

## The Oncogenic Role of Tissue Inhibitor of Metalloproteinase-1, TIMP-1, through Regulation of YAP in Head and Neck Squamous Cell Carcinoma

### ABSTRACT

**Background:** Head and neck squamous cell carcinoma (HNSCC) affects more than 600,000 patients per year in the world. Despite the treatment, about half of all patients will die of this disease. More advanced diagnosis and treatment are required. Tissue inhibitor of metalloproteinase-1 (TIMP-1) inhibits the degradation of extracellular matrix regulated by matrix metalloproteinases (MMP). Although this function is unfavorable for cancer cells to invade or migrate using MMP, TIMP-1 is controversially overexpressed in various types of cancer including HNSCC. Therefore, an oncogenic aspect of TIMP-1 has been featured, which can resolve the discrepancy. Actually, TIMP-1 binds to its receptor CD63 and activate integrin  $\beta$  1, leading to aberrant cell proliferation. However, a true role and its mechanism of TIMP-1 in HNSCC is still unclear. Yes-Associated Protein (YAP), a transcription co-activator, enhances transcription of specific genes related to cell proliferation, such as CTGF or Cyr61. YAP is inactivated due to its phosphorylation inducing localization in cytoplasm and degradation, which is regulated by Hippo-signaling pathway, composed of MST1/2 and LATS1/2, or Hippo-independent signaling pathway like actin dynamics by GPCR or integrin $\beta$ 1. YAP is dysregulated and contributes to aberrant cell proliferation in a variety of cancer including HNSCC. When I searched TIMP-1 expression by Oncomine data base analysis, I found that TIMP-1 was highly overexpressed in association with YAP-associated genes including CTGF and Cyr61 in HNSCC. Then, I hypothesized TIMP-1 might regulate YAP in HNSCC.

**Purpose:** To reveal an oncogenic role of TIMP-1 through regulation of novel downstream YAP in HNSCC.

**Method:** HNSCC cell lines (HSC-2, 3, 4 and KOSCC33A) and human embryonic kidney cells (HEK293) were used. Expressing or shRNA plasmids were transfected and stable clones were analyzed by western blotting, RT-PCR, Real time-PCR and proliferation assay. Data were evaluated by student's t-test with significant level set at  $P < 0.05$ .

**Results:** TIMP-1, YAP and CTGF expression were positively correlated among HNSCC cell lines. Stable overexpression or recombinant TIMP-1 induced YAP activation (dephosphorylation), CTGF expression and accelerated cell proliferation. In contrast, stable TIMP-1 knockdown inactivated (phosphorylated) YAP and inhibited proliferation. Furthermore, stable CD63 knockdown inactivated YAP and disturbed activation triggered by TIMP-1.

**Conclusion:** I revealed TIMP-1 with its receptor CD63 play an oncogenic role in HNSCC through regulation of YAP. This finding suggests that targeting of TIMP-1 and/or its new downstream YAP will be beneficial for the improvement of diagnosis and treatment for the patients with HNSCC.

**Keywords:** HNSCC cell lines (HSC-2,3,4 and KOSCC33A), TIMP-1, YAP