Conditional Knockout of Mouse *Insulin-Like Growth Factor-1* Gene Using the Cre/loxP System (44500)

JUN-LI LIU, SHOSHANA YAKAR, AND DEREK LEROITH[1]

Clinical Endocrinology Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892–1758

Abstract. **Insulin-like growth factor-1 (IGF-1) is an essential growth factor for normal intrauterine development and postnatal growth. Mice with a complete deficiency of IGF-1 (IGF-1–null mice), created by homologous recombination, were found to exhibit postnatal lethality, growth retardation, infertility, and profound defects in the development of major organ systems. Furthermore, IGF-1–null mice were resistant to growth hormone (GH) treatment in peri-pubertal somatic growth. Using the Cre/loxP-induced conditional knockout system, we generated a mouse that lacks IGF-1 specifically in the liver, the primary site of IGF-1 production. Interestingly, although circulating and serum levels of IGF-1 were decreased by ≈ 75% in these mice, they exhibited no defect in growth or development. When administered exogenously, GH stimulated IGF-1 production in several extra-hepatic tissues as well as body growth. The "Somatomedin hypothesis" originally proposed that circulating IGF-1 acting in various tissues mediate the effects of GH. These striking *in vivo* results, obtained using homologous recombination technology, call for a major modification of the Somatomedin hypothesis. These gene targeting studies confirm that IGF-1 is essential for GH-stimulated postnatal body growth. However, liver-derived (endocrine) IGF-1 is not essential for normal postnatal growth, though it does exert a negative feedback on GH secretion. Instead, local production of IGF-1, acting in a paracrine/autocrine fashion, appears to mediate GH-induced somatic growth. This review will discuss the effects of tissue-specific IGF-1 gene deficiency created by the Cre/loxP system versus the conventional IGF-1 knockout.** [P.S.E.B.M. 2000, Vol 223:344–351]

The role of a particular gene product *in vivo* is being studied increasingly by either deletion of the gene (gene targeting or knockout) or the gain of its function by transgenic overexpression. Gene targeting is the introduction of a defined modification at a specific location in the genome by homologous recombination. This powerful technique has followed from the classic experiments of Thomas and Capecchi in 1987 (1). Since then, numerous mouse models with defined genomic mutations have been developed.

Conventional and Cre/loxP-Induced, Conditional Gene Targeting

Conventional Gene Targeting. Embryonic stem (ES) cells can be isolated from mouse blastocysts and retain pluripotency. Gene targeting is achieved by transfecting ES cells with specific gene-targeting vectors. Figure 1 illustrates the schematic design of a typical gene-targeting vector. This vector contains a replacement construct, with two regions of homology to the target gene located on either side of a positive selectable marker (neo). When transfected into

[1] To whom requests for reprints should be addressed at CEB/NIDDK, Bldg. 10, Room 8D12, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892–1758. E-mail: derek@helix.nih.gov

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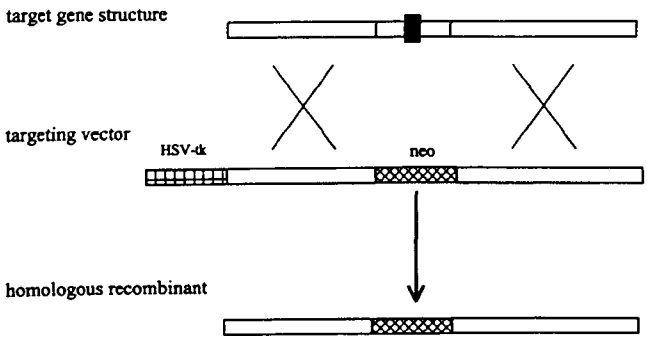

Figure 1. Conventional gene targeting strategy. A replacement type targeting vector (middle bar) consists of two segments of genomic DNA that are homologous to the flanking regions up- and downstream of the targeting exon, indicated by the two crosses. In this construct, the neomycin-resistant gene (neo) replaces the target exon. Embryonic stem (ES) cells are transfected with the vector and subjected to positive selection in the presence of neomycin. Outside of the homologous region, the *HSV-tk* gene is included in the construct to facilitate negative selection of the ES clones. A correct homologous recombination event will replace the target exon with the neomycin cassette, thereby permanently inactivating expression of the targeted gene, whereas the *HSV-tk* gene is not incorporated in correctly targeted recombinations.

ES cells, a double crossover event will replace the target gene sequences irreversibly with the replacement construct sequences. To direct selection of the correct clones, a positive-negative selection strategy is commonly used. Positive selection of the cells using neomycin eliminates most of the cells that have not stably incorporated the construct into the genome. Genomic integration can occur not only by homologous recombination, but also by random integration (insertion), and neomycin-resistant clones will also arise from this mechanism. To enrich for homologous recombinant clones, a second, negative selection marker (e.g., herpes simplex virus thymidine kinase, HSV-tk) is most commonly placed outside the region of homology to the target gene. In the presence of the *HSV-tk* gene, the cells are sensitive to acyclovir and its analogs (e.g., gancyclovir). HSV-tk enables these drugs to incorporate into DNA, resulting in DNA chain termination and cell death. During homologous recombination, vector sequences located outside the regions of homology to the target gene are lost due to crossover. In contrast, during random integration, all sequences in the vector tend to be retained because recombination usually occurs at the ends of the construct. Cells carrying the *HSV-tk* gene can thereby be selected against with gancyclovir. Thus, homologous recombinants will be resistant to both neomycin and gancyclovir, whereas clones in which the construct integrated randomly will be neomycin-resistant but gancyclovir-sensitive.

Finally, a correct homologous recombination event has to be confirmed by Southern blot hybridization. For this purpose, a DNA probe is designed using sequences from outside the region of homologous recombination. This probe is then hybridized to genomic DNA that has been prepared from the ES clones and digested with a restriction enzyme. The digestion creates specific DNA fragments that, based on the unique digestion pattern, can distinguish the correct homologous recombinant from the wild-type allele. Because ES cells are pluripotent, these genomic modifications can be introduced into the murine germ line by injecting ES cells into recipient blastocysts. Depending on the expression pattern of the particular target gene, disruption of a gene could have mild or severe consequences. For example, deletion of a gene that is expressed in a specific subset of cells could affect those cells and their downstream pathways (either within the same cells or their paracrine/postsynaptic target cells), whereas deletions of a ubiquitously expressed gene could have more severe phenotypic consequences, including embryonic lethality. Alternatively, deletion of many genes predicted to have important regulatory functions *in vivo* has been found to produce little or no observable phenotype, suggesting that many cellular functions are redundant, or can be compensated by other mechanisms. Interested readers are referred to excellent review articles on the principle and practice of gene targeting techniques (2–7).

Cre/loxP-Induced Conditional Gene Targeting. As described later in this review, although the conventional gene knockout approach demonstrated a crucial role for IGF-1 in intrauterine development and perinatal survival, it is not very useful for postnatal studies because most of the pups die after birth, and those that survive carry profound developmental defects (8, 9). Moreover, IGF-1 may exert different functions in various ontogenic stages and cell types. To overcome these obstacles and to allow us to dissect the role of IGF-1 in individual cell types (spatial) or developmental stages (temporal), we have used the Cre/loxP system to generate conditional gene knockouts (10, 11).

Site-specific recombination provides an efficient, complementary set of methods for manipulating the target gene, and is mediated by an exogenously supplied recombinase. The recombination occurs at specific sites on the interacting DNA molecules, where it induces strand exchange at small regions of DNA homology within the recombining sites and causes no degradation or synthesis of DNA (12). Cre-mediated loxP-specific recombination is achieved by the introduction of the loxP sites within the genomic DNA and expression of the Cre recombinase.

Two members of the integrase family of site-specific recombinases have been well characterized: the Cre (cyclization recombination) recombinase of bacteriophage P1 and the FLP recombinase from the 2-μ circle of *Saccharomyces cerevisiae*. Cre is a 38-kDa recombinase that recognizes the loxP (locus of crossover of P1) site, a 34-bp sequence consisting of two 13-base-pair inverted repeats, separated by an 8-base-pair, directional spacer. Intramolecular recombination results in either excision of intervening DNA (if the two loxP sites are tandem repeats) or DNA inversion (if the sites are positioned in opposing orientations). Cre recombinase activity requires no additional proteins or co-factors (11, 13).

To generate a conditional gene knockout using the Cre/loxP system, two lines of transgenic mice are required. The target mouse carries a modified allele of the gene to be ablated with two loxP repeats flanking critical exons, that are generated by homologous recombination in ES cells similar to the conventional gene targeting strategy (Fig. 2). A second Cre transgenic mouse line expresses the Cre recombinase under a promoter with the desired spatial and temporal pattern of expression. By crossing these two mouse lines, animals with tissue-specific gene deletion can be generated. Early studies demonstrated that the Cre/loxP system could mediate T-cell–specific deletion of DNA polymerase-β and *NMDAR1* gene deletion restricted to the CA1 region of the hippocampus (10, 14, 15). These mutant mice grow into adulthood without gross developmental abnormalities, therefore circumventing undesirable developmental and behavioral consequences caused by conventional gene deletion.

Using homologous recombination, we have established a mouse line in which Exon 4 of the *IGF-1* gene is flanked by two loxP sites (Fig. 2). Exon 4 encodes for amino acid residues 26–70 of IGF-1, including part of the B domain and entire C, A, and D domains. This region of the peptide is solely responsible for binding to the IGF-1 receptor (16, 17). Once the target gene is marked with the loxP sites, conditional knockout can be achieved by various approaches. The latter part of this review will discuss liver-specific IGF-1 deficiency using albumin promoter-directed Cre expression, as well as an interferon-inducible, liver- and spleen-specific IGF-1 knockout. Furthermore, one can activate Cre expression *via* a ligand-induced system or *via* local injection of an adenovirus-directed Cre expression system (18, 19).

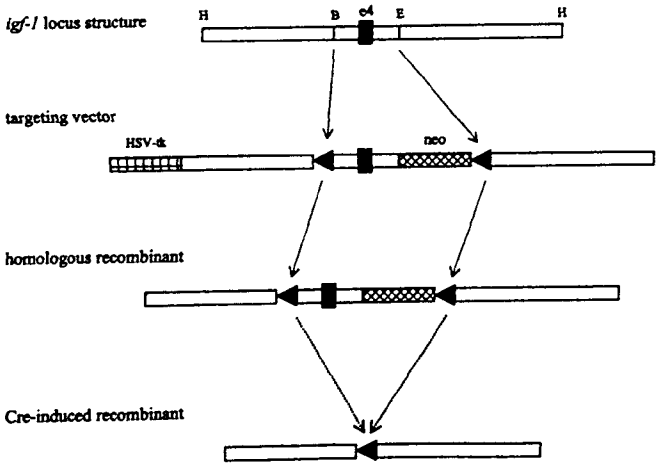

Figure 2. Strategy of Cre-induced *IGF-1* gene knockout. A 6.5-kb *Hind*III fragment of mouse genomic DNA containing the entire Exon 4 of the *IGF-1* gene (which encodes amino acid residues 26–70 of the IGF-1 peptide) was chosen as the targeting region. A replacement vector was designed to introduce two tandem repeats of loxP sites flanking both ends of Exon 4. Embryonic stem cells were transfected, selected, and injected into blastocysts for germline transmission of the homologous recombination. A positive (neomycin) and negative (HSV-tk) double selection strategy was employed. Mice homozygous for loxP-flanked IGF-1 locus (IGF-1/flox) were intercrossed with Cre-expressing transgenic mice that express Cre recombinase in restricted tissue (depending on the specific promoter used). Cre induces tissue-specific recombination of Exon 4, thereby inactivating the *IGF-1* gene.

Effects of Total IGF-1 Deficiency

The fundamental importance of the *IGF-1* gene has been well demonstrated by gene targeting studies using homologous recombination in mice and by a human case report (8, 9, 20, 21). Using conventional gene targeting technology, two groups have disrupted the *IGF-1* gene, by targeting the third and fourth exons. Liu *et al.* (8, 22) replaced part of Exon 4, encoding the residues 51–70, whereas Powell-Braxton *et al.* (9) targeted Exon 3 that only partially encodes the B domain of *IGF-1*. Both groups showed that a total deficiency of IGF-1 in mice is postnatally lethal, with severe growth retardation and profound defects in the development of major organ systems (bone, muscle, and reproduction).

Using homologous recombination, we established a mouse line in which the fourth exon of the *IGF-1* gene is flanked by two loxP sites (Fig. 2) (23). We have subsequently created a third line of IGF-1–deficient mice using the Cre/loxP system. Generation of these animals relied on the expression of Cre recombinase in germ cells driven by *EIIa-cre* transgene. This model is unique in that we induced deletion of the entire Exon 4 and removed the neomycin marker with the recombinase. In this way, our mice with complete IGF-1 deficiency have a postnatal survival rate of 42%, which enabled us to study their postnatal growth and the effect of growth hormone (21, 23).

Postnatal Lethality. The most obvious consequence of a total loss of *IGF-1* gene expression is postnatal death, which occurs within the first 24 hr. Depending on the genetic background of the mice, the death rate varies between 32%–95% (95% in C57BL/6 J, 90% in 129/sv, 84% in a mix of C57BL/6 J and 129/sv, and 32% in a mix of MF1 and 129/sv) (8, 9, 21). Close monitoring revealed that these pups were born alive and that most of them were able to breathe (8). The death at this early postnatal stage might be related to respiratory or feeding failure due to a severe muscle dystrophy (including the diaphragm) and atelectatic lungs (9). Powell-Braxton *et al.* (9) reported that whereas > 95% of newborn IGF-1–null pups die immediately after birth, the embryos were alive (heart beating) up to embryonic Day 18.5.

Growth Retardation. Another important aspect of *IGF-1* gene deletion is that these mice displayed marked intrauterine growth retardation and even greater postnatal growth retardation. At birth, the weight of viable IGF-1–null pups was only 65% of that of wild-type litter mates. Furthermore, this difference increased with age, as the average weight of these mice was only 50% of that of wild-type litter mates at age 2 weeks, and then growth slowed even more, with average IGF-1 knockouts body weight only ≈ 30% of that of wild-type litter mates at age 8 weeks. Average adult body weight was ≈ 1/3 and body length ≈ 2/3

of that of wild-type litter mates (21, 22). Thus although IGF-1 is essential for both intrauterine and postnatal development, the growth defect becomes more profound postnatally. This may indicate that the reduced fetal development of the IGF-1–null mice could be partially compensated by IGF-1 coming from the placenta or, more likely, the presence of IGF-2 and insulin. It is known that the placenta produces a large number of hormones including GH, IGF-1, and IGF-2 that may reach the fetus (24–26), and that deficiency in either IGF-2 or insulin contributes to intrauterine growth retardation. Birth weights of IGF-2 knockout and IGF-1/2 double knockout mice are 60% and 30%, respectively, of that of their wild-type litter mates (27, 28). In contrast, deletion of the insulin gene results in only a 15%–20% growth retardation in mouse embryos (29). The further growth retardation observed after birth in these animals might be due to the loss of influence by placental IGF-1, or to low levels of mouse IGF-2, which diminish rapidly postnatally. Furthermore, the role of insulin on postnatal growth is apparently also very limited (29).

Growth Hormone Resistance in Postnatal Growth. In addition to regulating IGF-1 function (i.e., the Somatomedin Hypothesis), GH has been shown to have direct actions on target tissues. For example, GH was found to stimulate the synthesis of the bone morphogenetic proteins and induce proliferation of prechondrocytes and pancreatic isletβ-cells in the presence of IGF-1 antiserum (30–32). To test whether GH had IGF-1–independent effects, we administered GH to IGF-1–null mice. Daily administration of GH to wild-type mice from 2–8 weeks of age accelerated their growth significantly, as measured both by body weight and length. In contrast, GH had no effect on the growth of IGF-1–null mice. Despite this lack of GH-induced growth, IGF-1–null mice had normal levels of GH receptor expression in the liver. Moreover, systemic treatment with GH induced *jun*B expression in liver and liver enlargement in these animals (21). *Jun*B is an established downstream target of GH receptor signaling (33, 34), suggesting that the GH signaling system is functional in these animals. It has also been reported that GH had no effect on peripubertal growth in a human case of IGF-1 deficiency (20). These results support an essential role for IGF-1 in GH-induced postnatal body growth.

Infertility. Both male and female IGF-1–null mice that survive to adulthood are infertile, primarily due to impairment of gonadal steroidogenesis (35). In males, the testes are reduced in size, but proportionally less than the degree of overall dwarfism. However, these animals have only ≈ 20% of the normal level of spermatogenesis. Despite the impact of IGF-1 gene deletion on the structure of the epididymis, sperm from mutant mice are able to fertilize wild-type eggs *in vitro*. The vast majority of males that were caged with wild-type females did not exhibit mating behavior. Nevertheless, an exceptional case of mating has been recorded (35). Thus it seems that the infertility of the IGF-1–null males is largely a consequence of the absence of sex drive, which is apparently due to inadequate serum testosterone levels (≈ 20% of normal) (35).

In wild-type mice, IGF-1 and IGF-1 receptor transcripts can be detected in female reproductive tissues, and gene-targeting experiments have demonstrated that IGF-1 is required for ovulation (35). IGF-1–null female mice fail to ovulate even after administration of gonadotropins. Furthermore, these animals have an infantile uterus that exhibits a dramatic hypoplasia especially in the myometrium.

A reduced level of granulosa follicle stimulating hormone (FSH) receptor expression in the ovaries may partly explain the infertility of the IGF-1–null females. It has been shown that the IGF-1 and FSH receptors are selectively co-expressed in healthy, growing murine follicles (irrespective of cycle stage). However, whereas FSH does not affect *IGF-1* gene expression, IGF-1 augments granulosa cell FSH receptor expression. This suggests that ovarian IGF-1 expression enhances granulosa cell FSH responsiveness by augmenting FSH receptor expression (36). Indeed, FSH receptor mRNA levels were significantly reduced in ovaries from IGF-1-null versus wild-type mice. Furthermore, FSH receptor mRNA was restored to wild-type levels by exogenous IGF-1 treatment. IGF-1–knockout mice displayed reduced aromatase expression and failed to develop normal follicles beyond the early antral stage, which may have represented functional consequences of reduced ovarian FSH receptor gene expression. Interestingly, the ovaries of IGF-1–knockout and FSH–knockout mice appeared very similar in terms of arrested follicular development (36).

Organ Enlargement. IGF-1–deficient dwarf mice are overall proportionally smaller in body weight, body length, and size of most viscera. Nevertheless, close examination revealed a relative enlargement of multiple organs (9, 21). To exclude the influence of an overall change in body weight, we expressed the organ weight as a percentage of body weight and found that adult IGF-1–null mice have significant increases in the weight of the kidney (20%), heart (30%), liver (40%), and brain (100%) (21). Wang *et al.* (37) have reported significant enlargement of the heart, spleen, and kidney in 40-day-old IGF-1–null mice.

As the relationship of GH to organ growth has been well-characterized, the enlargement of those organs may be the result of a sustained GH hyper-secretion (except for the brain). For example, prolonged excessive secretion of GH by implanted GH3 tumor cells in rats was associated with an increase in DNA synthesis and induced wide-spread visceromegaly affecting the liver, kidney, spleen, heart, and adrenal glands (38). Furthermore, transgenic mice overexpressing GH have been reported to have selective splanchnomegaly coupled with hepatomegaly (39), and others have reported that GH-transgenic mice have accelerated liver, kidney, and skeletal growth (40). Finally, pulsatile administration of GH in broiler pullets induced hepatomegaly largely due to cellular hypertrophy (41). On the other hand, diphtheria toxin–induced GH deficiency caused a 24% reduction in the relative liver size (42). We propose an

Table I. Major Abnormalities of Total IGF-1 Deficiency

IGF-1–deficient phenotype	Reference
Postnatal lethality, 32%–95%	(8, 9, 21)
Intrauterine growth retardation, 35%	(9, 21, 22)
Postnatal growth retardation, up to 70%	(21, 22, 37)
Infertility (both sexes)	(35, 36)
Relative organomegaly (brain, liver, heart, kidney)	(9, 21, 37)
Increase in serum GH, decreases in testosterone, and alkaline phosphatase	(21, 35)
Muscle dystrophy	(9)
Increased blood pressure and myocardial contractility	(45)
GH-resistance in postnatal growth	(21)

IGF-1–independent, direct action of GH on target cell growth and proliferation in IGF-1 deficiency. We base this hypothesis on the facts that IGF-1–null mice have no IGF-1 production, and liver does not normally express the IGF-1 receptor postnatally, and because IGF-1 over-expression cannot correct GH-deficiency–induced liver hypotrophy (43). However, we cannot exclude the possibility that the effects of GH could be mediated through other extracellular growth factors (such as hepatocyte growth factor, transforming growth factor, or epidermal growth factor) (44).

The relative enlargement of the brain in these IGF-1–null mice is less easily explained since brain overgrowth can be caused either by IGF-1 overexpression or GH-deficiency (43). Based on the more profound growth retardation in adults (only 30% of normal body weight) versus newborn (65% body weight) IGF-1–null mice, a relatively enlarged brain does not reflect growth of the brain itself, but rather a shrinkage of the rest of the body (Table I).

Liver-Specific IGF-1 Gene Inactivation

Albumin-Cre-Induced Liver-Specific IGF-1 Gene Targeting. The Somatomedin Hypothesis, proposed 3 decades ago, includes two central issues (in today's terminology): first, that GH action on somatic growth is dependent on an endocrine factor (termed variously, sulfation factor, Somatomedin C, or IGF-1); and second, that IGF-1 is mainly produced by the liver. The first aspect has been demonstrated by the classic experiments of Daughaday *et al.* (46) and is widely accepted. The second issue is derived from the following observations: (i) When hypophysectomized rats are treated with GH, IGF-1 secretion from perfused liver is sufficient to maintain a close to normal IGF-1 concentration in the serum (47); (ii) IGF-1 mRNA is most abundant in liver compared with other tissues, including adipose tissue (48, 49); (iii) the postnatal increases in hepatic expression of IGF-1 are more closely correlated with circulating IGF-1 levels than in other tissues (50, 51); (iv) during the peri-pubertal stages of development there is a close correlation with increases in hepatic GH receptor expression, circulating GH, and IGF-1 levels (48, 49); and (v) the levels of IGF-1 mRNA in the liver, but not in other tissues, were stimulated ≈ 10-fold by GH administration in GH-deficient *lit/lit* mice (52). Despite these lines of evidence, direct testing of the second part of the Somatomedin Hypothesis was not possible without an *in vivo* model such as tissue-specific gene knockouts.

Liver-specific *IGF-1* gene deficiency created a unique opportunity to test the Somatomedin Hypothesis. These animals were generated by intercrossing two lines of mice. The IGF-1/flox line, carries Exon 4 of the *IGF-1* gene flanked by two direct repeats of loxP sites through homologous recombination (23). IGF-1/flox mice are crossed with Alb-cre mice, which postnatally express the Cre recombinase specifically in hepatocytes as driven by expression of the albumin promoter (53). As reported earlier (53, 54), the resulting mice exhibited a complete deletion of the fourth exon of the *IGF-1* gene specifically in liver without affecting IGF-1 levels in other tissues such as the heart, brain, kidney, and fat. Excision of the *IGF-1* gene is detectable as early as 10 days after birth. Expression of IGF-1 mRNA is decreased 50–100-fold in the liver (Fig. 3), which results in a 75% reduction in circulating IGF-1 levels compared with wild-type mice (53). Our results confirmed the second aspect of the Somatomedin Hypothesis (e.g., IGF-1 is mainly produced in the liver).

To our surprise, as measured by body weight, overall body length and bone length in both male and females, these IGF-1/floxed Alb-cre mice grew normally, following identical growth curves with wild-type mice between the ages of 2 and 7 weeks. They were fertile and behaved normally (53). The female mice responded normally to exogenous

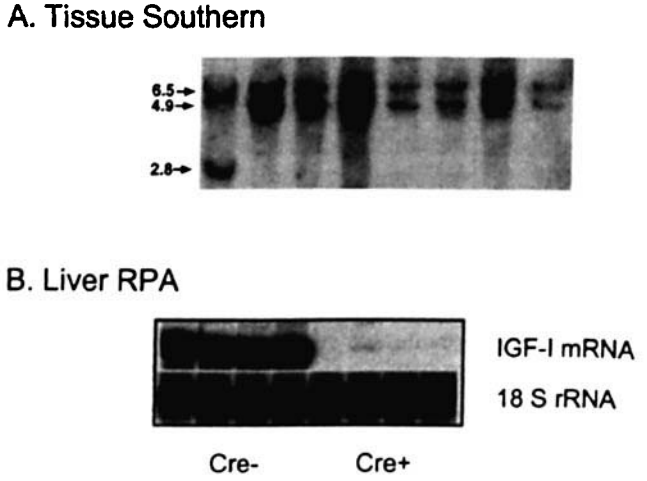

Figure 3. Liver-specific *IGF-1* gene deletion induced by albumin-cre transgenic mice. (A) Liver-specific *IGF-1* gene recombination. Genomic DNA prepared from heterozygous mice (wild-type and IGF-1/flox) were digested with *Hind*III and probed with *p*SP-3. The wild-type allele yielded a 6.5-kb band, whereas digestion of the IGF-1/flox allele yielded a 4.9-kb band. Digestion of genomic DNA from IGF-1–deficient mice yielded a 2.8-kb band, which was only found in the liver. From left the tissues are liver, heart, brain, kidney, muscle, spleen, lung, and fat. (B) IGF-1 gene inactivation in the liver. IGF-1 levels were measured in wild-type and IGF-1 deficient mice using RNase protection assays with total RNA isolated from the liver. Albumin-cre induced a 99% reduction in liver IGF-1 expression.

GH in postnatal growth (unpublished data). Therefore, our results suggest that endocrine IGF-1 may be relatively unimportant in postnatal growth. These results are supported by some earlier reports by others. For example, Orlowski and Chernausek (55) treated hypophysectomized rats for 4 days with GH and showed that the significant increase in body weight and epiphyseal width was not accompanied by an increased concentration in serum IGF-1. Furthermore, a role for paracrine IGF-1 in GH-mediated target tissue growth was demonstrated by Isaksson *et al.* in 1982 (56) and supported by the pattern of IGF-1 gene expression (57). Our studies indicated that such a paracrine mechanism may be the primary pathway by which GH stimulates postnatal growth.

Detailed analysis did reveal several abnormalities in the liver-specific IGF-1–deficient mice (21, 53, 54). First, serum GH levels were significantly elevated, due to diminished negative feedback by serum IGF-1. Second, the livers of these animals were slightly enlarged, which is more pronounced in females. As discussed earlier, in IGF-1–null mice, this could be a result of elevated GH levels in the circulation, as it has been demonstrated that GH, but not IGF-1, increases liver size relative to body weight (43, 58–60). Our results are consistent with a classic negative feedback loop between the liver and the pituitary, such that liver-derived circulating IGF-1 modulates GH secretion, which in turn regulates liver size. Finally, the kidney and spleen were slightly hypotrophic in these mice, which may reflect a role of circulating IGF-1 in maintaining normal growth of these tissues (21, 53, 54).

Interferon-Induced Liver-Specific IGF-1 Gene-Deficiency. A liver-specific IGF-1 knockout mouse has also been generated using an interferon-inducible system (54). Mx-cre transgenic mice express Cre recombinase specifically in the liver and spleen in response to administration of interferons or a synthetic double-stranded RNA (pI-pC), that induces interferons (61). We intercrossed Mx-cre mice with IGF-1/flox mice and selected those homozygous for IGF-1/flox and positive for Mx-cre (L/L + Mx-cre). Administration of multiple doses of interferon at postnatal Days 24–28 (P24 to P28) induced > 90% recombination of the IGF-1 locus in intact liver, 100% recombination in purified hepatocytes, 65% in the spleen, and < 20% in other tissues examined. As a result, IGF-1 mRNA was decreased by > 95% in the liver and 60% in the spleen, and serum IGF-1 levels were decreased by 75%. Once again, these results confirmed that the liver is the main source of circulating IGF-1.

Interferon-induced liver IGF-1 deficiency had no significant effect on body growth between P24 to P77, as measured by body weight, and femoral and tibial length in either males or females (54). This supports the notion that liver-derived endocrine IGF-1 may not be essential for normal postnatal growth. As expected, the decrease in endocrine IGF-1 levels resulted in a 3-fold increase in serum GH concentration and a 14% increase in liver mass.

In conclusion, liver-derived IGF-1 is the main determinant of serum IGF-1, but is not required for postnatal growth, indicating that autocrine/paracrine-derived IGF-1 is more important than liver-derived IGF-1 for body growth. This represents a major modification to the classic Somatomedin Hypothesis.

Perspectives

The fundamental importance of IGF-1 in development, normal metabolism, and abnormalities is well understood. The dramatic phenotype of IGF-1–null mice clearly demonstrated the essential role of IGF-1 in embryonic development and postnatal survival. The role of liver-produced, endocrine IGF-1 can now be studied, although our recent studies suggest that its role in postnatal growth may be of little importance. As IGF-1 is expressed extensively in many other tissues and in various stages of development, this newly developed technology will enable us to evaluate the role of IGF-1 in a spatial- and temporal-manner.

The introduction of the Cre/loxP model in IGF-1 research has prompted more questions and opportunities to address them. Why does the liver produce so much IGF-1? The high levels of IGF-1 produced by the liver suggest that it has other functions besides feedback inhibition on GH secretion. What other tissues secrete IGF-1 into the circulation, and does this IGF-1 mediate GH action? Why are there gender-specific responses to GH treatment in Alb-cre mice? What is the role of endocrine versus paracrine IGF-1 in tumor formation and development?

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