Journal of Bone Oncology 3 (2014) 18–24

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

Research Article

Targeting syndecan-1 in breast cancer inhibits osteoclast functions through up-regulation of osteoprotegerin

Peggy Benad-Mehner [a], Stefanie Thiele [a], Tilman D. Rachner [a], Andy Göbel [a], Martina Rauner [a], Lorenz C. Hofbauer [a,b,*]

[a] Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine III, Technical University Dresden, Fetscherstr. 74, D-01307 Dresden, Germany
[b] Center for Regenerative Therapies Dresden, Technical University, Dresden, Germany

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form
25 October 2013
Accepted 5 November 2013
Available online 15 November 2013

Keywords:
Breast cancer
Osteoclast
Osteoprotegerin
Syndecan-1

ABSTRACT

Background: Breast cancer often metastasizes into bone and leads to osteolytic lesions. The underlying mechanisms, however, are complex and not fully understood. Syndecan-1 is a proteoglycan that has various functions relevant for tumor progression including cell–cell communication and cell–matrix interactions. Moreover, its two glycosaminoglycan-binding sites suggest that it may interfere with glycoproteins such as osteoprotegerin, a potent inhibitor of osteoclastogenesis. Thus, we hypothesize that tumor-derived syndecan-1 alters osteoclast biology by modulating osteoprotegerin.
Methods: Syndecan-1 expression was down-regulated via siRNA and the cell fate of the breast cancer cell lines MCF-7, T-47D, and MDA-MB-231 was investigated. Furthermore, we determined the regulation of syndecan-1 by dexamethasone, a commonly used antiemetic in breast cancer therapy. Additionally, we analyzed the genesis and activity of osteoclasts in indirect co-culture experiments using supernatants from MCF-7 cells with deficient and sufficient levels of syndecan-1.
Results: Dexamethasone time- and dose-dependently increased syndecan-1 expression up to 4-fold but did not alter cell behavior. Syndecan-1 up-regulation did not affect the survival or migration of breast cancer cells. Depletion of syndecan-1 using siRNA led to decreased vitality of progesterone receptor-positive cell lines. In MCF-7 cells osteoprotegerin production was up-regulated 2.5-fold after syndecan-1 knock-down. The culture of osteoclast precursors with the supernatant of MCF-7 cells with reduced syndecan-1 levels suppressed osteoclast formation and activity by 21% and 23%, respectively. Adding neutralizing antibodies to osteoprotegerin to the breast cancer supernatants reversed osteoclastogenesis.
Conclusion: Thus, we identified tumor-derived syndecan-1 as a novel positive regulator of osteoclastogenesis and new player in the tumor-bone dialog.

1. Introduction

The proteoglycan syndecan-1 (also known as CD138) interacts with a variety of proteins via its heparin sulfate side chains or the core protein itself and therefore regulates key cellular functions such as apoptosis, proliferation, and epithelial-mesenchymal transition [1–7]. Because of its various interactions, several studies have investigated the role of syndecan-1 in tumor progression. While in multiple myeloma, high serum levels of soluble syndecan-1 correlate with a poor prognosis, the association of syndecan-1 expression and clinical outcome was ambiguous in breast cancer [8–11]. One study found a correlation between stromal and epithelial syndecan-1 expression and poor prognosis, whereas another study observed a poor clinical outcome in breast cancer cases without syndecan-1 expression [9,10]. Although the exact reasons for these different findings remain unclear, it

Abbreviations: ACTB, β-actin; C, control; DEX, dexamethasone; ERBB2, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; ER, estrogen receptor; GAPDH, glyceraldehyde 3-phosphate-dehydrogenase; OPG, osteoprotegerin; PR, progesterone receptor; RANKL, receptor activator of NF-κB ligand; SDC1, syndecan-1

* Corresponding author at: Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine III, Technical University Dresden, Fetscherstr. 74, D-01307 Dresden, Germany. Tel.: +49 351 458 3173; fax: +49 351 458 5801.
E-mail addresses: Peggy.Benad@uniklinikum-dresden.de (P. Benad-Mehner), Stefanie.Thiele@uniklinikum-dresden.de (S. Thiele), Tilman.Rachner@uniklinikum-dresden.de (T.D. Rachner), Andy.Goebel@uniklinikum-dresden.de (A. Göbel), Martina.Rauner@uniklinikum-dresden.de (M. Rauner), Lorenz.Hofbauer@uniklinikum-dresden.de (L.C. Hofbauer).

2212-1374/$ - see front matter © 2013 Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
http://dx.doi.org/10.1016/j.jbo.2013.11.001

has been suggested that they may related to the presence of two functionally different syndecan-1 isoforms [5]. While membranous syndecan-1 facilitates the proliferation of breast cancer cells, the soluble form triggers invasion [5]. Despite extensive efforts to unravel the role of syndecan-1 in breast cancer, little is known about how it is regulated. Of note, zoledronic acid, a widely used drug against osteoporosis and skeletal metastases, was found to inhibit syndecan-1 expression in breast cancer cell lines [12].

In multiple myeloma, the interaction between syndecan-1 and osteoprotegerin (OPG), the physiological antagonist of the osteoclast promoting factor receptor activator of NF-κB (RANKL), has been investigated in more detail [13]. The observed syndecan-1-mediated internalization and degradation of OPG may explain low OPG serum levels in patients with multiple myeloma [13]. Furthermore, two other studies demonstrated that tumor-derived syndecan-1 affects bone physiology [14,15].

Here, we aimed to identify novel regulators of syndecan-1 in breast cancer. We hypothesized that changes in syndecan-1 expression affect osteoclastogenesis. Our results show that (i) dexamethasone increases syndecan-1 expression and that (ii) depletion of syndecan-1 decreases cell viability of hormone receptor-positive breast cancer cells and increases OPG expression, thus suppressing osteoclast differentiation and activation. Hence, syndecan-1 participates in the tumor-bone dialog and alters the bone microenvironment to stimulate osteoclastogenesis.

2. Methods

2.1. Cultivation and treatment of cells

All breast cancer cell lines (MCF-7, T-47D, and MDA-MB-231) were cultured in DMEM/Ham's F-12 (PAA, Pasching, Austria), 10% fetal calf serum (FCS) supreme (Lonza, Pasching, Austria) and 1% penicillin/streptomycin (PAA, Pasching, Austria). Cells were grown in a humidified atmosphere of 95% air and 5% CO_2. To assess the effects of dexamethasone (DEX), 70% confluent cells were serum starved for 12 h prior to DEX exposure (Sigma-Aldrich, Darmstadt, Germany) in various concentrations (10^{-9}–10^{-6} M). To antagonize the effects of DEX, RU-486 (Calbiochem, Darmstadt, Germany) was used at a concentration of 10^{-5} M. Cells were also treated with zoledronic acid (ZOL, provided by Novartis, Nürnberg, Germany) and the aromatase inhibitor (AI) 4-(imidazolyl)-1nitro-9H-9-xanthenone (Calbiochem, Darmstadt, Germany) in various concentrations (ZOL: 10^{-10}–10^{-6} M, AI: 7.5×10^{-8} M and 10^{-7} M).

To obtain osteoclasts, peripheral blood mononuclear cells (PBMCs) were isolated using Biocoll (1.077 g/ml, Biochrom, Berlin, Germany) from buffy coats obtained after informed consent and following IRB approval and plated at a density of 2×10^6 cells/cm^2 in α-MEM (Invitrogen, Darmstadt, Germany) containing 10% FCS and 1% penicillin/streptomycin. After attachment cells were cultured with 25 ng/ml M-CSF and 50 ng/ml RANKL for 21 d. For indirect cell culture, osteoclasts were cultured in 1/3 osteoclast culture media (α-MEM containing M-CSF and RANKL)+2/3 supernatant of MCF-7 cells from control cells and cells where syndecan-1 expression had been inhibited using siRNA.

2.2. Knock-down experiments

For knock-down experiments, SDC1 siRNA (ID 12527, Ambion, Applied Biosystems, Darmstadt, Germany) and for scrambled control Silencer® Select Negative Control #1 (Cat#4390844 Ambion, Applied Biosystems, Darmstadt, Germany) was introduced in cells with DharmaFECT 1 (Thermo Fisher Scientific, Schwerte, Germany). DharmaFECT1 reagent and siRNA were separately incubated in FCS-free medium (OPTI-MEM® I+GlutaMAX™—I, Invitrogen Karlsruhe, Germany) for 5 min and subsequently mixed and incubated for 20 min at room temperature. Thereafter, medium containing 10% FCS without penicillin/streptomycin was added to the siRNA and DharmaFECT-mixture to the final concentration of 50 nM. Breast cancer cells were washed with PBS and incubated with the transfection mixture for 5 h. Medium was change to stop the transfection and the cells were treated as described above.

2.3. RNA isolation and real-time PCR

RNA was isolated using HighPure RNA extraction kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. Five hundred ng RNA were reverse transcribed using SuperScript II (Invitrogen, Darmstadt, Germany) and subsequently used for SYBR green-based real-time PCR using a standard protocol (Roche, Mannheim, Germany). Primers (Sigma-Aldrich, Munich, Germany) used for semi-quantitative analyses of gene expression: ACTB (β-actin) [NCBI GenBank:NM_001101]: CCAACCGCGAGAAGATGA, CCAGAGGCGTACAGGGATAG, GAPDH (glyceraldehyde 3-phosphate-dehydrogenase) [NCBI GenBank:NM_002046]: AGCCACATCGCTCAGACAC, GCCCAATACGACCAAATCC, SDC1 (syndecan-1) [NCBI GenBank:NM_001006946]: TGGACAGGAAAGAGGTGCTG, GTTTGGTGGGCTTCTGGTAG, OPG (osteoprotegerin) [NCBI GenBank:NM_002546]: GAACCCCAGAGCGAAATACAG, TAGCAGGAGACCAAAGACACTG, RANK (receptor activator of NF-κB) [NCBI GenBank:NM_001270949]: ATCTGGGACGGTGCTGTAAC, CACAGGGCAGACATACACTG, RANKL (receptor activator of NF-κB ligand) [NCBI GenBank:NM_003701]: CTGATGAAAGGAGGAAGCAC, AGTAAGGAGGGGTTGGAGAC.

PCR conditions were 95 °C for 10 min followed by 40 cycles with 95 °C for 10 s, 56 °C for 10 s and 72 °C for 30 s. The melting curve was assessed with the following program: 60 °C for 1 min and 95 °C continuously. The results were calculated applying the ΔΔCT method, and are presented in *x*-fold increase relative to ACTB.

2.4. Tissue qPCR array

The Tissue Scan Breast Cancer Tissue qPCR Panel II (OriGene Technologies, Inc., Rockville, USA, Cat. no. BCRT 301) was performed according to the manufacturer's protocol. For this study syndecan-1 was analyzed and normalized to ACTB (see Section 2.3).

2.5. Protein analyses

For the detection of membrane-bound proteins, the cells were cultured on cover slips and treated as described above. Subsequently cells were washed with PBS and fixed with ice-cold methanol for 1 h and −20 °C. Afterwards cells were rehydrated with PBS for 10 min and 37 °C. To avoid nonspecific binding, cells were incubated in 1% BSA for 1 h prior incubation with specific primary antibodies over night at 4 °C. Cells were than washed with PBS and treated with secondary fluorescence antibody for 1 h, washed twice and nuclei were stained with DAPI (2.5 μg/ml). Cells were washed three times with PBS and mounted with Dako Fluorescence Mounting Medium (Dako, Dako Deutschland GmbH, Hamburg, Germany).

The following primary and secondary antibodies for immunofluorescence staining were used for detection of syndecan-1: AM00592SU-N (Acris), OPG (osteoprotegerin) AF 805, (R&D), goat anti-mouse Alexa Fluor 488 (green, for SDC1) and rabbit anti-goat Alexa Fluor 594 (red, for OPG). For fluorescence microscopy the Axio M1 microscope (Carl Zeiss, Jena, Germany) was used.

For soluble proteins ELISAs for syndecan-1 (Diaclone, Besancon, France) and OPG (Immundiagnostik, Bensheim, Germany), were used according to the manufacturer's protocol.