Structural differences in the cell binding region of human fibronectin molecules isolated from cultured normal and tumor-derived human cells

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Fibronectins isolated from human plasma (pFN) and from the conditioned media of normal (N-cFN) and tumor (T-cFN) human cells were compared by cathepsin D digestion followed by immunostaining of released fragments with the monoclonal antibody 3E3, specific for the cell binding site. Two different staining patterns were obtained, one specific for pFN and N-cFN, the second common to fibronectins from the 3 different kinds of tumors studied. This indicates structural differences between N-cFN and T-cFN in the cell binding region of the fibronectin molecule.

Fibronectin *Tumor cell* *Cell binding site* *Immunoblotting*

1. INTRODUCTION

Fibronectins (FNs) are high molecular mass adhesive glycoproteins present in soluble form in plasma and other body fluids and in insoluble form in the extracellular matrices and basement membranes. FN molecules act as bridges between cell and extracellular materials. In fact FN molecules contain a cell binding site and binding sites for collagen, heparin, ganglioside and fibrin. Due to their polyvalency FNs play an important role in diverse biological phenomena including cell adhesion, establishment and maintenance of normal morphology, cell migration, hemostasis and thrombosis, wound healing, ability to induce a more normal phenotype in transformed cells and metastatic processes (reviews on distribution, structure and biological functions [1–8]).

Recently a highly sensitive and specific procedure has been developed to compare the domain structure of FNs from different sources based on cleavage of FN by a proteolytic enzyme, fractionation on SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting analysis with domain specific monoclonal antibodies [9–10].

Using this procedure and a monoclonal antibody specific for the cell binding site we compared FNs released by cultured normal human fibroblasts and by 3 cell lines originating from human tumors. The results indicate structural differences in the cell binding segment between FNs from normal and tumor cells.

2. MATERIALS AND METHODS

2.1. *Cell lines*

Cultured normal fibroblast cell lines (GM-5659, human skin; GM-5386, fetal human skin, Human Genetic Mutant Cell Repository, Camden, NJ) and tumor cell lines derived from human fibrosarcoma, HT-1080, embryonal rhabdomyosarcoma, RD and melanoma, IgR3 were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland) which had been depleted of bovine FN by passage through a large capacity gelatin-Sepharose column.

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FN was purified from human plasma and from the conditioned media of the various cell lines as in [11]. Cathepsin D (Sigma, St. Louis, MO) digestion of FN was performed according to [12]. The monoclonal antibody 3E3, directed to the cell binding site [13,14] was a gift of Dr E. Ruoslahti (La Jolla Cancer Research Foundation, La Jolla, CA). SDS-PAGE was carried out as in [15] and immunoblotting as in [16,17].

3. RESULTS

Fig.1 shows the polypeptide patterns on 4–18% SDS-PAGE gradients of FNs from human plasma and from the tissue culture media of normal and tumor-derived human cells digested for different times by cathepsin D (see legend to fig.1). 4–18% SDS-PAGE gradients identical to those shown in fig.1 were blotted onto a nitrocellulose sheet and reacted with the monoclonal antibody 3E3 which is specific for the cell binding site of human FN (fig.2). This monoclonal antibody reacts, in all the 6 FNs analyzed, with 2 high-molecular-mass fragments of 200 and 130 kDa which correspond to domains 1-2-3 and 4 (200 kDa) and 3 and 4 (130 kDa) (see fig.3) [10] and a low-molecular-mass fragment (24 kDa). Furthermore this monoclonal antibody reacts with a 43 kDa fragment only present in FNs from tumor cells (fig.2).

In a range of digestion times from 15 min to 24 h we have been unable to observe the 43 kDa fragment in FNs from plasma or from 6 different normal human fibroblast lines (not shown). In FN from tumor cells the 43 kDa fragment appears after 1 h of digestion and starts to decrease after 5–6 h of digestion (not shown).

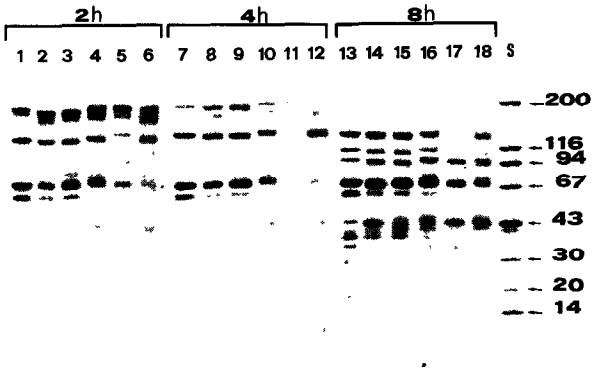

Fig.1. 4–18% SDS-PAGE gradients of FNs from different sources digested for 2, 4 and 8 h by cathepsin D. FNs (200 μg) were digested with cathepsin D at an enzyme/substrate ratio of 1:300 in 1 ml of 50 mM sodium acetate (pH 3.5) containing 1.8 mM CAPS, 0.5 mM EDTA and 0.2 mM phenylmethylsulfonyl fluoride at 37°C. Lanes: (1,7 and 13) human plFN, (2,8 and 14) FN from conditioned medium of GM-5659 normal human fibroblasts, (3,9 and 15) FN from conditioned medium of GM-5386 normal human embryo fibroblasts, (4,10 and 16) FN from conditioned medium of IgR3 human melanoma cells, (5,11 and 17) FN from conditioned medium of RD human rhabdomyosarcoma cells, (6,12 and 18) FN from conditioned medium of HT-1080 human fibrosarcoma cells. The values on the right are the molecular masses (in kDa) of the standards (S).

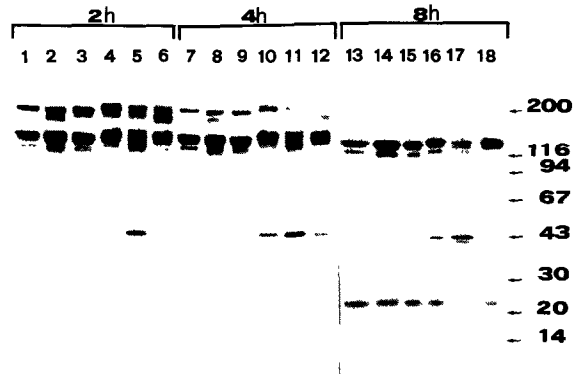

Fig.2. Immunoblot analysis of a 4–18% SDS-PAGE gradient identical to that shown in fig.1, using the monoclonal antibody 3E3. The values on the right are the molecular masses (in kDa) of the standards (S).

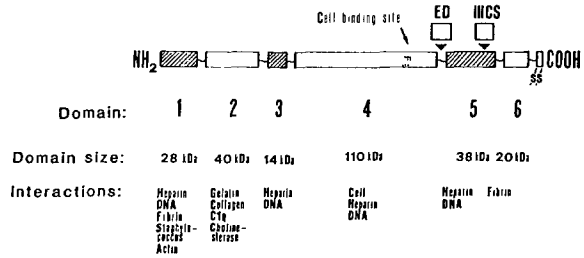

Fig.3. Model of the domain structure of a subunit of human FN. ED and IIICS represent the 2 regions of variability due to alternative splicing of primary transcript. The model is mainly based on [20,21,28,29].

4. DISCUSSION

Domain-specific monoclonal antibodies combined with well characterized proteolytic fragmentation provide a highly sensitive and specific procedure to compare the structure of FNs from different sources [9,10,18,19]. Using this procedure Sekiguchi et al. [9,10] have been able to observe differences between human FNs from plasma and from normal or SV40 transformed fibroblasts. Using the same procedure and a monoclonal antibody directed to the cell binding site here we compared human FNs from plasma, normal fibroblasts and 3 different types of human tumors. The immunoblots of these 6 different FNs clearly show 2 different kinds of patterns (fig.2): one specific for pFN and N-cFN, the second one common to FNs from the 3 different kinds of tumors. This suggests structural differences between pFN, N-cFN and T-cFN in the cell binding region either due to differences in the primary sequence or in post-translational processing.

In fact, the heterogeneity of FN seems to be due to multiple FN mRNAs arising from a single gene, localized on chromosome 2 [17], by differential splicing in at least 2 different regions of the primary transcript [20,21] (see fig.3) and by post-translational processing (glycosylation, phosphorylation and sulfation) [22–27]. Most probably FN represents a mixture of a number of structurally different molecules whose make-up varies depending on the FN sources.

In any case, structural modification in the cell binding region, either due to differential splicing of the primary transcript or to post-translational modification, may have important biological consequences which could interfere with FN's ability to interact with its cellular receptor.

This study clearly needs to be extended to a larger number of tumor cell lines before the difference observed can be considered a general phenomenon of tumor cells. Purification and characterization of the 43 kDa fragment are under way to determine the exact nature of the modifications observed.

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