabetes mellitus." Antioxidants & redox signaling **10**(1): 43-50.

Yao, K.-M., et al. (1997). "Molecular Analysis of a Novel Winged Helix Protein, WIN EXPRESSION PATTERN, DNA BINDING PROPERTY, AND ALTERNATIVE SPLICING WITHIN THE DNA BINDING DOMAIN." Journal of Biological Chemistry **272**(32): 19827-19836.

Ye, H., et al. (1997). "Hepatocyte nuclear factor 3/fork head homolog 11 is expressed in proliferating epithelial and mesenchymal cells of embryonic and adult tissues." Molecular and cellular biology **17**(3): 1626-1641.

Yoshihara, E., et al. (2010). Thiol redox transitions by thioredoxin and thioredoxin-binding protein-2 in cell signaling. Methods in enzymology, Elsevier. **474:** 67-82.

Yoshihara, E., et al. (2014). "Thioredoxin/Txnip: redoxisome, as a redox switch for the pathogenesis of diseases." Frontiers in immunology **4**: 514.

Yuan, Z., et al. (2010). "Phosphoinositide 3-kinase/Akt inhibits MST1-mediated pro-apoptotic signaling through phosphorylation of threonine 120." Journal of Biological Chemistry **285**(6): 3815-3824.

Zhang, H., et al. (2006). "The FoxM1 transcription factor is required to maintain pancreatic β-cell mass." Molecular Endocrinology **20**(8): 1853-1866.

Zhang, H., et al. (2010). "Gestational diabetes mellitus resulting from impaired β-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen." Diabetes **59**(1): 143-152.

Zhao, B., et al. (2007). "Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control." Genes & development **21**(21): 2747-2761.

Acknowledgements

I thank God for this great blessing, his faithfulness and good health to finish this project.
I owe a deep sense of acknowledgment to my supervisor Prof. Dr. Kathrin Maedler. You guided my progress with a keen eye for detail and style during my whole Ph.D. project. Thank you for giving me an opportunity to work in your lab and learn from you in scientific lifestyle.
I want to thank my co-supervisor to Dr. Amin Ardestani. Thank you for having set me on this path, and also involving me in different excellent projects and supervising me very wisely for each step.
Here, I would like to show my greatest appreciation to Prof. Dr. Andreas Birkenfeld and Prof. Dr. Janine Kirstein for your precious time to review my thesis and be my kind examiners.
I would also like to thank all the past and new members of islet' lab: Delsi, Hazem, Andrea, Federico, Niels, Wei, Ting, Payal, Anasual, Sushil, Zahra, Blaz, Shirin, Katrischa, Durga, Karthika, Huan, Murali and other nice colleges.
I would like to thank all my nice and supportive friends in Iran and Germany.
Finally, to my family, who have always been there for me: I am grateful in more ways than I can express for your pure love, support and encouragement over the years. To my sisters Maryam and Mina, cousin Mahsa, beloved nephew Arsam, brother and brother in low, thank you for helping me to become the person I am today. And most importantly to my dear parents, without whom none of this would have been possible, I owe you an immeasurable debt of gratitude for the long hours you worked to give me and my sibling the opportunity to follow our dreams. I dedicate this dissertation in loving memory of my cousin Gohar, grandmother, grandfather and all relatives who passed away at Bam earthquake and sadly did not live to see me complete it: I love and miss you.