Mouse models of insulin resistance and type 2 diabetes

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INTRODUCTION

Type 2 diabetes is a polygenic disease characterized by impaired insulin stimulated glucose uptake in skeletal muscle and adipose tissue, increased hepatic glucose production and inadequate compensation of insulin secretion from pancreatic β-cells, ultimately leading to fasting hyperglycemia [11] (fig. 1). To fully understand the pathogenesis of type 2 diabetes and to develop new approaches of treatment or prevention of this disease one must understand what is the relative order of these defects in the progressive deterioration of glucose homeostasis seen in the disease. In addition one must elucidate what are the molecular mechanisms of insulin resistance and impaired β-cell function and what are the genetic factors that might influence them. Several studies obtained from genetically engineered mice have helped dissecting the pathogenesis of this disease. Transgenic and knockout models with monogenic impairment in insulin action and insulin secretion have highlighted potential molecular mechanisms for insulin resistance and suggested a mechanistic for the development of MODY in humans. Polygenic models have strengthened the idea that minor defects in insulin secretion and action can lead to diabetes when combined, pointing out the importance of epistatic interactions of different genetic loci in the production of diabetes.

The aim of the present study is not to provide an exhaustive list of all the transgenic and knockout models of type 2 diabetes generated in the recent years but to discuss some of the best examples that have helped understand the important sites and levels of insulin resistance.

USE OF TISSUE-SPECIFIC KNOCKOUT MOUSE MODELS TO DISSECT OUT THE PATHOGENESIS OF DIABETES

The development of conditional gene knockout approaches [12, 35] (figure 2) has helped assessing the contribution of individual insulin sensitive tissues in the regulation of glucose homeostasis and to the development of type 2 diabetes. For example, homozygous insulin receptor (IR) knockout mice die within the first week of life from diabetic ketoacidosis, preventing detailed analysis of glucose homeostasis and insulin action at a tissue specific level [2, 27]. Tissue-specific knock-outs of the insulin receptor using the Cre-loxP system have been generated and challenged current concepts on the regulation of glucose homeostasis [31]. These mouse models have highlighted the importance of insulin action in several tissues [28] including liver [43], brain [6] and pancreatic β-cells [32] (figure 3). β-cell insulin receptor knockout (βIRKO) mice exhibit a selective loss of acute insulin release in response to glucose resulting in a progressive and severe impairment of glucose tolerance, a defect similar to that seen in type 2 diabetes.

Figure 1: Glucose homeostasis in type 2 diabetes.
More recent studies were undertaken in βIRKO mice to define the mechanism underlying the defect in insulin secretion and showed that the loss of IR in β-cells leads primarily to profound defects in postnatal β-cell growth [50]. All together these studies indicate that IR is critical for glucose sensing by pancreatic β-cells which suggests that defects in insulin action at the level of the β-cell may contribute to the impaired insulin secretion in type 2 diabetes.

DELETION OF THE INSULIN RECEPTOR OR GLUT4 IN SKELETAL MUSCLE PRODUCES DIFFERENT PHENOTYPES

Since muscle accounts for 80% of post-prandial glucose uptake in humans and is a site of insulin resistance early in the pre-diabetic state [11, 16, 29], the impact of deleting key genes of insulin action in skeletal muscle was investigated using the Cre-loxP system [28]. Surprisingly, skeletal muscle-specific insulin receptor knockout (MIRKO) mice, which exhibit no early insulin signaling events in skeletal muscle, show no alteration in glucose homeostasis but have elevated serum triglycerides, free fatty acids and develop increased visceral fat mass [7]. These characteristics are hallmarks of the metabolic syndrome X. Interestingly, during a glucose tolerance test, MIRKO mice maintain near normal glucose uptake in skeletal muscle which may explain why these animals do not become diabetic [42]. Interestingly, exercise can activate postreceptor insulin signaling and increase glucose transport in muscle from MIRKO mice independently of insulin [67]. Thus, contraction of postural muscles could be the explanation for the maintenance of normal glucose uptake in muscle of MIRKO mice, which emphasizes the importance of non insulin-dependent mechanisms of glucose uptake to maintain normoglycemia.

Also of interest MIRKO mice show a shift of glucose utilization towards adipose tissue with a resulting increased fat mass [7]. This 40% increase in epididymal WAT mass results from a 40% increase in adipocyte number without modification in cell size [8]. This WAT hyperplasia is accompanied by a 40% increase in serum adiponectin without modification in any of other adipocyte cytokines or hormones such as leptin, resistin or TNF-α. Therefore, although MIRKO mice display muscle insulin resistance, visceral obesity and dyslipidemia they do not develop global insulin resistance or diabetes. These studies confirm the qualitative importance of WAT, regardless of its mass, in the maintenance of whole body insulin sensitivity and the
development of insulin resistance and suggest that there is a cross-talk between skeletal muscle and fat in order to maintain euglycemia. One of the factors responsible for cross-talk could in fact be Myostatin since Myostatin gene knockout causes a switch between myogenesis and adipogenesis. Myostatin gene knockout mice have decreased fat depots and a decreased in serum leptin concentration. Both CCAAT/enhancer binding protein-alpha (C/EBPα) and PPARγ levels in adipose tissue are significantly lower in Myostatin KO mice compared to controls suggesting that increased muscle development in Myostatin knockout mice is associated with reduced adipogenesis and consequently, decreased leptin secretion [36, 37]. The existence of such a cross-talk between skeletal muscle and fat, which if it would occur in humans, could contribute to the increase adiposity in patients with insulin resistance in muscle. In agreement with the notion of communication between muscle and other insulin sensitive tissues selective insulin resistance in muscle also seems to modify glucose sensing from pancreatic β-cells by a yet unknown mechanism that could also involve a circulating factor. Indeed, double tissue specific βIRKO-MIRKO mice show improved glucose tolerance compared to βIRKO mice due to improved glucose-stimulated acute insulin release [42].

GLUT4 is known as the insulin-sensitive glucose transporter since it is mainly expressed in skeletal, heart and adipose tissue [9] and mediates glucose transport stimulated by insulin and contraction [61]. Unlike the phenotype observed in MIRKO mice [7], selective disruption of GLUT4 glucose transporter in muscle (MG4KO mice) leads to glucose intolerance and insulin resistance secondary to a marked decrease in muscle glucose uptake [44, 70]. Indeed hyperinsulinemic-euglycemic clamp studies show a 55% decrease in insulin-stimulated whole body glucose uptake in MG4KO mice compared to controls demonstrating their severe insulin resistance. The two examples of MIRKO and MG4KO mice reveal that different sites of insulin resistance in the same tissue can produce quite different phenotypes. These findings, along with studies performed in humans [9] bring a new vision on the role of skeletal muscle in diabetes: glucose uptake rather than early insulin signaling events is of critical importance for the maintenance of normal glucose homeostasis by this tissue.

WHITE ADIPOSE TISSUE AS A DYNAMIC ENDOCRINE TISSUE

Adipose tissue has long been considered as most important for storage than for glucose uptake. More recently, it has become clear that the adipocyte is an active endocrine secretory cell releasing non only free fatty acids but also producing several cytokines and hormones, including tumor necrosis factor α (TNF-α), interleukines, leptin, adiponectin and resistin [21] (figure 4).

The importance of white adipose tissue (WAT) in the regulation of glucose homeostasis has been assessed in various animal models and seems to be related to leptin secretion. Indeed, leptin acts primarily as a satiety factor acting on the hypothalamus, decreasing food intake and increasing energy expenditure, thereby preventing excessive increase in body weight. Selective deletion of IR expression in WAT (FIRKO mice) does not alter glucose homeostasis despite a 90% decrease in fat mass but protects mice from obesity [5]. Other features of FIRKO mice include high levels of leptin for their fat mass and an unexpected increase in their life span, suggesting that insulin signaling pathways may be involved in both leanness and longevity [4]. A transgenic mouse model that was generated without WAT depots and with undetectable leptin levels leads to insulin resistance, elevated plasma lipid levels, and diabetes, a phenotype similar to that of generalized lipoatrophic diabetes in humans [45, 56]. Interestingly, restoration of near normal circulating leptin levels in these mice either by leptin infusion [55] or by surgical implantation of WAT [10], reverses the diabetic phenotype. These results indicate that leptin acts as an adipocyte-derived anti-diabetic hormone in vivo and that its deficiency

Figure 4: White adipose tissue as a dynamic endocrine tissue. White adipose tissue, previously considered as a static fat store is now envisioned as a dynamic endocrine tissue playing a critical and central role in the regulation of glucose homeostasis and energy metabolism, through release of modulators of insulin action, glucose uptake and insulin secretion.
leads to insulin resistance and diabetes. Therefore, understanding the mechanisms of leptin-induced glucose disposal in peripheral tissue is of critical importance to the understanding of the regulation of glucose homeostasis and to the development of new hypoglycemic agents.

Tissue-specific knockout of GLUT4 in WAT (GA4−/−) was also created [1] (fig. 5). These mice exhibit a decrease in both basal (40%) and insulin-stimulated (70%) glucose transport in WAT. Hyperinsulinemic-euglycemic clamp studies of GA4−/− mice revealed a 50% reduction in whole body glucose uptake that was caused not only by insulin resistance in WAT but more surprisingly in both skeletal muscle and liver [1]. Therefore, the selective ablation of GLUT4 in WAT leads to secondary insulin resistance in other insulin target tissues, providing another example of cross-talk communication between insulin-sensitive tissues in vivo. Thus, as we discussed for skeletal muscle, insulin resistance at different levels, IR versus GLUT4, provides quite distinct phenotypes in mice.

**ROLE OF THE LIVER IN THE PATHOGENESIS OF INSULIN RESISTANCE AND TYPE 2 DIABETES**

The liver plays a unique role in controlling carbohydrate metabolism by maintaining glucose concentrations in a normal range over both short and long periods of times. In the postabsorptive state, hepatic glucose production ensures a sufficient supply of glucose to the central nervous system at the same time as it regulates fasting plasma glucose concentrations. In postprandial conditions, the liver takes up a portion of ingested carbohydrates to restore glycogen stores. This net hepatic glucose uptake, which results from simultaneous suppression of glucose-producing pathways and stimulation of hepatic glucose uptake and anabolic pathways of glucose disposal, restricts postprandial increases in plasma glucose concentrations. In type 2 diabetes, alterations in hepatic glucose metabolism are observed, i.e. increased postabsorptive glucose production and impaired suppression of glucose production together with diminished splanchnic glucose uptake following carbohydrate ingestion.

The key role of the liver in controlling glucose homeostasis in vivo was confirmed by several transgenic and knockout models. Transgenic mice overexpressing key enzymes of gluconeogenesis (de novo glucose synthesis), phosphoenolpyruvate carboxykinase (PEPCK) [63] or glucose-6-phosphatase (G6Pase) (fig. 6) [62] show unsuppressed hepatic glucose production in the fed state despite hyperinsulinemia resulting in hyperglycemia which confirms the central role of the liver in causing fasting hyperglycemia. Disruption of insulin action in liver by tissue specific knockout of the insulin receptor (LIRKO mice) also leads to severe glucose intolerance (fig. 7), resistance to the blood glucose lowering effect of insulin with an increased hepatic glucose production with elevated G6Pase and PEPCK expression levels in liver [43]. Because of the absence of insulin receptor-
mediated clearance of insulin by the liver, insulin concentrations are extremely high in LIRKO mice. In addition, LIRKO mice exhibit a 6-fold increase in pancreatic β-cell mass [43]. All together, analysis of LIRKO mice have revealed the critical role for the liver for the maintenance of normal glucose homeostasis and hepatic function [43]. Given that mice with a muscle-specific insulin receptor knock-out (MIRKO) have normal fasting glucose levels and normal glucose tolerance [7] this mouse model suggests that the liver may play a more important role in the control of glucose homeostasis than it has been previously thought (fig. 7). Furthermore, a considerable portion of the decrease in blood glucose following insulin administration may due, at least in mice, to a suppression of hepatic glucose production rather than an increase in muscle glucose uptake [43].

The three genetic models presented here demonstrate that the ability of the β-cell to compensate for insulin resistance in liver and to prevent the resulting hyperglycemia is somehow limited. In addition, the compensatory hyperinsulinemia that occurs in insulin resistant states may further increase insulin resistance in the liver via downregulation of the insulin signaling cascade itself. Indeed, transgenic mice overexpressing insulin in the liver develop hyperinsulinemia, glucose intolerance with aging as a result of a insulin receptor downregulation in absence of any defect in insulin secretion [39, 40].

The possibility of controlling hepatic glucose utilization as a treatment of type 2 diabetes has been also explored through the modulation of expression of one the key glycolytic enzyme, glucokinase (GK) (fig. 6) [38, 53]. Both overexpression and conditional gene knockout strategies proved to be valuable for determining the effects of GK gene dosage on blood glucose concentrations [38]. GK, which is the predominant glucose-phosphorylating enzyme in hepatocytes exhibits kinetic properties quite distinct from the those of the other members of the mammalian hexokinase (HK) family [41, 66]. GK is not inhibited its end-product reaction, glucose 6-phosphate (G6P), and has a relatively low affinity for glucose (S0.5 8 mM), so that the flux through GK is sensitive to fluctuations in the concentration of its substrate in the physiological range [41]. In transgenic mice over-expressing hepatic GK, there is an activation of glycolysis and glycogen synthesis [17, 22, 47]. GK overexpressing transgenic mice have reduced glycemia, insulinemia and blood glucose after a glucose tolerance test indicating that GK overexpression in liver increases glucose disposal by the liver [18]. Similar results have been obtained with mice we generated that express one or more copies of the entire GK gene locus [46, 47]. Furthermore, these mice overexpressing one extra copy of the GK gene locus [57] are protected against high-fat diet induced diabetes. In these mice, increased GK activity reduces hyperglycemia and hyperinsulinemia induced by high-fat diet. Ferre et al. [18] had also reported that the streptozotocin-induced increase in glucose and glycogen concentrations, are normalized in GK overexpressing mice. These studies demonstrate that the modulation of expression of a single hepatic gene is able to revert the diabetic phenotype. This suggests that hepatic GK a good gene candidate for therapy-based approach and indeed, allosteric activators of the enzyme recently proven to be efficient in mice to decrease blood glucose concentrations [19] could be tested in a near future in type diabetic patients.

**MOUSE MODELS FOR MODYS**

Maturity-onset diabetes of the young (MODY) is characterized by autosomal dominant inheritance, onset of diabetes usually before 25 years of age, and deficient insulin secretory response [15]. Genetic studies have identified mutations in at least six genes associated with different forms of MODY. The MODY2 gene encodes the glycolytic enzyme glucokinase (GK), and MODY subtypes 1, 3, 4, 5, and 6 are caused by mutations in transcription factors hepatocyte nuclear factor (HNF)-4, -1, PDX-1, HNF-1β, and NEURO-D/BETA-2, respectively [24, 58, 64, 68, 69]. In the recent years, the generation and characterization of mouse models for MODYs have been very useful in determining the genetic basis of this form of diabetes. Although a lot of efforts have been directed at studying β-cell function, the liver may play an important role in the development of the disease. Indeed, with the exception of PDX-1,
which is exclusively expressed in β-cells, all of the other identified MODY genes are expressed in both β-cells and liver.

Mutations in glucokinase (MODY2) result in mild chronic hyperglycemia due to reduced pancreatic β-cell responsiveness to glucose as well as decreased net accumulation of hepatic glycogen and increased hepatic gluconeogenesis following meals. Mice either globally deficient in GK, or lacking GK only in β-cells, die soon after birth from severe diabetes pointing out the importance of this enzyme in insulin secretion and glucose homeostasis [20, 54, 60]. Moreover, a rescue experiment in which GK was only re-expressed in β-cells of the GK deficient mice reverts the diabetic phenotype of the mice, although rescued mice remain mildly hyperglycemic due a residual defect in hepatic GK expression [20]. Heterozygous mice with one functional GK allele, either globally or in β-cells, develop impaired insulin secretion in response to glucose and mild diabetes [54], a phenotype in complete agreement with the phenotype of MODY2 patients [64].

In contrast to MODY2, MODY1 and MODY3 are characterized by severe insulin secretory defects and severe hyperglycemia associated with microvascular complications. Mutations in the transcription factor HNF-1α gene cause MODY 3 in humans [69] and animal models have shed light on the mechanism of impaired insulin secretion observed in this form of diabetes [13, 52]. Mice lacking the HNF-1α gene show impaired insulin secretion in response to both glucose and arginine but normal response with non-nutrient stimuli such as potassium chloride, without alteration in β-cell mass. In these mice, diabetes results from defective β-cell glycolytic signaling which is potentially correctable using substrates that bypass the defect [13, 52]. These results are consistent with that fact that the expression of L-pyruvate (L-PK) and glucose transporter GLUT2 is both reduced in islets from HNF-1α−/− mice [51] and in pancreatic cell lines that express a dominant negative form of HNF-1α [65].

In humans, heterozygosity for a point mutation in the gene of the β-cell transcription factor PDX1 gene is associated with a strong family history of MODY4 [58]. The importance of PDX-1 in pancreatic development was demonstrated by the consequences of the loss of PDX-1 function. Targeted disruption of the pdx-1 gene in mice and an inactivating mutation of pdx-1 in a human infant [59] manifest as agenesis of the pancreas [26, 49]. Conditional disruption of the pdx-1 gene selectively in β-cells of mice using the Cre-loxP approach results in a progressive loss of β-cells and the development of diabetes in mice by age 5 – 6 months [3]. Mice [14] and humans [59] with a haploinsufficiency for PDX-1 develop diabetes and/or glucose intolerance. These models provide a mechanistic for the development of MODY4 in haploinsufficient individuals.

Although the role of the known MODY genes in susceptibility to the more common late-onset from of type 2 diabetes remains to be determined they may play a minor role in a polygenic context of type 2 diabetes or a major role in particular populations.

**IMPACT OF THE GENETIC BACKGROUND ON INSULIN ACTION, INSULIN SECRETION AND THE INCIDENCE OF DIABETES**

Insulin resistance is known to have an important genetic component but effort to identify predisposing genes has been so far unsuccessful. For example, when double heterozygous knockout insulin receptor/insulin receptor substrate-1 (IR/IRS-1) mice are generated on different genetic strains of mice, the incidence of diabetes for the same given mutation varies greatly depending on the genetic background used [33]. To identify such genetic loci predisposing to insulin resistance a genome wide scan with polymorphic markers between different strains of mice with a targeted null allele of the IR gene was performed [30]. They identified a locus on chromosome 2 that show significant linkage to plasma insulin levels [30]. Thus, it is clear that modifier genes present in the genetic background influence β-cell function, degree of insulin resistance and finally the incidence of diabetes. This may help to explain why phenotypical features in the onset and outcome of type 2 diabetes vary among the various human population. The impact of the genetic background on insulin action is therefore highly important as revealed by two recent mouse models of PPARγ gene knockout in muscle [23, 48]. Previous studies have shown that PPARγ is implicated in whole body glucose homeostasis and insulin sensitivity. The involvement of this transcription factor in systemic insulin sensitisation is further supported by the fact that thiazolidinediones (TZDs), a class of antidiabetic agents, are high affinity PPARγ ligands [34]. Compared to its high expression in adipose tissue, PPARγ is barely detectable in skeletal muscle, therefore it is unclear whether TZDs act directly in skeletal muscle or indirectly through the activity of PPARγ in fat. Surprisingly, while the targeted deletion of PPARγ in muscle in mice on a pure C57BL/6J background (MKO mice) causes a profound insulin resistance with no improvement after TZD treatment [23], the deletion of the same gene, but this time on a mixed genetic background (MuPPARγKO mice) causes a very different phenotype [48]. Although insulin-stimulated glucose uptake in muscle was not impaired, MuP-
PARγKO mice had whole-body insulin resistance with a 36% reduction in the glucose infusion rate required to maintain euglycemia during hyperinsulinemic clamp, primarily due to dramatic impairment in hepatic insulin action. When placed on a high-fat diet, MuPPARγKO mice developed hyperinsulinemia and impaired glucose homeostasis identical to controls. More importantly, treatment withTZD ameliorated high fat-induced defects in MuPPARγKO mice similarly to controls, suggesting that muscle PPARγ is not required for the antidiabetic effects of TZDs [48]. The discrepancy in the phenotypes observed is very surprising and likely due to the difference in genetic background. These findings indicate that one must be very cautious before interpreting phenotypes of gene knockouts and they underline how genetic predisposition contributes to the development and/or the severity of diabetes.

CONCLUSION

Although the precise determinants of type 2 diabetes are still unclear, major advances have been provided by these animal models in understanding the in vivo mechanism of regulation of insulin action, the determinants of insulin secretion and the potential dysregulation that lead to hyperglycemia [25]. From the tissue-specific knockouts we have learned that different tissues contribute uniquely to the pathogenesis of type 2 diabetes, but not always in the predicted way. Indeed, insulin resistance at different levels in the same tissue may produce different phenotypes and tissues possess mechanisms of communication such that insulin resistance in one tissue affects insulin signaling or metabolism in others, including the brain and β-cells. The results of the studies presented have led to new hypotheses about the nature of insulin action network. White adipose tissue, previously considered as a static fat store, is now envisioned as a dynamic endocrine tissue playing a critical and central role in the regulation of glucose homeostasis and energy metabolism, through release of modulators of insulin action, glucose uptake and insulin secretion. The finding that insulin action in pancreatic β-cells is important for normal glucose sensing brings up a unifying hypothesis in which insulin resistance at the level of muscle, adipose, liver and pancreatic β-cells may lead to type 2 diabetes. Also of interest is the emerging idea that insulin-sensitive tissues can communicate between each other to regulate glucose homeostasis. Muscle seems to communicate to fat and liver and in addition, muscle, liver and fat can all modulate insulin secretion from pancreatic β-cells possibly via endocrine or metabolic mechanisms. Thus, impaired communication between insulin sensitive tissues could also contribute to the pathogenesis of type 2 diabetes. In addition, the recent development of inducible gene knockouts, in which the deletion of a given gene in controlled in both a tissue-specific and time-dependent manner, is likely to improve dissecting out the pathogenesis of insulin resistance and diabetes.

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