Human Pathology (2011) **42**, 269–278

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Latent TGF-β binding proteins (LTBPs) 1 and 3 differentially regulate transforming growth factor-β activity in malignant mesothelioma☆

Piia Vehviläinen MSc[a], Katri Koli PhD[a], Marjukka Myllärniemi MD, PhD[b], Pamela Lindholm PhD[b], Ylermi Soini MD, PhD[c,d], Kaisa Salmenkivi MD, PhD[a], Vuokko L. Kinnula MD, PhD[b], Jorma Keski-Oja MD, PhD[a,*]

[a]*Departments of Pathology and Virology and Molecular Cancer Biology Research Program, University of Helsinki and HUSLAB, Helsinki University Hospital, 00014 Helsinki, Finland*
[b]*Department of Medicine, Pulmonary Division, University of Helsinki and Helsinki University Central Hospital, 00014 Helsinki, Finland*
[c]*Department of Clinical Pathology and Forensic Medicine, University of Eastern Finland, 70211 Kuopio, Finland*
[d]*Department of Pathology, Oulu University Hospital, 90220 Oulu, Finland*

Received 31 May 2010; revised 29 June 2010; accepted 21 July 2010

Keywords:
LTBP;
Mesothelioma;
Stroma;
TGF-β;
Tumor biopsy

Summary Malignant mesothelioma is an aggressive cancer of the pleura with poor prognosis. There is a need to identify new biomarkers and therapeutic targets for this invasive and fatal disease. Transforming growth factor β (TGF-β) can promote mesothelioma tumorigenesis through multiple mechanisms. Latent TGF-β binding proteins (LTBPs) regulate TGF-β activation by targeting the growth factor into the extracellular matrix from where it can be released and activated. We investigated here the expression patterns of different LTBP isoforms in malignant mesothelioma tissues and in 2 established malignant mesothelioma cell lines. All LTBPs were expressed, but LTBP-3 was the main isoform in healthy pleura and in cultured nonmalignant mesothelial cells. We observed down-regulation of LTBP-3 expression in malignant mesothelioma, which was associated with high P-Smad2 levels indicative of TGF-β activity specifically in the tumor tissue. Small interfering RNA-mediated suppression of LTBP-3 expression in mesothelioma cells increased the secretion of TGF-β activity. Immunoreactivity of LTBP-1, on the other hand, was markedly strong in the tumor stroma, which showed significantly lower levels of P-Smad2. A strong negative correlation between LTBP-1 and P-Smad2 immunoreactivity was found, implying that LTBP-1 is not likely to contribute directly to the increased levels of TGF-β activity in malignant mesothelioma.

Abbreviations: EMT, epithelial-to-mesenchymal transition; LLC, large latent TGF-β complex; MM, malignant mesothelioma; SLC, small latent TGF-β complex; TGF-β, transforming growth factor-β; LTBP, latent TGF-β binding protein.

☆ This work was supported by the Academy of Finland, the Finnish Cancer Foundation, the Sigrid Juselius Foundation, Biocentrum Helsinki, Ida Montin Foundation, Paulo Foundation, Foundation for the Finnish Cancer Institute, Helsinki University Hospital Fund, Helsinki Biomedical Graduate School and the University of Helsinki, and the Jalmari and Rauha Ahokas Foundation.

* Corresponding author. University of Helsinki, Biomedicum/A506, P.O. Box 63, Haartmaninkatu 8, 00014 Helsinki, Finland.
E-mail address: jorma.keski-oja@helsinki.fi (J. Keski-Oja).

0046-8177/$ – see front matter
doi:10.1016/j.humpath.2010.07.005

Current results suggest that LTBPs 1 and 3 may have specific roles in malignant mesothelioma pathogenesis through the regulation of TGF-β activation in the tumor tissue and the structure of the tumor stroma.

1. Introduction

Malignant mesothelioma (MM) is an aggressive tumor of the pleura and serosa. It arises from normal cells of pleural and peritoneal cavity and spreads locally into surrounding tissues. Mesothelioma is frequently associated with occupational exposure to asbestos fibers (reviewed in Jaurand and Fleury-Feith [1]). Due to the long latency (20-40 years) between asbestos exposure and tumor development, the incidence of MM is expected to increase worldwide, even though the use of asbestos has been banned in most industrial countries. Mesothelioma is classified as epithelial, biphasic, or sarcomatous according to the histologic appearance [1]. It is resistant to conventional therapies, both to chemotherapy and radiotherapy, and its prognosis is dismal. Other characteristic features of MM comprise high local invasiveness, whereas it typically does not metastasize until at very late stages. These features probably associate with specific growth factors and stromal proteins, components that have not been profoundly investigated or their significance understood in MM.

MM expresses some stromal components such as tenascin C and fibronectin [2]. In MM tissues, tenascin C is localized especially in the stroma and invasive border of the tumor having also association with poor prognosis [3]. MM is strongly associated with various growth factors including PDGF, IGF, FGF-2, and transforming growth factor β (TGF-β) [4]. Most human MM tumor biopsies reveal immunoreactivity for TGF-β [3,5,6], and it is secreted by human and rodent MM cells in culture [7-9]. TGF-β is thought to enhance tumor growth and invasion through regulation of immune functions, angiogenesis, as well as the production of stromal components. Tumor microenvironment and cancer-associated fibroblasts play an important role in the promotion of tumor invasion [10]. Malignant cells can activate fibroblasts by expressing tumor-promoting growth factors, and the activated fibroblasts, in turn, secrete various soluble factors such as proteases, interleukins, hepatocyte growth factor, insulin-like growth factors, and TGF-βs, which all contribute to stromal alterations toward more permissive environment for tumor growth. These stromal interactions and growth factors very likely contribute to the high invasiveness of MM.

Three isoforms of TGF-β (1-3) exist in humans. TGF-β is secreted from cells noncovalently bound to its propeptide (latency-associated peptide). In most cells, this small latent complex associates with a large glycoprotein, latent TGF-β binding protein (LTBP) [11,12]. The large latent complex is targeted into the extracellular matrix from where TGF-β is released upon activation (for review, see Annes et al [13] and Hyytiäinen et al [14]). Activation can occur via diverse mechanisms including proteases, integrins, and reactive oxygen species. Of the 4 LTBP isoforms, all but LTBP-2 can associate with the small latent TGF-β [15]. Their tissue distribution varies, and there might be different mechanisms for TGF-β activation from distinct complexes [16].

Understanding the molecular mechanisms that promote MM invasion is important for the development of diagnostic and therapeutic tools. LTBPs are important regulators of the targeting and activation of TGF-β, which, in turn, has multiple tumor-promoting effects on mesothelioma cells [8]. However, the roles of LTBPs in MM have not been explored. This study was carried out to identify the LTBPs that play a role in TGF-β activation/storage in MM. We analyzed here TGF-β activity and the expression of different TGF-β and LTBP isoforms in human MM tissue biopsies and in 2 established MM cell lines. Furthermore, we explored the role of LTBPs in the regulation of TGF-β activity in MM cell lines by small interfering RNA (siRNA) silencing.

2. Methods

2.1. Cell culture and reagents

Met5A cells (immortalized with SV40 large T antigen) were from American Type Culture Collection (Manassas, VA). These cells are near-diploid nontumorigenic cells exhibiting typical mesothelial cell characteristics. The mesothelioma cell lines M14K and M38K were established as described [17]. Cells were cultured in RPMI supplemented with 10% fetal calf serum (Gibco, Gaithersburg, MD), 100 IU/mL penicillin, and 50 μg/mL streptomycin. Rabbit polyclonal antibody against phosphorylated Smad2 (AB3849) was from Millipore (Temecula, CA). Mouse monoclonal anti–LTBP-1 (MAB388) antibody was from R&D Systems (Minneapolis, MN).

2.2. RNA isolation and quantitative reverse transcriptase polymerase chain reaction

Total cellular RNA was isolated from 7 MM tissue samples using RNeasy Mini kit (Qiagen, Valencia, CA). A total of 4 tumor samples containing more than 90% of tumor tissue met the criteria and were used for the analysis. Due to