PLASMA FIBRONECTIN IN RHEUMATOID ARTHRITIS

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الله

بسم الله الرحمن الرحيم

سبحانك

لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم

صدق الله العظيم

بسم الله الرحمن الرحيم

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Zeinab Galal

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Introduction

Fibronectin is a cell surface glycoprotein of fibroblasts and other cells which is necessary for the adherence and spreading of the cells on collagenous substractes (Klebe, 1974). It can function as an opsonin (Pommier et al.,1983) it has chemotactic properties for monocytes and fibroblasts [not neutrophils and lymphocytes],(Norris etal.,1982). Furthermore, it binds to the C_{1q} component of complement (Bing et al.,1982) and to bacteria (Kuusela,1978). Finally it may also help in the adhesion and spreading of cells during inflammatory and immune responses.

In rheumatoid arthritis [RA], the concentration of fibronectin has been known to be increased in synovial fluid (Carsous et al.,1981) and massive deposition of fibronectin has been observed in synovial tissue (Shiozawa & Ziff,1983).

Fibronectin may play a role in promoting the assembly of collagen fibrils, and in this way may provide an interstitial frame work for the attachement and extension of inflammatory cells in RA.

Aim of the work

To determine the level of plasma fibronectin in patients having either definite or classical RA, who are diagnosed according to American Rheumatism Association criteria [Ropes et al.,1958].

FIBRONECTIN

The term fibronectin describes a family of structurally and immunologically related high molecular weight glycoproteins that are present in blood, other body fluids and tissue, (Mosesson MW. et al.,1980 ; Cosio FG. et al.,1986; Hynes RO.,1986).

The name from the latin roots, fibra = fiber, nectere = to bind, or to contact. (Mosesson MW. et al.,1980 ; Saba TM. et al.,1986 ; Hynes Ro.,1986).

It has been known by many other names including cold-insoluble globulin,(Morrison PR. et al.,1948 ; Chen AB. et al.,1976), Large external transformation sensitive Protein(Hynes Ro. et al.,1974), cell surface Protein (Yamada KM. et al.,1974), cell adhesion factor,(Pearlstein E. 1976), soluble fibroblast antigen,(Ruoslahti E. et al., 1974), opsonic protein,(Saba TM. 1970), cell spreading factor,(Grinnell F.1976).

Historical events

Mossison. et al.,in 1948, described a protein component of fibrinogen-containing fraction, that was cold-insoluble and, unlike fibrinogen, was not thrombin coagulable, displayed a more rapid anodal electrophoretic migration rate, higher sedimentation coefficient then did fibrinogen. Mossison named it "cold-insoluble globulin of plasma".

Later, physicochemical analyses reported in 1955 by Edsall et al., led to the suggestion that cold-insoluble globulin was a modified dimer of fibrinogen. Shortly thereafter, Smith & Von Korff in 1957, discribed a protein with properties similar to those of cold-insoluble globulin that they had found in a heparin-induced cold precipitate of either normal or pathologic plasma.

Subsequent clarification of these observation began by Mosesson et al., in 1968, with investigation of a patient presenting with a chronic intravascular coagulation syndrome secondary to an occult neoplasm. The illness was characterized by the persistence of a pathologic cold-induced plasma precipitate termed "cryofibrinogen". The solubilized cryofibrinogen was partially coagulable by thrombin, thus proving that fibrinogen was present. Another major component resembling cold-insoluble globulin was also found in the fractions & was shown to be immunochemically identical with a normal serum protein unrelated to fibrinogen.

in the early 1970' a number of investigation had focused on the changes that occured in all surface proteins of fibroblasts as a consequence of transformation by oncogenic viruses . Particular attention was paid to a large transformation - sensitive glycoprotein of high molecular weight,(Hynes et al.,1974 ; Ruoslahti et al., 1974 ; Yamada et al.,1974 ; Blumberg et al.,1975 ; Ruoslahti et al.,1973), that was released from the fibroblast cell surface into the culture media. The report by Ruoslahti & Vaheri in 1975 that cold-insoluble globulin was antigenically identical to large transformation-sensitive glycoprotein brought to light the uniqueness of fibronectin as both a cell-surface/ matrix protein & a blood protein and has stimulated numerous investigations on all forms of this protein.

A great deal of biochemical work done in laboratories through out the world has led to model of the fibronectine molecule in which the protein's binding functions and its structure are clearly corelated. Structural studies of fibronectin derived from cell surface, tissue culture medium, or extracellular fluids have been complicated by the presence of molecular heterogeneity,(Chen et al.,1976 ; Yamada et al.,1977 Jaffe et al.,1978). Despite this a fairly detailed outline of the basic molecular architecture can be sketched. Since there are many similarities and only a few significant difference among the various fibronectins, it is convenient to present this subject mainly in terms of fibronectin .

The molecule is a dimer : it consist of two similar subunits. Each subunit has a molecular weight of about 250,000 daltons,(one dalton is the mass of a hydrogen atom), and they are joined at one end by disulfide bonds. (Mosher.,1975 ; Mosesson MW et al.,1975 ; Hynes,1986). These disulfide bridges are located very close to one end of the molecule probably at the CooH-terminus (Hynes et al., 1978 ; Wagner et al.,1980). The protein chain of each subunit forms an elongated structure 60 to 70 nanometers thick ; that structure in turn is subdivided into a series of smaller domains, within each of which the protein chain is tightly folded. The domains are defined by the action of proteolytic enzymes : when the fibronectin chain is treated with such enzymes, it is cut only in the extended and flexible segments joining the domains, leaving the domains intact.(Hynes

1986).

Each domain, it appears, is responsible for one of fibronectin's binding functions. For example, earlier work had shown that fibronectin binds to collagen and fibrin, to other glycoproteins that are important constituents of extracellular matrices. By degrading fibronectin with proteolytic enzymes and exposing it to matrices of collagen and fibrin, one can identify different domains that bind specifically to each glycoprotein.(Hynes,1986).

Recently , fibronectin domains may be fractionated by a hydroxyapatite chromatography column (HACC) using sodium phosphate linear gradients.(Zardi et al.,1985).

Fibronectin is able to link callagen & fibrin with the all surface and so it must also have a domain capable of binding to cell. One can isolate fragment of fibronectin that bind cells to plastic (to which most proteins stick, regardless of structure). These fragments contain the cell-binding region only , they cannot link cells to fibrin or collagen (Hynes,1986). Fibronectin mediates the adhesion of fibroblast to collagen (Cold et al.,1980) and it is mediator of the attachment of denatured collagen to macrophages (Stathakis et al.,1981).

Several other binding functions have also been mapped onto the fibronectin molecule, it can bind to cell surfaces and many different macromolecules including heparin, DNA, actin,(Saba et al.,1986), ganglioside (Borsi et al.,1986), also it can bind to the Clq component of complement(Pearlstein et al.,1982), and to bacteria,(Kuusela,1978 ; Mosher et al.,1980).

To examine the structure of fibronectin at a higher resolution, it is necessary to determine the linear sequence of the amino acids making up the molecule. Since each protein subunit includes more than 2000 amino acids, sequencing the protein directly is not easy. For a protein as large as fibronectin, a faster way to find the amino acid sequence is to decipher it indirectly from the DNA sequence of the gene that encodes it. The DNA sequence can be reverse-tranlated according to the genetic code to deduce the amino acid sequence of the protein.(Hynes,1986).

The amino acid data show that the fibronectin molecule is made up mostly of short amino acid sequences repeated many times. The sequences are of three general types; the members of each set are similar but not identical. Each of the structural and functional domains of fibronectin contains one or more of the repeats.

For example, there are two fibrin binding domains on each subunit of the protein; each consists of the sequence known as type I ,repeated three times in one domain and five times in the other. The repeats are themselves functional : individual type I sequences are probably cabable of binding to fibrin, for instance, and a single type III repeat serves as the functional core of the cell-binding domain. (Hynes,1986).

Plasma fibronectin is comprised of about 5% carbohydrate as is the cell surface form,(Vuento et al.,1977). The role of carbohydrate in fibronectin molecules has been investigated by treating fibroblasts with tunicamycin, which is an antibiotic that induces synthesis of proteins deficient in asparagine-linked oligosaccharide units. (Tracz et al.,1975).

N.B.: All oligosaccharide units are linked to the piptide backbone by asparagine residues at 4-6sites along the middle protein of the peptide chain. (Carter et al., 1979).

Fibronectin synthesized in the presence of tunicamycin was not glycosylated. The rates of synthesis and secretion were not changed, although its proteolytic degradation in culture was increased 2-3 fold,(Olden,et al.,1978), but its effect in promoting normal fibroblast morphology, and in mediating all attachment to collagen was the same. It thus appears that carbohydrate units in fibronectin are not essential for the biologic activities, but instead may serve to increase its resistance to proteolysis.

Several minor fibronectin components of smaller size than the two-chain structure have been identified in plasma molecules.(Chen et al.,1977). most of them range in size from 235,000-146,000 ,but even smaller fibronectin peptides may exist, some or even all of which may be derived by catabolic processes from larger parents molecules. (Mosesson et al.,1980).

Fibronectin appears in most instances to be dimeric, but unreduced fibroblast cell surface fibronectin preparation also exhibit species of very high molecular weight. These multimeric forms may indicate a higher degree of interchain disulfide bridging than is found in plasma forms and could account, at least in part, for low solubility of the cell surface form in aqueous buffers at neutral pH.(Yamada et al.,1977 ; Alexander et al.,1978). However, dimeric species present in cell surface fibronectin preparations are still considerably less soluble than the dimeric molecules found in plasma fibronectin preparations. (Yamada et al., 1977 ; Cohler et al., 1985 ; Borisl et al., 1986).

Mosher (1981) found that cell culture fibronectin was 100 fold less soluble in physiological saline then was plasma fibronectin. Yamada & Kennedy(1979) found that plasma fibronectin was 50 times less active then fibroblast fibronectin in restoring morphology and alignment to transformed fibroblast cell line, and 150 times less active in agglutinating formalin-fixed sheep erythrocytes.

Yamaguchi & Lsemura (1986) found that amniotic fluid fibronectin had different carbohydrate moieties from plasma fibronectin and that amniotic fluid fibronectin showed a significantly lower gelatin-binding affinity than plasma fibronectin at 25 degree C.

Crouch & Co-workers(1978) have reported that fibroblast cell surface fibronectin,as well as amniotic fluid form, possess a subunit polypeptide chain that is significantly larger than that found in plasma molecules. It appears unlikely that variation in the type or degree of glycosylation explains the apparent size differences, because the number and structure of oligosaccharide units constituting these various molecular forms are quite similar (Wrann,1978 ; Carter et al.,1979).

Mosher(1981) reported that the amino acid compositions of fibronectin from a variety of species and sources are very similar. In contrast, there are reproducible differences in carbohydrate composition between plasma and cell culture fibronectins. Cell culture fibronectins generally contain more total carbohydrate but less sialic acid than do the plasma forms.

These differences between cellular and plasma forms of fibronectin were explained by Mosesson & Amrani(1980): (A) that, the cell surface and plasma forms represent the products of different structural genes, (B) that, they reflect differences in gene processing, or (C)that, they reflect post-translation modifications of otherwise identical gene products.

Finally, Hynes in 1986 reported that the gene itself does not vary, but studies of messenger RNA's that direct the assembly of fibronectin molecules, have shown that it can give rise to at least 12 versions of the protein and that different cell types synthesize different sets of variation. For example, liver cells make only plasma fibronectin. This explanation was confirmed by Cutman & Yamada (1986).