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Identification of miR-1293 potential target gene: TIMP-1

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Abstract Tissue inhibitor of metalloproteinases 1 (TIMP-1) is a glycosylated protein with multiple activities in the regulation of biological processes, such as cell growth and apoptosis as well as tumor invasion and metastasis. Bioinformatics analysis using TargetScan and miRanda suggested tissue inhibitors of TIMP-1 are among the targets of miR-1293. To confirm this, we cloned both wild-type and mutant TIMP-1 3′UTR fragments by overlap extension PCR, constructed the recombinant plasmids pGL3-TIMP-1-wt, -mut, and pcDNA 3.1(+)/TIMP-1-CDS and, respectively, co-transfected them into 293T cells with the miR-1293 inhibitor, mimics or the miR inhibitor-NC using a BTX ECM 2001 square-wave electroporator. We used a luciferase assay to investigate binding of miR-1293 to the 3′UTR of TIMP-1. Effects on the levels of the TIMP-1 protein were analyzed by Western blot experiments. The luciferase reporter assay showed a statistically significant ($P < 0.05$) upregulation of activity. Western blot analysis showed a significant increase of expression of the TIMP-1 gene co-transfected with the miR-1293 inhibitor, and demonstrated direct binding of miR-1293 to the 3′UTR of TIMP-1. In this study, we identified TIMP-1 as a novel direct target for miR-1293, which provides the basis for further study of the multifunctional mechanisms of miR-1293 and TIMP-1 in the regulation of a variety of diseases.

Keywords Tissue inhibitor of metalloproteinases 1 (TIMP-1) · MiR-1293 · Overlap extension PCR · Luciferase reporter assay

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Introduction

Tissue inhibitor of metalloproteinases 1 (TIMP-1), a glycosylated protein with a molecular mass ranging from 28.5 to 34 kDa, is expressed in numerous human cells and tissues [1, 2]. TIMPs 1–4 are a group of endogenous inhibitors that control the activity of matrix metalloproteinases (MMPs) and other metalloproteinases [3]. TIMPs are thought to act as biological regulators of the turnover of the extracellular matrix (ECM) by inhibiting MMP activity. TIMP-1 was identified as a humoral protein that promotes proliferation of human erythroid progenitor cells [4]. Since then, TIMP-1 has been shown to exhibit multiple activities in the regulation of various biological processes involved in both normal and pathological events, such as cell growth, apoptosis, and differentiation, which are independent of its metalloproteinase inhibitory activity [5], tissue remodeling of the ECM [6, 7] as well as tumor invasion and metastasis [8–12]. However, little is known about the upstream mechanisms mediating TIMP-1.

Non-coding RNA molecules known as micro-RNAs (miRNAs) have gained increasing attention because of their important roles as post-transcriptional gene regulators in numerous biological processes, including development, stem cell regulation [13, 14], and human diseases [15, 16].