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Transforming growth factor β regulates the levels of different fibronectin isoforms in normal human cultured fibroblasts

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Fibronectin (FN) polymorphism is caused by alternative splicing patterns in at least three regions of the primary transcript of a single gene. Using a monoclonal antibody (Mab) specific for an FN segment (ED-A), that can be included or omitted from the molecule depending on the pattern of splicing, we have examined whether transforming growth factor β (TGF-β) and dexamethasone, which are both known to increase the level of total FN, regulate the levels of different FN isoforms. We found that, while dexamethasone does not significantly change the ratio between the total FN and the ED-A containing FN, TGF-β preferentially increases the expression of the FN isoform containing the ED-A sequence.

Transforming growth factor β; Fibronectin isoform; RNA splicing

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. FN molecules are involved in diverse biological phenomena including the establishment of maintenance of normal cell morphology, cell migration, hemostasis and thrombosis, wound healing, and oncogenic transformation [1-3].

It has previously been demonstrated that FN polymorphism is at least partially caused by alternative splicing patterns in three regions of the primary transcript of a single gene (see fig.1A) [4-6].

Recently it has been reported that dexamethasone, a synthetic glucocorticoid, and human platelet-derived transforming growth factor β (TGF-β) may increase levels of FN in both the media and the extracellular matrix of a variety of cultured cells [7,8]. TGF-βs are hormonally active polypeptides consisting of two 12-kDa chains linked by disulfide bonds [9,10]. Because of its abundance in platelets, bone and developing tissues [11], TGF-β is likely to function in a broad range of processes involved in tissue development and repair.

Using two Mabs, one (IST-4) specific for all FN isoforms (tFN) and another (IST-9) specific only for ED-A (fig.1) containing FN molecules (A-FN), we have studied whether dexamethasone and TGF-β may preferentially induce the expression of the ED-A containing FN isoforms. The results demonstrate that while dexamethasone does not change the ratio between tFN and A-FN, TGF-β preferentially increases the expression of the FN isoforms containing the ED-A sequence.

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2. MATERIALS AND METHODS

Cultured normal human fibroblasts from adult human skin (GM-3440) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% FN-free fetal calf serum (FCS) (Flow Laboratories, Irvine, Scotland) as reported [12].

Cells were grown to confluence in 35-mm wells, then the medium was replaced with 3.5 ml MEM supplemented with 0.5% FCS. After 3 days different sets of cells were treated for 4 days with low-serum medium containing: (i) 450 pM human

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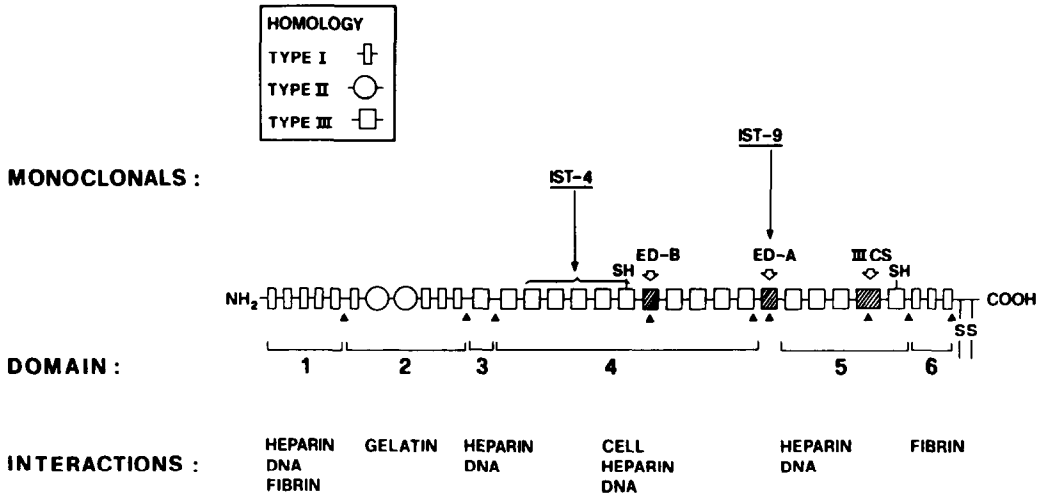

Fig.1. Schematic representation of the structure of a subunbit of human FN. ED-A, ED-B and IIICS represent the three known regions of variability due to alternative splicing of mRNA precursors. The sites of specificity of the two Mabs used are indicated. The three types of internal homology are also depicted. Arrowheads indicate the sites sensitive to the proteolytic enzyme thermolysin.

platelet-derived TGF-β (R and S Systems, Minneapolis, MN); (ii) 10 μM dexamethasone (Sigma, St. Louis, MO) and (iii) respective solvents. Cell counting after this treatment showed that each well contained between 350000 and 400000 cells without significant variations among the different sets. After this treatment media were quantitatively collected from each well, 350 μl of a 10× sample buffer for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (100 mM Tris-HCl, pH 6.8, 10% SDS, 20% mercaptoethanol, 50% glycerol) was added. To estimate the total level of FN in the cultures (FN in media + FN in the extracellular matrix), cultures were solubilized by directly adding 350 μl of 10× sample buffer for SDS-PAGE. 80 μl of samples prepared in this way were directly analyzed on a 4–18% SDS-PAGE gradient followed by immunoblotting. All experiments were carried out in triplicate. SDS-PAGE and immunoblotting were carried out as in [15].

Mabs IST-4 and IST-9 respectively specific for all FN isomers (IST-4) and for ED-A sequence (IST-9) were prepared and characterized as in [14–16]. Densitometric scanning of immunoblots was carried out as in [12].

Table 1
Areas (cm^2) of FN peaks from densitometric scanning of immunoblots using the Mabs IST-4 and IST-9[a]

	Media			Total extracts[b]		
	IST-4	IST-9	$\frac{\text{IST-9}}{\text{IST-4}}$	IST-4	IST-9	$\frac{\text{IST-9}}{\text{IST-4}}$
Controls	1.29	0.345	0.27	1.39	0.365	0.26
Dexamethasone	2.03	0.4	0.20	2.01	0.43	0.21
TGF-β	1.87	0.8	0.43	3.35	1.82	0.54

[a] Values represent the average of three independent experiments. For experimental procedures see section 2
[b] Total extracts: FN in cells plus FN released into the media

3. RESULTS

Fig.1 shows a schematic representation of the domain structure of human FN and the localization within the molecule of the epitopes recognized by the two Mabs used, IST-4 and IST-9. IST-4 recognizes all different FN isoforms (total FN = tFN) while IST-9 recognizes only FN molecules containing the ED-A sequence (A-FN). In order to establish whether treatment of normal human fibroblasts by TGF-β dexamethasone (which both increase the level of tFN) may increase specifically one FN isoform, we have treated cultures for 4 days with TGF-β and dexamethasone. Using the above-mentioned Mabs we have studied the relative amounts of tFN and A-FN in total extracts and in the media of controls, TGF-β-treated cells and dexamethasone-treated cells by quantitative densitometric scanning of immunoblots. The results are summarized in table 1.

Fig.2 shows a typical pattern of gel and immunoblots of the total extracts stained with IST-9 and IST-4. The results indicate that both dexamethasone and TGF-β treatments increase the tFN in total extracts about 2–3-fold, as previously reported [7,8]. The increase of tFN in conditioned media is almost identical in both dexamethasone- and TGF-β-treated cells (see table 1). However, while dexamethasone induces a very low increase in A-FN in both media and total extracts, TGF-β

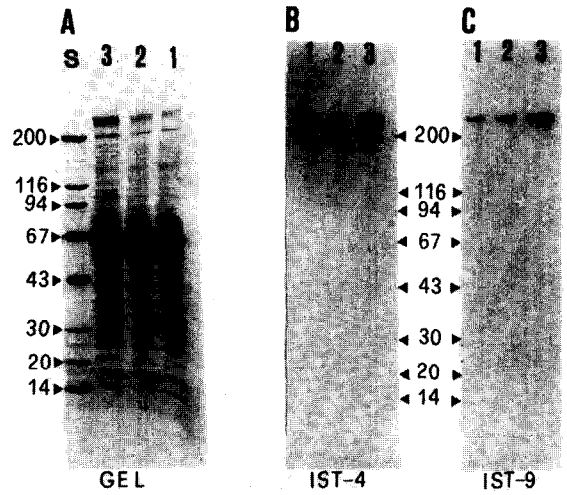

Fig.2. (A) 4–18% gradient SDS-PAGE of total extracts of GM-3440 normal human cells: control (lane 1), treated with dexamethasone (lane 2), and treated with TGF-β (lane 3). The detailed procedure is described in section 2. The values on the left are the molecular masses (in kDa) of the standards (S). (B,C) Immunoblot analysis of a 4–18% SDS-PAGE as in (A) using Mabs IST-4 (B) and IST-9 (C). The values between (B) and (C) are the molecular masses (in kDa) of the standards.

increases A-FN more than 2-fold in media and 5-fold in total extracts (see table 1).

4. DISCUSSION

Expression of the ED-A sequence in FN has been shown to be tissue-specific. The ED-A sequence is absent in the FN mRNA of liver [17] which is the source of plasma FN [18]. Using a rabbit antiserum to the rat ED-A sequence, Paul et al. [19] demonstrated that this sequence was not present in plasma FN but was expressed in FN released by cultured fibroblasts and in FN from blood platelets. More recently Borsi et al. [15] demonstrated that in FM from the tissue culture medium of tumor-derived human cells the percentage of A-FN is about 10-times higher than in FN released by normal human fibroblasts. Similar results were obtained for the other two sites of alternative splicing, ED-B [16] and IIICS [12].

Here we report that while both dexamethasone and TGF-β are able to increase the accumulation of tFN in normal human fibroblasts, dexamethasone does not change the ratio A-FN/tFN while TGF-β dramatically increases this ratio since it preferentially augments the level of the A-FN (see table 1).

This increased accumulation of A-FN induced by TGF-β in normal human fibroblasts could be mediated by (i) differential changes in the rate of synthesis and/or stability of the various FN isoforms, (ii) preferential accumulation in the cytoplasm of ED-A containing FN mRNA either due to differential stability of the various FN mRNAs or to modification in the mechanisms regulating RNA splicing. At present studies are underway to establish at which level TGF-β acts.

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