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Review Article

Two years in IGF research

Briony E Forbes

Department of Medical Biochemistry, School of Medicine, Flinders University of South Australia, Bedford Park 5042, South Australia, Australia

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ABSTRACT

The last two years of insulin-like growth factor (IGF) research has yielded a vast literature highlighting the central role IGFs factors play in processes such as development, growth, aging and neurological function. It also provides our latest understanding of how IGF system perturbation is linked to diseases including growth deficiency, cancer, and neurological and cardiovascular diseases. A snapshot of the highlights is presented in this review, focussing on the topics of IGFs and growth, comparative and structural biology to understand insulin-like peptide function, IGFs and cancer, and IGFs and neurological function. New revelations in the IGF field include the unexpected discovery that the gut microbiome has a remarkable influence on the GH/IGF axis to influence growth, that the insulin of cone snails provides novel insight into the mechanism of receptor binding, and that macrophages in the tumour microenvironment can provide IGF-I to promote drug resistance. These advances and many others provide the exciting basis for future development of disease treatments and for biomarkers of disease.

1. Introduction

The last two years of insulin-like growth factor (IGF) research, from late 2014 to the end of 2016, has yielded close to 4000 publications with insulin-like in the title or abstract. This vast literature highlights the central role these growth factors play in processes such as development, growth, aging and neurological function. It also describes the latest understanding of how IGF system perturbation is linked to disease including cancer, growth deficiency, neurological and cardiovascular diseases, and how IGF system components may be targeted for treatment or used as biomarkers of disease. While many of these themes in the field have been developing over the past 10–15 years, specific advances and exciting discoveries have been made recently that open up new areas of understanding. This brief review will serve to point the reader to a small number of studies that are highlights of the past two years, have made significant contributions to our basic understanding of IGF biology and have already had an impact on the field. Specific review of IGF binding proteins (IGFBPs) will not be included, although reference to some studies where IGFBPs play a role in modulating IGF action will be made.

2. IGFs in growth

The role of IGF-I in postnatal growth has been well studied for many years. Mutations in genes of the GH/IGF-I axis have previously been described and growth deficiencies have been attributed to these mutations. These include mutations in the growth hormone (GH) receptor which lead to severe growth retardation and Laron Syndrome, the growth hormone releasing hormone (GHRH) and its receptor, GH itself, as well as mutations in the *STAT5B*, *IGFALS*, *IGF-I* and *IGF-1R* genes, which cause varying degrees of growth retardation [1]. Of particular note, two studies in the last two years stand out as they have reported novel mutations in the IGF axis that have revealed new insights into the role of the less well studied IGF, namely IGF-II, and the role of proteases in controlling IGF action in growth and development.

Perturbation of mechanisms controlling *IGF2* expression through imprinting has previously been associated with prenatal growth deficiency (Silver-Russell syndrome) or overgrowth (Beckwith Wiedemann syndrome). The *IGF2* gene is an imprinted gene that is expressed from the paternal allele in most tissues, including the placenta, although it is expressed from both alleles in the liver [2]. Recently and for the first time a paternally inherited nonsense mutation in the *IGF2* gene (c.191C → A, p.Ser64Ter) was reported by Begemann et al. [3]. The p.Ser64Ter mutation led to premature termination within the protein leader sequence and before the sequence coding for the mature peptide and the receptor binding sequences. Severe prenatal and postnatal growth restriction was seen in 4 individuals from the one family. Low circulating IGF-II levels were detected and most likely were derived from the maternal allele expressed in the liver. This report confirms the importance of IGF-II in prenatal development and growth. It also surprisingly provides evidence for a role of IGF-II in postnatal growth, with the mechanisms remaining to be elucidated.

Interestingly, no mutations have been reported in the IGF binding proteins (IGFBPs) that act as carrier proteins for the IGFs, prolonging

E-mail address: Briony.forbes@flinders.edu.au.

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their half-life and delivering them to target tissues. The majority of IGFs in circulation is bound to IGFBP-3 or IGFBP-5 in complex with acid labile subunit (ALS). Proteolysis of IGFBPs is a mechanism by which their affinity is lowered for IGFs and by which the ligands are released, thus allowing them to be free to interact with the type 1 IGF receptor (IGF-1R) [4]. Until recently the in vivo evidence for this mechanism came from animal studies of the metalloproteinase pregnancy associated plasma protein-A (PAPP-A) and its homolog PAPP-A2, which both regulate levels of bioactive IGF through controlling IGFBP proteolysis [5,6]. Dauber et al. [7] have now identified two mutations in the human *PAPPA2* gene (p.D643fs25* and p.Ala1033Val) that cause short stature. PAPP-A2 is highly specific for the IGF binding proteins-3 and -5 (IGFBP-3 and -5). Both *PAPPA2* mutations led to a loss of proteolytic activity and a concomitant increase in intact IGFBP-3 and -5 with a decrease in free IGF-I. Overall levels of IGFBP bound IGF-I and IGF-II were increased as a compensatory mechanism to the lack of free IGF-I. The resultant phenotype mirrored that seen in knockout mice with postnatal growth retardation and confirmed an important role for proteolysis in regulating the bioavailability of IGFs.

Many other mutations and single nucleotide polymorphisms (SNPs) in genes regulating the expression or activity of key GH/IGF system components have also been reported [1]. Many of these act indirectly on the GH/IGF system to affect growth. However, one of the polymorphisms identified recently [8] acts directly to regulate IGF levels. The SNP (rs116891695C → T) in the gene *HSP90B1* encoding the glucose-regulated protein 94 (GRP94) leads to a P300L substitution. This reduces the ability of GRP94 to act as a chaperone that normally directly assists the expression of IGF-I and IGF-II. While this SNP was identified in patients with primary IGF deficiency, a heterozygous mutation is unlikely to provide a sole cause of growth deficiency, most likely due to compensatory feedback mechanisms coming into play. However, this study highlights the fact that there are likely to be many other proteins that directly control IGF levels and the function of other components of the GH/IGF-I axis. For further discussion of mutations and SNPs identified in the last 10 years refer to Wit et al. [1].

Of great interest is the discovery by Schwarzer et al. [9] that intestinal microbiota can influence growth (both weight and bone length) in infant mice under both normal and starvation conditions. Initially a significantly lower growth rate was seen in germ free (GF) mice than wild type (WT) mice and this related to lower circulating IGF-I levels. Post weaning, the GF mice lost significantly more weight than WT mice when under starvation conditions. Colonisation of GF mice with *Lactobacillus plantarum* led to improved growth similar to WT mice, particularly under starvation conditions. Apparently the microbiota influences the GH/IGF-I axis increasing the expression of the *GHR*, *Igf1*, *Igfbp-3* and *Socs3* in the liver and *Igf1* in muscle, thus improving the sensitivity to GH, which is diminished during prolonged starvation. The mechanisms underlying this interplay between microbiota and the GH/IGF-I axis are yet to be revealed.

3. Comparative and structural biology to understand insulin-like peptide function

3.1. Comparative biology of insulin and IGF signalling (IIS)

The value of comparative biology has been recognised for decades as it has provided us with clues of how human insulin and insulin-like peptides (including IGFs, relaxin and INSL3-7) bind to their receptors and subsequently influence biological outcomes such as growth and aging. A recent comprehensive review by Nässel and Vanden Broeck in 2016 [10] highlighted the explosion of research in flies, nematodes and yeast that has defined common insulin-like peptide (ILP) signalling pathways involved in growth and aging

The complexity of the insulin/insulin-like peptide system in insects is evident with, for example, *Drosophila* expressing 8 ILPs. DILP6 is structurally and functionally like IGF and is produced in the adipose tissue. It regulates lifespan through regulation of *DILP2* and *-5* expression in the brain [11]. Chatterjee et al. [12] discovered that DILP6 promotes insulin/IGF1 signalling in oenocytes (functionally equivalent to hepatocytes) leading to lipid accumulation. Under starvation DILP6 induces lipid turnover in oenocytes thus providing a mechanism to adapt or become tolerant to starvation. These findings raise the possibility that insulin signalling regulates the interplay between adipocytes and the liver in conditions of starvation.

Until recently it has been difficult to understand how apparently only a single receptor tyrosine kinase could act as the sole receptor for the Drosophila ILPs to promote a wide range of functions from cell and organism growth to metabolic homeostasis. However, in 2015 almost simultaneously Vallejo et al. [13] and Colombani et al. [14] discovered that DILP8 binds the Lgr3, a G protein coupled relaxin-like receptor to promote growth coordinated with homeostatic control through central nervous system (CNS) neuronal activation. Subsequently, mutational studies of Lgr3 in Drosophila confirmed the role of Lgr3 in CNS neuronal DILP8 signalling but also suggested the need to define whether this is via solely a direct interaction or if it involves other receptors including an insulin-like receptor (the known DILP6 receptor) [15]. Therefore, while there is still a lot to understand about the complex Drosophila ILP system this latest research suggests ILP signalling via a relaxin-like receptor co-ordinates growth and maintains body size.

Complexity in ILP signalling in insects is also achieved through temporal expression of different insulin-like receptors. In planthoppers (*Nilaparvata lugens*) alternative wing morphology is determined through expression and activation of two insulin receptors (*Nl*InR1 and *Nl*InR2), which have opposing roles [16]. *Nl*InR1 activation by *NlILP3* (one of the four *Nl*ILPs) promotes PI3K/Akt signalling and inhibits the Foxo transcription factor activity leading to long-wing morph (proposed to be the default developmental morph). Expression of *Nl*InR2 leads to formation of hybrid *Nl*InR1/*Nl*InR2 receptors and inhibition of PI3K/Akt signalling leading to short-winged morph. This study not only highlights a unique IIS signalling regulatory mechanism but also demonstrates the importance of understanding hybrid receptor signalling, a topic that is technically difficult to address in mammalian cells.

In nematodes, of the 40 insulin-like peptides, several are involved in regulation of the growth arrest dauer phase. Matsunaga et al. [17] demonstrated that polarity and timing of secretion of INS-35 and INS-7 by intestinal epithelial cells is changed during dauer such that the peptides are degraded during dauer arrest and then later secreted into the body cavity during growth.

These findings in insects and worms provide significant advances in our understanding of how ILPs are signalling to coordinate metabolic control and growth. Study of how these insect peptides promote different biological actions ultimately may by comparison shed light on how human insulin and insulin-like peptides function.

3.2. Molecular mechanisms underlying IIS

Few studies have provided any molecular detail of the interaction between the insect and worm ILPs and their insulin-like receptors, despite comparative studies previously being very informative for our basic understanding of the way in which human insulin engages with the human IR. For example, many years ago sequence comparisons between hystrichomorph insulins and human insulin combined with mutational studies enabled the definition of two receptor-binding surfaces, providing an insight into how insulins interact with the insulin receptor [18].

Much of our understanding of how human IGFs and insulin engage with the IGF-1R and IR has come from the Lawrence et al. through crystal structures of human insulin bound to a fragment of the human insulin receptor [19]. In the last two years they discovered a key mechanism of engagement of insulin with the IR [20]. A protective hinge at the end of the insulin B chain needs to swing away from the insulin core to allow engagement with the αCT peptide and L1 domains of the IR. TyrB24

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