INVESTIGATION INTO THE MOLECULAR SPECIFICITY OF THE IGFs: A CHIMERIC APPROACH

By

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ABSTRACT

Insulin-like growth factors (IGF)-I and -II induce a variety of cellular outcomes including cell proliferation, differentiation, cell migration and protection from apoptosis. IGF-I and IGF-II share a high degree of structural similarity reflecting their high level of sequence similarity. Despite this both IGF-I and IGF-II exhibit different binding affinities to almost all proteins with which they both interact. To date few studies have investigated the structural basis whereby IGF-I and IGF-II bind with different affinities to the type-1 IGF receptor (IGF-1R), insulin receptor (IR) isoforms, type-2 IGF receptor (IGF-2R) and IGF binding proteins (IGFBPs). The major objective of this thesis is to investigate the molecular basis for the unique receptor and binding protein binding properties of IGF-I and IGF-II

To address the question of what parts of IGF-I and IGF-II confer their unique receptor and binding protein binding affinities, chimeras of IGF-I and IGF-II were engineered where their C and D domains were exchanged either singly or together. These are the first ever whole domain chimeras of IGF-I and IGF-II. Recently the alternatively spliced isoform of the insulin receptor lacking 12 amino acids encoded by exon 11 (IR-A) has been shown to be overexpressed in foetal and cancer tissues and bind IGF-II but not IGF-I with high affinity. Binding analysis of the engineered chimeras to the IR-A showed that the C and D domains of IGF-II allow high affinity binding whereas the same domains of IGF-I preclude high affinity binding to the IR-A. The C and D domains also regulate the differential interaction of IGF-I and IGF-II with the insulin receptor isoform containing the amino acids encoded by exon 11 (IR-B). The C domain of IGF-II, in the background of IGF-II or IGF-I, also allows potent induction of phosphorylation of various tyrosine residues in the intracellular β

subunits of the IR-A and IR-B, whereas the same domain of IGF-I in the background of IGF-II did not. In addition this same domain also is the molecular basis for the differential ability of IGF-II and IGF-I to phosphorylate IRS-1 and activate PKB/Akt. Furthermore, the differential ability of IGF-II and IGF-I to induce cell survival and promote cell migration via the IR-A is due to their C domains.

The IGF-1R binds IGF-I with a 3-5 fold higher affinity than IGF-II. Here in this thesis the differential affinity of IGF-I and IGF-II for the IGF-1R is shown to be in a large part due to their C and D domains.

The IGF-2R is a receptor that does not contain any intrinsic signaling activity and in the context of the IGF system appears to regulate the concentration of IGF-II. IGF-2R binds IGF-II with high affinity but binds IGF-I with very low affinity. Reported here is the novel identification of the IGF-II C domain as an important contributor for wildtype IGF-2R binding. IGFBPs act to both potentiate and inhibit the cellular actions of the IGFs. Almost all IGFBPs bind IGF-I and IGF-II with different affinities, particularly IGFBP-6 which has a 10-60 fold higher affinity for IGF-II compared to IGF-I. Binding analysis of IGF chimeras to IGFBP-1, -2, -3 revealed that the C and D domains of the IGFs do not regulate any IGFBP affinity difference, however the IGF-II C domain may play a small role in binding to IGFBP-6.

Recently a patient exhibiting mental retardation, short stature and gondal dysfunction was shown to be homozygous for a mutation in his IGF-I gene resulting in a protein with a methionine instead of a valine at position 44. Here in this thesis the biochemical characterization of this mutant is reported. This mutation causes almost a 90-fold reduction in IGF-1R binding affinity, while completely abrogating binding to either IR isoform. Interestingly binding to IGFBP-2, -3 and –6 is unaffected. In addition, NMR analysis showed only small perturbations in the structure localized around the site of the mutation.

In conclusion this thesis has contributed to the understanding of ligand receptor interactions of the insulin/IGF system.

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