Insulin Resistance and Hepatocarcinogegesis

Yutaka Sasaki

Department of Gastroenterology & Hepatology

Graduate School of Medical Sciences

Kumamoto University

1-1-1　Honjo, Kumaoto City, Kumamoto 860-8556, JAPAN

Tel; 81-96-373-5146

Fax; 81-96-371-0582

E-mail; sasakiy@kumamoto-u.ac.jp

Summary

Hepatocellular carcinoma (HCC) accounts for 85-90% of liver cancers and one of the most frequent carcinoma in the world. HCCs classically develop against the background of chronic liver diseases. Common causes of such liver diseases are viral hepatitis, alcoholic hepatitis, or immune-related diseases. However, 15-50% of patients with HCCs have none of these classic antecedents, especially in developed countries. In this context, obesity and diabetes mellitus have been found to exhibit an increased risk of HCC. Both conditions are associated with insulin resistance. The tumorigenic effects of insulin resistance and complementary hyperinsulinemia could be mediated directly by insulin signaling, or indirectly related to changes in endogeneous hormone metabolism, particularly IGF-1. Conversely, insulin resistance may be a consequence of obesity and hepatic inflammation, both of which can themselves promote tumorigenesis, mainly through cytokine production and /or generation of oxidative stress.

Because the prevalence of obesity is now increasing throughout the world, insulin resistance is sure to be put more forth as a central factor for hepatocarcinogenesis in the foreseeable future,

Key Words: insulin resistance, obesity, insulin, IGF-1, oxidative stress, HCC

Introduction

Primary liver cancer is the 5th most common malignancy worldwide, and the third leading cause of cancer-related death, exceeded only by lung and stomach cancer (1). Hepatocellular carcinoma (HCC) accounts for between 85-90% of these primary liver cancers. The estimated incidence of new cases is approximately 0.5-1 million per year, causing 0.6 million deaths per year in the world (2). Importantly, difference of HCC burden has been noted among countries and /or regions; more than 80% of HCC cases

occur in developing countries, particularly in either Africa or in Eastern Asia. Especially, China alone provides more than 50% of the whole world' cases.

However, the incidence of HCC in these areas is now decreasing. Conversely, the incidence has been recently rising in developed countries. Indeed, there has been an increase about 80% in the annual incidence during the past two decades in the United States **(**3).

HCCs develop against the background of chronic liver diseases. Common causes of such diseases are viral hepatitis (HBV or HCV), alcoholic hepatitis, or immune-related diseases (primary biliary cirrhosis and autoimmune hepatitis). However, 15-50% of patients with HCCs have none of these classic antecedents, especially in developed countries (4).

Emerging evidences suggest metabolic factor as a risk factor for HCC in the developed countries. In this context, prevalence of obesity has been increasing rapidly in the world. Epidemiological studies clearly indicate association of obesity with development of a variety of cancers, although the mechanisms whereby obesity induces or promotes tumorigenesis vary among cancers. Indeed, obesity and diabetes mellitus have been found to be associated with an increased risk of HCC in several epidemiologic studies. Large population studies have reported that obesity increases the risk of development and death due to HCC by 2-5 times (5-9).
In addition, a number of case-control and cohort studies have linked diabetes to a 2-fold increased risk of HCC (10-13).

With the increasing prevalence of obesity and diabetes, it is important to elucidate the complex relationship between these 2 factors as well as other metabolic factors and the risk of HCC.

Obesity and Insulin resistance

It has been clarified that adipose tissue constitutes an endocrine and metabolic

organ that can exert a wide range of physiological effects (14). In response to the signals from other tissues, adipose tissue responds by increase or decrease in the release of free fatty acid (FFA). In addition, adipose tissue is involved in energy balance and lipid metabolism in terms of releasing adipocytokines. Obesity is associated with increased release of FFA and multiple pro-inflammatory cytokines including TNF α, leptin, IL-6, resistin, reduced release of adiponectin, an anti-inflammatory polypeptide, from adipose tissue, and gives rise to insulin resistance. These processes will develop hepatic steatosis and inflammation in the liver (15).

Insulin resistance is defined as a situation of reduced sensitivity to insulin in insulin-responsive tissues, and its main consequences include an impaired ability of insulin to suppress hepatic glucose production and stimulate peripheral glucose elimination. Providing that β-cell function is preserved, insulin secretion increases to overcome insulin resistance, and glucose levels are normalized. Thus, the resulting compensatory hyperinsulinaemia is a hallmark of insulin resistance. Chronic hyperinsulinemia has been associated with a variety of cancers (16-20). Evidence has emerged that chronic insulin therapy significantly increases the risk of colorectal cancer among type 2 diabetes mellitus patients. In addition, cancer risk increased with increasing duration of insulin therapy (21). These clinical observations will highlight the potential importance of insulin as a cancer risk factor.

It is important to note that promotion of cancer by insulin resistance needs to be discriminated from promotion of cancer by the conditions that coincidently induce insulin resistance.

How does insulin resistance participate in hepatocarcinogenesis?

The tumorigenic effects of insulin resistance could be mediated directly by insulin signaling, or indirectly related to changes in endogeneous hormone metabolism, secondary to hyperinsulinemia.

1) Direct action of insulin on growth promotion via classical insulin signaling

Insulin exerts its action upon the binding to its specific receptor with tyrosine kinase activity. In turn, the activated insulin receptor promotes the phosphorylation of IRS-1 (insulin receptor substrate-1) and transmission of the insulin signal through two major phosphorylation cascades: PI3K (phosphoinositide 3-kinase) and MAPK (mitogen-activated protein kinase) cascades **(Figure 1)**. PI3K cascade induces translocation of the serine/threonine protein kinase Akt, from cytoplasm to the cell membrane, where Akt stimulates the phosphorylation and consequent inhibition or activation of a variety of proteins involving cell growth, division, survival as well as lipid and carbohydrate metabolisms, for example, the pro-apoptotic Bcl-2 family member BAD and the growth-related mTOR.

The role of PI3K cascade for cell proliferation and survival has been clarified by the high prevalence in a variety of cancers with a loss-of-function of PTEN (phosphatase and tensin homologue deleted on chromosome 10), which enhances PI3K cascade (22).

On the other hand, phosphorylated IRS-1 can also mediate the formation of a complex between the adaptor protein Grb2 (growth factor-receptor-bound protein 2) and the guanine nucleotide-exchange factor mSos (mammalian Son of sevenless). The Grb2–mSos complex can then activate p21Ras. In turn, activated p21Ras induces the activation of MAPK cascade, leading to activation of transcription factors involved in cell proliferation. Importance of the Ras/MAPK cascade for cell proliferation is supported by the finding that the prevalence of p21Ras over-expression has been reported to be quite high in a variety of cancers (23).

Although *in vitro* studies exhibit that insulin can promote cell proliferation, supra-physiological insulin concentrations have been used in these studies. Thus, it remains obscure whether insulin can exert any growth-promoting effect or not.

On the other hand, insulin can induce other changes to amplify growth-promoting properties. In this regard, chronic hypeinsulinemia increases the intracellular content of

farnesylated p21Ras and loading of p21Ras with GTP, which may amplify growth factor signaling through MAPK cascade (24).

Hepatocarcinogenesis is a multi-factorial multistage process, and one of these stages can include over-expression of insulin signal components. In this regard, IRS-1 has been shown to be up-regulated in HCC (25, 26). Thus, the compensatory hyperinsulinemia in insulin resistance might provide an additional stimulation to cell proliferation and survival in the conditions where insulin signaling has already been rendered hypersensitive by over-expression of insulin signal components.

2) Indirect action of insulin on growth promotion

Previous studies have described that insulin and IGF-1 act as growth factors, leading to the cell proliferation and inhibition of apoptosis (27). It has been clarified that hyperinsulinemia can promote the synthesis and biological activity of insulin-like growth factor I (IGF-1)(28). Liver is the source of over 80 percent of circulating IGF-1, and the principal stimulus for IGF-1 synthesis in the liver is provided by GH signaling. Insulin can up-regulate human hepatic GH (growth hormone) receptors (29). Hyperinsulinemia, thus would produce and release the large amount of IGF-1 from the liver. Indeed, in patients with type II diabetes, hyperinsulinemia accompanied by up-regulation of hepatic GH-receptor, enhances IGF-1 production.

IGF-1 signaling via the IGF-1R has effects on cell proliferation and survival, which is obviously stronger than those of insulin (30). IGF-1 can act as a potent growth factor for cancer cells both *in vivo* (31) and *in vitro* (32), and *in vivo* over-expression of IGF-1 can promote tumor formation (33). Conversely, its down- regulation can inhibit tumorigenesis (34). Thus, the role of IGF system and IGF-1 receptor (IGF-1R) signaling has been emphasized in tumorigenesis. Epidemiological evidences have described elevated circulating IGF-1 levels in the development of a variety of cancers, including colorectal, prostate and breast cancers, and HCC (35-37).

In addition, hyperinsulinemia could enhance IGF-1activity by means of modulating

the availability of IGF binding proteins (IGFBPs). Over 80% of IGF-1 in the circulation is bound to IGFBP3, while the remainder of IGF-1 is bound to at least five IGFBPs. The actions of IGFBPs vary among their subtypes. Because IGFBP3, having anti-tumorigenic property, is up-regulated by GH signaling, hyperinsulinemia is often associated with higher levels of IGFBP3. Conversely, IGFBP1 and IGFBP2, which play an important role in regulating IGF-1 bioactivity, are suppressed by insulin. Hyperinsulinemia, thus reduces liver synthesis and blood levels of IGFBP1 and IGFBP2, leading to increase in bio-available IGF-1. In accordance with this, an inverse relationship has been reported between cancer risk and blood levels of IGFBP1 and IGFBP2 (38)

Since considerable homology has been identified between insulin receptor and IGF-1 receptor, insulin can bind to IGF-1 receptor and enhance IGF-1 signaling. Provided that signaling through IGF-1 receptor is more tightly linked to growth promo tion than that through insulin receptor, enhancement of IGF-1 receptor-mediate signaling by insulin would contribute much to cell proliferation and survival **(Figure 2)**.

Obesity and Inflammation may promote hepatocarcinogenesis independently of their effects on insulin resistance

As described above, insulin resistance and its complementary hyperinsulinemia can promote hepatocarcinogenesis, and such effect can interact with other growth-promoting signals.

Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions that can themselves promote tumorigenesis by other pathways. In this context, two key conditions have been clarified; obesity and inflammation in the liver. Both of which can induce insulin resistance and both of which may induce the initiation and promotion of hepatocarcinogenesis, independently of their effects on insulin resistance.

1) **Obesity changes adipocytokine levels, leading to cell proliferation and survival**

In obesity, adipose tissues increased release a variety of adipocytokines, including TNF α, IL-6, both of which have pro-oncogenic effects, leading to insulin resistance. Obesity is also associated with leptin resistance and hyperleptinemia. Leptin has been clarified to have pro-oncogenic effects and enhance proliferation and angiogenesis (39). In addition, obesity is associated with reduced levels of anti-inflammatory pro-apoptotic adiponectin. Adiponectin has anti-proliferation effect through activation of AMPK (AMP-activated protein kinase), and is inversely associated with insulin resistance. Taken these findings together, changes in adipocytokine levels associated with obesity would lead to cell proliferation and survival, independently of the effect on insulin resistance.

2) **Hepatic steatosis induced by insulin resistance generates reactive oxygen species (ROS), accompanied by cytokine production**

Obesity is often associated with hepatic steatosis through insulin resistance and hyperinsulinemia; increased levels of insulin and glucose enhance fatty acid and triglyceride (TG) synthesis. In addition, insulin resistance hampers the inhibitory action of insulin on hormone- dependent lipase, thus increasing TG hydrolysis and free fatty acid (FFA) release from the adipose tissue. Increased plasma FFA levels are associated with higher hepatic uptake of FFA. Increase in hepatic uptake and synthesis of FFAs are, in turn, compensated by a faster removal of fatty acids. Apparently, this will take place via increased mitochondrial β-oxidation of FA. In accordance with increased levels of TNF α released from adipose tissue, increased β oxidation will enhance reactive oxygen species (ROS) production in the liver. Consequently, ROS overproduction generates chemically reactive lipid peroxidation products such as 4-hydorxynonenal (4-HNE), and also increases the expression of cytokines in the liver, such as TNF α, TGF β, IL-8. Among them, TGF- β, IL-8, 4HNE are chemoattractants for neutrophil, which may account, in part for

neutrophil infiltration and inflammation in the liver (40).

3) Inflammation in the liver generates ROS and RNS

Inflammation in the liver has been considered to contribute to the initiation and progression of HCCs. In this regard, oxidative stress that occurs through overproduction of ROS or reactive nitrogen species (RNS), is recognized to play an important role in the initiation and promotion events of carcinogenesis (41, 42). Indeed, oxidative stress generated not only by obesity but also HBV infection, HCV infection and alcohol, has emerged as a key player in the pathogenesis of chronic liver diseases and pre-cancerous lesions. PMNs (polymorphonuclear neutrophils) infiltrated into the liver, produce and release a vast amount of oxidants (43), and the whole spectrum of oxidants generated by PMNs is due to the actions of four different enzymes. Among these enzymes, NADPH oxidase is the one by which the oxidant generation is initiated. In addition, PMNs also produce RNS, which is generated by inducible nitric oxide synthase (iNOS). In any case, the four types of oxidants including O2-, H_2O_2, constantly interact with each other, causing the formation of a myriad of oxidants among which the hydroxyl radical (·OH) is the most DNA-reactive compound (44). Furthermore, non-parenchymal cells including Kupffer cells and macrophages, which can release pro-inflammatory cytokines, are another source to induce ROS in the liver (44).

Oxidative stress generates intracellular responses leading to carcinogenesis

Oxidative stress can react with a wide range of intracellular molecules, and elicit cytostatic/cytotoxic damage to cellular DNA, protein and lipids (45) **(Figure 3)**

1) Nuclear DNA damage

As described above, the hydroxyl radical (·OH) in particular has been shown to generate a number of oxidized DNA lesions. Recent attention has focused on the formation of 8-hydroxy deoxyguanosine (8-OHdG) in the DNA. This 8-OHdG

lesion results in site-specific mutagenesis that is widely found in mutated oncogenes and tumor suppressor genes (46). Further support for the involvement of 8-OHdG in the carcinogenesis comes from the studies showing that 8-OHdG produces dose-related increases in cellular transformation, which can be prevented by anti-oxidants (47).

2) DNA methylation

DNA methylation is an important regulator of gene expression, decreased methylation being associated with increased gene expression. In this context, many cancer cells have been shown to exhibit global hypomethylation of DNA compared with control cells. In particular, hypomethylation of tumor promoting genes has been proposed as a possible mechanism for cancer development. On the contrary, hypermethylation of genes may inhibit transcription. Indeed, tumor suppressor genes are methylated, resulting in their inactivation (48). ROS can modify DNA methylation, and particularly oxidative DNA damage elicited by ROS can result in decreased DNA methylation. For instance, the formation of 8-OHdG in DNA can lead to hypomethylation. Additionally, 8-OHdG formation can interfere with the normal function of DNA methyltranseferase and alter DNA methylation (49).

3) Signal pathway

Mitogen-activated protein kinases (MAPKs) are divided into three subfamilies based on the structural differences: the extra-cellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNK) and the p38kinases (p38MAPK). The latter two are categorized as stress-activated protein kinases (SAPKs). The ERK pathway is most linked to the regulation of cell proliferation, while the SAPKs (JNK and p38MAPK) pathways are more strongly tied to stress

The SAPK pathways are noted for their activation by a wide range of stresses. For oxidative stress-induced activation of these pathways, change in the cellular redox state appears to be a key factor. Under normal condition, the redox regulatory

protein thioredoxin (Trx) has been shown to bind and inhibit apoptosis signal-regulating kinase (ASK1), which is involved in both JNK and p38 MAPK activation (50). However, oxidative stress causes dissociation of the Trx-ASK1 complex, leading to activation of JNK and p38MAPK. As is the case with Trx, under non-stressed conditions, glutathione S-transferase (GST) binds to JNK and inhibits its activity, but this interaction is disrupted by oxidative stress (51).

Thus, oxidative stress may act at multiple levels in the SAPK pathways to modulate their activities. The influence of JNK activation on cell survival following oxidative stress is complex and controversial. Many studies have shown that JNK activation is correlated with cell death or apoptosis. The role of p38MAPK is also controversial; previous studies have yielded evidence for pro-apoptotic (52) as well as anti-apoptotic (53).

4)Gene expression

The most significant effects of oxidative stress on gene expression have been observed in expression of transcriptional factors including AP-1 and NFκB(54). Activation of these transcriptional factors is involved in both cell proliferation and apoptosis. The cellular redox state appears to influence the selective activation of these transcriptional factors and therefore, may explain the observation that either cell death or cell proliferation might result from exposure to oxidative stress.

One of the target genes of AP-1 is cyclinD1, which is supporting the fact that AP-1 promotes entry into the cell division cycle (55). AP-1 proteins also participate in oncogenic transformation, through interaction with activated oncogenes (56).

On the other hand, the NFκB family of transcriptional factors is composed of homodimers or heterodimers of Rel proteins (57). Virtually, every step of NFκB signaling cascade consists of redox-sensitive proteins whose activities are modulated by oxidative stress (58). Activation of NFκB has been considered to be linked to the carcinogenesis, because NFκB regulates several genes involved in cell

transformation, proliferation, angiogenesis and cell survival (59). In this context, a large number of NFκB target genes have anti-apoptotic functions. These include TNF α, TNF receptor-associated factor1 (TRAF1), TRAFS, cellular inhibitors of apoptosis proteins(CIAPs) (60). NFκB is also involved in regulating the expression of Bcl-Xl, anti-apoptotic member of Bcl-2 family.

Activation of p53 by oxidative stress can either result in growth arrest or apoptosis. Oxidative stress contributes to p53 activation through SAPK cascade. Downstream targets of p53 activation are including p21/Waf1, GADD45, 14-3-3、which are important in mediating G2/M arrest (61), while genes linked to apoptosis include Bax, a pro-apoptotic Bcl-2 family member, and Fas. P53 activation can also interfere with survival signals to render cells permissive to apoptosis (62).

Although the above events may be derived by different mechanisms, a commonality is the involvement of ROS in development of HCCs. Especialy, un-repaired damage to DNA may result in mutation, provided that cell replication ensues prior to repair of modified bases. In addition to oxidative nuclear DNA damage, formation of mitochondrial DNA damage and mutation and alteration of mitochondrial genomic function have been proposed to contribute much to the process of carcinogenesis.

At least three distinct stages of carcinogenesis process, including initiation, promotion and progression, have been identified. Aside from a role of oxidative stress in the induction of mutation, it is apparent that ROS and cellular redox state mediate cell signaling pathways that are involved in cell growth and survive, leading to promotion and progression of HCCs.

Conclusion

Insulin resistance and its complementary hyperinsulinemia can promote growth by insulin action, and also amplify growth by other growth factors, particularly IGF-1.

Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions, including obesity and inflammation in the liver, that can themselves promote tumorigenesis, mainly through cytokine production and /or generation of oxidative stress. Because insulin itself does not induce somatic mutations, intracellular responses to oxidative stress induced by inflammation and/or obesity, are indispensable for hepatocarcinogensis. Thus, the above components need to work together to increase cancer risk beyond that of the individual component alone **(Figure 4)**.

Metabolic syndrome has been considered as the association between obesity, insulin resistance and the risk of a variety of chronic diseases, including cancers. Because the trend for increasing obesity, which began in the West, is now spreading throughout the world, insulin resistance is sure to be put more forth as a central factor for hepatocarcinogenesis in the foreseeable future, not only in developed countries but also in developing countries.

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Figure legends

Figure 1. Insulin signaling and down-stream intracellular events

Insulin action begins with binding to its specific receptor with tyrosine kinase activity. Signaling by the activated insulin receptor then promotes the phosphorylation of IRS-1 (insulin receptor substrate-1) and transmission of the insulin signal via two major phosphorylation cascades: PI3K (phosphoinositide 3-kinase) and MAPK (mitogen-activated protein kinase) cascades.

P: phosphorylation, mSos; mammalian son of sevenless.

Figure 2. Effect of Obesity on cell growth and survival through insulin and IGF-1 signaling

Obesity is associated with increased release of FFA, multiple pro-inflammatory cytokines including TNF α , leptin, IL-6, resistin, reduced release of adiponectin, an anti-inflammatory polypeptide, from adipose tissue, which gives rise to insulin resistance and compensatory hyperinsulinemia. Hyperinsulinemia, in turn, promotes the synthesis and biological activity of insulin-like growth factor I (IGF-1) through GH signaling, and reduces IGFBP1 and IGFBP2 levels, leading to increase in bio-available IGF-1. Since considerable homology has been identified between insulin receptor and IGF-1 receptor, insulin can bind to IGF-1 receptor and enhance IGF-1 signaling.

IR; insulin receptor, IGF-1; insulin-like growth factor 1, IGF-1R; IGF-1 receptor, GHR; growth hormone receptor, IRS-1; insulin receptor substrate 1.

Figure 3. Oxidative stress generates a variety of intracellular responses

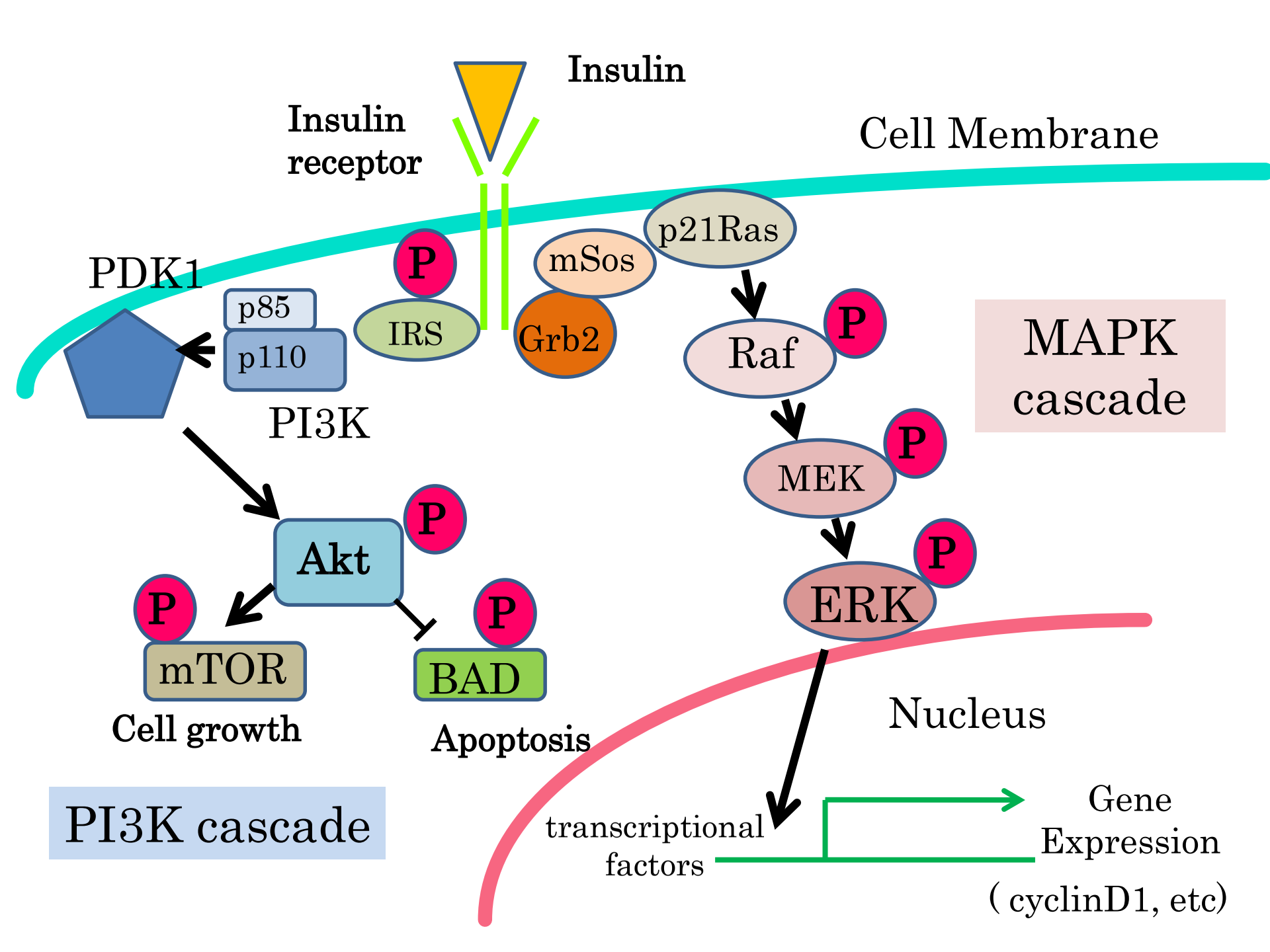
Reactive oxygen species (ROS) encompass a variety of partially reduced metabolites of oxygen possessing higher reactivities than molecular oxygen, and are generated endogeneously as a consequence of normal cell functions or derived from external sources. A number of antioxidant defense systems have evolved to combat the accumulation of ROS. These include enzymatic and non-enzymatic molecules.

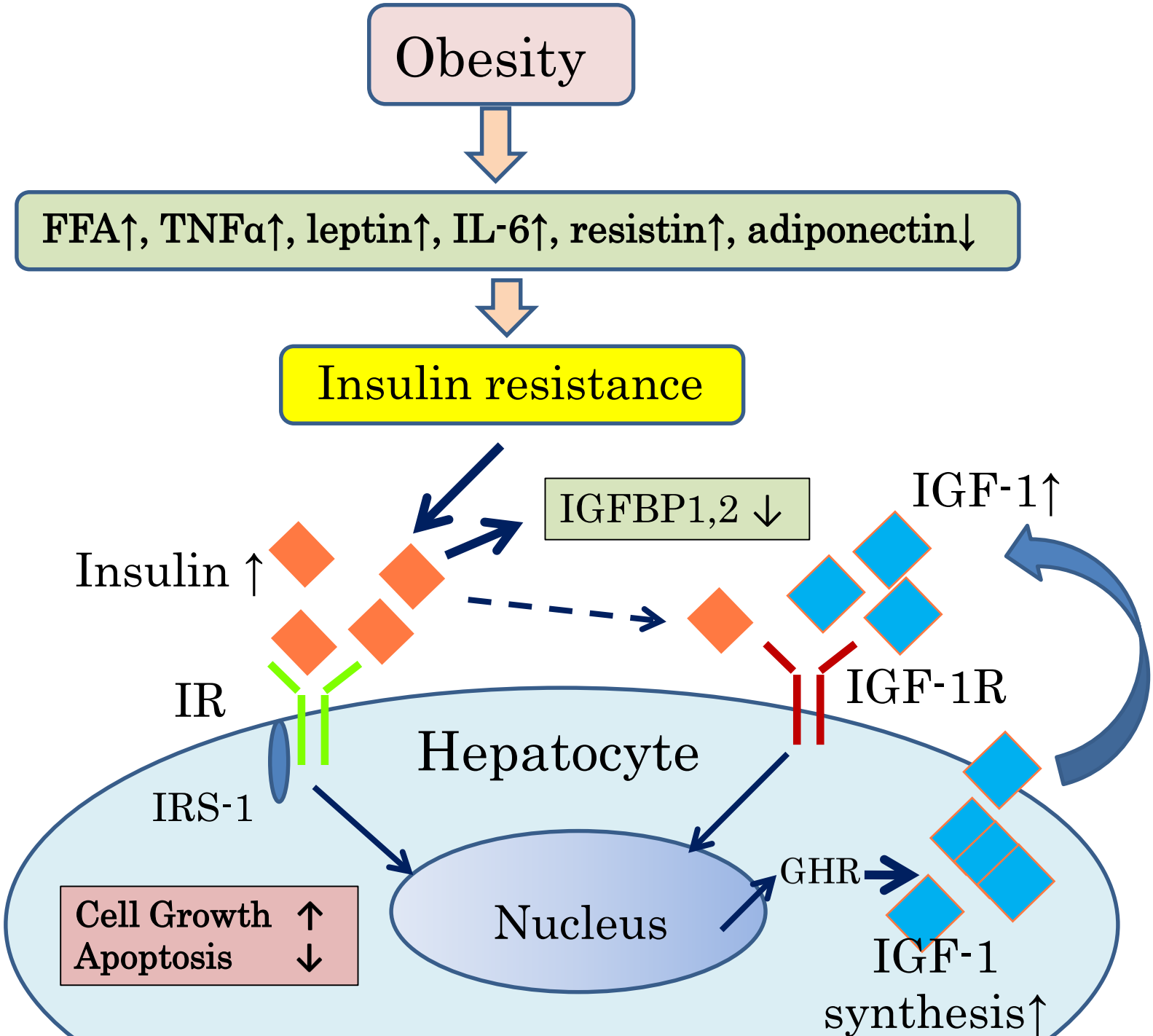
Oxidative stress can occur through overproduction of ROS or reactive nitrogen species (RNS), and interacts with a wide range of intracellular molecules.

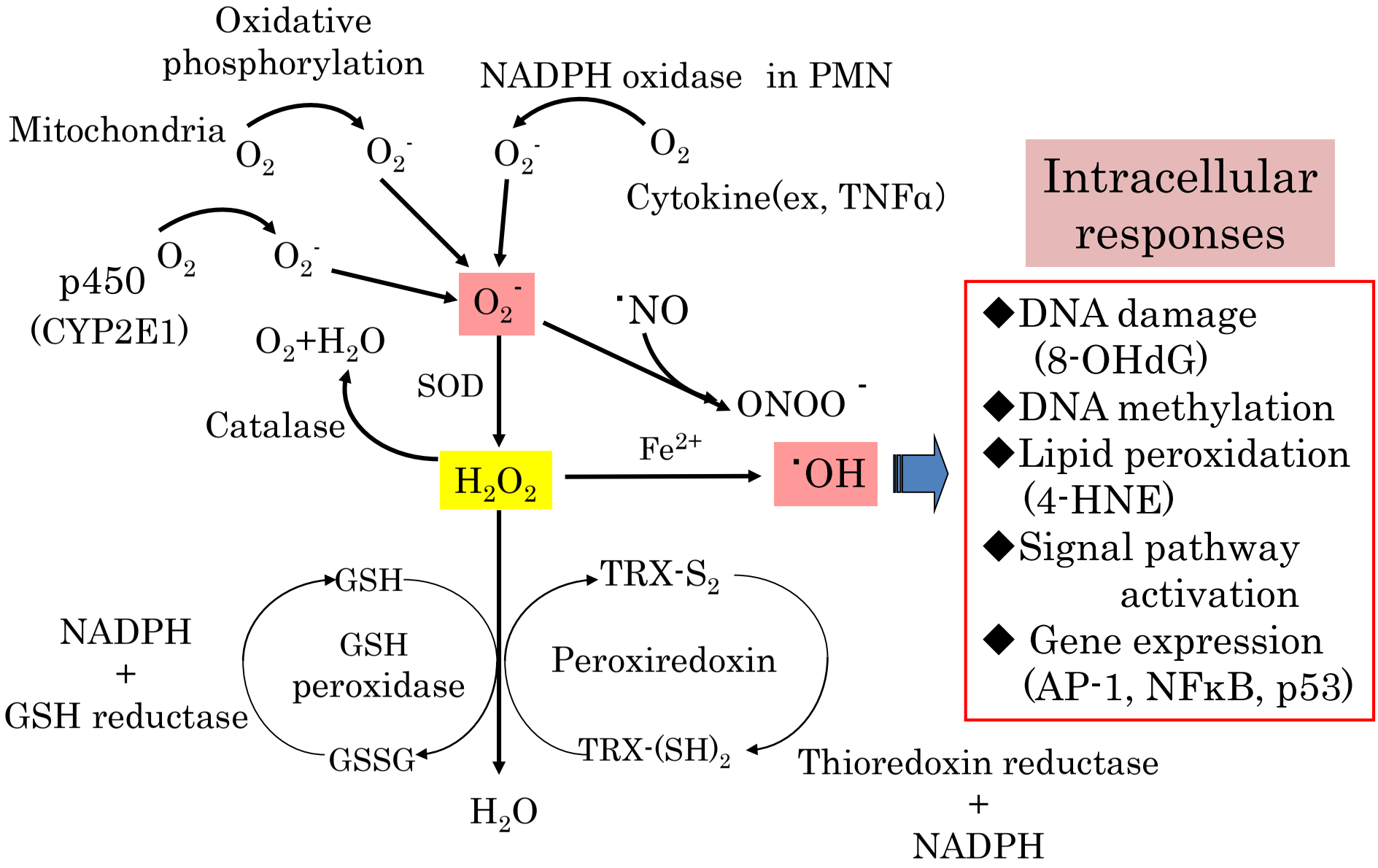
Abbreviations: CYP2E1, cytochrome p450 2E1: SOD, superoxide dismutase:GSH, reduced glutathione: GSSG, oxidized glutathione.

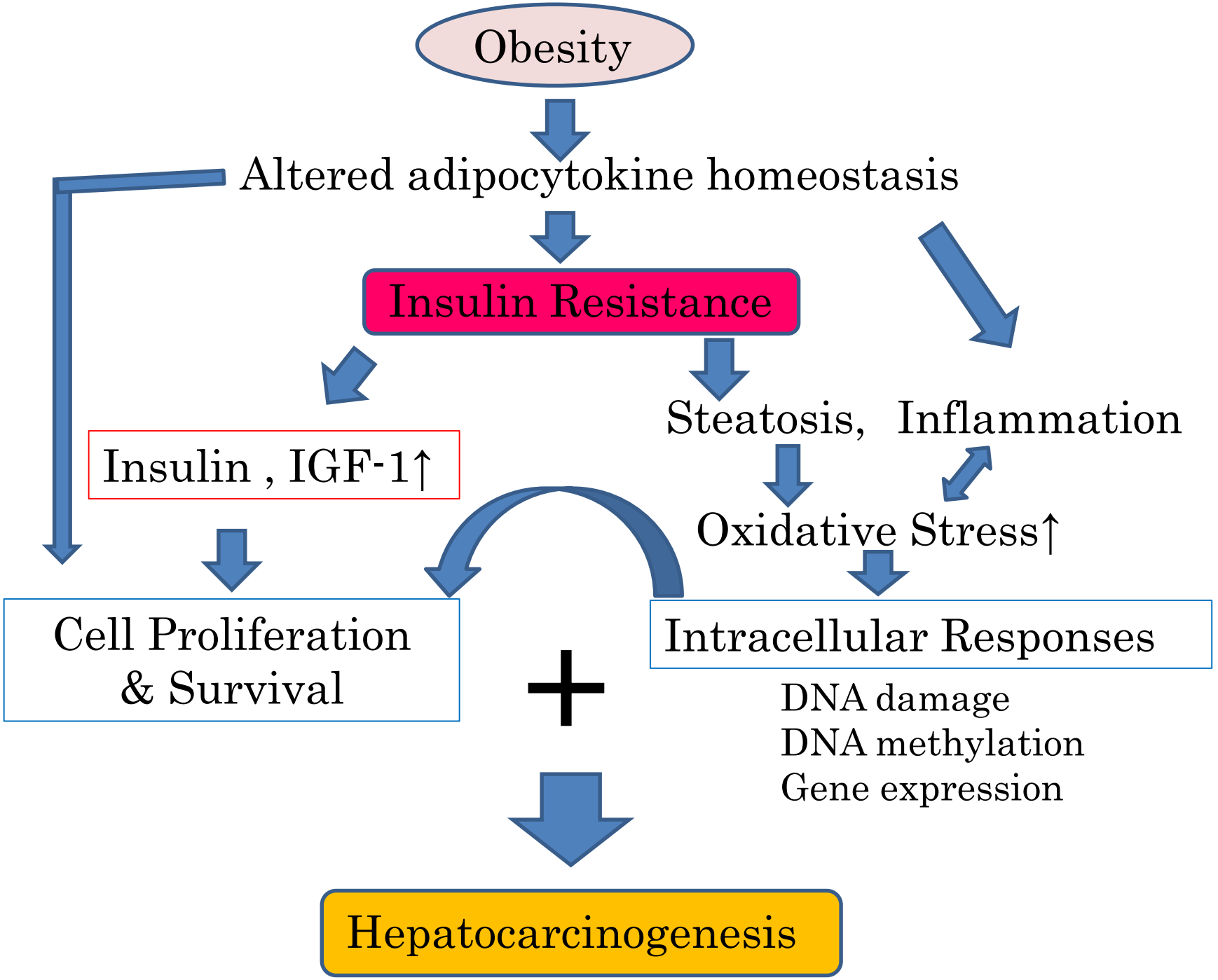
Figure 4. Interaction of insulin resistance induced by obesity and hepatocarcinogenesis

Insulin resistance can promote growth by the action that insulin may have, and also amplify growth by other growth factors, particularly IGF-1. Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions, including obesity and inflammation in the liver, that can themselves promote tumorigenesis, mainly through cytokine production and /or generation of oxidative stress. Because insulin itself does not induce somatic mutations, intracellular responses to oxidative stress induced by inflammation and/or obesity, are indispensable for hepatocarcinogensis.


Insulin
Insulin receptor
Cell Membrane
PDK1
p85
p110
PI3K
P
IRS
mSos
Grb2
p21Ras
Raf
P
MAPK cascade
MEK
P
ERK
P
Akt
P
P
mTOR
BAD
P
Cell growth
Apoptosis
Nucleus
PI3K cascade
transcriptional factors
Gene Expression
(cyclinD1, etc)


Obesity
FFA↑, TNFα↑, leptin↑, IL-6↑, resistin↑, adiponectin↓
Insulin resistance
IGFBP1,2 ↓
IGF-1↑
Insulin ↑
IR
IGF-1R
Hepatocyte
IRS-1
Cell Growth ↑
Apoptosis ↓
Nucleus
GHR
IGF-1
synthesis↑


Oxidative phosphorylation
NADPH oxidase in PMN
Mitochondria
O_2
O_2^-
O_2^-
O_2
Cytokine(ex, TNFα)
p450 (CYP2E1)
O_2
O_2^-
O_2^-
·NO
O_2+H_2O
SOD
ONOO $^-$
Catalase
Fe^{2+}
H_2O_2
·OH
GSH
TRX-S_2
NADPH + GSH reductase
GSH peroxidase
Peroxiredoxin
GSSG
TRX-$(SH)_2$
H_2O
Thioredoxin reductase + NADPH
Intracellular responses
◆DNA damage (8-OHdG)
◆DNA methylation
◆Lipid peroxidation (4-HNE)
◆Signal pathway activation
◆ Gene expression (AP-1, NFκB, p53)


Obesity
Altered adipocytokine homeostasis
Insulin Resistance
Steatosis, Inflammation
Insulin , IGF-1↑
Oxidative Stress↑
Cell Proliferation
& Survival
Intracellular Responses
DNA damage
DNA methylation
Gene expression
Hepatocarcinogenesis