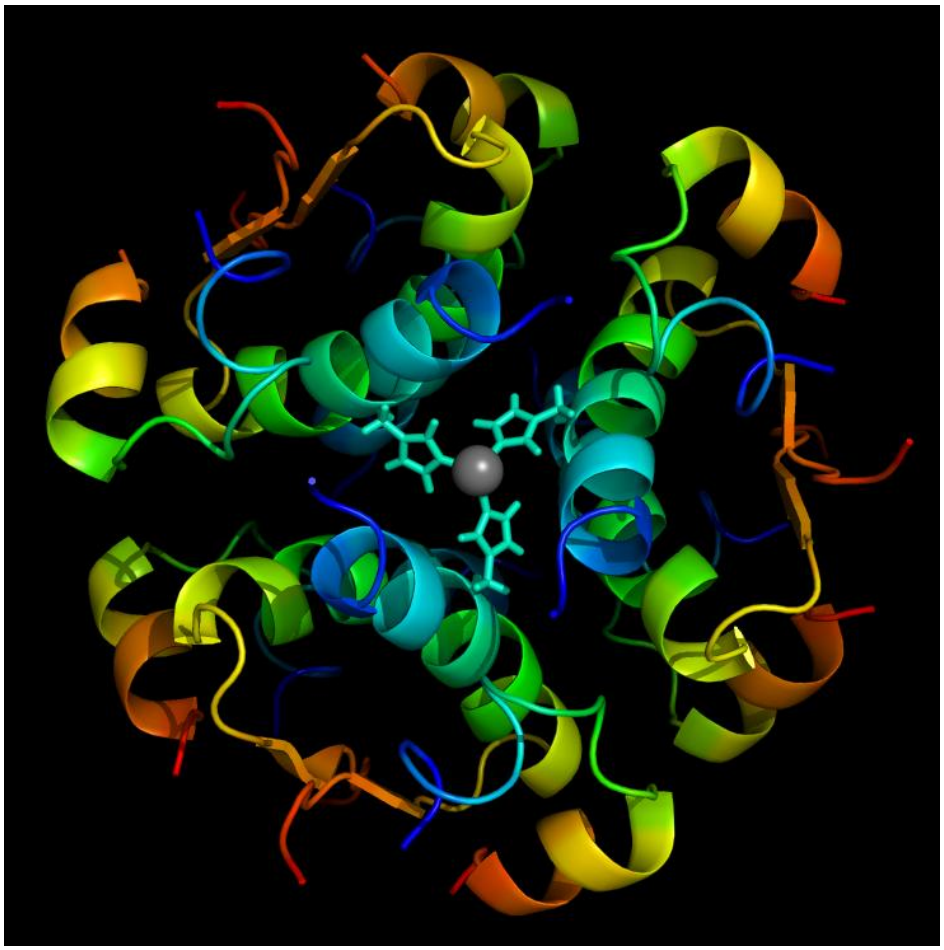
Diabetes, Insulin and Cancer risk

The molecular role of insulin signaling and hyperinsulinemia in cancer development and progression in diabetes patients



Literature review

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Picture on the front page represents the hexamer molecule of human insulin (Chang et al., 1997).
Source: Mike Tyke from beautifulproteins.blogspot.nl

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Introduction

Cancer is one of the most common and most deathly diseases of our time. The disease is very complex and for the past decades much research has been done to better understand its causes and development. In the famous article "The hallmarks of cancer" written by Hanahan and Weinberg in 2000, and its follow-up from 2011, it is described how a normal cell can become a cancer cell (Hanahan and Weinberg, 2000, 2011). They suggest that eight essential alterations are needed; concerning avoidance of apoptosis, self sufficiency in growth factors, insensitivity to anti-growth factors, metastases and the ability to invade tissue, limitless cell reproduction, sustained angiogenesis, deregulation of cellular energetics and avoidance of immune destruction. Genome instability and tumor-promoting inflammation enable the cell to acquire these alterations (Hanahan and Weinberg, 2011); this enhances the chance to obtain mutations in important pathways leading to an upregulation of oncogenes or a downregulation of tumor suppressor genes (Weinberg, 2007). Because some other diseases also cause alterations in gene expression or homeostasis, they can be associated with a higher risk of cancer formation.

One example is the coincidence of cancer and the metabolic disease diabetes mellitus. The name diabetes mellitus describes the sweet urine of the patients, due to failure to store glucose. This failure is caused by a deregulation in insulin signaling, due to either the inability of the pancreas to produce insulin (as found in type 1 diabetes mellitus, T1DM) or by insulin resistance (as found in type 2 diabetes mellitus, T2DM). The disease was already described by the Egyptians 1500 B.C. and it was clinically recognized in 1812. For the past 200 years much research has been done regarding this disorder, which has greatly induced our knowledge of the disease and the prospects and treatment of the patients (Polonsky, 2012).

The relationship between diabetes and the risk for cancer development seems to be more and more confirmed. An important link between these two is the hyperinsulinemia found both in patients with T1DM and patients with T2DM. T1DM patients cannot produce insulin anymore and are therefore dependent on insulin administration via injections. This causes an abnormal, evenly distributed insulin concentration throughout the body; for tissues that normally receive low amounts of insulin, insulin administration can lead to hyperinsulinemia. T2DM patients have a reduced responsiveness to insulin, the β-cells in the pancreas try to compromise for this by producing more, which results in hyperinsulinemia. (Vigneri et al., 2009). Hyperinsulinemia can lead to an increased risk for cancer formation (Belfiore and Malaguarnera, 2011). In this literature study I will review the molecular aspects of the relationship between cancer risk and hyperinsulinemia, to clearly overview to what extent a malfunctioning in the insulin system can lead to cancer formation. In the end I want to answer the question: To what extent does hyperinsulinemia, caused by T2DM or by insulin administration, lead to changes in molecular pathways involved in cancer formation?

To answer this question I will first look more closely at the metabolic disorder diabetes mellitus and its molecular causes and consequences, thereby answering the question: What is diabetes mellitus and how does this disorder change the insulin homeostasis?

Then I will look more closely at the insulin signaling pathway, which has not only metabolic, but also mitogenic effects, to help understand the molecular basis of insulin actions and insulin

resistance. The main questions will be: What are the molecular pathways of insulin signaling leading to both metabolic and mitogenic effects and how can a cell become insulin resistant?

Thereafter I will describe the underlying mechanisms of cancer formation, mainly focusing on the hallmarks of cancer as described by Hanahan and Weinberg and their extension published in 2011 (Hanahan and Weinberg, 2011), to answer the questions: How can a normal cell become a cancer cell and how can the insulin signaling pathway be involved in this process?

And in the end I will look further into the coincidence of diabetes and cancer formation to understand how diabetes patients can have a greater risk to develop cancer. Hereby I will try to answer the questions: What are the consequences for diabetes patients on their risk for cancer formation and how does this work on the molecular level of insulin signaling?

With this review I hope to clarify the relationship between diabetes and cancer risk. I choose this subject because I am very interested in both metabolic diseases and cancer development. When I read about the fact that diabetes patients have a higher risk to develop cancer, I wanted to find out how this works on the molecular level. With the help of Dr.Tobias Dansen I could write this review as my master thesis. I think that this particular combination of diseases is very interesting in the world we live in today, since both diabetes and cancer are diseases with a high prevalence that affect many people worldwide.

Chapter 1: Diabetes mellitus

Metabolism

From our diet we can gain nutrients which contribute to the pool of free fatty acids, sugars or amino acids. These nutrients we can use as building blocks or for energy production. In the nutrient pools they are ready to use for metabolism. Instead of entering the nutrient pools they can also undergo some reactions to be stored in the body for later use. Under normal circumstances the brain can only use glucose as an energy source; to make sure the brain is never out of energy the metabolism of glucose is strictly regulated. Once taken up from the digestive system, glucose will be phosphorylated by hexokinase to produce glucose-6-phosphate, now it can either enter the glycolysis to produce energy or it can be stored as glycogen for later use (Silverthorn et al., 2009).

Insulin and glucagon are the main regulators of metabolism

The hormone insulin, secreted by the β-cells of the islets of Langerhans in the pancreas, promotes restoration of the blood glucose levels after food intake. It induces glucose uptake in the liver, muscles and adipose tissue by stimulating the translocation of *glucose transporter* 4 (GLUT4)-containing vesicles to the plasma membrane. Other important members of the GLUT family are GLUT1, which is expressed in the brain; and GLUT2 which is among others expressed in the pancreatic β-cells and in the liver and which has a low affinity for glucose and thus only responds when blood glucose levels are high. In contrast with GLUT4, both GLUT1 and GLUT2 are localized at the plasma membrane and therefore do not need to be transported and are not insulin dependent (Watson and Pessin, 2001).Once inside the cell glucose can be used for oxidation, glycogen storage, fat synthesis and protein synthesis; this will either result in energy production or energy storage (Silverthorn et al., 2009).

The release of insulin by the pancreatic β-cells is dependent on glucose; after food intake, when there is a sufficiently high blood glucose level (higher than 100 mg/dL), glucose will enter the β-cells via GLUT2 (Berg et al., 2012). This process is called the stimulus-secretion-coupling (figure 1) (Leibiger et al., 2008). Inside the cell the glucose will be used for cellular respiration to produce ATP (and the byproducts CO_2 en H_2O). The cellular ATP/ADP levels will now become higher, as a result the ATP-dependent K^+-channels will close. The consequent depolarization of the cell causes the Ca^{2+}-channels to open; Ca^{2+} can now enter the cell. Ca^{2+} is the trigger for intracellular vesicles containing insulin to fuse with the membrane so that insulin is released (Berg et al., 2012).

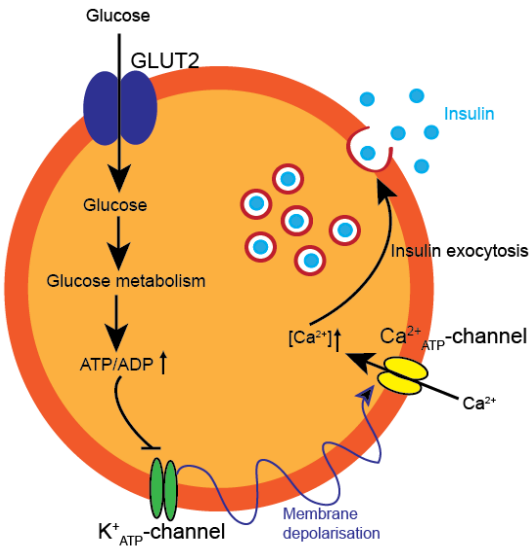

Figure 1: Insulin secretion is induced by glucose. When glucose levels are above 100mg/dL, GLUT2 will transport glucose into the pancreatic β-cell. After glucose metabolism, the ATP/ADP levels will go up, which closes the K^+_{ATP}-channels. Membrane depolarization will follow, which opens the Ca^{2+}-channels. Ca^{2+} can now enter the cell, were it will initiate insulin exocytosis.

Insulin release can also be stimulated by increased plasma amino acid levels, by *gastrointestinal* (GI) hormones like *glucagon-like protein 1* (GLP-1) (Gromada et al., 2004) and by the parasympathetic neurons. The autonomic nervous system contains glucose-excited neurons (part of the parasympathetic system) and glucose-inhibited neurons (part of the sympathetic system), which are located mainly in the brainstem and the hypothalamus. They are activated by hyper- or hypoglycemia respectively and can thereafter stimulate or inhibit insulin secretion by the pancreatic β-cells (Thorens, 2011).

After a period of fasting, the α-cells of the islets of Langerhans will secrete glucagon, which has an opposite function to insulin. Glucagon will promote energy release by stimulating glycogenolysis (the breakdown of glycogen), gluconeogenesis (the generation of glucose from noncarbohydrate precursors) and ketogenesis (the breakdown of fatty acid) (Silverthorn et al., 2009).

The balance between insulin and glucagon release determines which metabolic pathways are stimulated and which are attenuated.

Insulin signaling in glucose metabolism

Insulin signaling works via two pathways: the *phosphatidylinositol 3-kinase* (PI3K)-pathway, which triggers both metabolic and mitogenic downstream effectors; and the *mitogen-activated protein kinase* (MAPK)-pathway, which has mainly mitogenic effects triggering cell proliferation, but also plays a role in cell survival (Leibiger et al., 2008). Both the PI3K- and the MAPK-pathway may play a role in cancer formation; this will be discussed the following chapters. In this paragraph the metabolic effects of insulin signaling will be described; which is important for glucose metabolism.

The metabolic signaling pathway of insulin is found mainly in the liver, adipose tissue and muscles. Since the brain expresses GLUT1, which does not need to be translocated to the plasma membrane, the brain is not dependent on insulin for glucose uptake (Watson and Pessin, 2001). Interestingly, muscles only depend on insulin for their glucose uptake when they are in rest; during exercise insulin is not required for the muscles (Silverthorn et al., 2009). In the liver insulin reduces gluconeogenesis and glycogenolysis and it induces glycogenesis (the production of glycogen). In the adipose tissue insulin induces glucose uptake and reduces lipolysis (the breakdown of lipids). In the muscles insulin induces glucose uptake and glycogenesis (Belfiore and Malaguarnera, 2011).

The signaling in the different tissues proceeds similarly, though with small differences in the factors involved, like expression of different isoforms or family members, which can result in different outcomes. In all cells signaling starts with the binding of insulin to its receptor (IR). The receptor can now homodimerize and cross phosphorylate, which makes the binding of *insulin-receptor substrates* (IRSs) possible. In the muscles and adipose tissue IRS1 is active, in the liver IRS2. IRS can now be phosphorylated, so it can bind to and activate PI3K. Active PI3K can convert *phosphoinositides-4,5-biphosphate* (PIP_2) into *phosphoinositides-3,4,5-triphosphate* (PIP_3). PIP_3 activates *PIP_3-dependent protein kinase* (PDK), which in turn activates multiple other kinases, but most importantly AKT (also known as *protein kinase B*, PKB; figure 2) (Berg et al., 2012).

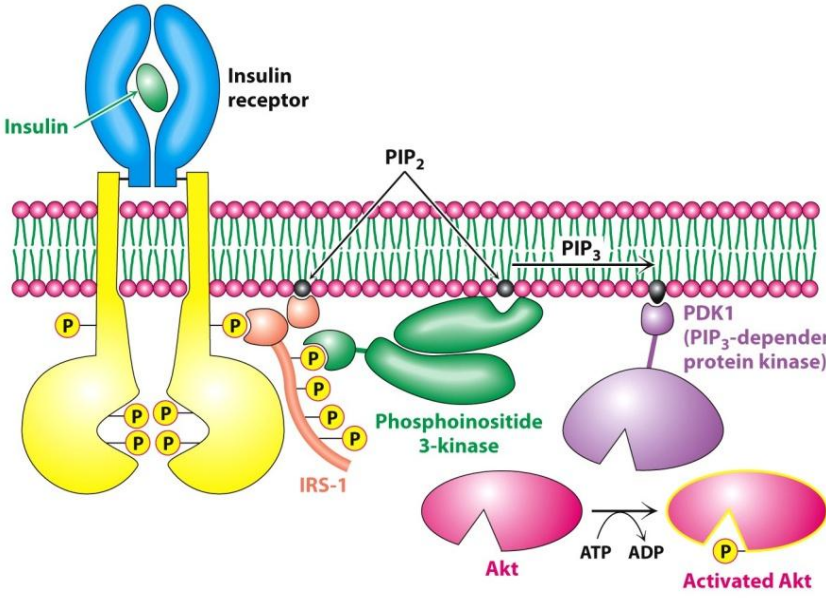

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Figure 2: Activation of AKT by the insulin signaling pathway. When insulin binds to its receptor, homodimerization and cross phosphorylation of the intra cellular domains can take place. Now IRS1 will be phosphorylated so it can bind and activate PI3K. PI3K will now convert PIP_2 into PIP_3. PIP_3 can activate PDK1, so that PDK1 in turn can activate AKT by phosphorylation. (Berg et al., 2012)

AKT acts on various factors; in the muscles and adipose tissue it facilitates the translocation of vesicles containing GLUT4 to the membrane, to enhance glucose uptake (Silverthorn et al., 2009). In the liver and the muscles AKT inhibits *glycogen synthase kinase 3* (GSK3), to reduce its inhibition on glycogen production (Belfiore and Malaguarnera, 2011). Furthermore AKT can inhibit *forkhead box O 1* (Foxo1), to reduce gluconeogenesis (Taniguchi et al., 2006), and stimulate *phosphodiesterase-3B* (PDE3B) to inhibit lipolysis (Belfiore and Malaguarnera, 2011). Altogether, activation of AKT will stimulate glucose metabolism and storage and it will inhibit glucose generation.

To make sure insulin is kept in balance its actions can be attenuated in three ways. First, the *insulin receptor* (IR) and PIP_3 can be inhibited when their phosphoryl groups are removed by *protein tyrosine phosphatase-1B* (PTP-1B) and *phosphatase and tensin homolog* (PTEN) respectively. Second, IRS can be phosphorylated on a serine residue to inactivate it. It is suggested that this inhibition is activated by overnutrition and that it could be important in the development of insulin resistance. And third, the proteolytic degradation of both IR and IRS1 can be induced by *suppressor of cytokine signaling* (SOCS) proteins (Berg et al., 2012). The attenuation of insulin signaling will be discussed in more detail in chapter 2.

Diabetes mellitus

According to the International Diabetes Federation (IDF) there were over 371 million people worldwide diagnosed with diabetes in 2012, that is 5,2% of the human population. The real number is even higher, since another estimate percentage of 2,7% of all people have diabetes without being diagnosed with the disease yet (Harris et al., 1998). This makes diabetes the most common metabolic disease of the world (Leibiger *et al.*, 2008). Of all patients with diabetes, about 10% has type 1 diabetes mellitus (T1DM) and 90% has type 2 diabetes mellitus (T2DM).

T1DM is an autoimmune disease, which results in the destruction of the β-cell of the pancreas. People with T1DM often develop the disease before the age of 20. Because they can no longer produce insulin themselves they are dependent on insulin administration.

T2DM is caused by a resistance to insulin, due to which the body no longer responds to this hormone. However, the hormone levels are mostly normal or elevated. The disease is commonly considered to be a result of an unhealthy lifestyle and obesity; therefore it often develops later in life (Berg et al., 2012). However, it can also be a result of an inherited genetic mutation; in this case the disease can develop at younger age (Taylor, 1999). Many different gene mutations have been found, none of them seems to be present in a majority of patients. For example, development of diabetes in infants can be the result of a mutation in the *insulin* gene; insulin resistance can be caused by mutations in the *insulin receptor* gene or in the *AKT2* gene; and furthermore, T2DM can be the result of a mutation in *IRS1*, *IRS2* or *PI3K*, among others (Doria et al., 2008).

Type 1 diabetes mellitus

T1DM is an autoimmune disease in which the pancreatic β-cells of the islets of Langerhans are destroyed, resulting in little to no insulin production (Mathis et al., 2001). The low levels of insulin cause the body to be in a constant fasting mode even though there are high glucose levels. As a result of the fasting mode, the liver starts to produce glucose by gluconeogenesis and glycogen breakdown (Berg et al., 2012). This will lead to a state of hyperglycemia (Silverthorn et al., 2009).

Excess glucose will be excreted by the kidneys together with more water than usual. This deregulated metabolism results in the patient being thirsty (Berg et al., 2012) and can result in a decreased blood pressure (Silverthorn et al., 2009).

In the brain, most neurons are insulin independent. However, the neurons of the satiety center are dependent on insulin to measure the blood glucose levels. As a result, they signal to increase the food intake; therefore the T1DM patients are often hungry and eat excessively (Silverthorn et al., 2009).

Because the body cannot produce enough energy from carbohydrates anymore, the adipose tissue it will shift to burn fat and the muscle will start to breakdown proteins (Silverthorn et al., 2009). When fat is burned free fatty acids will enter the blood circulation and will be taken up by the liver. The liver can break down the free fatty acids via oxidation, to produce ketone bodies, which can be used as an energy source. Under normal circumstances this production is stimulated by glucagon during a state of fasting, in which especially the brain is dependent on ketone bodies (Foster and McGarry, 1982). High levels of ketone bodies lead to a toxic state of ketosis, this will lower the pH-level of the blood and can ultimate result in a coma or even death (Berg et al., 2012).

Treatment of T1DM mainly consists of insulin administration and the monitoring of blood glucose levels. Furthermore, patients with T1DM are recommended to live healthy and exercise regularly (Berg et al., 2012). Research is being done to produce a therapy in which β-cells are implanted, so that the patient can live without insulin injections (Smink et al., 2013).

Since only 5-10% of the diabetes patients suffers of T1DM, not much research has been done on T1DM and cancer risk. Some articles describe an induced risk of cancer in the stomach, cervix, endometrium (Zendehdel et al., 2003) and pancreas (Stevens et al., 2007). However, these limited studies are not enough to confirm any relationships (Shikata et al., 2013).

Type 2 diabetes mellitus (T2DM)

T2DM is considered to be a result of both insulin resistance, and later of pancreatic β-cell failure (Prentki and Nolan, 2006). The symptoms in T2DM patients can vary from hyperinsulinemia, as a result of insulin resistance, to decreased insulin secretion, due to pancreatic β-cell failure (Silverthorn et al., 2009). Most of the time, the disease begins with insulin resistance due to unhealthy eating habits (overnutrition) and obesity due to too little exercise (Prentki and Nolan, 2006). Some patients have gene mutations, for example in the insulin gene, which cause them to have a genetic predisposition to develop diabetes type 2 (Taylor, 1999).

T2DM can be diagnosed by looking at the response to glucose; T2DM patients have a delayed response due to their insulin resistance (Silverthorn et al., 2009).

It is important to know that insulin signaling is tissue specific; insulin target tissue will activate different pathways upon insulin binding compared to non-target tissue. Due to this difference insulin resistance is often found in the target tissue only, meaning that non-target tissue can have an overactivation of insulin signaling due to hyperinsulinemia (Vigneri et al., 2009), which can lead to dangerous changes. This phenomenon will be explained in later chapters.

Hyperinsulinemia in T2DM patients is caused by overproduction of insulin in the pancreatic β-cell to make up for the insulin resistance in its target tissue. Eventually this can lead to failure of the pancreatic β-cells when the cells can no longer keep up with the need for insulin production (Taylor, 1999).

As long as the pancreatic β-cells are able to produce enough insulin, the glucose levels will not become too high. However, when the β-cell failure starts, patients often suffer from hyperglycemia, just like T1DM patients (Taylor, 1999). In T2DM hyperglycemia is not only a result of reduced insulin production, but it is more importantly a result of constant glucagon production. The pancreatic α-cells produce glucagon as long as they do not sense any glucose. Since insulin is needed to facilitate the translocation of GLUT4, and since the patient is insulin resistant, the α-cells cannot sense that the blood glucose levels are high. Glucagon will be produced even in a fed state of the body and glucagon will stimulate glycogenolysis and gluconeogenesis, which will lead to more glucose production and to hyperglycemia (Silverthorn et al., 2009).

Later in the development of the disease, patients often start to suffer from failure of their β-cells in the pancreas due to endoplasmic reticulum (ER) stress. In healthy β-cells large amounts of proinsulin is created which is folded in the ER to form vesicles with insulin ready to release (Berg et al., 2012). However, in a T2DM β-cell more insulin is created to try to make up for the insulin resistance. Now the ER has to fold many more proteins this leads to stress and unfolded or misfolded proteins. The cell initially will go into cell senescence to stop the cell cycle and to try to restore the stress causing situation. However, when the stress is too high apoptosis is triggered and the pancreatic β-cells die (Eizirik et al., 2008).

ER stress will lead to a switch in the disease characteristics form hyperinsulinemia to reduced insulin production (Prentki and Nolan, 2006). Often the first phase is not yet considered to be real T2DM, since the high insulin levels can still manage to keep the glucose levels normal. Once the pancreatic β-cells start to fail to produce insulin the patients are called diabetic (Taylor, 1999).

The symptoms of T2DM are most of the time less severe than those of T1DM, because the glucose metabolism does not shut off completely. Therefore it is not necessary for the liver to produce

ketone bodies; ketosis is uncommon in T2DM patients. However, patients do encounter many additional problems as a result of a disfunctioning glucose and fat metabolism. These problems vary from atherosclerosis, renal failure and blindness to an increased cancer risk. Up to 70% of all T2DM die due to the effects of cardiovascular disease (Silverthorn et al., 2009).

More and more evidence is found that diabetes type 2 can be linked to a higher risk of developing cancer (Vigneri et al., 2009). Meta-analyses to induced cancer risk have confirmed a higher risk for diabetes patients to develop cancer in the liver (El-Serag et al., 2006), pancreas (Huxley et al., 2005), endometrium (Friberg et al., 2007), colon-rectum (Larsson et al., 2005), bladder (Larsson et al., 2006) and breast (Larsson et al., 2007) and a reduced risk to develop prostate cancer (Kasper and Giovannucci, 2006).

The higher risk to develop cancer can be due to multiple factors including hyperinsulinemia, hyperglycemia and chronic inflammation (Giovannucci et al., 2010). In T2DM patients the state of hyperinsulinemia can last for several years before the pancreatic β-cell begin to fail and die (Vigneri et al., 2009). It was found that the insulin resistance normally has only an effect on the PI3K-pathway and does not influence the mitotic effect of the MAPK-pathway (Cusi et al., 2000; Jiang et al., 1999). In the next chapters the induced cancer risk due to hyperinsulinemia will be further described by looking deeper into the signaling pathways of insulin.

Treatment for T2DM patients mainly consists of a change in lifestyle; regular exercise and a healthy diet can cure most cases. When there is pancreatic β-cell failure due to ER stress, administration of insulin can be needed (Berg et al., 2012). Hyperglycemia can be reduces by regular exercise, because active muscles are not dependent on insulin to burn glucose (Silverthorn et al., 2009).

Also an effective medicine is created called Metformin (commercial name Glucophage), which activates AMPK (Berg et al., 2012) to reduce the hyperglycemia by decreasing gluconeogenesis (Silverthorn et al., 2009).

Diabetes is a serious disorder which can be due either to a failure to produce insulin or to insulin resistance. In the next chapter insulin signaling will be further discussed to highlight the most important players of its pathway and to clarify how a cell can become insulin resistant.

Chapter 2: Insulin signaling

The insulin signaling pathway

Insulin signaling consists of two main routes: the PI3K/AKT pathway, which is mainly involved in glucose metabolism; and the Ras/MAPK pathway, which regulates expression of certain genes that are involved in proliferation, differentiation, cell growth and cell survival. The PI3K/AKT pathway is connected to the Ras/MAPK pathway by some cross regulation between these two (Taniguchi et al., 2006). In chapter 1 the insulin pathway concerning glucose metabolism was already described shortly. Here the molecular pathways of insulin signaling will be discussed in more detail, describing the most important factors leading to both metabolic and mitogenic effects of insulin on the cell. Figure 3 shows a simplified, schematic overview of the insulin signaling pathway. The total picture of insulin signaling is very complex and not all aspects have been researched in the same level of detail, much is still left to be clarified. This review will focus on the two most important branches of the pathway: the recruitment and activation of PI3K and Ras by the insulin receptor, which stimulate the AKT and MAPK pathways respectively.

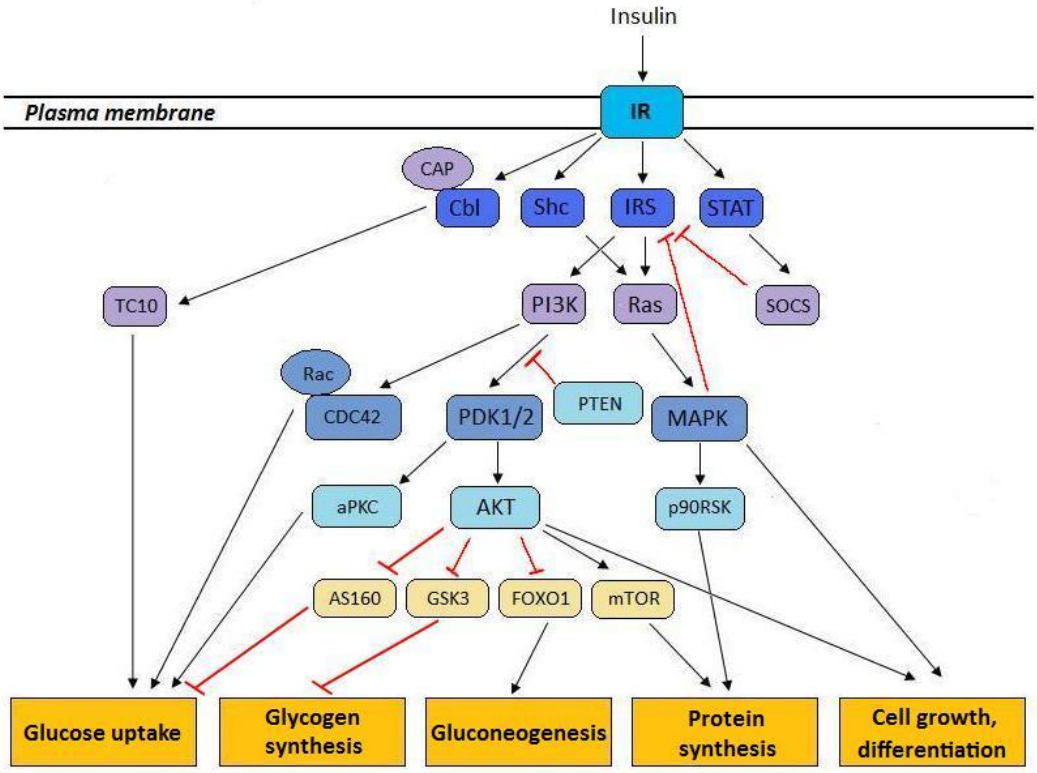

Figure 3: Schematic overview of the most important insulin signaling pathways. Insulin signaling starts with the binding of insulin to its receptor (IR). Now several adaptor molecules can be recruited among which Cbl, Shc, STAT and most importantly IRS. These in turn can recruit downstream effector molecules. In the end insulin binding will stimulate glucose uptake via TC10, CDC42, aPKC and inhibition of AS160. It will stimulate glycogen synthesis via GSK3 inhibition. It will down regulate gluconeogenesis via inhibition of FOXO1. It will stimulate protein synthesis via mTOR and p90RSK. And it will stimulate cell growth and differentiation via MAPK and AKT. This figure represents a simplified overview, not all contributing factors are included.

The PI3K/AKT pathway induces both metabolic and mitogenic effects

As discussed in chapter 1, activated PI3K can convert PIP_2 into PIP_3. PIP_3 activates PDK, which in turn can recruit, phosphorylate and activate AKT and other kinases, including *atypical protein kinase C- ζ* (aPKCζ) (Taniguchi et al., 2006). aPKCζ can inhibit IRS1 by serine phosphorylation and is therefore part of a negative feedback loop to attenuate insulin signaling (Liu et al., 2001), this will be discussed later. AKT is a serine/threonine kinase which in mammals has three isoforms: AKT1-3. AKT1 is mostly involved in growth regulation, AKT2 is mainly responsible for the metabolic effects and AKT3 predominantly expressed in the nervous system. All isoforms consist of two important domains: the *pleckstrin-homology* (PH) domain, which binds to the phospholipids of the membrane, and the *catalytic* (Cat) domain, which becomes activated after phosphorylation (Taniguchi et al., 2006).

Activation of AKT has many important consequences. Firstly, AKT will phosphorylate and inactivate AS160 so it can no longer block GLUT4 translocation. In this way AKT is responsible for glucose uptake. Secondly, AKT will phosphorylate and inactivate GSK3, which will lead to increased glycogen synthesis. Thirdly, AKT will phosphorylate *forkhead box O* (FOXO) transcription factors to initiate their translocation to outside the nucleus and thus inactivate them. As long as the FOXO transcription factors are phosphorylated, they will be kept in the cytosol by 14-3-3 phosphoserine binding protein (Taniguchi et al., 2006). The family of FOXO transcription factors control the expression of genes involved in apoptosis, DNA repair, cell cycle, metabolism and oxidative stress resistance (Taguchi and White, 2008). Fourthly, Akt can phosphorylate *tuberous sclerosis complex-1* and *-2* (TSC1/2), which stops it from inhibiting *Ras homologue enriched in brain* (Rheb). Rheb can now stimulate *mammalian target of rapamycin* (mTOR), which in turn stimulates translation initiation factor *4E-binding protein 1* (4EBP1) and *p70 ribosomal protein S6 kinase* (p70S6K) to initiate protein synthesis (Taniguchi et al., 2006). The mRNA that is now translated into proteins mainly manifests in cell growth (Weinberg, 2007). These four actions of AKT are summarized in figure 4.

AKT plays a regulatory role on many cellular processes; these four examples do not include everything, just the ones that are most important for insulin signaling. AKT can also act as an anti-apoptotic factor by inhibiting pro-apoptotic molecules and activating anti-apoptotic ones. Furthermore AKT inhibits several anti-proliferative factors, thereby stimulating proliferation. As will be discussed in chapter 3, AKT is thus an important oncogene which is often upregulated in cancer cells (Weinberg, 2007).

The Ras/MAPK pathway influences many non-metabolic processes

The MAPK pathway starts with the recruitment of *growth factor-bound protein 2* (Grb2); this can be done by both the adaptor proteins IRS or Shc, which are activated by phosphorylation after insulin binding to the IR. Activated Grb2 can recruit *son of sevenless* (SOS) to the plasma membrane, so that SOS can activate Ras (De Luca et al., 2012). In quiescent cells Ras is found in its GDP-coupled form (Ras^{GDP}), SOS can induce the exchange of GDP for GTP with the help of *guanine nucleotide exchange factors* (GNEFs). The backward reaction is facilitated by *GTPase activating proteins* (GAPs) (Santarpia et al., 2012). Ras^{GTP} can crosstalk with the AKT pathway by activating PI3K (De Luca et al., 2012). More importantly, Ras^{GTP} activates Raf (also known as MAPKKK) by recruiting it from the cytosol to the plasma membrane (Santarpia et al., 2012). Activated Raf can phosphorylate MEK (MAPKK), which in turn can phosphorylate MAPK (originally known as *extracellular signal-regulated protein kinase*,

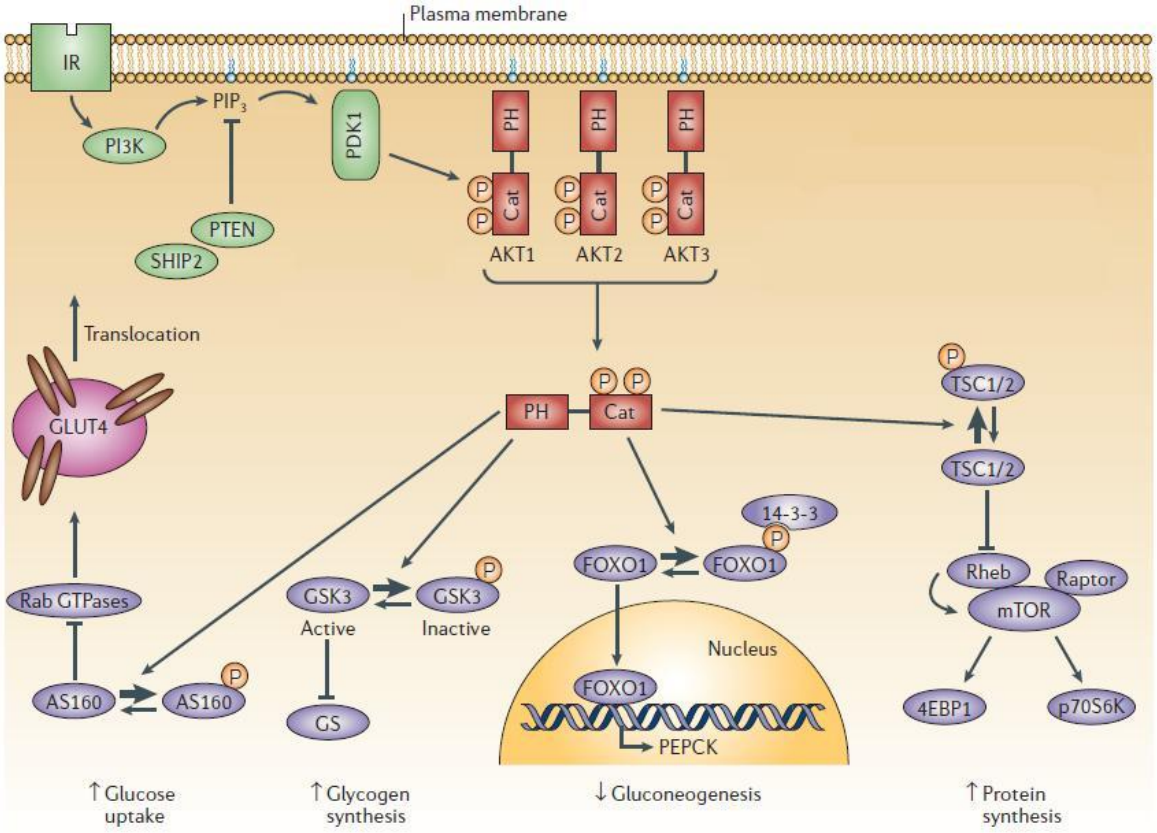

Figure 4: Insulin activates the PI3K/AKT pathway. Upon insulin binding IRS can activate PI3K, which then converts PIP_2 into PIP_3. Now PDK1 can be recruited to phosphorylate AKT on its catalytic subunit. Activated AKT phosphorylates several downstream molecules. First, AS160 is phosphorylated and inactivated; this induces GLUT4 translocation to the cell membrane and thus glucose uptake. Second, GSK3 is phosphorylated and inactivated, this induces glycogen synthesis. Third, FOXO1 is phosphorylated and kept outside the nucleus, this reduces gluconeogenesis. Fourth, TSC1 is inactivated to activate mTOR, this will induce translation of certain proteins. (Adapted and adjusted from **(Taniguchi et al., 2006)**).

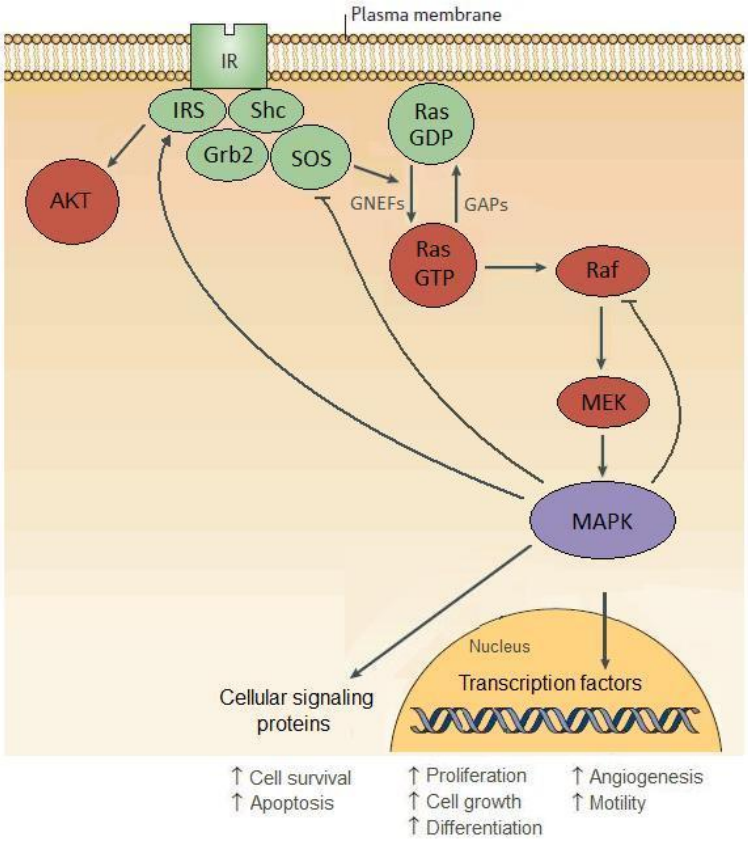

Figure 5: Insulin activates the Ras/MAPK pathway. Upon insulin binding IRS or Shc can recruit Grb2. Grb2 can now phosphorylate SOS, which exchanges the GDP bound to Ras with GTP, with the help of GNEFs. Ras^{GTP} is the active state of Ras which can activate Raf (MAPKKK). Raf can phosphorylate MEK (MAPKK), which in turn phosphorylates MAPK. MAPK can now be translocated from the plasma membrane to the cytosol or the nucleus, where it can activate certain cellular signaling proteins and transcription factors. Activation the cellular signaling proteins by MAPK can lead to both cell survival and apoptosis, depending on the situation. Activation of the transcription factors leads to expression of genes involved in proliferation, cell growth, differentiation, angiogenesis and motility.

ERK) on its tyrosine and threonine residues in the activation loop (De Luca et al., 2012). In quiescent cells, MAPK is bound to the plasma membrane, when it gets phosphorylated by MEK, it can be translocated to the nucleus (Mebratu and Tesfaigzi, 2009). MAPK can phosphorylate many factors; most of them are cellular signaling proteins or transcription factors. In the end, activation of the MAPK pathway will lead to proliferation, differentiation, cell growth, cell survival, induced motility and angiogenesis (Santarpia et al., 2012). Even though MAPK has always been associated with cell survival, it can also under specific conditions induce apoptosis (Mebratu and Tesfaigzi, 2009). Figure 5 shows a schematic summary of the Ras/MAPK pathway stimulated by insulin. As will be clarified in the next chapter, Ras is a very important oncogene which is mutated in nearly 25% of all human tumors.

IR and IRS functions in insulin signaling

Both the insulin receptor (IR) and IRS play key roles in the regulation of insulin signaling. Expression of specific isoforms or family members can determine which branch of the pathway is activated. Also IRS is found to be involved in the development of insulin resistance and is thus an important player in diabetes characterization. These interesting roles of the IR and IRS will now be described in more detail.

The insulin receptor exists in two isoforms

The IR is a receptor tyrosine kinase which can be found in two isoforms: IR-A and IR-B; in most cells they are co-expressed, however, their expression concentrations can vary (Taniguchi et al., 2006). The two isoforms stimulate different insulin signaling pathways and therefore might play a role in the selective insulin actions (Leibiger et al., 2001). IR-A is mostly expressed in fetal tissue, the central nervous system and hematopoietic cells (Taguchi and White, 2008) and is often overexpressed in cancer cells (Frasca et al., 1999). This isoform can be activated by both insulin and *insulin-like growth factor 2* (IGF2) and mainly stimulates the mitogenic pathway which, among others, results in anti-apoptotic signals (Sciacca et al., 2003). IR-B differs from IR-A in twelve amino acids at the carboxyl terminus; this difference is caused by an alternative splicing of exon 11 when IR-A is generated (Frasca et al., 1999). IR-B is mostly expressed in differentiated tissue, mainly insulin sensitive target tissues like the liver, muscles and adipose tissue (Taguchi and White, 2008); where it initiates metabolic and differentiation signals upon insulin binding (Belfiore and Malaguarnera, 2011). IR-B can only poorly bind IGF2 (Sciacca et al., 2003).

Both isoforms of the insulin receptor consist of two extracellular α-subunits and two transmembrane β-subunits. Insulin will bind to the α-subunits which will then homodimerize; as a result the β-subunits will cross phosphorylate each other on their tyrosine residues (Gual et al., 2005). Unlike other receptor tyrosine kinases, the insulin receptor does not bind directly to downstream effectors, but needs satellite proteins like IRS (Taniguchi et al., 2006). After autophosphorylation of the receptor, these proteins can be phosphorylated (Sesti et al., 2001). IR signaling can be negatively regulated by dephosphorylation of its tyrosine residues, for example via PTP1B; by blocking IRS interaction with IR, for example via SOCS1 and -3; or by receptor internalization and degradation. Both SOCS proteins and receptor internalization are often found to be upregulated in insulin resistant diabetes patients (Taniguchi et al., 2006).

IRS proteins are important adaptor molecules in insulin signaling

After IR activation several adaptor molecules, including IRS, Shc, APS and *c-Cbl-associated protein* (CAP) can be phosphorylated at one of their tyrosine phosphorylation motifs. Depending on which tyrosines are phosphorylated, specific downstream effectors are being recruited and activated. IRS can recruit *Src-homology-2 domain-containing tyrosine phosphatase 2* (SHP2), Nck and PI3K. PI3K can, in turn, activate the AKT pathway; this will lead to GLUT4 translocation, glucose transport, protein synthesis and reduced glycogen synthesis. APS and CAP will recruit Cbl, which will ultimately also lead to GLUT4 translocation. Shc will recruit Grb2/SOS complex which can activate Ras and consequently the MAPK cascade, which leads to phosphorylation of transcription factors and consequently to transcription of specific genes (figure 6) (Schmitz-Peiffer and Whitehead, 2003).

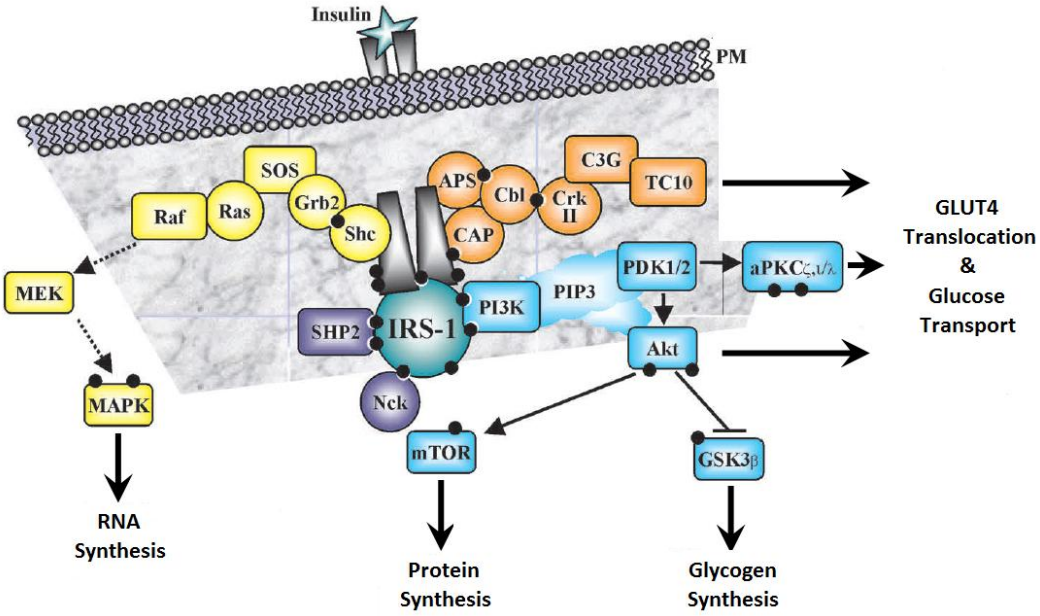

Figure 6: insulin receptor adaptor molecules and their downstream effectors. Activation of the insulin receptor makes the phosphorylation of adaptor molecules IRS1, Shc, APS and CAP possible. These adaptor molecules can in turn activate several downstream effectors for further signaling. IRS1 activates the PI3K pathway and thereby AKT. Shc activates the Ras-MAPK pathway. And APS and CAP together activate Cbl. Ultimately these pathways can lead to metabolic effects like GLUT4 translocation, glucose transport and glycogen synthesis or mitogenic effects via gene transcription and protein synthesis. (Adapted and adjusted from (Schmitz-Peiffer and Whitehead, 2003)).

For many actions of insulin signaling IRS proteins play a key role, even for the recruitment of downstream effectors by other adaptor molecules. The other adaptor molecules often function only as modulators and compensators for IRS proteins (Taguchi and White, 2008). The IRS protein family consists of six members; IRS1-6. IRS1 and IRS2 are the best known, since they are by far the most distributed members. IRS1 is mainly found in the muscles and adipose tissue and IRS2 is mainly found in the liver, however their distribution is far from exclusive (Taniguchi et al., 2006). Experiments with small interfering RNA (siRNA) to silence the IRS1 and IRS2 genes showed that IRS1 is more involved in GLUT4 translocation and glucose uptake by regulating AKT phosphorylation and that IRS2 signaling is more directed towards MAPK phosphorylation and thus RNA synthesis (Huang et al., 2005). IRS3 is mostly found in adipose tissue and the brain, while IRS4 is expressed almost exclusively in embryonic tissue. The expression of IRS5 and IRS6 seems to be limited, there is not much known about their function (Taniguchi et al., 2006). In this review we will focuss on IRS1 and IRS2.

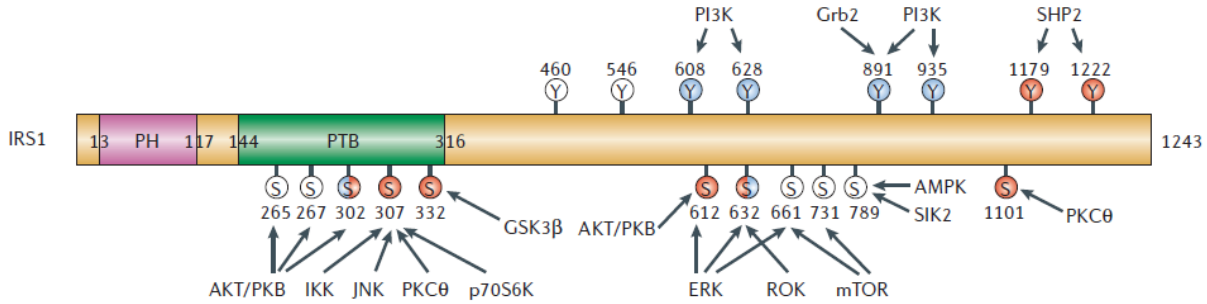

Figure 7: Schematic drawing of the IRS1 protein. The pink PH and the green PTB domains can bind to the insulin receptor. Phosphorylation of one of the tyrosine phosphorylation sides (Y) can lead to the recruitment and binding of specific downstream effector molecules like PI3K, Grb2 and SHP2, for positive (blue circles) or negative (red circles) regulation of IRS1. Phosphorylation of the serine residues (S) often leads to negative regulation of IRS1. The half blue, half red circles represent phosphorylations which have been found to either positively or negatively regulate IRS1. It is still unknown what the effects are of phosphorylation of the white circles. Note that many kinases that can phosphorylate the serine residues represent downstream molecules of IRS1 itself, which may represent a negative feedback loop. (Taniguchi et al., 2006).

IRS activity can be regulated by phosphorylation

Figure 7 shows a schematic drawing of the IRS1 protein, which is in many aspects comparable to the other IRS proteins. The PH and *phosphotyrosine-binding* (PTB) domains around the N-terminus are responsible for the interaction between IRS1 and IR. IRS1 has up till 20 tyrosine phosphorylation sides (Y), some of which can be phosphorylated by IR. This makes it possible for intracellulair molecules, like PI3K and Grb2, to bind and for insulin signalling to continue. IRS1 can also be phosphorylated at its serine phosphorylation sides (S)by different kinases ,often this will have an inhibiting effect on IRS1 functioning. Important kinases that phosphorylate IRS to inhibit it are *protein kinase C-ϑ* (PKCθ, on residue 307 or 1101), mTOR (on residue 661 or 731), MAPK (on residue 612 or 632) and AKT (on residue 612) (Taniguchi et al., 2006).

Interestingly, it was found that IRS phosphorylation on specific serine residues can lead to insulin resistance (Taniguchi et al., 2006), this will be discussed later.

Regulation of the IRS proteins plays an important role to attenuate insulin signaling. Some of the kinases that phosphorylate the IRS1 serine residues, thereby inhibiting IRS1, are activated by insulin signaling (Gual et al., 2005). Two examples of these kinases are aPKCζ and mTOR, they work partly by reducing the interaction between IRS and IR (Liu et al., 2001). In this way insulin is responsible for its own negative feedback loop (Gual et al., 2005). On the other side, AKT, also part of the insulin signaling pathway, can phosphorylate one or more IRS1 serine residues and thereby stimulate its actions by preventing tyrosine phosphatases from dephosphorylating IRS1 (Paz et al., 1999).

Excessive insulin treatment (hyperinsulinemia) was shown to reduce the expression levels of IRS1, due to degradation (Taniguchi et al., 2006); and IRS2, due to reduced transcription (Hirashima et al., 2003). IRS1 and IRS2 can both be ubiquitinated for degradation upon initiation by SOCS1 and SOCS3. Expression of SOCS1 and SOCS3 is upregulated by inflammation (Rui et al., 2002), a stage often found in diabetic β-cell destruction (Eizirik et al., 2008).

IRS protein action can also be regulated by cellular translocation. To be activated quickly after insulin binding to IR, IRS1 needs to be located close to the cell membrane in a region called the *high speed pellet* (HSP). Translocation of IRS1 outside the HSP to a more intracellular position can be a

way to attenuate insulin signaling (Schmitz-Peiffer and Whitehead, 2003); it was found that this translocation is directed by 14-3-3 phosphoserine binding protein (Xiang et al., 2002).

Deregulation of IRS1 can lead to insulin resistance

Insulin resistance is characterized by a decreased glucose uptake by the liver, muscles and adipose tissue; for example because of a reduced translocation of glucose transporters to the plasma membrane. Multiple factors can induce insulin resistance, some of them involve a decreased phosphorylation of the IRS1 tyrosine residues (Sesti et al., 2001) or an increased phosphorylation of the IRS1 serine residues (Tanti et al., 1994). Both *tumor necrosis factor-α* (TNFα) and hyperinsulinemia are known to induce insulin resistance by activating serine/threonine kinases that phosphorylate IRS1 and thereby inhibit its function (Gual et al., 2005; Kanety et al., 1995). Other factors that can induce insulin resistance are *inducible nitric oxide synthase* (iNOS), MAPK and *IκB kinase β* (IKKβ), the latter two can both stimulate *protein kinase C* (PKC) to inhibit IRS1 by blocking phosphorylation of the tyrosine residues (Schmitz-Peiffer and Whitehead, 2003). Experiments with okadaic acid, a serine/threonine phosphatase inhibitor, resulted in a constitutive inhibition of IRS1, decreased PI3K activation and decreased translocation of glucose transporters. This suggests that specific serine residues of IRS1 need to be unphosphorylated for translocation of GLUT by insulin signaling (Tanti et al., 1994).

Together this tells us that there needs to be a balance between activation and inhibition of IRS1 for insulin signaling, by phosphorylation of its tyrosine or serine residues respectively. When this balance switches towards inhibition of IRS1, insulin resistance could develop and when it switches towards overactivation there could be a sustained signal for cell growth and avoidance of apoptosis (Gual et al., 2005). Insulin resistance is a characteristic of T2DM which is often considered as the cause of an induced risk to develop cancer. This may seem contradictive at first, but makes more sense when you know that insulin resistance mainly affects the PI3K/AKT pathway and has much less effect on the Ras/MAPK pathway (Cusi et al., 2000; Jiang et al., 1999) and that hyperinsulinemia reaches not only insulin target tissue, but also normal tissues, where insulin signaling predominantly works via the Ras/MAPK pathway (Vigneri et al., 2009). This will be discussed in more detail in chapter 4.

Figure 8 shows the difference between normal insulin signaling attenuation and insulin resistance. In a normal cell situation, IRS1 activity can be regulated by translocation from the HSP to the cytosol and back. This translocation can be triggered by PI3K through phosphorylation the IRS1 serine residues, which will cause dissociation from the IR and possibly translocation to the cytosol. If needed, additional phosphorylation of IRS1 serine residues can further induce translocation. The translocation can be facilitated by 14-3-3 phosphoserine binding protein, which also keeps IRS1 in the cytosol. IRS1 can go back to the HSP and the IR when the serine residues are dephosphotylated by Ser/Thr phosphatases. There is a balanced equilibrium between the phosphorylation and dephosphorylation of the serine residues. However, when a cell is in a state of insulin resistance, phosphorylation is highly favored, leading to increased concentrations of IRS1 in the cytosol and consequently ubiquitination and degradation of IRS1 (Schmitz-Peiffer and Whitehead, 2003).

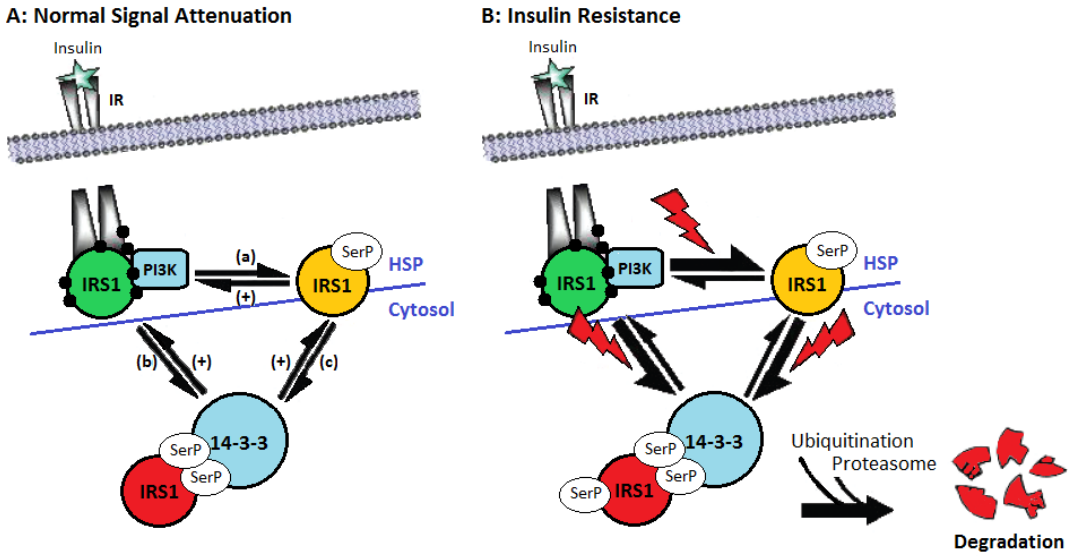

Figure 8: Deregulation of IRS1 can lead to insulin resistance due to IRS1 degradation. In a normal cell situation (**A**), insulin signaling is attenuated by translocation of IRS1 to the cytosol. Herefore IRS1 needs to be phosphorylated on its serine residues. This can be initiated by PI3K (a), which can be enough for translocation to the cytosol in some situations (c), in others 14-3-3 phosphoserine binding protein needs to help by more phosphorylation. 14-3-3 phosphoserine binding protein is also responsible for keeping IRS1 inside the cytosol. When IRS1 is dephosphorylated again, it can move back to the IR (+). The equilibrium between phosphorylation and dephosphorylation is very important. In a situation of insulin resistance (**B**), the balance between phosphorylation and dephosphorylation is shifted. This results in an induced translocation of IRS1 to the cytosol, where it will ultimately be ubiquitinated for degradation by the proteasome. (Adapted and adjusted from (Schmitz-Peiffer and Whitehead, 2003)).

Insulin signaling is not only important for the metabolism of glucose, but also functions in other pathways. This way insulin can stimulate cell growth, proliferation, differentiation, angiogenesis and motility and it can inhibit apoptosis. These are all important characteristics of cancer development and progression. In the next chapter the hallmarks of cancer will be described with the prospects as to how they are influenced by insulin signaling.

Chapter 3: The development of cancer

The hallmarks of cancer

In 2000, Hanahan and Weinberg wrote a famous review describing six essential, acquired capabilities for a normal cell to become a cancer cell: The Hallmarks of Cancer. These alterations help the cell to survive, proliferate and spread through the body; and can be gained through mutations in the genome (Hanahan and Weinberg, 2000). In 2011, they wrote a follow up for the article describing four more alterations, two of which enable the cell to get the acquired capabilities and the other two describe alterations that should be added to the list of acquired capabilities (Hanahan and Weinberg, 2011). In this chapter the hallmarks of cancer (figure 9) will be described shortly, to answer the question: How can a normal cell become a cancer cell? And to better understand the link between insulin signaling and cancer development and progression.

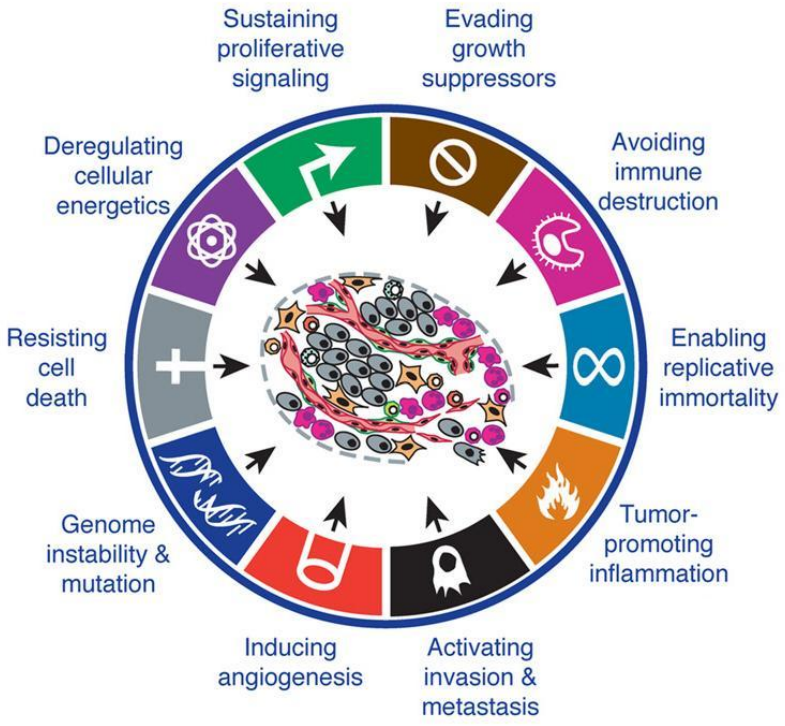

Figure 9: The hallmarks of cancer. This figure shows the ten essential alterations for a normal cell to become a cancer cell. The original six hallmarks of cancer are sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis. Genome instability and tumor promoting inflammation enable the cell to acquire the other alterations. And finally, two emerging hallmarks are avoiding immune destruction and deregulating cellular energetics. (Adapted and adjusted from (Hanahan and Weinberg, 2011))

Acquired capabilities

There are eight capabilities a cell must acquire to become a cancer cell and to able to grow into a macroscopic tumor. These hallmarks include induced proliferation, reduced sensitivity to growth suppressors, escaping cell death, cell immortality, vessel formation, metastasis, deregulation of cellular energetics and avoidance of the immune system. Most of these alterations are gained by genetic mutations or cellular deregulations leading to activation of oncogenes and inhibition of tumor-suppressor genes. Every cell has some protection mechanisms against such alterations, including a DNA repair system; cancer cell therefore often first acquire enabling changes to from a tumor promoting environment.

One of the enabling alterations is genome instability, leading to more mutations and thus a greater

chance to obtain a necessary mutation. This involves the downregulation of DNA damage-detecting genes or of genes of the DNA repair system and upregulation of genes causing DNA damage (Hanahan and Weinberg, 2011). The most commonly found mutation, in over 50% of all human cancers, is the loss of *protein 53* (P53) (Sigal and Rotter, 2000). p53 is often called the "Guardian of the Genome", because it puts the cell cycle to a halt when DNA is damaged, to make sure there is time for repair. When the damage is so severe that reparation is not possible it will save the genome by inducing apoptosis (Lane, 1992). When p53 is mutated, DNA damage is recognized less often and genome instability is easier to obtain.

The other enabling alteration is tumor-promoting inflammation, which creates a tumor-promoting environment in multiple ways. Firstly, it can supply growth factors, proliferation promoting signals, survival factors and angiogenesis promoting factors, originally meant to restore injured tissue. Secondly, inflammatory cells can release reactive oxygen species (ROS), which can cause mutations and are thus helpful for a cancer cell to acquire genetic alterations(Hanahan and Weinberg, 2011). Chronic inflammation is often found in diabetes patients (Giovannucci et al., 2010), this thus may enable cancer formation in diabetes patients.

With these enabling alterations it is easier of a cell obtain the eight hallmarks of cancer. Some of these hallmarks are promoted by insulin signaling and are therefore very important to understand the relationship between insulin signaling, diabetes and cancer risk.

Sustaining proliferative signals

For a tumor to grow bigger, cancer cells must proliferate more than a normal cell would. However, proliferation is tightly regulated, to make sure cells do not come out of the quiescent state and proliferate without the right signals (Hanahan and Weinberg, 2000). Cancer cells must avoid this regulation to sustain the proliferative signal by growth factors. To do so they can act through various ways; by producing their own growth factors; by producing a signal to induce neighboring cell to release growth factors; by elevating the number of growth factor receptors on their cell membrane, to react sooner at lower concentrations; by a constant activation of one of the downstream signaling components through a mutation, so that growth factor activation is not needed; or by reducing the negative feedback which controls proliferation (Hanahan and Weinberg, 2011).

One mutation often found in cancer cells, which can disrupt the negative-feedback mechanisms, involves the inactivation of Ras GTPase and thereby the activation of oncogene *Ras* (Hanahan and Weinberg, 2011). As was described in chapter 2, Ras is part of the insulin signaling pathway and activated Ras will activate the MAPK cascade which leads to the transcription of genes that stimulate cell proliferation. For example, the Ras/MAPK pathway activates transcription factors like *serum response factor* (SRF) and *activating transcription factor 2* (ATF2), which leads to the expression of cyclin D1 and consequently cell cycle progression. Also, Ras/MAPK signaling induces the expression of the growth factors *heparin-binding epidermal growth factor-like growth factor* (HBEGF) and *transforming growth factor-α* (TGFα) (Pylayeva-Gupta et al., 2011). Furthermore, both Ras and insulin can activate the PI3K/AKT pathway. Induced activation of AKT can also cause sustained proliferation by upregulation of cyclin D1 expression via activating the transcriptional co-activator *protein 300* (p300) (Martelli et al., 2012).

Another important mutation often found in cancer cells is the loss of PTEN (Hanahan and Weinberg, 2011). As mentioned in chapter 1, PTEN can remove phosphoryl groups of PIP_3 to keep it in its inactive PIP_2 mode. In this way PTEN counteracts with PI3K and thereby attenuates the pathway

(Berg et al., 2012). When PTEN is lost, as in many cancer cells, PIP_3 is not inhibited and consequently AKT will be overactivated.

Together this shows that Ras or AKT overactivation can lead to cell proliferation. In diabetes patients, both Ras and AKT can be overactivated due to hyperinsulinemia, this may thus result in a higher change to develop cancer.

Resisting cell death

When a cell becomes under high stress, mechanisms within the cell can trigger apoptosis, to prevent it from becoming a threat to the organism's health (Weinberg, 2007). The first signs of cancer development, elevated levels of oncogenes and DNA damage leading to induced proliferation, are such stress factors. The normal response to these alterations would be for the cell to induce apoptosis. Therefore, cancer cells can only survive if they have a way to resist cell death (Hanahan and Weinberg, 2011).

Apoptosis can be triggered both by extrinsic and intrinsic pathways. The extrinsic pathway is triggered by signals from outside the cell via death receptors like *cluster of differentiation 95* (CD95). The intrinsic pathway is triggered by signals from within the cell and often involves activation by p53. Both the extrinsic and intrinsic pathways will lead to activation of caspases to induce apoptosis (Roos and Kaina, 2013). Cancer cells need to find a way to avoid apoptosis.

In insulin signaling, both the Ras/MAPK pathway and the PI3K/AKT pathway can lead to anti-apoptotic signals. As mentioned in chapter 2, the Ras/MAPK pathway can both inhibit and induce apoptosis; this depends on the situation in the cell. Apoptosis is induced via stimulation of p53; and it is inhibited by downregulation of *prostate apoptosis response 4* (PAR4) and upregulation of *B-cell lymphoma 2* (Bcl-2), which will both lead to phosphorylation and inactivation of the pro-apoptotic factor *Bcl-associated agonist of cell death* (BAD) and thus will stimulate cell survival. Again, Ras can also activate the PI3K/AKT pathway (Pylayeva-Gupta et al., 2011). AKT can inhibit apoptosis by preventing nuclear localization of p53 (Roos and Kaina, 2006), by regulation of pro-apoptotic and anti-apoptotic gene expression and by preventing chromosome condensation and DNA fragmentation, which is needed for apoptosis (Martelli et al., 2012).

When the insulin signaling pathways are overactivated, as found in a state of hyperinsulinemia in diabetes patients, anti-apoptotic signals and processes can be stimulated. This may inhibit apoptosis in situations that normally trigger cell death and can therefore be favorable for tumor development and growth; thus again linking diabetes to an induced risk to develop cancer.

Inducing angiogenesis

When a tumor is growing bigger, eventually it will need to induce angiogenesis, to be able to receive nutrients and oxygen and to get rid of the waste products. In many cancer cells the shift towards angiogenesis is acquired through altered gene expression (Hanahan and Weinberg, 2000).

The most important inducer of angiogenesis is vascular endothelial growth factor-A (VEGF-A). VEGF-A can be upregulated by certain oncogenes and by a state of hypoxia, to try to save the tissue with oxygen supply. Direct upregulation of VEGF-A is often under control of oncogenes like Ras (Hanahan and Weinberg, 2011). Ras can stimulate the transcription of *VEGF-A* by recruiting the transcription factors *specificity protein 1/2* (SP1/2), *activating protein 2* (AP2) and *E-twenty six* (ETS) to the promoter of the *VEGF-A* gene; or Ras can stabilize the *VEGF-A* mRNA and stimulate its

translation (Pylayeva-Gupta et al., 2011). Indirect upregulation of VEGF-A can be induced by signals which come from immune inflammatory cells (Hanahan and Weinberg, 2011).

This hallmark of cancer might be activated in diabetes patients via upregulation of Ras during hyperinsulinemia. Also, many diabetic patients are in a state of chronic inflammation due to pancreatic β-cell death (Mathis et al., 2001), this may cause an induced stimulation of VEGF-A. Together we can state that both direct and indirect overactivation of VEGF-A can be found in diabetic patients, making them more vulnerable for angiogenesis and subsequently cancer development.

Activating invasion and metastasis

Most tumors eventually start to spread through the body to find new locations to grow without a shortage of space and nutrients (Hanahan and Weinberg, 2000). For a cancer cell to start metastasizing it has to undergo several alterations concerning their attachment to other cells and to the surrounding extracellular matrix (ECM). Herefore it is important that certain cell-adhesion molecules are lost or downregulated (Hanahan and Weinberg, 2011); cancerous mutations often involve cadherins (most importantly E-cadherin), *cell adhesion molecules* (CAMs) and integrins (Hanahan and Weinberg, 2000).

Ras oncogene signaling is known to influence many processes concerning metastasis. It can induce the expression of the *Snail* and *Slug* genes, which code for repressors of E-cadherin. Ras signaling will also stimulate E-cadherin degradation, downregulate integrin subunits and help the cell to become polymerized for motility. For these alterations Ras works via multiple pathways including the one via MAPK, as found in insulin signaling (Pylayeva-Gupta et al., 2011). In this way again we can couple an induced insulin signaling, as found in diabetes patients with hyperinsulinemia, to a higher chance of developing cancer.

Metastasis is a really important subject for cancer research and treatment, since metastasized tumors account for 90% of all human deaths caused by cancer (Hanahan and Weinberg, 2000).

Deregulating cellular energetics

To account for their induced proliferation and cell growth cancer cells have to make adjustments in their energy metabolism. It was found that many cancer cells reprogram their glucose metabolism to mostly convert glucose into pyruvate via glycolysis and to not continue energy metabolism via the citric acid cycle and oxidative phosphorylation (Hanahan and Weinberg, 2011). This situation is normally only found in cells which are in a state of hypoxia, because oxygen is needed for the citric acid cycle. These cells then produce lactate from pyruvate, which generates only 2 ATP per glucose molecule, while oxidative phosphorylation generates around 36 ATP per glucose molecule. However, most cancer cells have access to enough oxygen, but still use most of their glucose to produce lactate (aerobic glycolysis; figure 10) (Vander Heiden et al., 2009). This phenomenon is also called the Warburg effect, after Otto Warburg who first observed it (Warburg, 1956).

It is hard to understand why a cancer cell would switch to such an inefficient energy metabolism. The current hypothesis is that a cell can get more of the necessary requirements for proliferation and growth when they do not completely break down their carbohydrates to CO_2 and H_2O. Since cancer cells have a greatly induced proliferation and growth, they can benefit from producing building block like nucleotides, amino acids and lipids from glucose instead of producing large amounts of energy. From these building blocks they can then produce macromolecules and organelles needed for the generation of new cells (Vander Heiden et al., 2009). To promote this process cancer cells often

increase their expression of glucose transporters (GLUT) by upregulation of the *hypoxia inducible factor 1α* and *-2α* (HIF1α/HIF2α) transcription factors (Hanahan and Weinberg, 2011).

This hallmark might be interesting with regard to insulin signaling, since mTOR is responsible for the transcription of HIF1α and HIF2α, and mTOR itself is stimulated by the PI3K/MAPK pathway, downstream of insulin signaling. Furthermore, Ras signaling was found to be able to increase the expression of GLUT1 (Pylayeva-Gupta et al., 2011). Also, T2DM patients often are in a state of hyperglycemia, which can favor the high needs for glucose of cancer cells (Giovannucci et al., 2010). Finally, insulin stimulated translocation of GLUT4-containing vesicles to the plasma membrane might play a role. Together this suggests that hyperinsulinemia and hyperglycemia in diabetes patients can favor the deregulation of their cellular energetics, making them more vulnerable to develop cancer.

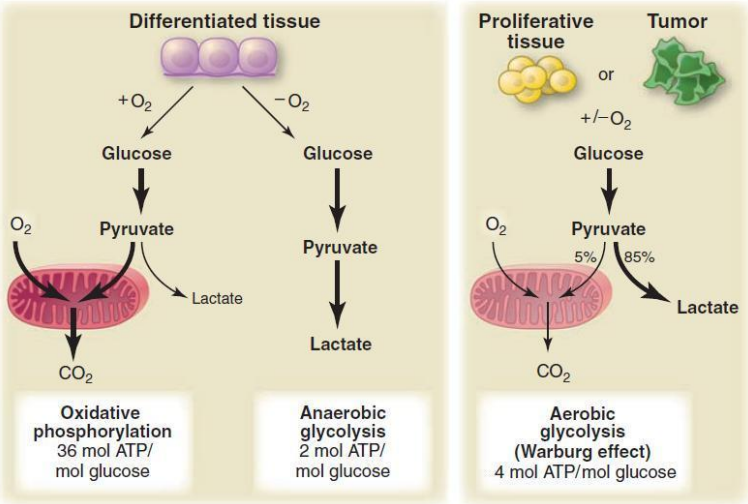

Figure 10: The Warburg effect. In differentiated tissue energy is normally gained via the citric acid cycle and oxidative phosphorylation in the mitochondria. Hereby large amounts of energy are produced. When there is no oxygen available, the cells use anaerobic glycolysis to produce lactate, this generates less energy. In proliferative and tumor tissue glucose is mainly used to produce lactate, despite there being enough oxygen. This is called aerobic glycolysis or the Warburg effect and can be beneficial for proliferative tissue because it produces more building blocks for the generation of new cells. (Vander Heiden et al., 2009)

Avoiding immune destruction

A well known theory describes that all cells are under constant monitoring of the immune system; in this way most cells that have developed certain dangerous mutations are eliminated before they can become a cancer cell. This means that cancer cells that do survive must have developed a way to evade destruction by the immune system (Hanahan and Weinberg, 2011).

Three essential phases have been proposed for the interaction between tumor cells and the immune system: elimination, equilibrium and escape. In the elimination phase cancerous cells are cleared by the immune system; in the equilibrium phase there is a balance between the production of cancerous cells and their elimination by the immune system; and in the escape phase the cancer cells become resistant to the immune system (Kim et al., 2007).

Ras signaling can influence avoidance of the immune system by reducing the activity and expression of *transporter associated with antigen processing 1/2* (TAP1/2), which are involved in antigen presentation by *major histocompatibility complexes* (MHC). This will result in a decreased recognition of the cancer cells by the immune system (Pylayeva-Gupta et al., 2011). Interestingly, polymorphisms in both the *TAP1* and *TAP2* genes have been shown to be associated with T1DM, due to failure to present antigens of the pancreatic β-cells, which can lead to autoimmune cell destruction, they major characteristic of T1DM (Qu et al., 2007; Yan et al., 1997). This could mean that T1DM patients with mutations in the *TAP1* or *TAP2* gene can have an induced risk to develop cancer due to facilitated avoidance of the immune system.

Evading growth suppressors

All cells of the body have protections to avoid extensive cell growth and proliferation. For a cancer cell, just stimulating proliferation is not enough; it must also evade the growth suppressors (Hanahan and Weinberg, 2011). The antigrowth factors can work via two mechanisms; they may induce a cell to enter the quiescent state, to avoid proliferation; or they can push the cell into a permanent postmitotic state (Hanahan and Weinberg, 2000).

The two most important anti growth factors are the tumor suppressors *retinoblastoma protein* (pRb) and p53. pRb is mainly stimulated by signals from outside the cell and decides whether or not a cell can pass the restriction point , while p53 is activated upon DNA damage and can induce cell senescence or apoptosis (Hanahan and Weinberg, 2011). In almost all human cancer cells either *pRB* or *p53* is mutated; this implicates the importance for a cancer cell to evade growth suppressors.

There does not seem to be an obvious link between insulin signaling and tumor promotion by evasion of growth suppressors. Therefore this hallmark of cancer will not be further considered as a possible cause of induced cancer risk in diabetes patients.

Enabling replicative immortality

Tumors often keep on growing unlimited. Normal cells can only undergo a limited amount of growth and division cycles, thereafter senescence will be initiated and if this is circumvented, cell crisis and ultimately cell death will follow (Hanahan and Weinberg, 2011). The protection against unlimited cell replication is mainly mediated by telomeres (Hanahan and Weinberg, 2000). Telomeres are structures at the end of all chromosomes, which prevent these ends to be recognized as double-strand DNA breaks. They consist of a repeated DNA sequence (TTAGGG), which ends in a T-loop. With every growth and division cycle the length of the telomeres becomes shorter, until they are too short and the cell will go into senescence (Weinberg, 2007). Eventually, all cancer cells must escape from senescence and prevent cell crisis. Herefore, they restore a small part of their telomeres. This can be done either by expression of the enzyme telomerase (as found in 90% of all cancer cells), which elongates the telomeric DNA or they can use the *alternative lengthening of telomeres* (ALT) mechanism (as found in the remaining 10% of the cancer cells), which works through recombination of DNA sequences between different chromosomes (Hanahan and Weinberg, 2000).

The restoration of telomeres does not seem to be connected in any way to the insulin signaling pathway and the induced risk for diabetes patients to develop cancer is therefore most likely not directly due to immortality of their cells.

The hallmarks of cancer provide insight as to how a normal cell can develop into a cancer cell and eventually become a macroscopic tumor. Many of these hallmark are in some way connect to the insulin signaling pathway and may therefore be responsible for an induced risk of diabetes patients to develop cancer when their insulin signaling is overactivated due to hyperinsulinemia. Insulin signaling can stimulate proliferation, anti-apoptotic signals, angiogenesis, invasion and metastasis, a deregulation of the cellular energetics and avoidance of the immune system.

Oncogene Ras has been mentioned multiple times in this chapter, however also other mutations concerning insulin signaling are often found in human cancer cells; Raf can be mutated to a constant

active form and as a consequent, the MAPK-pathway is activated; the catalytic subunit of PI3K can be mutated, which results in a hyperactivation of AKT; and PTEN can be mutated to an inactive form, PTEN is a negative regulator in the PI3K pathway (Hanahan and Weinberg, 2011). Also, overexpression of IRS1 was found in some cases of pancreatic cancer (Bergmann et al., 1996). In the next chapter a closer look will be taken at these pathways and at the role of insulin signaling in cancer formation with regard to diabetic patients.

Chapter 4: Diabetes and cancer risk

Diabetes and cancer

As mentioned in chapter 1, T2DM patients have a higher risk for developing certain types of cancer (El-Serag et al., 2006; Friberg et al., 2007; Larsson et al., 2007; Larsson et al., 2006; Larsson et al., 2005). Up until today many reviewing articles can be found that confirm this epidemiological relationship between diabetes and especially pancreatic, liver, colorectal, breast, endometrium and kidney cancer (Shikata et al., 2013; Strickler et al., 2001; Vigneri et al., 2009). Also, a reduced risk for the development of prostate cancer is widely accepted, this reduced risk can possibly be explained by the low testosterone levels in diabetic men (Giovannucci et al., 2010). Furthermore, diabetes patients seem to have an increased risk for not only the development of cancer, but also for mortality due to cancer (Coughlin et al., 2004; Larsson et al., 2007; Larsson et al., 2005). This mortality could be because the tumors in diabetes patients are more aggressive or it could be due to the often more unhealthy lifestyle of the patients rather than the diabetes itself, which could lead to favorable conditions for tumor progression (Vigneri et al., 2009).

The relative risks for T2DM patients to develop different types of cancer vary from 1.18 till 2.50 times the risk for non-diabetic persons. According to the International Diabetes Federation (IDF), in 2012 over 371 million people worldwide had diabetes, 90% of them have T2DM. With so many people suffering of this disease, even an at first sight small increase in cancer risk can, in fact, affect many people (Vigneri et al., 2009), therefore it is important to understand how these cancer risks are induced, so it can be taken in account for future therapeutic tactics.

T1DM is associated far less with an induced cancer risk, still some evidence directs to an induced risk for stomach, cervix and endometrial cancer. However these findings are questionable, because little account was taken for shared risk factors and possibly some of the patients were classified as T1DM while they actually suffer of T2DM (Zendehdel et al., 2003).

The relationship between T2DM and cancer formation is not only found by case-control and cohort studies with patients, but also some experimental results with model organisms give this confirmation. One example is an experiment done with the MKR mouse model (a diabetic mouse created by a dominant negative *IGF1 receptor* (IGF1R) mutation expressed in the muscles exclusively, (Fernandez et al., 2001), which is insulin resistant, glucose intolerant and hyperinsulinemic. These mice were shown to have an induced risk to develop breast cancer over non-diabetic mice. This risk was correlated with an enhanced phosphorylation of the IR, the IGF1R and AKT, indicating an overactivation of the PI3K/AKT pathway (Novosyadlyy et al., 2010). However, experimental data explaining the molecular causes of an induced cancer risk in diabetic patients is limited and often based on just specific characteristics of the disease without looking at the overall picture. This results in confusing and seemingly contradicting stories. In this chapter a clear overview will be given of the mechanisms by which diabetes can lead to an enhanced risk of cancer development.

Hyperinsulinemia

It is long known that insulin can affect cancer progression and in chapter 3 it was shown that the insulin pathway has multiple oncogenic factors which are often overactivated in cancer cells. In 1972, an experiment with insulin deficient rats (made with alloxan, a toxic glucose analogue that kills pancreatic β-cells), showed that tumor formation regressed in 90% of the mammary carcinomas. The 10% of the tumors that did not show any regression were found to be insulin independent (Heuson and Legros, 1972). Also, by the same researchers it was found that insulin administration in rats enhances tumor growth of mammary carcinoma's (Heuson et al., 1972). Together these results show that some tumors are insulin dependent, maybe for their growth and development, but at least for their glucose uptake. These tumors can thus not grow in insulin deficient states; this phenomenon was also found in several cancer cell lines (Frasca et al., 2008).

A cohort study in non-diabetic women showed that hyperinsulinemia can indeed result in a higher risk to develop breast cancer (Gunter et al., 2009). Hyperinsulinemia can be caused by compensation for insulin resistance in T2DM and by insulin administration in T1DM; it can affect the insulin pathways in several ways. Firstly, it is important to understand that hyperinsulinemia can lead to abnormal distributions of insulin through the body and different tissue types can react differently to high levels of insulin. Secondly, when insulin is available in high concentrations, it can bind to not only its own receptor, but also the IGF-IR, consequently activating its cellular pathways. Thirdly, hyperinsulinemia was found to change the expression levels of the insulin receptor, favoring expression of the IR-A isoform (Vigneri et al., 2009). These three important findings can all lead to an enhanced risk for cancer development; however the influence of insulin depends greatly on the cancer type (Piatkiewicz and Czech, 2011). Each of these three findings will be explained separately below.

Interestingly, high insulin concentrations can additionally increase androgen levels (Giovannucci et al., 2010) and induce DNA damage by enhancing ROS production (Othman et al., 2013), both could result in an induced cancer risk, however in this review we will focus on the more direct effects of insulin.

Hyperinsulinemia and insulin administration can lead to abnormal insulin distribution

T1DM patients are insulin deficient and are therefore dependent on insulin administration to survive. However, insulin administration cannot mimic natural conditions, since the insulin is injected into the blood stream and will arrive throughout the body in high concentrations. In normal situations insulin is secreted by the pancreas and reaches the liver first, there most of the insulin is administered or degraded. This means that, after insulin injection most tissues are under higher levels of insulin than they will ever be in normal situations (two till five times higher) (Vigneri et al., 2009).

T2DM patients are exposed to high levels of insulin for a period that can last several years before they become insulin deficient. During this time, like in the normal situation, the liver is the first tissue to receive the insulin. However due to sometimes extremely high insulin production, also the rest of the body is exposed to elevated levels of insulin (Vigneri et al., 2009). Later in the development of the disease the pancreatic β-cells start to fail and the patient might need exogenous insulin administration like in T1DM (Prentki and Nolan, 2006).

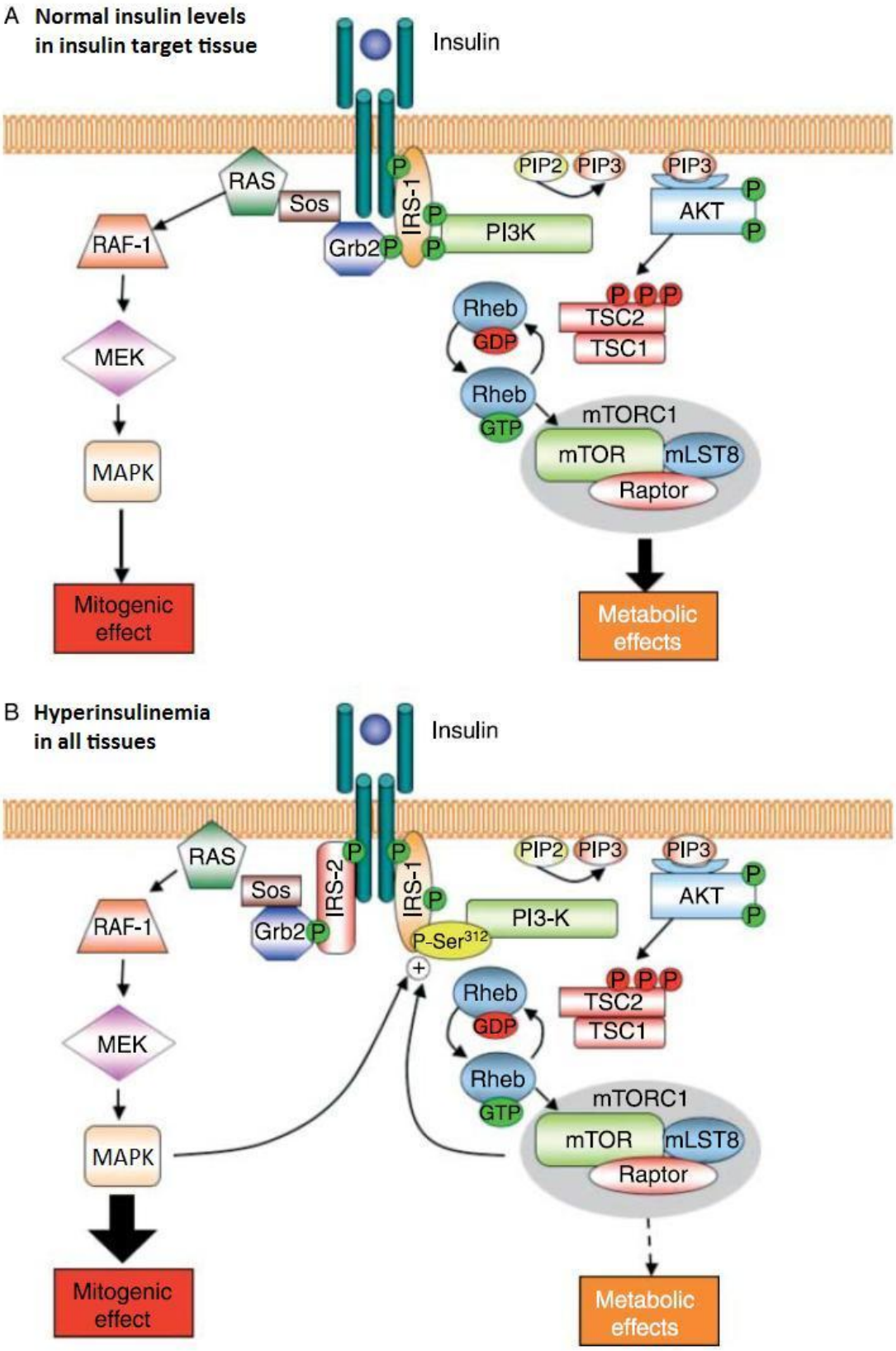

Figure 11: Hyperinsulinemia can result in a reduction of metabolic effects and an induction of mitogenic effect. A) In a normal insulin situation, insulin target tissue mainly reacts to insulin binding via activation of IRS1 and consequently the PI3K/AKT pathway which will result in metabolic effects. In smaller amounts, also the mitogenic Ras/MAPK pathway is stimulated via Grg2 recruitment. **B)** During hyperinsulinemia, the PI3K/AKT pathway is attenuated by IRS1 phosphorylation on its 312 serine residue, this results in reduced metabolic effects. However, IRS2 is not attenuated during hyperinsulinemia. As a result IRS2 can become overactivated and consequently the Ras/MAPK pathway is induced, leading to enhanced mitogenic effects. Especially in non-insulin target tissues this can result in cancer formation, since these tissue are not adapted to high insulin concentrations. (**Vigneri 2009**)

In chapter 2 it was discussed that expression of both the IR isoforms and IRS family members can vary between different tissues and that they can favor different reactions after insulin binding. IR-A is mainly found in fetal tissue, in the central nervous system and in hematopoietic cells while IR-B is mainly found in insulin responsive tissue like the liver, muscles and adipose tissue (Taniguchi et al., 2006). Activation of the IR-A by insulin or IGF2 binding favors proliferative and cell survival effects, while activation of IR-B mainly results in metabolic effects (Sciacca et al., 2003). For the IRS family was explained that IRS1 preferentially stimulates the PI3K/AKT pathway while IRS2 mostly activates the Ras/MAPK pathway (Huang et al., 2005). Also, it was described that insulin resistance is often caused by a decreased activity of IRS1 (Sesti et al., 2001; Tanti et al., 1994). Together this results in the knowledge that, under normal circumstances, insulin binding in its target tissue will lead to the activation of IRS1 and consequently the PI3K/AKT pathway, resulting in mostly metabolic effects like glucose uptake and glycogen synthesis (figure 11a). In the situation of hyperinsulinemia, due to either insulin resistance or insulin administration, IRS1 activity is respectively inhibited or attenuated via phosphorylation of the its serine 312 residue. Consequently the activation of the PI3K/AKT pathway goes down (Vigneri et al., 2009).

From these findings we can conclude that insulin resistance might reduce the responsiveness of insulin target tissue to insulin, but does not attenuate overactivation of the Ras/MAPK pathway due to hyperinsulinemia in other tissues. Especially in combination with specific gene mutations in cells that are now under higher insulin concentrations than normally, this can lead to cancer promoting conditions. For example, loss of PTEN would in most cells not have great consequences since its function to attenuate the PI3K/AKT pathway is not needed when this pathway is not activated. However, in a situation of hyperinsulinemia loss of PTEN, as found in many tumor cells (Hanahan and Weinberg, 2011), can be critical since the overactivation of PIP_3 will not be attenuated leading in turn to an overactivation of AKT. In this way hyperinsulinemia can contribute significantly in the process of a normal cell turning into a cancer cell.

Hyperinsulinemia favors Ras/MAPK activation via IGF1R

Insulin cannot only activate its own receptor, but is involved in a complex interplay between insulin, IGF1, IGF2, IR-A, IR-B, IGF1R and *IGF binding protein 1-6* (IGFBP1-6). As a result, insulin can bind to IGF1R if it this present in high concentrations (Piatkiewicz and Czech, 2011), just like IGF2 can bind to IR-A (Sciacca et al., 2003), as mentioned in chapter 2. Stimulation of IGF1R also results in activation of the Ras/MAPK and PI3K/AKT pathways in a similar way as insulin signaling (Frasca et al., 2008), only favoring proliferative effects over stimulation of glucose metabolism (Frasca et al., 1999). The fact that some cancer cell lines are dependent on insulin for cell growth has been attributed to the overactivation of the IGR1R as a result of hyperinsulinemia (Frasca et al., 2008).

Hyperinsulinemia can also enhance the activity of *growth hormone* (GH), which is among other functions responsible for the stimulation of IGF1 production in the liver (Braun et al., 2011). And hyperinsulinemia can decrease the blood and tissue levels of IGFBP1 and IGFBP2, these binding proteins attenuate the actions of IGF1 by preventing its receptor binding. Insulin can thus increase the levels of free IGF1 (Inoue and Tsugane, 2012).

Together this indicates that hyperinsulinemia can lead to an induced cancer risk via upregulation of the IGF-pathway either by activating the IGF-1R directly, by indirectly inducing IGF1 expression or by increasing the levels of free IGF1.

Hyperinsulinemia changes IR expression levels

As a reaction to insulin resistance, many cells start to express more insulin receptors (Vigneri et al., 2009). As mentioned earlier, the IR-A isoform mainly activates the Ras/MAPK pathway and thus the proliferative and cell survival effects of insulin signaling (Sciacca et al., 2003). Also, previous research has shown that this pathway is not influenced by insulin resistance (Cusi et al., 2000; Jiang et al., 1999). Interestingly, 50% of all cancer cells overexpress the IR (Piatkiewicz and Czech, 2011), especially the IR-A isoform (Giovannucci et al., 2010; Papa et al., 1990). This suggests an important role for the IR in tumor formation (Frasca et al., 2008).This could also be a result of IR-A activation by IGF2, since it has a high affinity for this growth factor (Frasca et al., 1999).

Taken all together this means that hyperinsulinemia, as found in diabetic patients, can lead to an induced risk to develop cancer via overactivation of the Ras/MAPK pathway, via stimulation of the IGF-pathway and via IR overexpression.

Hyperglycemia and inflammation

Diabetes can not only favor cancer formation via hyperinsulinemia, but also via other characteristics of the disease, most importantly hyperglycemia and chronic inflammation (Giovannucci et al., 2010).

As described as one of the hallmarks of cancer in chapter 3, cancer cells have to change their metabolism to favor production of building blocks for new cells over energy production. Therefore they reduce their oxidative phosphorylation and switch to aerobic glycolysis; this is called the Warburg effect (Vander Heiden et al., 2009). For this process cancer cells need to increase their glucose uptake, this is often done by induced expression of GLUT1 (Piatkiewicz and Czech, 2011) and seems to be insulin independent (Giovannucci et al., 2010). Hyperglycemia is a characteristic of both T1DM and T2DM due to reduced glucose metabolism (Taylor, 1999) and induced glucagon production (Silverthorn et al., 2009). High blood glucose levels facilitate an increase of glucose uptake by cancer cells and can thus be considered as cancer progressive. Thus, in this case hyperglycemia rather than hyperinsulinemia seems to be an important risk factor for cancer development in diabetes patients.

One of the enabling hallmarks of cancer is tumor-promoting inflammation which recruits growth, proliferative, survival and angiogenesis promoting factors and can cause DNA mutations by the release of ROS (Hanahan and Weinberg, 2011).Chronic inflammation is often found in diabetes patients as a result of pancreatic β-cell death (Mathis et al., 2001). This means diabetes can provide a tumor-promoting environment through inflammation, this might especially account for the pancreas, which is one of the cancer types with an induced relative risk in T2DM patients.

Diabetes therapy

A very important and also disturbing risk factor for cancer formation in diabetes patients is their therapy. As mentioned earlier, insulin administration can lead to hyperinsulinemia and an abnormal distribution of insulin concentrations. Multiple experiments can confirm the induced cancer risk after exogenous insulin administration. For example, rats treated with Ultralente bovine insulin (an insulin suspension containing zinc to extend its active half life) injections had a significantly higher

change to enhanced colon carcinogenesis (Corpet et al., 1997). Also patients using insulin glargine (a long-working insulin analogue) were shown to have an increased risk to develop pancreatic cancer (Colmers et al., 2012). Furthermore, T2DM patients treated with Sulphonylurea (a drug that increase insulin secretion by the pancreatic β-cells, (Anisimov and Bartke, 2013)) show an increased risk for cancer mortality compared to patients treated with Metformin (Bowker et al., 2006). However, this study does not tell whether this difference is due to a negative effect of Sulphonylurea or to a positive effect of Metformin (Monami et al., 2009).

Metformin does indeed seem to reduce the risk for cancer development. It is a widely used medicine for T2DM patients which works by inducing *AMP activated protein kinase* (AMPK) activity (Evans et al., 2005). AMPK activation mainly works on key metabolic tissues like the muscles, the liver and adipose tissue, where it mimics the situation of muscle exercise which results in higher insulin sensitivity and glucose uptake to lower the blood glucose levels and can thereby reduce cancer progression (Zhang et al., 2009). This is in line with the finding that insulin-sensitizing treatment of mice with mammary tumors due to T2DM stops the tumor progression (Fierz et al., 2010). Furthermore, Metformin can inhibit mTOR independently of insulin (Anisimov and Bartke, 2013), resulting in reduced protein production and consequently less cell growth and proliferation (Braun et al., 2011).

These results tell us that diabetes treatment must be approached with care, since it can easily influence the cancer risk of the patient. The positive effects of Metformin are promising for both diabetes treatment and reduction of cancer risk. Future treatments could possibly be searched in the same direction, working on insulin target tissue specifically instead of the whole body and thereby reducing the risk to overstimulate non-insulin resistant tissues.

Diabetes can lead to an induced risk to develop cancer or to provide tumor-progressive environments. The most important causations are hyperinsulinemia, hyperglycemia, inflammation and diabetes treatment. To better understand these factors it is very important that more specific research is done to the molecular mechanisms behind the induced cancer risk. Only then anti-diabetic therapies can be developed that do not by accident induce cancer risk.

Discussion

In this thesis the role of insulin signaling and diabetes on cancer risk was reviewed. Although the existence of an induced risk to develop cancer for diabetic patient is widely accepted, there are still many questions unanswered. Firstly, observational research that is done concerning diabetic patients is limited by several factors: control groups can be polluted by undiagnosed diabetes patients; there is often a poor discrimination between T1DM and T2DM patients; and independence of commonly shared risk factors for diabetes and cancer is not always noted. Secondly, research to the molecular origin of an induced cancer risk for diabetes patients is very scarce; this is very problematic since diabetes therapy sometimes results in an even higher risk for cancer development. This can be prevented if we better understand the link between diabetes, insulin signaling, glucose metabolism and cancer. These two attention points will be further discussed below.

Limitations in the patient database

An estimate percentage of 2,7% of all Americans have diabetes without being diagnosed (Harris et al., 1998), this quit a high percentage, which means that it is highly likely that in most studies the control group, classified as non-diabetic, do contain undiagnosed diabetic patients. This could heighten the average cancer risk in the control group and unjustly reduce the difference between control and patients (Vigneri et al., 2009). Also, many studies do not or poorly distinguish between T1DM and T2DM, even though the two diseases are quite different in their characteristics. Furthermore most studies do not look at the phase of T2DM the patients are in; this makes big differences since the symptoms vary from hyperinsulinemia to insulin deficiency (Vigneri et al., 2009). These limitations make it important that we interpret relative risks with caution.

Additionally, in the case of pancreatic cancer, reversed causality can be unnoticed; this means that a higher cancer risk is said to be caused by diabetes while it is actually the other way around. Pancreatic cancer could lead to β-cell failure and consequently to diabetes (Giovannucci et al., 2010). It should be clarified which is the cause and which is a result before the patients are classified as a diabetics that developed pancreatic cancer.

What deserves maybe even the most attention are the common risk factors of diabetes and cancer. Many risk factors that can increase the chance to develop T2DM are also risk factor to develop cancer. They make it hard to see the cause of an induced cancer risk; is it the diabetes itself, or the thing that causes the diabetes? The most important of these common risk factors are obesity, age, insulin blood levels, physical activity, smoking, alcohol consumption and diet (Giovannucci et al., 2010; Inoue and Tsugane, 2012). Especially obesity is very interesting in this dilemma, because it is one of the most important risk factors causing insulin resistance and later T2DM; of course obesity is directly linked to some of the other risk factors like diet, physical activity, age and insulin blood levels. The link between obesity, T2DM and cancer development will be explained below.

Obesity can lead to insulin resistance and thereby to T2DM and cancer development

Excess body weight is characterized by an increased amount of adipose tissue. This will result in a higher production of *free fatty acids* (FFA), *tumor necrosis factor-α* (TNFα) and resistin and a reduced production of adiponectin (Calle and Kaaks, 2004). Increased FFA and TNFα can both induce insulin resistance. FFA do this via the activation of the kinases *protein kinase C-ϑ* (PKC-θ), *c-jun N-terminal kinase* (JNK) and *IκB kinase* (IKK) (Capurso and Capurso, 2012), which can all phosphorylate IRS on its 307 (and 1101 for PKCθ) serine residue, thereby inhibiting it (figure 7) (Taniguchi et al., 2006), this can lead to insulin resistance. TNFα can cause IRS inhibition by both tyrosine-dephosphorylation and serine phosphorylation (Capurso and Capurso, 2012). Compensation of the insulin resistance leads to hyperinsulinemia and, as known, this can lead to induced insulin signaling as well as decreased IGFBP1 and -2 levels. Consequently, the availability of free IGF1 rises, which leads to even more stimulation of the Ras/MAPK pathway and thus its tumor promoting effects (figure 12) (Calle and Kaaks, 2004).

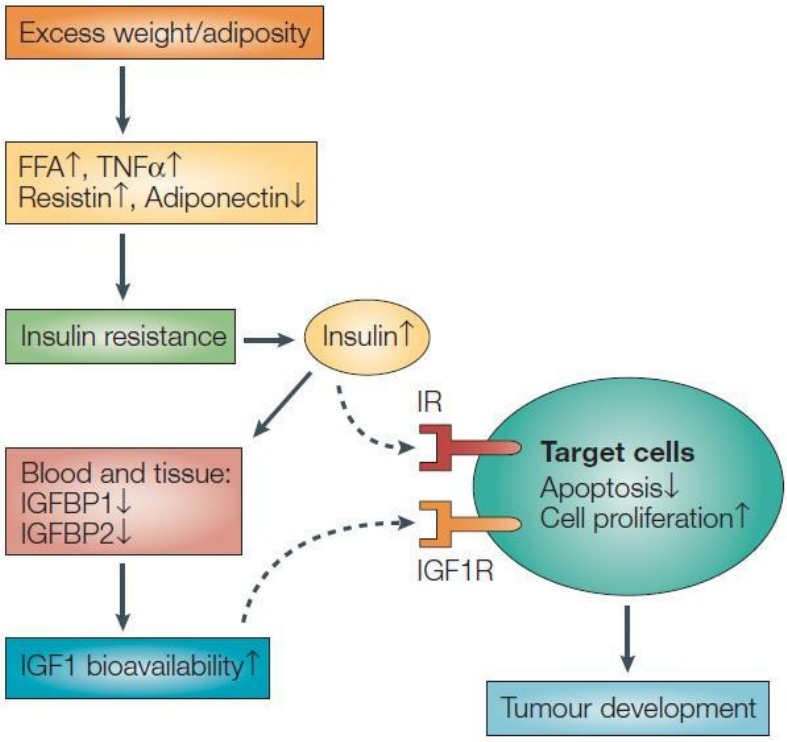

Figure 12: Obesity can lead to hyperinsulinemia and consequently to tumor development. Excess body weight and induced amounts of adipose tissue can lead to an induced release of FFA, TNFα and resistin and a reduced release of adiponectin. FFA and TNFα can cause insulin resistance by inhibition of IRS. To compensate, the body starts to produce more insulin, which in turn reduces the levels of IGFBP1 and -2, leading to more free IGF1. High levels of insulin and IGF1 can increase insulin and IGF1 signaling, both leading to, among others, reduced apoptosis and increased proliferation and thus favorable conditions for tumor development. (Calle and Kaaks, 2004)

It is very important to understand that the scheme of figure 12, initialized by excess body weight is almost exactly the same for T2DM. This means that it is hard to make sure that an increased cancer risk in T2DM patients is indeed caused by the diabetes or if it is a result of obesity. It is therefore important that cohort and case control studies with T2DM patients pay good attention to the physical conditions of the patients and include this in the data analysis.

Limited molecular research

Up untill today the amount of molecular research done on the relationship between diabetes, insulin and cancer risk is limited. There is much known about the three individual players, but the combination is far from completely understood. Aditionally most research is done in cell lines, however both diabetes and cancer are systematic diseases, it is therefore important that they are studied in model organisms to understand the complete picture.

Since both diabetes and cancer are growing problems in the modern world with millons of people affected by the diseases, it deserves our attention. Especially disturbing is the increased risk to develop cancer for diabetes patients that are treated by exogenous insulin administration. Such risks due to therapy can be evaded when we understand better the underlying mechanisms of diabetes on insulin and IGF signaling and, in turn, their signaling on cancer development and progression.

Furthermore, little is known about T1DM and cancer risk. Case control studies explicitly for T1DM are very rare. It would be interesting to know of these patients have a decreased risk for cancer development because they are insulin deficient. However, it would be hard to examine this because they are dependent on insulin administration to survive; experiments with type 1 diabetic mice might be an interesting option.

Since T1DM almost always has a genetic bakground, it would be interesting to see if any genes mutations causing T1DM can also lead to an increased risk to develop cancer. For example, as mentioned in chapter 3, Ras oncogene reduces the expression of TAP1 and TAP2 to reduce antigen recognition by the immune system, thereby promoting tumor progression (Pylayeva-Gupta et al., 2011). TAP1 and TAP2 gene mutations are also associated with T1DM. These mutations lead to polymorphisms in the gene which can result in different antigen presentations. In pancreatic β-cells this can lead to failure to be recognized by the immune system as cells of the body and therefore result in autoimmune destruction (Qu et al., 2007; Yan et al., 1997). Since mutations in the *TAP1* and TAP2 genes are found in both cancer cells and in T1DM patients this could mean that there is a relationship between these two. However, they do represent different kinds of mutations, leading to either reduced expression or polymorphisms, which means that they are not directly comparable. Still, these and other T1DM gene mutations can be interesting for future research.

Diabetes, insulin and cancer treatment

Taken all together, this review has showed that there is a clear link between the metabolic disorder diabetes mellitus and the risk to develop cancer. We now know that an induced insulin signaling due to more insulin production or to insulin administration as a therapy can lead to overactivation of its cellular pathways, leading to an induced change to develop cancer. This tells us to be careful with the treatment of diabetes with regard to cancer risk; however we can also use this knowledge the other way around. Insulin signaling could be an interesting target for cancer therapy, since mutations in this cellular pathway are often found in tumor cells.

Therapy against the insulin signaling pathway should be approached with care, since insulin is critical for glucose metabolism. Targeting the IR-A receptor might be an effective approach because this receptor isoform mainly activates non-metabolic processes and is overexpressed in many cancer cells (Giovannucci et al., 2010; Papa et al., 1990). This strategy is under development, but up till today no suitable way has been found to only block the IR-A receptor (Belfiore and Malaguarnera, 2011).

Another approach that is being investigated is the blockage of IGF1 and IGF2 with antibodies (Feng et al., 2006; Miyamoto et al., 2005), this could be effective since IGF1 and IGF2 are known to activate the insulin signaling pathway via the IR-A receptor (Sciacca et al., 2003). This strategy seems to be very promising in selective targeting of the IGF-1R and IR-A pathways (Gao et al., 2011). Earlier approaches blocking IGF-1R specifically were not very effective when used as a single treatment and could only give good results when used in combination with other treatments (Belfiore and Malaguarnera, 2011; Hofmann and Garcia-Echeverria, 2005). Together this shows us that the insulin signaling pathway is a potent target for cancer therapy and already much research is being done to try and put this idea into practice.

Conclusion

The metabolic disorder diabetes mellitus exists in two types: t*ype 1 diabetes mellitus* (T1DM) and *type 2 diabetes mellitus* (T2DM). In T1DM, the pancreas cannot produce insulin and therefore the patients are dependent on treatment to survive; insulin administration is a very common therapy. However, this can lead to an abnormal, evenly distributed insulin concentration throughout the body; for tissues that normally receive low amount of insulin this can lead to hyperinsulinemia (Vigneri et al., 2009). In T2DM, an unhealthy life style, obesity or certain gene mutations can lead to insulin resistance. To compensate for this, the pancreatic β-cells start to produce more insulin, leading to hyperinsulinemia. Eventually the β-cells can undergo failure and die (Eizirik et al., 2008), the patient will now be insulin deficient like T1DM patients and can become dependent on insulin administration.

Changes in insulin concentrations and distribution in diabetes patients can have important effects on molecular insulin signaling pathways. Insulin signaling consists of two main routes: the PI3K/AKT pathway and the Ras/MAPK pathway. The PI3K/AKT pathway leads mainly to glucose metabolism, but can also inhibit apoptotic factor, DNA repair and oxidative stress resistance and can stimulate cell progression and proliferation (Taguchi and White, 2008; Taniguchi et al., 2006). The Ras/MAPK pathway is a well know oncogenic pathway resulting in gene expression of factors involved in proliferation, differentiation, cell growth, cell survival, cell motility and angiogenesis. All these effects are tumor progressive and an therefore induce the risk to develop cancer (Pylayeva-Gupta et al., 2011). Tumor cells are often found to have a mutation in components of insulin signaling.

T2DM patients are insulin resistant, but this does not affect all tissues and also the Ras/MAPK pathway is not affected by insulin resistance. Their state of hyperinsulinemia can result in cancer progression via three ways: overactivation of the Ras/MAPK pathway in non-insulin target tissues, cross activation of the IGF pathway and overexpression of the IR, mainly the IR-A isoform (Vigneri et al., 2009).

Taken all together, it is clear that diabetes patients are at higher risk to develop certain kinds of cancer. This is often due to characteristic of the disease like hyperinsulinemia, hyperglycemia and chronic inflammation. Together this can lead to overactivation of insulin signaling pathways, inducing the change for more proliferation, anti-apoptotic signals, angiogenesis, invasion and metastasis, deregulation of the cellular energetics and avoidance of the immune system. All these effects can contribute to cancer development.

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