INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS AND THEIR FUNCTIONS (MINIREVIEW)

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Insulin-like growth factor (IGF) action is influenced by the insulin-like growth factor binding proteins (IGFBPs). Since 1988 eight forms of IGFBPs have been found which differ in molecular weight, amino acid composition, distribution in biological fluids and influence upon IGF activity. An important biological property of the IGFBPs is their ability to increase the half-life of the IGFs in the blood. They are able to act as potentiators of IGFs activity on the cell proliferation. As IGFBPs bind to cell surfaces, they may act either to deliver the IGFs to those surfaces either for the activation of specific receptors or cell responses independently of receptor activation. Posttranslation modification such as phosphorylation, glycosylation and proteolysis of IGFBPs influence their affinity to IGFs. In addition, the IGFBPs may also act as inhibitors to block the activity of the IGFs by preventing cell-surface binding.

Key words: IGF – IGF binding proteins – Receptors – Minireview

One variable that provides IGFs control at the extracellular level is the presence of high-affinity, soluble IGFBPs. Their ability to form complexes with IGFs influences IGFs transport to membrane receptors and thus also IGFs effects on cell proliferation (McCUSKER and CLEMMONS 1992). IGFBPs are proteins of different size which are produced by many different tissues and they bind to IGF-I, IGF-II, but not to insulin. The affinity constants of the six IGFBPs are similar for IGF-I and IGF-II (2-20 and 3-30 x 10^9 l/mol, respectively) with the exception of IGFBP-6, which has a 20- to 70-fold higher affinity for IGF-II than for IGF-I (ZAPF 1995). IGFBPs molecules contain 18 cysteine residues, six of them being located on carboxy terminus and twelve on amino terminus. IGFBPs modulate IGFs effects by endocrine, paracrine and autocrine mechanisms. The origin of this regulation may depend on IGFBPs recognition such as glycosylated binding protein or posttranslation modification such as phosphorylation (MARTIN and BAXTER 1992).

Individual IGF Binding Proteins

There are eight well characterized forms of IGFBPs with different molecular weight, amino acid composition, binding properties and distribution in biological fluids.

IGFBP-1 (25-34 kDa). Growth hormone independent IGFBP-1 was originally isolated from human placenta as placental protein 12. PP 12 has the same amino acid sequence on N-terminus (Ala-Pro-Trp-Gln-) as IGFBP-1 obtained from human amniotic fluid (KOISTINEN et al. 1986). IGFBP-1 was found also in serum in 100-fold lower concentration than IGFBP-3. It seems that complex IGF-IGFBP-1 (30-40 kDa) in amniotic fluid is identical with a similar complex in serum. IGFBP-1 (28 kDa) of amniotic fluid binds with both IGFs with high affinity: $K_a = 6.55 \pm 2.24$ l/nmol for IGF-I and $K_a = 3.23 \pm 1.05$ l/nmol for IGF-II (BAXTER et al. 1987).

Non-phosphorylated and phosphorylated forms of IGFBPs have already been found in human amniotic fluid, fetal serum and decidua. Degree of phos-

phorylation of IGFBP-1 is increasing with time of pregnancy (Koistinen et al. 1993). Busby et al. (1988) isolated two forms of IGFBP-1 (31 kDa) from amniotic fluid with different biological effects: the first one had inhibitory effect on ^{3}H-thymidine incorporation into smooth muscle cell DNA and the second one worked as activator.

In animals (sheep and goats) there are besides IGFs also proteins with higher molecular weight, which are mitogenically active in BP-A31 cell culture (Blahovec et al. 1997). It seems that their properties are the same or very similar to that of human IGFBP-1 and IGFBP-3 (Blahovec et al. 1999).

IGFBP-2 (32-34 kDa) is present in human cerebrospinal fluid, seminal plasma and lymph, in rat amniotic and cerebrospinal fluid, in chicken vitreal fluid (Schoen et al. 1992) and in porcine follicular fluid (Mondschein et al. 1990).

High concentrations of IGFBP-2 in rat fetal tissues and in porcine fetal serum decrease in postnatal life (Russell and **Van Wyk** 1995; Lee et al., 1993). IGFBP-2 preferentially binds to IGF-II which is the important regulator in fetal growth (Hossner et al. 1997).

IGFBP-3 (53 kDa) is growth hormone dependent. Its acid stable subunit of 150 kDa complex binds to IGF-I or IGF-II with high affinity and can function either as inhibitor or activator of IGF-I stimulated DNA synthesis. Molecular weight of IGFBP-3 depends on glycosylation degree. Thus, non-glycosylated form has 29 480 Da, while glycosylated one has 54 kDa (Russell and Van Wyk 1995). Glycosylated IGFBP-3 is able to bind to cell surfaces by a weak non-covalent sugar-sugar interaction. Free IGFBP-3 has 3- to 10-fold higher affinity to ligand than cell surface-associated IGFBP-3 (McCusker and Clemmons 1992). IGFBP-3 is the dominant binding protein in blood in 40-fold higher concentration than IGFBP-1 and with higher affinity to IGF-I. The majority of circulatory IGF-I is bound to IGFBP-3. IGFBP-3 serum levels are 10-fold higher than these in lymph (McCusker and Clemmons 1992). IGFBP-3 is present also in cerebrospinal fluid, in human and rat lymph, in porcine and rat colostrum and milk, in human and porcine follicular fluid, in human seminal plasma and last trimester amniotic fluid. Human amniotic fluid IGFBP (28 kDa) and IGFBP (53 kDa) show a high affinity to IGF-I and IGF-II and their concentrations decrease with fetal development (Baxter et al., 1987). IGF-I and IGFBP-3 concentrations in porcine serum are low during fetal life, but they increase in postnatal life. During the whole fetal development IGF-II levels are higher than IGF-I levels (Lee et al. 1993).

IGFBP-4 was isolated in two forms with different molecular weight (29 kDa and 24 kDa) from ovine blood plasma. IGFBP-4 with higher molecular weight is N-glycosylated like ovine IGFBP-3 (Hossner et al. 1997). IGFBP-4 (24 kDa) was found also in human, rat and porcine serum (McCusker and Clemmons 1992). Smooth muscle cells of rat vessels produce IGFBP-4 (24 kDa) and its 5- to 6-fold increasing is caused by PDGF isoforms (Gianella-Neto et al. 1992). IGFBP-4 blocks the effects of exogenously added IGF-I to the cells (Russell and Van Wyk 1995).

IGFBP-5 (23 kDa) was purified by Andress and Birnbaum (1992) from human osteoblast derived culture which increases mitogenesis by coincubation with IGF-I or IGF-II. IGFBP-5 stimulates osteoblast mitogenesis not only by association with IGF, but also with osteoblast surfaces (extracellular matrix) either without IGF presence or interaction with IGF receptor. IGFBP-5 is present in rat blood serum together with IGFBP-6 and also in the kidney, bone and endocrine tissues. It is important for lung development (Wallen et al. 1997).

IGFBP-6 (30-32 kDa) was isolated from human cerebrospinal fluid and his affinity is 10-fold higher to IGF-II than to IGF-I. It is present in human and rat tissues (Russell and Van Wyk 1995). It contains O-glycosidic bond. Enzymatic deglycosylation does not change affinity properties of IGFBP-6 to IGFs. Glycosylation is probably important for the secretion, stability and localization of IGFBP-6 (Bach et al. 1992).

IGFBP-7 together with IGFBP-8 show a low affinity to IGFs. IGFBP-8 is probably connective tissue growth factor (Kim et al. 1997).

Functions of IGF Binding Proteins

IGFBPs carrier IGFs in circulatory system, they can caused inhibition of IGFs effects or total inactivation of growth factor. In certain situation they are able to potentiate the IGFs effects, too. They not only transport IGFs molecules to their receptors but also adhere to cell surface.

1. Carrier function. IGFs circulate in blood serum as macromolecules. As much as 75-90 % of total IGF-I (1 μg/ml in human adults) in serum are bound to ternary protein complex with molecular weight about 150 kDa and with long half-life (12-16 hours). 10-25 % of total IGFs form binary complex with molecular weight 28-35 kDa and with short half-life (about 30 min) (Martin and Baxter 1992; Gourmelen et al. 1994; Zapf 1997). Less than 1 % of total IGF-I circulates in free form (Blum et al. 1989) and its half-life is about 10 minutes (McCusker and Clemmons 1992).

Ternary complex consists of IGFBP-3 (binding or ß-subunit), acid-labile subunit (ALS, α-subunit) and IGF-I or IGF-II (γ-subunit). α-Subunit is N-glycosylated protein with molecular weight 84-86 kDa. It is able to form complexes not only with IGFBP-3 (50 kDa), but also with smaller forms of IGFBP-3 which explains the existence of 120-kDa ternary complex.

Binary complex consisting of one form of IGFBPs and IGF-I or -II, is able to cross capillary barrier and transport IGFs to target cells where the IGFs effect is modulated by IGFBPs (stimulation or inhibition), either directly by interaction with IGF-receptor or by the change in phosphorylation (Zapf 1995).

High levels of IGFBP-3 and IGFBP-1 in chorion fluid and low levels in amniotic fluid mean that amnion is an effective barrier for transport of binding proteins from chorion to amniotic fluid (Nonoshita et al. 1994).

IFGBP-2 is able to cross endothelial barriers and it works as IGFs transporter (McCusker and Clemmons 1992).

2. Potentiation of IGFs effects. Potentiation phenomenon of IGFs effects is characterized by (Zapf 1995): 1. phosphorylation (IGFBP-1); 2. association with cell surface (IGFBP-3); 3. association with extracellular matrix (IGFBP-5); 4. proteolysis (IGFBP-3 and -5).

Posttranslation modifications of IGFBPs can influence their biological activity. These modifications of IGFBPs such as phosphorylation result in the change of their affinity to IGFs. More phosphorylated form of IGFBP-1 of human amniotic fluid and decidua has higher binding affinity to IGFs than less phosphorylated form (Koistinen et al. 1993). There are known phosphorylated forms of IGFBP-1 which potentiate mitogenic activity of IGF-I.

There is more and more information about possible mechanisms which explain how IGFBPs contact to cells. Then it leads to signal transmission either by means of IGF receptor type I, or IGF receptor type I independent pathways of mitogenic signal transduction (Hossner et al. 1997). IGFBP-1 and -2 contain integrin recognition sequence Arg-Gly-Asp (RGD) which causes their binding to $\alpha_5\beta_1$integrin (fibronectin) receptor. Both these effects depend on presence of RGD sequence but not IGF-I (Jones et al., 1993).

On the other hand, IGFBP-3 and -5 do not contain RGD sequence and are able to associate with the cell surface. IGFBP-5 binds to extracellular matrix and IGFBP-3 interacts with membrane glycosaminoglycans. Cell-associated IGFBPs show lower affinity to IGFs and potentiate IGFs effects (Hossner et al. 1997).

Proteolysis of IGFBP-3, -4 and -5 decreases their affinity to IGF-I for 50-100-fold. IGFBP-3 protease which is present in chorion fluid and maternal serum, but not that in amniotic fluid, cleaves IGFBP-3 into two fragments with molecular weight 18 and 15 kDa, which further regulate IGF-II amounts for target chorion and amnion cells (Nonoshita et al., 1994). IGFBPs proteolysis may be an important mechanisms in releasing of biological active molecules of IGFs which increases their ability of interaction with their receptors on cell surfaces.

3. Inhibition of IGFs effects. The binding of IGFBPs to IGFs inhibits IGFs association with target cells and thus the resulting IGFs effect is weak (Russell and Van Wyk 1995).

IGFBP-1 inhibits IGF effect on human osteosarcoma cells (Campbell and Novak 1991). Rat serum IGFBP-3 shows an inhibitory effect on the growth

of chicken embryonal fibroblasts which was stimulated by IGF-I, IGF-II and whole human blood serum (Liu et al. 1992).

Increased concentration of IGFBP-3 inhibits the proliferation of breast cancer cell line MCF_7 either directly or by competition for IGF receptors. Thus, IGFBPs possibly work as antimitogens and IGFs are potential promotors of cancer growth (Zapf 1995).

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