



Article

Systems Biology and Experimental Validation Enable Discovery of MMP9-Centered Networks, Anticancer Activity, and Pharmacodynamic Signature in Non-Small Cell Lung Cancer

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Abstract

Matrix metalloproteinase-9 (MMP9) is involved in extracellular matrix remodeling, inflammation, and metastasis, and its overexpression is associated with poor prognosis in lung cancer. However, the systems-level effects of MMP9 inhibition remain incompletely understood. We combined systems biology with experimental validation to characterize MMP9-centered signaling in non-small cell lung cancer (NSCLC) and identify downstream pharmacodynamic biomarkers. Network and pathway

analyses revealed extracellular matrix- and inflammation-related interaction modules and prioritized an *MMP9*-associated gene panel. Synthesized compounds were evaluated using purified enzyme assays and A549 NSCLC cells. At 50 μM , compounds M34 and M33 showed the strongest inhibition of *MMP9* activity (65.01% and 61.32%, respectively). Seven compounds (M1, M2, M8, M9, M10, M27, and M34) demonstrated antiproliferative activity at 72 h (IC_{50} = 40–115 μM), suppressed migration and colony formation, induced apoptosis, and reduced *MMP9* protein expression. Quantitative PCR confirmed coordinated downregulation of *MMP9*, *VEGFA*, *APP*, and *ETV4*, with upregulation of *CDH1*, *COL5A1*, *COL6A2*, *CCL2*, and *CCL17*. Enrichment analysis linked these changes to inflammatory signaling, immune activation, and extracellular matrix remodeling. These findings establish an experimentally supported eight-gene pharmacodynamic biomarker signature and support biomarker-guided development of *MMP9*-targeted strategies in NSCLC.

Keywords: matrix metalloproteinase-9 (*MMP9*) inhibitors; non-small cell lung cancer (NSCLC); systems biology; pharmacodynamic biomarker; extracellular matrix remodeling; apoptosis

1. Introduction

Matrix metalloproteinase-9 (*MMP9*; gelatinase B) is a zinc-dependent endopeptidase that participates in extracellular matrix (ECM) remodeling and thereby facilitates key steps in tumor progression, including invasion and metastasis [1,2]. *MMP9* expression is regulated at multiple levels, including epigenetic control and non-coding RNA-mediated mechanisms, which collectively shape its cell- and context-specific activity [1,3]. In addition, inflammatory mediators can amplify *MMP9* expression through coordinated signaling and transcriptional programs, including NF- κ B-dependent regulation, linking *MMP9* activity to tumor microenvironment crosstalk [4,5]. Given these regulatory layers and the dynamic nature of ECM remodeling, *MMP9* is increasingly recognized as a mechanistically important node in cancer biology and a promising target for inhibitor development [6].