WE DANCE ROUND *IN A RING AND SUPPOSE,*
BUT THE SECRETSITS INTHE MIDDLE AND KNOWS.
ROBERT FROST

INSULIN-LIKE GROWTH FACTOR-11 IN THE CYCLE OF LIFE

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• The insulin-like growth factors (IGF-I and IGF-II) play important roles in growth and development. They are structurally homologous to proinsulin and can interact with the type I and type II IGF receptors and, to a lesser extent, the insulin receptor (1). IGF have potent hypoglycemic activity, which is modulated by the presence of specific IGF-binding proteins, six of which (IGFBP-1 to IGFBP-6) have been cloned to date (2,3). Postnatally, IGF-stimulated growth is merely regulated by pituitary-derived growth hormone (GH). However, prenatal growth regulation by IGF is GH-independent (4). IGF and IGFBP are produced by many tissues and circulate in plasma, suggesting that they act both by endocrine and autocrine/paracrine mechanisms (5). In spite of the vast literature about IGF, their physiological implications in prenatal and postnatal growth regulation are insufficiently known. This is especially true for IGF-II. Therefore, this article will *dance round* a few selected aspects relevant to the biological effects of IGF-II *in vivo*.

IGF-II IN PHYLOGENY AMD ONTOGENY

• IGF-like molecules are found in species whose origins extend back over 550 million years. A hybrid insulin/IGF cDNA has been cloned from the protochordate *Amphioxus californiensis,* an invertebrate from which the vertebrates emerged. In the Atlantic hagfish, one of the most primitive vertebrates belonging to the group of the agnathans, a prototype IGF molecule has been cloned beside insulin, with a remarkable degree of sequence identity to the human IGF. This ancestral IGF molecule gave rise to two distinct IGFs in the elasmobranchs, a major group of vertebrates that lies between agnathans and teleosts on the phylogenetic tree (6). To date molecules with obvious sequence identity to either IGF-I or IGF-II have been demonstrated in teleosts, amphibians, birds and mammals (7).

IGF-I and IGF-II are polypeptides of 70 and 67 amino acid residues, respectively, encoded by separate genes, whose transcription and mRNA translation are developmentally regulated (8,9). Both IGF-I and IGF-II are already expressed during preimplantation embryogenesis (10,11). In rodents, IGF-II is synthesized primarily during the fetal and neonatal period but disappears from the serum in the early postnatal period. This maybe due to absence in these animals of one of the four promoters in the IGF-II gene, which directs "adult specific" expression of the IGF-II gene in man as well as in cattle, dogs, monkeys, guinea pigs and others (9). In these species, IGF-II production continues throughout life, with relatively high concentrations of the peptide being found in adult serum (1), whereas in the adult rat tissue specific expression can only be demonstrated in brain, growth plate cartilage and bone (12-14). Most studies regarding a possible physiological role of IGF-II *in vivo* have been performed in rodents, since they are much easier to manipulate by transgenesis or gene targeting than others. It is wise to keep in mind these differences in postnatal IGF-II concentrations before extrapolating data obtained from rodents to man and other species.

IGF-II RECEPTORS

• The type I IGF receptor appears to mediate most physiological responses of IGF-II on cell growth. This receptor is stractually related to the insulin receptor and belongs to the class of growth factor receptors with intrinsic tyrosine kinase activity (7). IGF-II also binds to the type II IGF receptor which is identical to the cation-independent mannose-6-phosphate receptor. Its physiological role as a cell surface receptor for IGF-II is currently unclear. Ligand binding to this type II receptor seems to be coupled to G-protein activation and Ca^{2+}-influx (15,16). Its main function appears to be targeting of lysosomal enzymes (17).

IGF-II-BINDIMG PROTEINS

• Like the IGF, their binding proteins are widespread in the animal kingdom. Not only do they function as carrier proteins, but they can inhibit or stimulate IGF-mediated actions. However, the precise mechanism by which IGFBPs modulate IGF signalling remains speculative (5). IGFBP-1, -3 and -4 bind IGF-I and IGF-II with approximately equal affinities, whereas the others have a higher affinity for IGF-II. IGFBP-3, the predominant serum IGFBP, is unique in that it binds to an additional 85 kD protein known as the acid-labile subunit to form a 150 kD complex that does not cross the vascular epithelium thereby increasing the half life of serum IGF (2).

IGF-II IM PRENATAL AMD POSTNATAL GROWTH

• IGF-II is a pleiotropic growth factor influencing growth, glucose metabolism, organ homeostasis, the immune and nervous systems and the fetoplacental unit (18). Exogenous IGF-II and IGF-I are important regulators of fetal growth from the earliest preimplantation stages of embryonic development onwards (19,20).

Postnatally, systemic administration of recombinant IGF-II promotes the growth of normal rats and growth hormone-deficient rats and mice, although IGF-II is generally less potent than IGF-I (21-23). Both IGF-I and IGF-II, alone or in combination with growth hormone (GH), show distinct effects on growth of the liver in pituitary-deficient Snell dwarf mice (23). In animals with high levels of IGF-I and IGF-II, alteration at the levels of one IGF results in a reciprocal change at the level of the other one (24). Thus, growth will not be altered by treating these animals with either IGF-I or IGF-II as has been observed in guinea pigs (25).

Although IGF-II is, in general, a poor stimulator of growth, it is an important regulator of intermediary metabolism in chickens (26) and sheep (27). In the latter, coadministration of IGF-II completely blocks the anabolic effect of IGF-I (27). Like-

wise in man, IGF-II influences both lipid and carbohydrate metabolism as shown by the induction of sustained insulin sensitivity in two patients with insulin resistance and hyperlipidemia. In this regard IGF-II may prove to be of benefit in the treatment of lipodystrophy (28).

In the normal neonate, colostrum- and milk-derived growth factors have great implications for overall growth and development (29,30). IGF-I, and to a lesser extent IGF-II, increase milk secretion by direct infusion in the mammary gland in goats (31).

In the immune system, the predominance of IGF-II in the thymus suggests a role for IGF-II (32). Indeed, transgenic mice overexpressing IGF-II under the transcriptional control of the H2Kb promoter show increased growth of the thymus due to an increase in thymocyte number (33,34).

IGF-II is produced throughout life in rat brain (12,13) and its expression is induced following hypoxic-ischemic injury. Whereas IGF-II may modulate the response of glial cells during recovery from cerebral infarction, IGF-I appears to reduce the incidence of infarction and thus may act as an neuronal survival factor (35). By itself, IGF-II stimulates motor nerve regeneration in rats and spontaneous regeneration can be inhibited by anti-IGF-II antiserum (36). Another fimction of IGF-II in the central nervous system may be in the control of food intake, since intracerebroventricularly injected IGF-II in rats results in a decrease in food intake, whereas IGF-I has no effect (37).

New insights for a possible physiological role of IGF-II were obtained from studies in transgenic mice overexpressing or lacking IGF-II. In IGF-II transgenic mice with elevated serum IGF-II levels body growth was either decreased (38) or not significantly influenced (33,39,40). Overexpression in specific organs may result in disproportionate growth as reported for the thymus (33), uterus (39), kidneys and testis (40). These data indicate distinct local and systemic actions for IGF-II in these animals.

By contrast, mice lacking IGF-II are 60% of the size of their wild-type littermates at birth, whereafter growth continues parallelly to that of normal mice, suggesting an important role in fetal growth. This contrasts with the growth pattern of IGF-I knock-out mice. At birth these animals are also 60% of normals in size, but they continue to be growth retarded postnatally reaching a maximum weight of 25-30% of wild-type siblings. Their survival rate is lower compared to IGF-II knockouts. This is indicative for the importance of IGF-I in both prenatal and postnatal growth regulation (41). Surprisingly, loss of the type II IGF receptor results in fetal overgrowth and perinatal lethality. This phenotype is probably caused by an

excess of IGF-II because introduction of an IGF-II null allele rescues the type II IGF receptor mutant mice (42). This indicates that expression of this receptor is essential for late embryonic development and growth regulation. The same is true for the type I IGF receptor, inactivation of which leads to growth retardation and perinatal lethality (43). Insulin receptor substrate-1 (ERS-1) is the major substrate both for insulin and type I IGF receptors. Mice homozygous for a targeted disruption of this gene were born alive but were retarded in embryonal and postnatal growth. This suggests the existence both of IRS-I-dependent and IRS-I-independent pathways for signal transduction of insulin and both IGF-I and IGF-II (44).

IMPRINTING

• Both IGF-II and type II IGF receptor are genetically imprinted in mice; the paternal allele of the IGF-II gene is expressed, whereas of the type II IGF receptor the maternal allele is the active copy (41,43). In man, IGF-II is parentally imprinted as in mice. The type II IGF receptor is imprinted only in a minority of individuals (45). The phenotypic expression of gene imprinting is subject to tissue specificity and developmental regulation. It has been suggested that imprinting may have occurred during evolution because of conflicting parental interest in the growth rates of offspring, male gene transmission being favorable for competition with other males by increasing embryonic growth rate, whereas female gene transmission is favorable for maximizing group size of litters, in competition with other females. Therefore, IGF-II, which is important for growth of the animal, is paternally expressed, whereas the type II IGF receptor should suppress growth by removing extracellular IGF-II and consequently is maternally expressed in order to meet these evolutionary considerations

(46).

IGF-II AMD TUMORIGEHESIS

• IGF-II has been implicated in tumor growth, since its production is enhanced in a number of embryonal and adult tumors (47,48). It is now generally accepted that most cases of the syndrome of non-islet cell tumor hypoglycemia are caused by hypersecretion of IGF-II by the tumor. Due to abnormalities in expression of both IGFBP-3 and its acid-labile subunit, the elevation of free IGF-II in serum leads to an increased bioactiviry, explaining the marked hypoglycaemia of these patients (49).

IGF-II is not only involved in controlling the proliferation of previously transformed cells but also in the transformation process itself. Loss of the genetic imprinting of IGF-II occurring in adult-onset malignancy as well as in embryonic tumors results in an increased expression of IGF-II (50,51). Thus, alterations in imprinting may prove to be an important epige-

netic mechanism for the formation or maintenance of neoplasms throughout life. In this respect, it has been demonstrated that transgenic mice, in which expression of IGF-II was targeted to the mammary gland by placing it under control of the sheep 6-lactoglobulin promoter, develop an excess of mammary tumors (52). In addition, transgenic mice in which IGF-II expression was targeted to the liver develop a variety of tumors with a preponderance of hepatocellular carcinomas (38). These studies are the first *in vivo* experiments to suggest a direct role for IGF-II in the malignant transformation of cells

With respect to the development of metastases, proliferation and cell motility are required. Tumorigenesis and metastatic progression seem to be independent events. The mitogenic signal of IGF-II is mediated by the type I IGF receptor, whereas locomotion acts by the type n IGF receptor, as shown in rhabdomyosarcoma cells (47). Thus, although expression of IGF-II is important in tumorigenesis, the type I IGF receptor is one of the most promising targets for effective antimetastatic therapy. This receptor can act as a ligand-dependent oncogene (53,54). Evidence comes from studies with rat glioblastoma cells which are IGF-I receptor-deficient. When injected into rats these cells are unable to form tumors in contrast with the wild-type cells, which grow into large tumors (55). These and other results suggest that type I IGF receptors are extremely important in establishing and maintaining the transformed phenotype as well as in regulating metastases (54). Recently it has been demonstrated that C-terminal truncation of the type I IGF receptor is essential for transformation but not for mitogenesis, thus these two functions can be dissociated at an intramolecular level (56).

IGF-II AND APOPTOSIS

• Growth of tumors depends not only on cell proliferation, but also on the rate of programmed cell death, or apoptosis (57). Autocrine production of IGF-II may support cell transformation by either enhancing proliferation or increasing resistance to apoptosis. In this regard, it has been shown that transgenic mice homozygous for a disruption of the IGF-II gene develop tumors with a reduced grade of malignancy and a higher incidence of apoptosis (58). In addition, the type I IGF receptor protects tumor cells from apoptosis *in vivo* (59).

CONCLUSIONS

• IGF-II is involved in a variety of cellular responses, such as proliferation, differentiation, intermediary metabolism, migration, and transformation. Although its role in growth regulation seems to be well established, several major questions still remain, e.g. the function of IGF-II in regeneration as well as its interplay with other growth factors. The specific

role of the high postnatal IGF-II levels in humans and other mammals remains puzzling and the question arises as to whether IGF-II is merely a regulator of metabolic processes or a modulator of IGF-I functions. Furthermore, the cellular mechanism responsible for the translation of a specific signal into either a metabolic, mitogenic, differentiating or transforming response needs elucidation (Fig.l).

Much work has to be done to better understand the complex regulatory mechanisms of IGF-II in the process of growth and development. Refinements in the technology of homologous recombination by knocking genes "in" instead of "out" can be a helpful tool in untangling this complex system (60). In addition, non-rodent animal models overexpressing IGF-II will be most helpful to find out whether or not IGF-II is of vital importance in animals with high concentrations of IGF-II postnatally.

Research in an attempt to unravel the function of IGF-II in the cycle of life evolves like a mandala (61): it started many years ago and although a circle with increasing diameter has developed around this starting point, the final appearance of the IGF-II masterpiece is at present still an enigma.

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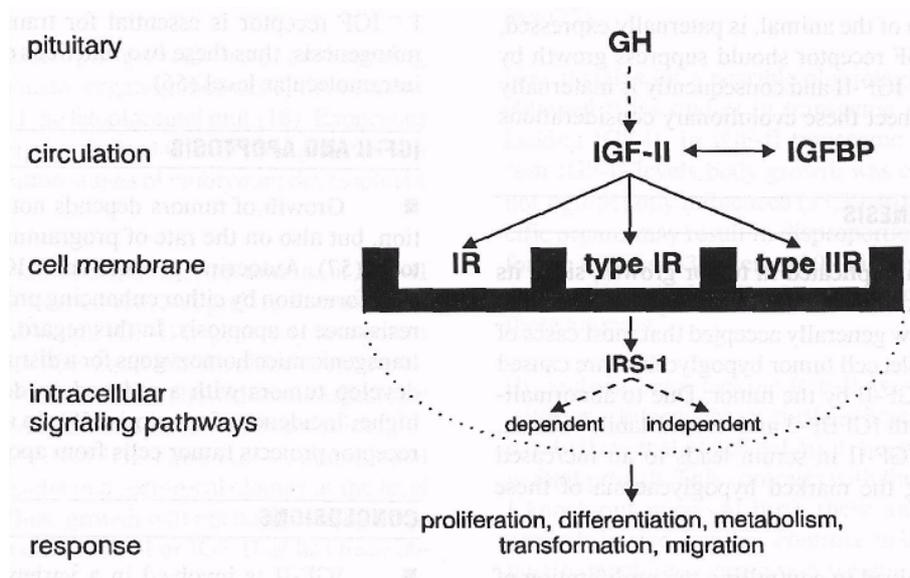
Figure 1. *Shematic diagram of actions and interactions of lGF-IL ---- possible pathway; ——— known pathway; GH - growth hormone; IGF-II - insulin-like growth factor-II; IGFBP - IGF-binding proteins; IR - insulin receptor; type IR - type I IGF receptor; type IIR - type II IGF receptor; IRS-1 - insulin receptor substrate-1.*

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