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Original article

Desmoid and fibroma tumors differently respond to TGFβ$_1$ stimulus and ECM macromolecule accumulation

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Abstract

Desmoid and fibroma tumours are characterized by cell proliferation, glycosaminoglycan and collagen fibre accumulation, high levels of transforming growth factor β$_1$ (TGFβ$_1$) and different patterns of tissue infiltration. TGFβ$_1$ is related to extracellular matrix (ECM) composition which, in turn, regulates cell functions and cell migration. In this study we report changes in cell proliferation, glycosaminoglycan (GAG) and collagen synthesis, TGFβ$_1$ mRNA expression and fibronectin levels in normal, desmoid and fibroma fibroblast cultures before and after TGFβ$_1$ stimulation. Our data showed cell proliferation, GAG and collagen synthesis, transforming growth factor β$_1$ mRNA expression and fibronectin levels were significantly higher in desmoid than in fibroma cultures. TGFβ$_1$ treatment had no effect on cell proliferation, but increased TGFβ$_1$ mRNA expression, GAG, fibronectin and collagen synthesis in desmoid and fibroma fibroblasts. Its effects were more marked in desmoid cells. Fibronectin favours cell migration, while changes in GAG composition alter cell behaviour and ECM organization. In conclusion our data suggest that the different patterns of infiltration in desmoid and fibroma tumours are due to changes in ECM components and cell–ECM interactions which can be ascribed to altered TGFβ$_1$ mRNA expression and TGFβ$_1$ activity.

Keywords: Desmoid; Fibroma; Extracellular matrix

1. Introduction

Extracellular matrix (ECM) composition plays a major role in cell behaviour as glycosaminoglycan (GAG) classes such as hyaluronic acid, chondroitin sulphate and heparan sulphate are related to cell migration [1,2], tissue specific gene expression [3] and modulation of cytokine activity [4]. In the ECM abnormal interactions among cells and proteoglycans, collagen and fibronectin change cell adhesive properties and facilitate tumour progression [5,6].

Transforming growth factor β$_1$ (TGFβ$_1$), which plays a central role in normal tissue homeostatic regulation, stimulates the production and secretion of ECM macromolecules, such as GAG and matrix proteins, and inhibits protease production which regulates ECM turnover [7,8]. In tumour cells TGFβ$_1$ is significantly higher than in normal cells [9] and modifies cell proliferation and differentiation, angiogenesis, collagen, proteoglycan and GAG composition [10,11], fibronectin levels and ECM polymerisation [12,13]. TGFβ$_1$ deregulation can be related to the invasive metastatic potential of tumour cells.

Benign pathologies such as fibroma and desmoid tumours are caused by an autosomal dominant gene mutation [14–16] and are characterized by cell proliferation and collagen fibre accumulation. Unlike fibroma, a well-encapsulated tumour

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that does not metastasise and does not recur after surgical exportation [17,18], the desmoid tumour has a high rate of recurrence after surgical removal because massive infiltration into surrounding tissues [19] prevents full asportation.

To investigate the relationships between ECM composition, tumour invasiveness and TGFβ_1 activity, we evaluated cell proliferation, GAG, collagen, fibronectin accumulation and TGFβ_1 mRNA transcripts in human normal, fibroma and desmoid fibroblasts before and after TGFβ_1 stimulation.

2. Materials and methods

2.1. Cell cultures

Normal GMO 6965, fibroma GMO 6855 and desmoid GMO 6888 fibroblast cell lines were provided by NIGMS (Camden, NJ). All cell lines were cultured at a density of 1×10^6 cells/ml in Eagle's MEM (Sigma, St. Louis, MO), supplemented with 20% foetal calf serum (FBS; GIBCO BRL, Grand Island, NY), 2% nonessential amino acids (GIBCO), 2 mM L-glutamine, 100 U/ml penicillin and 100 U/ml streptomycin, in a humidified 5% CO_2 atmosphere at 37 °C. Sub-confluent cultures were obtained after 24 h. Cells were cultured for the next 12 h in MEM, which was then discarded to avoid serum factor contamination. Cultures were analysed for cell proliferation, GAG and collagen synthesis, TGFβ_1 activity and TGFβ_1 mRNA expression after 24 h in Eagle's MEM in the presence or absence of TGFβ_1 as described below. Viable cells (trypan blue negative) and dead cells (trypan blue positive) were counted by Burker chamber [20]. Protein concentration was determined using Lowry et al. method [21].

2.2. TGFβ_1 assay

Fibroma, desmoid and normal fibroblasts were cultured for 24 h in Eagle's MEM and analysed for TGFβ_1 level using an ELISA assay (ICN, Costa Mesa, CA) according to the manufacturer's instructions. Samples were read at 450 nm. To determine TGFβ_1 concentration, the standard curve was linear for TGFβ_1 concentrations between 1 and 1000 pg/ml. Values were expressed as pg/μg protein.

2.3. Cell proliferation

Sub-confluent normal, fibroma and desmoid cultures were maintained for 24 h in Eagle's MEM 1 μCi/ml supplemented with ^{3}H-thymidine (s.a. 13.4 Ci/mmol, Amersham, Freiburg, Germany), in the presence or absence of 8 ng/ml TGFβ_1. After incubation, the medium was discarded and an aliquot of cells solubilised in 0.5 M NaOH, precipitated with 10% TCA (30 min at 4 °C), filtered through 0.45 μm glass fibre pore filters (Millipore Spa, Milan, Italy) and washed with cold 5% TCA. Filters holding the acid-insoluble fraction were dried and counted in 10 ml of Instagel scintillation fluid (Packard, Meriden, CT) in an LKB scintillation counter. Total cell radioactivity (uptake) was measured in separate aliquots of solubilised cells counted in 10 ml of scintillation fluid. The results were expressed as dpm/10^6 cells. Another cell aliquot was used for counting the cells [22].

2.4. Newly synthesized GAG

Confluent normal, fibroma and desmoid cultures were maintained in Eagle's MEM, supplemented with 8 ng/ml TGFβ_1 and 5 μCi/ml ^{3}H-glucosamine (s.a. 30 Ci/mmol, Amersham). After 24 h media were recovered and GAG were precipitated as previously described [23]. Aliquots of ^{3}H-labelled GAG were mixed with 10 ml of Pico-fluor 40 (Canberra Packard, Zurich, Switzerland) and counted in a Packard Tri-Carb 2000 CA liquid scintillation counter. Radioactivity incorporation was expressed as cpm/mg protein.

2.5. Collagen synthesis

Fibroma, desmoid and normal fibroblasts were cultured for 24 h in Eagle's MEM without FBS supplemented with 50 μg/ml L-ascorbic acid, 50 μg/ml β-aminopropionitrile fumarate and 10 μCi/ml of ^{3}H-proline (s.a. 35 Ci/mmol, Amersham) with or wihout 8 ng/ml TGFβ_1. Collagen was extracted from cells and media using Webster and Harwey method [24]. Total radioactivity was counted in a liquid scintillation counter. Results were expressed as cpm/mg protein.

2.6. RNA extraction and Northern blot analysis

Confluent fibroma, desmoid and normal cultures were maintained in Eagle's MEM alone or supplemented with 8 ng/ml TGFβ_1 for 24 h. RNA was extracted using the Chomczynski and Sacchi method [25]. For Northern blot analysis equal amounts of total RNA (15 μg) were electrophoresed on a 1% agarose gel containing 0.66 M formaldehyde and transferred onto nylon filters (Hybond N; Amersham). Before blotting, the gel was rinsed in water for 15 min at room temperature and then in 20× SSC [1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate (pH 7)] for 10 min. Blots were prehybridised (0.2 ml/cm^2) in a cocktail containing 50% formamide, 6× SSC, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA and 5× Denhardt's solution for 4 h at 42 °C. Probes were labelled with [α-^{32}P]dCTP (3000 Ci/mM) by random priming. Hybridisation was performed at 42 °C overnight using a 10^6 cpm/ml probe in the same buffer as used for prehybridisation. After hybridisation was completed, the nylon membrane was washed once in 2× SSC, 0.1% SDS at 65 °C for 15 min each. Filters were stripped and rehybridised with a guanidine phosphate (GAPDH) probe to assess blot loading. For autoradiography, membranes were exposed to preflashed Kodak (Rochester, NY) X-Omat film at −80 °C for 4 days with an intensifying screen. Autoradiograms were analyzed by computerized scanning densitometry. The absolute counts were corrected for GAPDH mRNA expression and converted to percentages assuming the level of normal fibroblasts as 100.