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Regulation of hyaluronan and versican deposition by growth factors in fibrosarcoma cell lines

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Abstract

Versican, a large chondroitin sulphate proteoglycan and hyaluronan (HA), a non-sulphated glycosaminoglycan are major constituents of the pericellular matrix. In many neoplastic tissues, changes in the expression of versican and HA affect tumour progression. Here, we analyse the synthesis of versican and hyaluronan by fibrosarcoma cells, and document how the latter is affected by PDGF-BB, bFGF and TGFB2, growth factors endogenously produced by these cells. Fibrosarcoma cell lines B6FS and HT1080 were utilised and compared with normal lung fibroblasts (DLF). The major versican isoforms expressed by DLF and B6FS cells were V0 and V1. Treatment of B6FS cells with TGFB2 showed a significant increase of V0 and V1 mRNAs. Versican expression in HT1080 cells was not significantly affected by any of the growth factors. In addition, TGFB2 treatment increased versican protein in DLF cells. HA, showed approximately a 2-fold and a 9-fold higher production in DLF cells compared to B6FS and HT1080 cells, respectively. In HT1080 cells, HA biosynthesis was significantly increased by bFGF, whereas, in B6FS cells it was increased by TGFB2 and PDGF-BB. Furthermore, analysis of HA synthases (HAS) expression indicated that HT1080 expressed similar levels of all three HAS isoforms in the following order: HAS2> HAS3> HAS1. bFGF shifted that balance by increasing the abundance of HAS1. The major HAS isoform expressed by B6FS cells was HAS2. PDGF-BB and TGFB2 showed the most prominent effects by increasing both HAS2 and HAS1 isoforms. In conclusion, these growth factors modulated, through upregulation of specific HAS isoforms, HA synthesis, secretion and net deposition to the pericellular matrix.

Keywords: Fibrosarcoma; Versican; Hyaluronan; Basic fibroblast growth factor; Transforming growth factor B2; Platelet derived growth factor BB; Hyaluronan synthases

1. Introduction

The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells within tissues. ECM's main components are collagens, glycoproteins, proteoglycans (PGs) and glycosaminoglycans (GAGs). The ECM provides a physical framework for cell attachment and is crucial for normal cell function regulating proliferation, migration and differentiation.

Changes in the ECM composition occur in tumour formation and uncontrolled destruction of the ECM contributes to tumour invasion. During tumour growth, transformed cells remodel the extracellular space for dynamic growth conditions. Tumour stroma and fibrotic tissue often contain PGs and GAGs in higher proportion than in normal surrounding tissue [1] and correlate with the activity of numerous extracellular signalling proteins such as chemokines, cytokines and growth factors. Therefore, the identification of the full complement of factors that contribute to the remodelling of the ECM becomes of great importance.

PGs are ubiquitous components of the ECM and are necessary for tissue hydration, maintenance of structural integrity, facilitation of cellular adhesion and migration [2]. Among PGs, a chondroitin sulphate PG, versican, seems to have a prominent importance in modulation of cell adhesion and in the promotion of tumour cell proliferation and invasiveness [3,4]. Versican (hyalectan) interacts with hyaluronan (HA), a non-sulphated glycosaminoglycan, forming molecular bridges between cell surfaces and extracellular matrices [5]. In many neoplastic

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tissues expression of versican and HA correlates to tumour progression. Elevated versican levels are found in breast, brain, prostate tumours and melanoma [6–14]. Increased concentration of stromal and peri-tumoural versican is a predictor of outcome for patients with moderately differentiated prostate tumours and of survival in breast cancer respectively [15,16]. Versican has four distinct isoforms denoted V0, V1, V2, and V3. Indeed the expression of specific variants may be involved in tumour cell behaviour [17,18].

The interaction of versican with HA within the ECM is essential in the structure and assembly of tissues [19]. In addition, HA occupies an enormous hydrodynamic domain that influences the hydration and the physical properties of tissues [20]. The function of HA is highly dependent on its molecular weight. Increased synthesis of HA has often been associated with malignant progression in certain types of human tumours, including colon, lung, and breast cancers, and mesotheliomas and gliomas. Furthermore, the levels of HA in sera of some cancer patients were significantly elevated as compared to those of healthy individuals [20–25].

Fibrosarcomas, neurofibrosarcomas and other fibromatous neoplasms typically account for 10–20% of childhood soft tissue sarcomas [26]. Fibrosarcoma is a rare malignant tumour which originates from the fibroblasts and its ECM has been found to be rich in GAGs and PGs. In addition, it has been indicated that the content, cellular disposition and turnover of PGs are quite variable between different cell lines of fibroblastic origin [27]. Hence, we hypothesised that in different fibrosarcoma cell lines, versican and HA synthesis could be regulated by growth factors, which are also produced endogenously and serve as an important mechanism to support cell-shape changes as well as cell proliferation and migration. Therefore, the aim of the present study was (a) to analyse the capacity of fibrosarcoma cells to synthesize versican and HA and (b) to examine how versican and HA biosynthesis is affected by transforming growth factor B2 (TGFB2), basic fibroblast growth factor (bFGF) and platelet derived growth factor BB (PDGF-BB) which are produced by fibrosarcoma cells and are major effectors for cells of fibroblastoid origin.

2. Materials and methods

2.1. Materials

PDGF-BB, bFGF and TGFB2 were obtained from R&D diagnostics. The commercial name of the HA preparation used in the proliferation assay is Healon (10 mg/mL, also containing sodium chloride 8.5 mg, disodium hydrogen phosphate dihydrate 0.28 mg, sodium dihydrogen phosphate hydrate 0.04 mg and water for injection USP; Pharmacia AB Sweden). Chondroitinase ABC was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Cell culture reagents were obtained from Biochrom KG (Berlin, Germany). Polyclonal antibodies against versican as well as anti-rabbit and anti-goat HRP conjugated secondary antibodies were purchased from Santa Cruz Biochemicals (USA).

2.2. Cell culture

HT1080 human fibrosarcoma cells [28] and DLF human fibroblast cells (kindly provided by Dr. Kletsas, Demokritos, Athens, Greece) were grown in DMEM (Biochrom KG) supplemented with 10% fetal bovine serum (FBS) (GIBCO) and B6FS human fibrosarcoma cell line [29] were grown in RPMI (GIBCO) supplemented with 10% FBS. Prior to stimulation with growth factors, cells were incubated in 2% FBS medium (DLF) or in serum free medium (HT1080 and B6FS) for 24 h. PDGF-BB, bFGF and TGFB2 (final concentration 10 ng/mL which was selected after dose response experiments) were added in 2% FBS medium (DLF) or serum free medium (HT1080 and B6FS). Prior to RNA extraction, cells were treated with each factor for 24 h. Samples for protein detection were collected after 48 h incubation with each growth factor.

2.3. RNA isolation and real-time PCR

Total RNA was isolated with the TRIzol method (GibcoBRL) according to the manufacturer's instructions. Five micrograms of total RNA was used for cDNA synthesis using the ThermoScriptTM RT-PCRSystem (Invitrogen) according to the manufacturer's instructions. PCR reaction was performed using GoTaq Flexi DNA polymerase (Promega) and the conditions used for amplification were: 94 °C for 15 min, then 40 cycles at 94 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 10 min. Primers were mRNA specific to avoid traces of DNA contamination (Table 1). For semi-quantification of the genes of interest we utilized the QuantiTech SYBR Green master mix (Qiagen) performing real-time PCR reactions in an Mx300P cycler. Standard curves were run in each optimized assay which produced a linear plot of threshold cycle

Table 1
Sequence of primers for the genes of interest

Primer name	Sequence
Versican V0_F	5′ GAC CTC AGG CGC TTT C 3′
Versican V0_R	5′ CAG TGG TAA CGA GAT GCT TC 3′
Versican V1_F	5′ GCG CCA CCC TGT GAC 3′
Versican V1_R	5′ CAG TGG TAA CGA GAT GCT TC 3′
Versican V2_F	5′ GAC CTC AGG CGC TTT C 3′
Versican V2_R	5′ TAG CAC TGC CCT TGG A 3′
Versican V3_F	5′ TGA GAA CCC TGT ATC GTT TTG AGA 3′
Versican V3_R	5′ CGT TAA GGC ACG GGT TCA TT 3′
HA Synthase_1_F	5′ TGT GTA TCC TGC ATC AGC GGT 3′
HA Synthase_1_R	5′ CTG GAG GTG TAC TTG GTA GCA TAA CC 3′
HA Synthase_2_F	5′ GTG TTA TAC ATG TCG AGT TTA CTT CC 3′
HA Synthase_2_R	5′ GTC ATA TTG TTG TCC CTT CTT CCG C 3′
HA Synthase_3_F	5′ GGT ACC ATC AGA AGT TCC TAG GCA GC 3′
HA Synthase_3_R	5′ GAG GAG AAT GTT CCA GAT GCG 3′
GAPDH_F	5′ GGA AGG TGA AGG TCG GAG TCA 3′
GAPDH_R	5′ GTC ATT GAT GGC AAC AAT ATC CAC T 3′
bFGF_F	5′ GAA GAG CGA CCC TCA CAT CAA G 3′
bFGF_R	5′ CTG CCC AGT TCG TTT CAG TG 3′
TGFB1_F	5′ AAGGACCTCGGCTGGAAGTG 3′
TGFB1_R	5′ CCCGGGTTATGCTGGTTGTA 3′
TGFB2_F	5′ CTG TCC CTG CTG CAC TTT TGT 3′
TGFB2_R	5′ TCT TCC GCC GGT TGG TCT GTT 3′
TGFB3_F	5′ GGGCTTTGGACACCAATTAC 3′
TGFB3_R	5′ GCAGATGCTTCAGGGTTCAG 3′
FGFR1_F	5′ ACA ACC TGC CTT ATG TCC AGA TC 3′
FGFR1_R	5′ TGC GTC CTC AAA GGA GAC ATT3′
FGFR2_F	5′ GGT CAC CAT GGC AAC CTT GT 3′
FGFR2_R	5′ TCT GGT TGA GAG ATT TGG TAT TTG G 3′
FGFR3_F	5′ GGG CAA TTC TAT TGG GTT TTC TC 3′
FGFR3_R	5′ GAA GCC CAC CCC GTA GCT 3′
FGFR4_F	5′ TCA TCA ACG GCA GCA GCT T 3′
FGFR4_R	5′ CAG GAC CTC CAC CTC TGA GCT A 3′
PDGF-Rα_F	5′ CCT GGC TGA AAA ACA ATC TGA CT 3′
PDGF-Rα_R	5′ CAG CTT TAA TTT GCT TCG ATA CCT T 3′
PDGF-Rβ_F	5′ GCT CAC CAT CAT CTC CCT TAT CA 3′
PDGF-Rβ_R	5′ GGC CGT CAG AGC TCA CAG A 3′
TGFR1_F	5′ TCG TCT GCA TCT CAC TCA T 3′
TGFR1_R	5′ GAT AAA TCT CTG CCT CAC G 3′
TGFR2_F	5′ GCG GGA GCA CCC CTG TGT C 3′
TGFR2_R	5′ CCC GAG AGC CTG TCC AGA TGC 3′
TGFR3_F	5′ AAT CTG GGC CAT GAT GCA G 3′
TGFR3_R	5′ ACT GCT GTT TTC CGA GGC T 3′