EXPRESSION OF GNA12 AND IFITM3,

AND THEIR ROLES IN ORAL CARCINOGENESIS

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is a major health problem worldwide. The heterogeneity of the disease is the main challenge for the improvement of current treatment modalities. Efforts in our laboratory have focused on the molecular profiling of oral cancer in order to understand the mechanisms underlying this disease. Based on the previous microarray data, Guanine nucleotide binding protein alpha-12 (GNA12) and Interferon inducible transmembrane protein 3 (IFITM3) were identified to be up-regulated in oral cancer. **Objectives:** This study aims to validate the expression of GNA12 and IFITM3 at the mRNA and protein levels in oral cancer tissues and to determine the effects of their over-expression on the biology of oral cancer cells. **Methodology:** Real-time quantitative PCR (QPCR) was conducted for relative quantification of GNA12 and IFITM3 mRNA expression in 47 OSCC in comparison to 18 non-malignant oral tissues. GNA12 and IFITM3 protein expressions were accessed by immunohistochemistry (IHC) on tissue macro-arrays (TMaA) consisting of 44 tumours and 23 non-malignant tissues. Target molecules were exogenously expressed in oral cancer cell lines via virus-transduction, and further examined for *in-vitro* cell proliferation, migration and invasion to determine their functional roles in oral cancer. **Results:** In comparison to non-malignant tissues, OSCC tissues exhibited high mRNA levels of GNA12 ($p<0.001$) and IFITM3 ($p=0.003$). Over-expression of GNA12 was observed in 55% (n=26) OSCC tissues, and IFITM3 over-expression was found in 46% (n=21) OSCC tissues. Consistently, IHC analysis also detected high levels of GNA12 and IFITM3 protein expressions in 75% (n=33) and 79% (n=34) of OSCC, respectively. Their expression was primarily localized to the cytoplasm. Conversely, more than 80% of the non-malignant cells showed negative staining for GNA12 and IFITM3. Following this, the *in-vitro* functional studies showed that expression of activated

GNA12 (GαQ231L) in oral cancer cell line markedly increased cell migration in monolayer wound healing assay (p<0.001) and invasion through matrigel barrier (p=0.015) but have no effect on cell proliferation. However, IFITM3-transformed oral cancer cells lost the ability to form confluent monolayer and showed inhibition of cell growth. Moreover, over-expression of IFITM3 significantly reduced oral cancer cells migration (p=0.019) and invasion (p=0.004). **Conclusion:** To the best of our knowledge, this is probably the first study that demonstrated the expression of GNA12 and IFITM3 at the mRNA and protein levels in oral cancer. Over-expression of GNA12 and IFITM3 are associated with oral cancer, since high levels of these genes were found to be present in a large proportion of Malaysia's oral cancer patients. Expression of activated GNA12 induced oral cancer cell migration and invasion hence warrant further investigations in the *in-vivo* model to determine if it could be targeted for therapy to prevent the spread of oral cancer. Over-expression of IFITM3 has inhibitory effects on oral cancer cell growth, migration and invasion. Thus, its role as oncogene or anti-tumour gene remains unclear.

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List of Symbols and Abbreviations

ºC: Degree celcius

µg: Micro-gram

µl: Micro-litre

bp: Base-pair

cDNA: complementary DNA

CO_2: Carbon dioxide

Ct: Cycle threshold

DAB: 3, 3'-diaminobenzidine

DMEM/F-12 HAM's: Dulbecco's Modified Eagle's Medium/Nutrient mixture F-12

DNA: Deoxyribonucleic acid

dNTP: Deoxynucleotide Triphosphate

EDTA: Ethylene diamine tetra-acetic acid

FBS: Fetal bovine serum

FEP: Fibro epithelial polyp

FFPE: Formalin-fixed parraffin-embedded

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GDP: Guanosine diphosphate

GNA12: Guanine nucleotide binding protein alpha-12

GPCR: G-protein coupled receptor

GTP: Guanosine triphosphate

HNSCC: Head and neck squamous cell carcinoma

ICD: International classification of disease

IFITM3: Interferon inducible transmembrane protein 3

IHC: Immunohistochemistry

ISG: Interferon-stimulable gene

ISRE: Interferon-stimulable response element

LB: Luria-Bertani

mg: Milli-gram

$MgCl_2$: Magnesium chloreide

min: Minute

ml: Milli-litre

mm: Milli-metre

NaOH: Sodium hydroxide

ng: Nano-gram

nm: Nano-metre

OSCC: Oral squamous cell carcinoma

PBS: Phosphate buffer saline

PCR: Polymerase chain reaction

RGS: Regulators of G-protein signaling

RIN: RNA integrity number

RIPA: Radio-Immunoprecipitation Assay

RNA: Ribonucleic acid

rpm: Revolutions per minute

QPCR: Quantitative polymerase chain reaction

SDS: Sodium dodecyl sulfate

sec: Second

TAE: Tris-acetate-EDTA

TBS: Tris-buffered saline

TBST: Tris-buffered saline with 0.1% tween20

TMaA: Tissue macro-array

WHO: World Health Organization