Matrix metalloproteinase 12 promotes tumor propagation in the lung

Ezra Ella, PhD,[a] Yaniv Harel, MSc,[a] Michal Abraham, PhD,[a] Hanna Wald, PhD,[a] Ofra Benny, PhD,[b] Adi Karsch-Bluman, MSc,[b] Dive Vincent, PhD,[c] Devel Laurent, PhD,[c] Gail Amir, MD,[e] Uzi Izhar, MD,[d] Oz M. Shapira, MD,[d] David Yoon, BA,[f] Hyun-Sung Lee, MD, PhD,[f] David J. Sugarbaker, MD,[f] Bryan Burt, MD,[f] Amnon Peled, PhD,[a] and Ori Wald, MD, PhD[a,d]

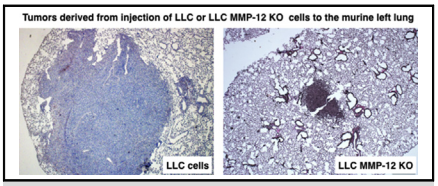
ABSTRACT

Objective: Past studies are inconsistent with regard to the role of matrix metalloproteinase 12 in lung tumorigenesis. This is due, in part, to differential tumorigenesis based on tumor-derived versus immune-derived matrix metalloproteinase 12 expression. Our study aims to thoroughly dissect the role of matrix metalloproteinase 12 in lung tumorigenesis.

Methods: We tested matrix metalloproteinase 12 expression and the association with prognosis using a tissue array and a published non–small cell lung cancer gene expression database. In addition, we characterized the contribution of matrix metalloproteinase 12 to tumor propagation in the lung using a series of in vitro and in vivo studies.

Results: Tumor cells of a diverse set of human lung cancers stained positive for matrix metalloproteinase 12, and high matrix metalloproteinase 12 mRNA levels in the tumor were associated with reduced survival. The lung microenvironment stimulated endogenous production of matrix metalloproteinase 12 in lung cancer cells (human 460 lung cancer cell line, Lewis lung carcinoma). In vitro, matrix metalloproteinase 12 knockout Lewis lung carcinoma and Lewis lung carcinoma cells had the same proliferation rate, but Lewis lung carcinoma showed increased invasiveness. In vivo, deficiency of matrix metalloproteinase 12 in Lewis lung carcinoma cells, but not in the host, reduced tumor growth and invasiveness.

Conclusions: We suggest that tumor cell–derived matrix metalloproteinase 12 promotes tumor propagation in the lung and that in the context of pulmonary malignancies matrix metalloproteinase 12 should further be tested as a potential novel therapeutic target. (J Thorac Cardiovasc Surg 2018;■:1-12)



Representative tumors derived from the injection of LLC and LLC MMP-12 KO cells to the murine left lung.

Central Message

In the current work, we show that MMP-12 drives tumor propagation in the lung. We submit that MMP-12 is a novel therapeutic target in the context of pulmonary malignancies.

Perspective

Controversy exists regarding the role of MMP-12 in lung tumorigenesis with past research showing that immune-derived MMP-12 is antitumorigenic and that tumor-derived MMP-12 is protumorigenic. We test the role of MMP-12 in lung tumorigenesis. We show that in NSCLC, high MMP-12 mRNA levels correlate with reduced survival and that in an orthotopic model of the disease, MMP-12 promotes tumor growth.

See Editorial Commentary page XXX.

BS

Matrix metalloproteinase 12 (MMP-12), also known as "macrophage elastase," is a metalloproteinase that cleaves elastin.[1] It is produced by activated macrophages in premalignant lung diseases such as chronic obstructive pulmonary disease and emphysema.[2] In these pathologic

Scanning this QR code will take you to a supplemental video and figure. To view the AATS Annual Meeting Webcast, see the URL next to the webcast thumbnail.

From the [a]Goldyne Savad Institute of Gene Therapy, Hadassah University Hospital, Jerusalem, Israel; [b]School of Pharmacy, Faculty of Medicine, The Hebrew University, Jerusalem, Israel; [c]CEA Saclay, SIMOPRO, Gif/Yvette, France; Departments of [d]Cardiothoracic Surgery and [e]Pathology, Hadassah University Hospital, Jerusalem, Israel; and [f]Division of General Thoracic Surgery, Michael E. DeBakey Department of General Surgery, Baylor College of Medicine, Houston, Tex.

Read at the 97th Annual meeting of The American Association for Thoracic Surgery, Boston, Massachusetts, April 29-May 3, 2017.

Received for publication March 17, 2017; revisions received Nov 9, 2017; accepted for publication Nov 17, 2017.

Address for reprints: Ori Wald, MD, PhD, Department of Cardiothoracic Surgery, Hadassah Hebrew University Hospital, Jerusalem, Israel (E-mail: ori.wald@mail.huji.ac.il).

0022-5223/$36.00

https://doi.org/10.1016/j.jtcvs.2017.11.110

Abbreviations and Acronyms

ELISA = enzyme-linked immunosorbent assay
FCS = fetal calf serum
GFP = green fluorescent protein
H460 = human 460 lung cancer cell line
HPRT1 = hypoxanthine phosphoribosyltransferase 1
HR = hazard ratio
KO = knockout
LLC = Lewis lung carcinoma
MMP-12 = matrix metalloproteinase 12
NSCLC = non–small cell lung cancer
PCR = polymerase chain reaction
RPMI = Roswell Park Memorial Institute

conditions, macrophage-driven overproduction of MMP-12 is considered to promote excessive degradation of elastin and, consequently, to mediate irreversible parenchymal damage.[3] Notably, MMP-12 is also highly expressed in primary malignant tumors of the lung.[4,5] Yet, distinct from its well-documented role in the pathogenesis of chronic obstructive pulmonary disease and emphysema, the role of MMP-12 in human lung cancer pathogenesis remains largely unknown.[6] Indeed, studies that looked at the expression and potential function of MMP-12 in early-stage non–small cell lung cancer (NSCLC) have yielded vastly inconsistent findings. For example, when characterizing the tissue localization of MMP-12 in the tumor, Acuff and colleagues[7] found that MMP-12 is mainly expressed by tumor-infiltrating macrophages, whereas Hofmann and colleagues[8,9] reported that these were the tumor epithelial cells themselves that were the main source of MMP-12 in the tumor. Further, when looking into the association between MMP-12 expression and NSCLC disease recurrence, Hofmann and colleagues,[8,9] Cho and colleagues,[10] and Lv and colleagues[11] found that high MMP-12 expression in the tumor is associated with reduced survival, whereas Shah and colleagues[5] detected similar MMP-12 expression levels in recurrent and nonrecurrent tumors. Thus, although there is sufficient evidence to conclude that MMP-12 is overexpressed in human lung cancer, the parenchymal source of MMP-12 in these tumors and the pro- or antineoplastic effects that MMP-12 may have on tumor progression remain an area of dispute.

Studies in murine lung-cancer models have yielded conflicting evidence regarding the effects of MMP-12 on tumor propagation. To illustrate, studies that tested the growth of tail vein–injected Lewis Lung carcinoma (LLC) cells in control and in MMP-12–deficient (MMP-12 knockout [KO]) mice demonstrated that LLC cells formed large and vascular tumors in the lungs of the MMP-12 KO mice but that they formed relatively smaller and avascular tumors in the lungs of control mice. It was proposed that increased MMP-12–driven generation of angiostatin in the control group was responsible for the delay in tumor growth.[7,12] In stark contrast, when Qu and colleagues[13] used a bitransgenic MMP-12 overexpression modeling system to test the effects of MMP-12 on lung tumorigenesis, they found that discrete overexpression of MMP-12 in myeloid cells induced immune suppression and enhanced lung tumorigenesis. They further found that when MMP-12 was specifically overexpressed in lung epithelial cells, it promoted emphysema to lung adenocarcinoma transition.[14] Taken together, on the one hand, these studies indicate an antiangiogenic role for MMP-12 in the lung microenvironment, and on the other hand, they propose that MMP-12 is directly protumorigenic. Thus, further research elucidating the role of MMP-12 in lung tumorigenesis is clearly warranted.

To more comprehensively dissect the role of MMP-12 in lung tumorigenesis, we first characterize MMP-12 expression and correlation with survival in human lung cancer tissue samples and databases.[15] Next, we test the distinct pro- and antitumorigenic roles of MMP-12 in a unique orthotopic model of the disease. We report that a diverse set of human lung cancers stained positive for MMP-12 and that high MMP-12 mRNA levels in NSCLC were associated with reduced survival. In addition, we show that MMP-12 is pivotal in promoting the invasion and growth of murine lung cancer cells both in vitro and in vivo. We conclude that in the context of lung tumors, tumor-derived MMP-12 is protumorigenic.

MATERIALS AND METHODS

Cell Lines

The human 460 lung cancer cell line (H460), large cell carcinoma, and murine LLC cell line were all purchased from the American Type Culture Collection. LLC green fluorescent protein (GFP) cells were generated in our laboratory as previously described.[16] MMP-12 KO LLC cells were generated for this study as described next. All cell lines were maintained in Roswell Park Memorial Institute (RPMI) medium (BD Biosciences, San Jose, Calif) containing 10% fetal calf serum (FCS), 1 mmol/L L-glutamine, 100 U/mL penicillin, and 0.01 mg/mL streptomycin (Biological Industries, Kibbutz Beth Haemek, Israel). All cell lines were tested for mycoplasma contamination and found to be negative.

Mice

C57BL/6 (Harlan Laboratories Ltd, Indianapolis, Ind) and C57BL/6-MMP-12 KO (provided by Dr Dive, CEA Saclay, France) mice were used in the experiments.

In Vivo Experimental Design

In brief, cultured LLC or H460 cells ($1*10^3$ per 1 μL) were harvested and suspended in RPMI-1640 (BD Biosciences). Before injection, the cells were mixed with Matrigel (BD Biosciences) at a 1:1 ratio and kept on ice until injection. For injection, the cell mixture was gently mixed and transferred into a 10-μL syringe (Hamilton, Reno, Nev) fitted with a