THE MECHANISM OF *WWP1* GENE IN ORAL SQUAMOUS CELL CARCINOMA

古乃威 **NAI-WEI KU[1#]**, 林如華 **JU-HWA LIN[1*]**

1DEPARTMENT OF BIOLOGICAL SCIENCE AND TECHNOLOGY, CHINA MEDICAL UNIVERSITY, TAICHUNG, TAIWAN

Abstract

The mortality of oral squamous cell carcinoma (OSCC) is one of the ten leading causes of cancer deaths in Taiwan. Environmental carcinogens such as betel quid chewing, tobacco smoking and alcohol drinking have been identified as major risk factors for OSCC. Our laboratory has found that WW domain containing E3 ubiquitin protein ligase 1 (*WWP1*) overexpressed in OSCC due to gene amplification. *WWP1* belongs to the C_2-WW-HECT type E3 family, and the involvement of the HECT-type E3s in crucial signaling pathways implicates in tumorigenesis. The amplification and overexpression of *WWP1* was also found in prostate cancer and breast cancer. Knockdown of *WWP1* suppressed cell proliferation and induced apoptosis. These finding suggest an oncogenic role of *WWP1* in carcinogenesis. In this study, we established the LacO/IPTG controlled shRNA inducible cell system to investigate the functional roles of *WWP1* in OSCC. To verify the shRNA effect, we examined *WWP1* mRNA and protein expression by real time PCR and western blotting. Inhibition of cell growth was examined by trypan blue counting and MTT assay. The effects on cell cycle phase and apoptosis were determined by flow cytometry. We will prove that knockdown of WWP1 would cause cell cycle arrest and induce apoptosis in OSCC cell line. These findings suggest that WWP1 could have an oncogenic role in OSCC.

Key word: *WWP1*、shRNA、OSCC

Result

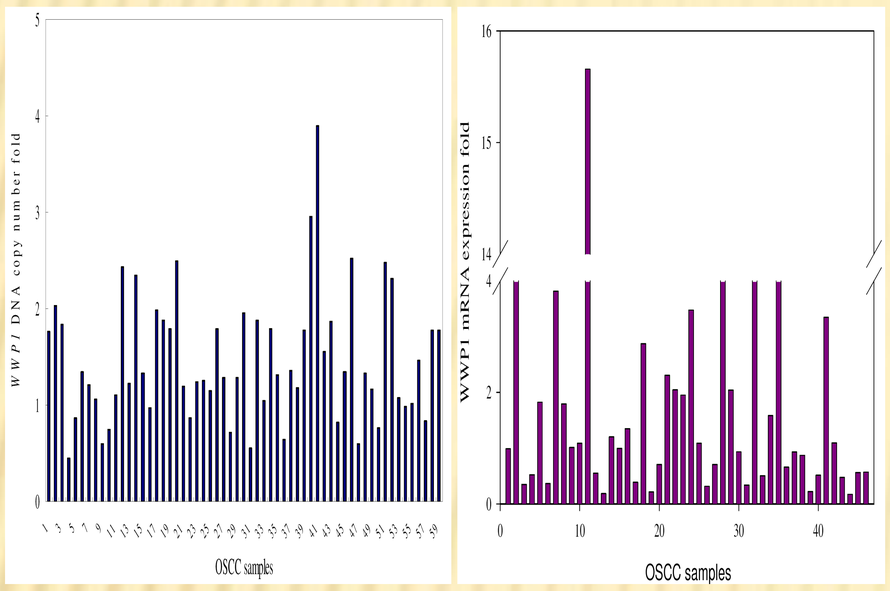


Fig1. *WWP1* represented DNA copy number amplification and mRNA overexpression in clinical OSCC samples.(A) 23 of 59 (39%) OSCC patients represented *WWP1* DNA amplification.(B) 16 of 46 (35%) OSCC patients represented *WWP1* mRNA overexpression.

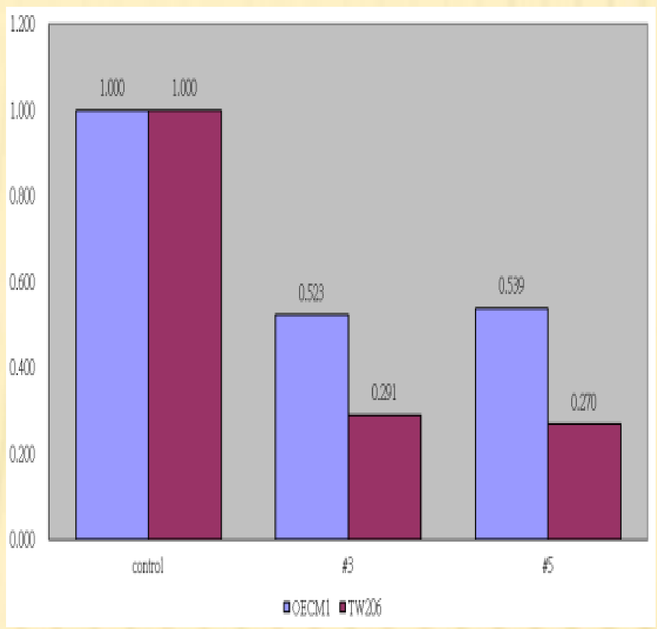


Fig4. MTT assay. Interference of *WWP1* expression suppresses the cell growth of OECM-1 and TW206.

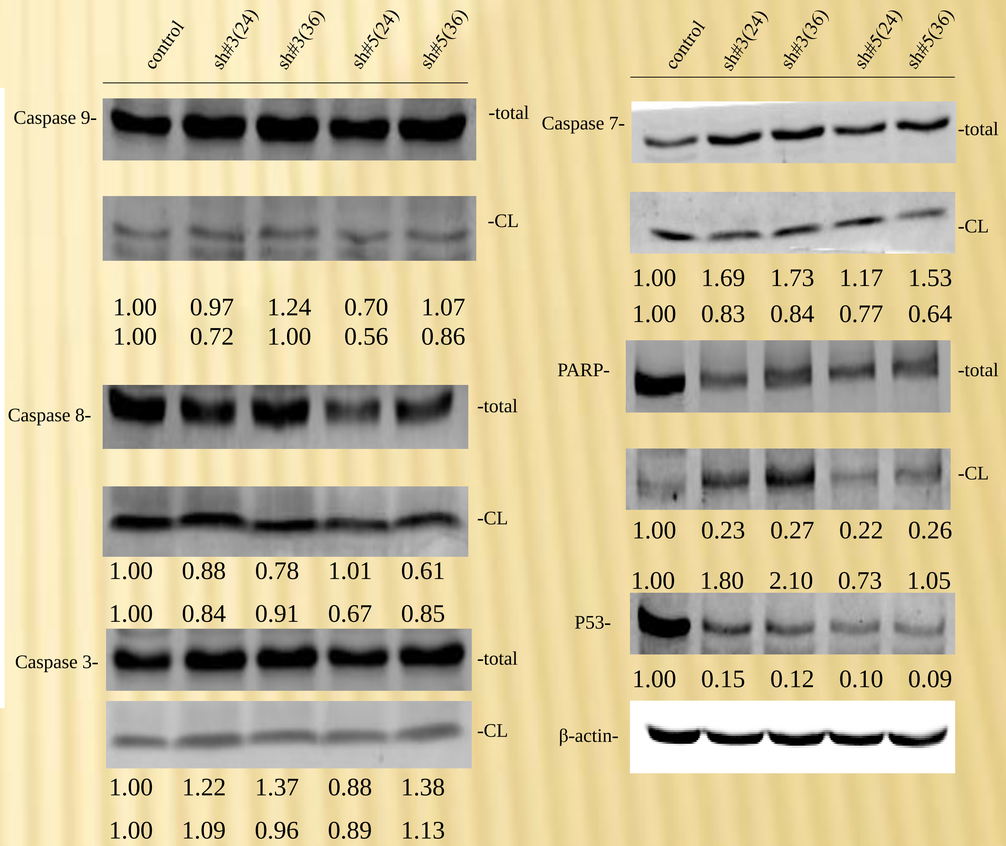
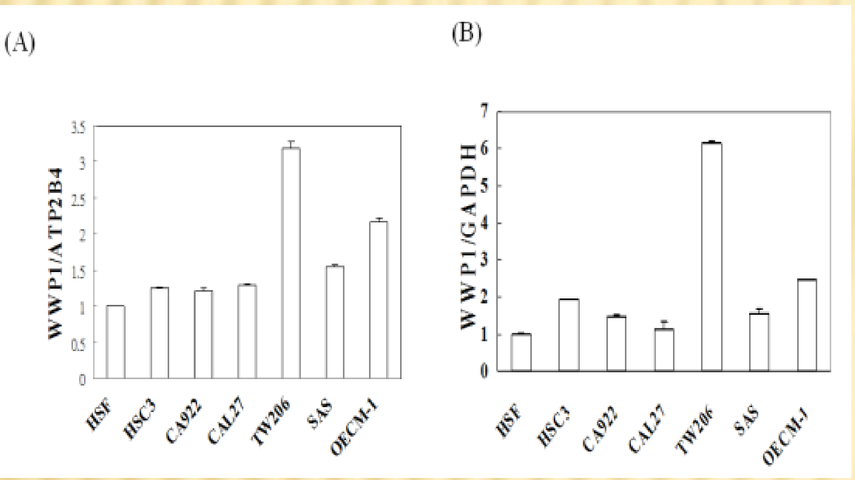
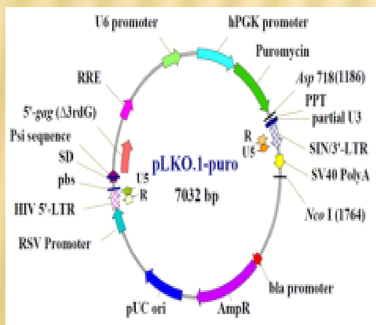


Fig6. The effects of *WWP1* Knockdown on the apoptosis-associated proteins in OECM-1 cells.



DNA copy number **mRNA level**

Fig2. Identifying *WWP1* DNA copy number change and *WWP1* mRNA expression for OSCC cell lines by Q-PCR.

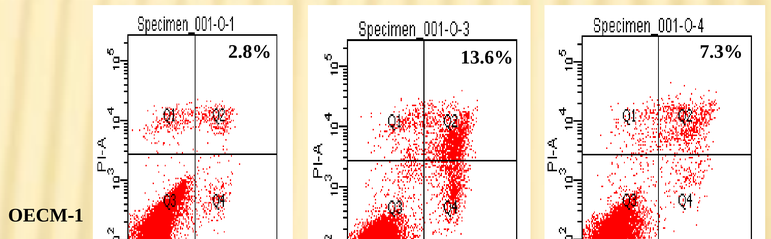


Gene: WWP1	Vector Name	Sequence
shRNA3	pLKO.1	TCTGTAACTAAAGGTGGTCCA
shRNA5	pLKO.1	GCTTATTTGAGTATGCGGGCA

(shRNA construction by National RNAi Core Facility)

Fig3. Knockdown of *WWP1* by shRNA.

Table1.Knockdown information.



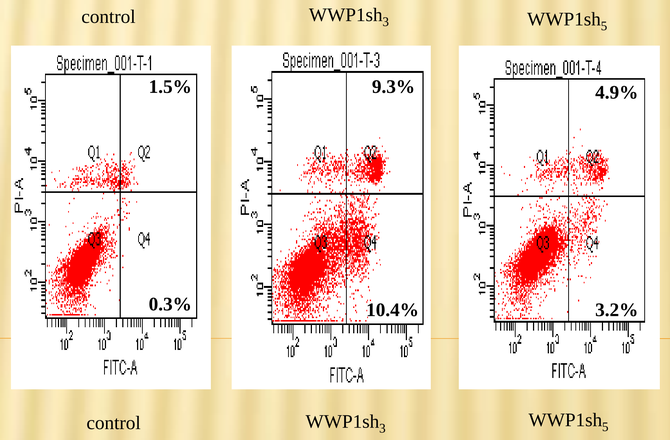


Fig5. *WWP1* knockdown induces apoptosis in OECM-1 and TW206 oral cancer cells.

Inducible system establishment

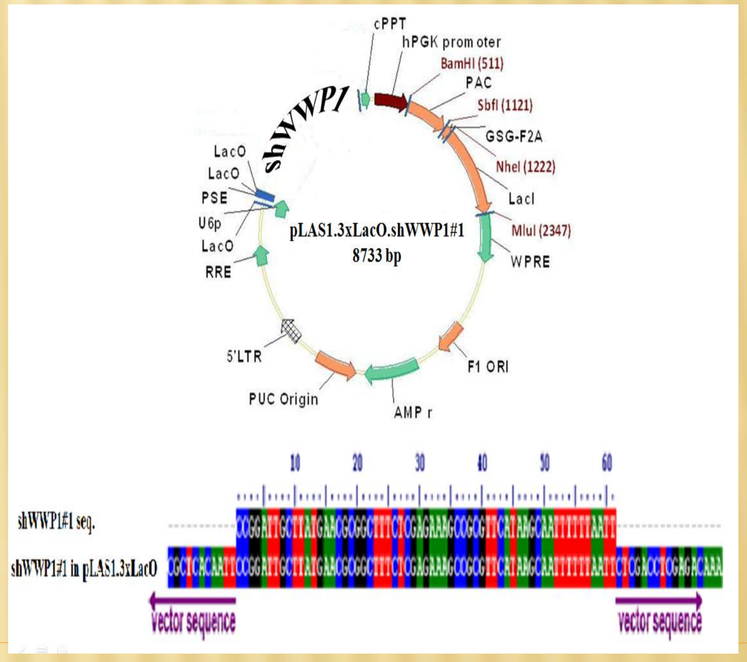


Fig7. Cloning shRNA sequence of interest into pLAS1.3xLacO .

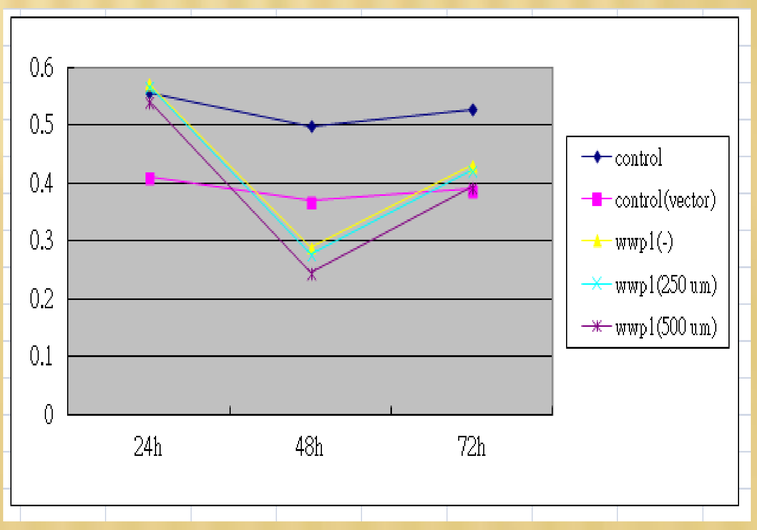


Fig8.The effects of *WWP1* knockdown on the viability from OECM-1 cells by IPTG inducing.