Minireview

Genetically engineered mice as animal models for NIDDM

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Abstract Genetically engineered animals carrying defined alterations in their genome can represent invaluable tools for better understanding complex polygenic diseases such as non-insulin-dependent diabetes mellitus (NIDDM) at the molecular level. The structure or expression of a number of genes potentially involved in insulin action or pancreatic β-cell function have recently been altered in the mouse using transgenic or gene-targeting approaches. The obtention of such mice is the first step towards the development of animal models carrying multiple gene defects which would be very useful in NIDDM research.

Key words: Transgenic mouse; Gene targeting; Diabetes; Insulin action; Insulin resistance; Insulin secretion

1. Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) affects several million people world-wide and is characterized by insulin resistance as well as β-cell dysfunction. Insulin resistance is also found associated with many disorders other than NIDDM. Several recent reviews have focused on various aspects of NIDDM (review [1] and refs. therein). This review attempts to summarize the recent application of transgenic and gene-targeting approaches to develop mouse models useful for NIDDM research.

1.1. Insulin secretion and action

Insulin is a polypeptide hormone which is synthesized, stored and secreted by the β-cells of pancreatic islets of Langerhans and which plays a vital role in glucose homeostasis. The β-cells possess a sophisticated glucose-sensing system, and insulin secretion by these cells is a highly regulated process (review [2]). The three major target tissues for insulin action are muscle, liver and adipose tissue. Besides stimulating the uptake, utilization and storage of glucose (i.e. as glycogen), insulin action affects several other processes at the cellular level such as amino acid uptake, lipogenesis/lipolysis, Na^+, K^+ pumps, protein synthesis, gene expression, DNA synthesis and apoptosis (review [3]).

1.2. Insulin receptor and signaling

Insulin action is mediated by the insulin receptor (IR) which belongs to the family of membrane receptor tyrosine kinases. It is composed of two subunits, designated α and β, that assemble into a heterodimeric α2β2 structure. Ligand binding to the α-subunits results in the autophosphorylation of certain tyrosine residues of the β-subunit and activation of the tyrosine kinase. One unique feature of the signaling mechanism used by IR is that its activated tyrosine kinase phosphorylates adaptor or docking proteins, such as IRS-1, IRS-2, Gab1 and Shc, which subsequently recruit various SH2-domain-containing effector proteins leading, for instance, to the activation of the MAP kinase or the PI-3 kinase pathways (reviews [4,5]). Some effector proteins can also bind directly to the cytoplasmic domain of IR. The links at the molecular level between the different pathways that are activated and the biological effects obtained in response to insulin are still poorly understood.

1.3. Potential diabetogenes

NIDDM is considered today as a complex syndrome. Molecular defects in different sets of genes would lead to the large spectrum of pathophysiologies seen in this heterogeneous disease (reviews [1,6]). In this respect, one could think of mutations in a variety of genes that could impair insulin action or β-cell function. Table 1 presents a non-exhaustive list of such potential diabetogenes. Much effort is being devoted to research focused on the search for candidate genes for NIDDM (reviews [7,8]). Since a limited number of animal models exist for NIDDM research (review [9]), the development of genetically engineered mice in which the effects of altered expression of a single gene or a set of genes could be studied in a systematic manner appears very compelling.

2. Altering the mouse genome

A brief outline of methods used for generating transgenic and 'knock-out' mice is depicted in Fig. 1. Transgenic mice are usually designed to examine the effects of overexpressing a specific gene (wild-type or mutated). The normal expression of an endogenous gene can also be inhibited in transgenic mice which produce an antisense RNA or a ribozyme directed against that gene. The gene-targeting approach generates mutant mice with specific genes disrupted. This approach also makes it possible to introduce point mutations in a gene of interest or to perform tissue-specific gene disruption with the use of site-specific recombination systems (i.e. the Cre/loxP system). Alternatively, the combination of transgenic and knock-out mice can be used to generate mice in which the expression of disrupted genes could be reconstituted in specific tissues or the effects of gene mutations could be studied in the absence of endogenous protein. Genetically engineered mice represent today powerful tools for studies in molecular medicine. The transgenic mouse approach has been used for a

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; IR, insulin receptor; MLC-2, myosine light chain-2; IGF, insulin-like growth factor; aP2, adipocyte P2; PEPCK, phosphoenolpyruvate carboxykinase; GK, glucokinase; P_{InsI}/P_{InsII}, rat insulin I or insulin II gene promoter; IAPP, islet amyloid polypeptide

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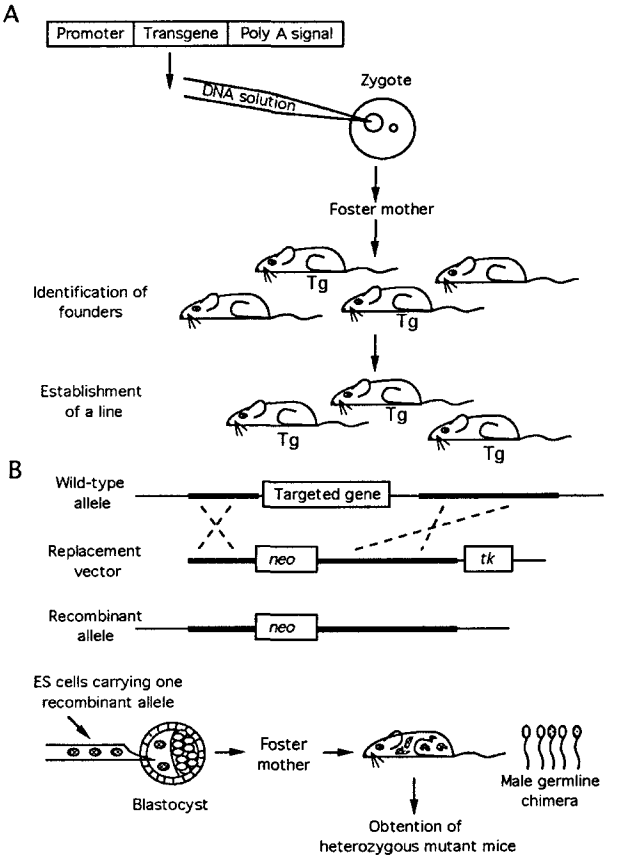

Fig. 1. Transgenic and gene-targeting approaches in the mouse. A: Transgenic mice are produced by microinjecting the DNA construct into male pronucleus of zygotes that are reimplanted into foster mothers. Founder transgenic animals in the progeny are identified by testing for the presence of the transgene in the genomic DNAs and mated with non-transgenic animals. The transgenic F1 animals are used for testing transgene expression and for establishing a line. B: The structure of the wild-type allele for the targeted gene of the replacement vector and of the recombinant allele after homologous recombination are shown. The targeting vector contains *neo* (neomycin phosphotransferase gene) and *tk* (thymidine kinase gene from herpes simplex virus type 1) cassettes that allow positive and negative selection in the presence of G418 and Ganciclovir, respectively, as well as regions of 5′- and 3′-homologies. After electroporation into male embryonic stem (ES) cells (derived from mouse strain 129 carrying the dominant agouti fur coat color gene), resistant clones that have undergone the right homologous recombination event are identified (i.e. by Southern blot analysis using appropriate restriction enzymes and probes or by PCR using appropriate primers). Recombinant ES cells are injected into blastocysts (i.e. from strain C57Bl/6) which are reimplanted into foster mothers, and generate chimeras (composite fur coat color) at term. The whole game consists in getting male germline chimeras which give agouti pups in the progeny when crossed with C57Bl/6 females for instance. Agouti animals are genotyped to identify heterozygous mutants which when intercrossed give homozygous mutant mice.

decade to develop animal models for NIDDM (review [10]). More recently, application of the gene-targeting approach (review [11]) has revived interests in mice as animal models for diabetes.

3. Manipulating insulin action in the mouse

3.1. Insulin receptor

Transgenic mice expressing human IR cDNA under the control of the myosin light chain-2 (MLC-2) gene promoter/enhancer have been generated [12]. Although the receptor number in skeletal muscle was increased, no significant changes in basal glucose and insulin levels were detected. However, glucose and insulin level regulation was affected after intraperitoneal glucose load. Other transgenic mice expressing human IR cDNA encoding a tyrosine kinase-deficient form under the control of the native promoter did not show any glucose intolerance, probably due to limited expression of the transgene [13]. Expression of human IR cDNA encoding another tyrosine kinase-deficient dominant negative form in skeletal and cardiac muscle was achieved in transgenic mice using the muscle creatine kinase gene promoter [14]. This resulted in impaired insulin-stimulated IR tyrosine kinase activity in muscle along with defective activation of downstream signaling proteins such as IRS-1 and PI-3 kinase [15]. Insulin-dependent glucose uptake and metabolism in skeletal muscle were impaired [16]. Stimulation of MAP kinase and $p90^{rsk}$ were not detectable, whereas activation of $p70^{S6k}$ and glycogen synthetase were preserved [17]. Finally, expression in transgenic mice of the extracellular domain of human IR in a soluble form using the mouse transferrin gene promoter resulted in altered glucose homeostasis [18].

More recently, mice carrying a null mutation in the IR gene have been reported [19,20]. Heterozygous animals showed normal glucose tolerance and were fertile. Homozygous pups were near normal at birth but rapidly developed metabolic disorders, such as hyperglycemia and elevated plasma triglyceride levels, which led to diabetic ketoacidosis and hepatic steatosis. The hyperglycemia also resulted in hyperinsulinemia. The glycogen content in the liver was reduced. Marked postnatal skeletal muscle hypotrophy and growth retardation were observed, and the IR-deficient pups finally died within 1 week after birth.

Insulin and insulin-like growth factors (IGF-1/IGF-2) and their receptors are structurally very similar and lead to some common as well as specific biological effects [21]. Genes encoding IGF-1 or IGF-2 or the IGF-1 receptor have already

Table 1
Proteins encoded by potential diabetogenes

Proteins involved in insulin action
The insulin receptor
Substrates of the receptor tyrosine kinase
Insulin-responsive enzymes of intracellular metabolism
Insulin-responsive effectors controlling gene expresssion
Enzymes of metabolic processes controlling glucose uptake and output by the liver
Proteins controlling the secretion of counterregulatory hormones
Transcription factors controlling the expression of such diabetogenes, etc.
Proteins involved in β-cell function
Proteins involved in the glucose sensing mechanism: transporter, glucokinase, other enzymes involved in β-cell glucose metabolism
Factors determining β-cell sensitivity to nonglucose secretagogues
Factors of processes controlling β-cell growth and regeneration
Proteins of processes involved in insulin synthesis and release
The insulin molecule itself
Factors controlling transendothelial transport of insulin, etc.

Adapted from [6].

been inactivated in the mouse [22–24]. Since these different ligands are known to bind to and activate the heterologous receptor, mice carrying single, double or multiple mutations will allow examination of the question of whether and to what extent insulin and IGF-1 receptors and their ligands can substitute for each other.

3.2. IRS-1

Homozygous mice carrying a null mutation in the IRS-1 gene showed intra-uterine and postnatal growth retardation [25,26]. However, IRS-1-deficient mice did not present any major metabolic disorders, indicating that IRS-1 can be replaced by other homologous docking proteins of insulin signaling.

3.3. Hexokinase II

Phosphorylation of glucose to glucose-6-phosphate, the first step of glucose metabolism, is performed by hexokinase. Its major isoform present in insulin-responsive tissues is hexokinase II. Transgenic mice overexpressing human hexokinase II using the promoter for the rat muscle creatine kinase gene have been produced [27]. These mice were unaffected for oral glucose tolerance, intravenous insulin tolerance and in their insulin and lactate levels.

3.4. GLUT4 and GLUT1

Glucose transport across the plasma membrane is facilitated by glucose transporters that represent a large family comprising GLUT1 to GLUT5 and GLUT7. These different transporters have different biochemical properties and correspond to different gene products differentially synthesized in various tissues. GLUT4, mainly synthesized in muscle and adipose cells, is particular in that it is present in intracellular vesicles that are translocated to the plasma membrane in response to insulin. A number of transgenic mice have been produced which overexpress the human, mouse or rat GLTU4 gene or the corresponding cDNA using either its own promoter or the promoters for the MLC-2 gene, the aldolase A gene (skeletal muscle-specific) or the adipocyte P2 (aP2) gene (specific for adipose tissue). Overexpression of GLUT4 under its own promoter resulted in increased whole-body insulin action [28–35] with an obesity that usually accompanies a high-fat diet but glycemic control was not impaired [36]. In the same line, overexpression of GLUT4 reduced hyperglycemia and improved glycemic control in diabetic *db/db* mice which carry a mutated leptin receptor gene [37], and overexpression of GLUT4 using the aP2 promoter resulted in increased adiposity in transgenic mice [38,39]. In skeletal muscle, overexpression of GLUT4 resulted in glycogen accumulation [40,41], and overexpression of GLUT1 reduced blood glucose levels [42–44].

Recently, targeted disruption of the GLUT4 gene was achieved and gave surprising results [45]. GLUT4-deficient mice did not develop diabetes as severe as one might have expected. Only male animals showed a mild fasting hypoglycemia and slight postprandial hyperglycemia. However, insulin resistance existed in both females and males as evidenced by postprandial hyperinsulinemia and impaired glucose tolerance tests. In addition, a number of disorders in lipid metabolism were observed such as raised serum levels of lactate, free fatty acids and β-hydroxybutyrate in the fasting state and marked reduction in fat tissue deposits. Finally, GLUT4-deficiency resulted in growth retardation and reduced longevity (5–6 months) due to cardiac hypotropy.

3.5. PEPCK

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the first step of gluconeogenesis in the liver. Transgenic mice expressing the rat PEPCK gene under its own promoter have been generated [46]. These animals presented with fasting hyperglycemia, reduced hepatic glycogen storage, reduced GLUT4 gene expression in muscle and altered glucose tolerance.

3.6. $G_{i\alpha 2}$

The G protein, $G_{i\alpha 2}$, has been implicated in the inhibition of adenylcyclase. Transgenic mice producing an antisense RNA directed against $G_{i\alpha 2}$ mRNA have been generated. These mice express an antisense sequence inserted in the 5′-untranslated region of rat PEPCK gene. $G_{i\alpha 2}$ gene expression was down-regulated in adipose tissue and liver, which resulted in hyperinsulinemia, impaired glucose tolerance and resistance to insulin affecting, for instance, glucose transporter activity and recruitment, counterregulation of lipolysis and activation of glycogen synthesis [47]. $G_{i\alpha 2}$ deficiency increased the activities of protein tyrosine phosphatases and reduced IRS-1 phosphorylation.

3.7. Manipulating adiposity

The mechanisms of insulin resistance associated with obesity, a major risk factor for NIDDM, are still poorly understood (review [48]). Recently, a few studies have attempted to manipulate adiposity in mice. Transgenic mice expressing a toxigene, encoding the diphteria toxin A chain, under the control of the aP2 gene promoter or the promoter for the mitochondrial uncoupling protein gene (brown fat tissue-specific) were obtained [49–51]. Overexpression of glycerol 3-phosphate dehydrogenase gene in transgenic mice was achieved using its own promoter [52]. Finally, targeted disruption of the gene encoding $R_{II\beta}$, one of the regulatory subunits of cAMP-dependent protein kinase A, has been performed [53]. These mice have abnormal brown and/or white fat tissues and will be useful models for studying the contribution of fat tissue in glucose homeostasis and the obesity associated insulin resistance leading to NIDDM.

4. Creating β-cell dysfunction in the mouse

As noted above, NIDDM patients present with β-cell defects in addition to insulin resistance, and it is still a matter of debate as to which might be the primary cause of NIDDM [54]. In this respect, transgenic mouse models in which β-cell function is impaired are potentially important to consider.

4.1. Insulin

The first transgenic mice expressing the human insulin gene under the control of its own promoter had normal insulin levels and glucose homeostasis [55,56]. Although total insulin mRNA levels could be elevated 2-fold or more, total serum and pancreatic insulin levels remained normal indicating the existence of post-transcriptional regulation for insulin production [57]. Two transgenic mouse lines carrying multiple copies of the human insulin gene were later reported which became hyperinsulinemic and showed glucose intolerance [58]. Trans-

genic mice for mutated human insulin genes were also produced. It was shown that a HisB10 to Asp mutation resulted in high levels of proinsulin secretion via the unregulated constitutive pathway [59]. Constitutive proinsulin release was also reported for SerB9 to Asp and ThrB27 to Glu mutations [60].

4.2. Glucokinase

A specific isoform of hexokinase, hexokinase IV or glucokinase (GK), is present in β-cells and liver, and has been proposed to act in β-cells in glucose sensing. In transgenic mice producing an antisense RNA encompassing a ribozyme element under control of the rat insulin II gene promoter (P_{InsII}), the GK activity in islets was reduced and insulin release in response to glucose from in situ-perfused pancreas was reduced [61]. However, plasma glucose and insulin levels remained normal. In another study, transgenic mice producing antisense RNA to the glucokinase gene under control of the human insulin gene promoter showed glucose intolerance that was mouse strain-dependent [62]. Transgenic mice overexpressing yeast hexokinase B under control of P_{InsII} showed increased insulin secretion and reduced blood glucose [63]. It was subsequently reported that overexpression of yeast hexokinase B caused significant pancreatic depletion of insulin [64]. The breeding of these mice overexpressing hexokinase B with transgenic mice made diabetic by overexpression of a chicken calmodulin gene in β-cells [65] transiently improved their diabetic symptoms. However, this protection was short-lived and did not protect against insulin depletion of β-cells.

More recently, mice carrying a disrupted GK gene have been generated [66–68]. Heterozygous animals showed mild diabetes. They were hyperglycemic with reduced capacity to secrete insulin and showed glucose intolerance. In their liver, glucose production persisted even under hyperglycemic and hyperinsulinemic conditions and glycogen content was reduced. Homozygous pups became extremely hyperglycemic and developed severe diabetes. The levels of cholesterol and triglycerides in the serum were elevated. Absence of GK in the liver resulted in reduced glycogen stores and in steatosis. GK-deficient pups were growth retarded and survived 3–5 days after birth. Reconstitution of GK activity using transgenic mice expressing GK cDNA in β-cells using P_{InsII} rescued the lethal phenotype.

4.3. GLUT2

GLUT2 has also been proposed to be part of the β-cell glucose sensing machinery. GLUT2 gene expression in β-cells could be reduced by 80% at the protein level in transgenic mice producing antisense RNA to GLUT2 using P_{InsII} [69]. Such mice were hyperglycemic and had impaired glucose tolerance tests. However, in transgenic mice expressing the human oncogene [Val^{12}]HRAS using P_{InsII} which resulted in down-regulation of GLUT2 in β-cells, glucose homeostasis remained unaffected in 2-month-old animals [70].

4.4. IAPP

Islet amyloid polypeptide (IAPP) is a normal constituent of β-cells. Transgenic mice expressing the human or rat IAPP gene using P_{InsI}, P_{InsII} or the human insulin gene promoter had increased IAPP levels in their blood [71–74]. In some reports, no amyloid deposits, characteristic of pathological states, were detected in the islets up to 7 months of age whereas accumulation of nonfibrillar human (but not rat) IAPP mass was reported in perivascular spaces in one study [74]. The animals were normoglycemic and normoinsulinemic.

5. Conclusion

It appears that both insulin action and β-cell function can be manipulated by altering the expression of many genes. Transgenic and gene-targeting approaches, therefore, offer great potential for further developing mouse models in which the effects of defined alterations in a set of genes could be examined. The study of such genetically engineered mice will help understanding the molecular basis of complex pathophysiologies in different kinds of NIDDM in humans. A systematic analysis of potential diabetogenes might also help the search for candidate genes in human disorders. Such mutant mice could also be used to test therapeutic strategies.

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