BIOMEDICINE

Association of Epstein-Barr Virus Latent Membrane Protein 1 (LMP1) Gene Expression and Caspase Activity in Normal Nasopharyngeal Cell

Mohd Aminudin Mustapha[1,2], Sai-Peng Sim[2]

ABSTRACT

Objective: **To assess *LMP1* gene expression-induced apoptosis in normal nasopharyngeal cells by measuring caspase activity.**

Material and Methods: ***LMP1*** **gene was subcloned into pTracer and pcDNA vector, producing pTracer-*LMP1* and pcDNA-*LMP1* expression plasmids. The plasmids were then transfected into normal nasopharyngeal cells. *LMP1* gene expression-induced apoptosis was accessed by measuring Caspase activities using Caspase-Glo ® 3/7 Assay kit following the manufacturer's protocol. The luminescence intensity was measured by microplate reader. Association of *LMP1* gene expression with caspase activation was analysed by independent Sample T test.**

Results: **LMP1 gene expression in normal nasopharyngeal cells is significantly associated with higher caspase activity of apoptosis compare to the vector control with t(-2.142), p value of 0.03.**

Conclusion: **Our results show that, there is association of *LMP1* gene expression and caspase activity level in normal nasopharyngeal cell thus support that *LMP1* gene expression is involved in apoptosis induction. This in turn suggests that the apoptosis process is potentially involved in the carcinogenesis of nasopharyngeal carcinoma.**

KEY WORDS

latent membrane protein 1, Epstein-Barr virus, caspase activity, apoptosis, nasopharyngeal cell

INTRODUCTION

Latent membrane protein 1 (LMP1), which is encoded by EBV, is the most important oncoprotein in EBV-related malignancies. Constitutive expression of *LMP1* contributes to the initiation and progression of NPC. *LMP1* is one of the seven informative genes that can accurately predict the survival of NPC patients (Wang *et al*., 2011). However, the exact mechanism of how *LMP1* contributes to carcinogenesis is largely unclear. The oncogenic potential of *LMP1*, which results in B cell transformation, is suggested by its high functional similarity to the tumor necrosis factor receptor (TNFR) family members, CD40 and TNFR1 (Kulwichit *et al*., 1998).

EBV infection occurs mostly during childhood and remains asymptomatic due to immune-related activities. Nearly 90% of the adult population worldwide is currently EBV infected, and the most common route of infection is through intimate contact with saliva from an EBV-infected person (Kgatle, Spearman, Kalla, & Hairwadzi, 2017). In NPC, EBV is harboured in a latent stage with restricted viral gene expression of *LMP1/2*, *EBNA1*, *EBER1/2*, and *BARTs*. *LMP1* can activate cellular DNA methyltransferase via c-Jun NH2-terminal kinase signaling (Tsai *et al*., 2006) and upregulate BMI1 expression (Dutton *et al*., 2007), which is associated with epigenetic changes in NPC.

Although chromosomal abnormalities are commonly found in NPC, the detail molecular mechanisms leading to these abnormalities remain elusive. However, there are increasing evidences that the apoptotic nuclease is responsible for the initial event of chromosome translocation, that is the breakage of the chromosome (Betti, Villalobos, Diaz, & Vaughan, 2003; Sim & Liu, 2001). Although apoptosis is a cell death process, it has also been implicated in chromosome rearrangement. Chemotherapeutic drug-induced apoptosis has been implicated in the introduction of chromosome break within the Mixed Lineage Leukaemia (MLL) gene, a gene frequently involved in chromosome translocations (Sim & Liu, 2001). More recently, the apoptotic nuclease was suggested to be involved in oxidative stress-induced and bile acid-induced chromosome breaks in normal nasopharyngeal epithelial cells (Tan, Sim, & Khoo, 2016). Apoptosis is a naturally occurring programmed cell death process that is characterised by a series of distinct morphological changes which result from the activity of caspases, a class of cycteine protease (Hengartner, 2000). The current study was designed to assess *LMP1* gene expression-induced apoptosis by measuring caspase activity in nasopharyngeal cells expressing *LMP1* and com-

Table 1: Mean and Standard Deviation of Caspace Activity in transfected cell line

Plasmid Transfection in Cell Line	N	Mean	Std. Deviation	Std. Error Mean
pTracer-LMP1	100	513.18	413.327	41.333
pTracer	100	529.99	322.257	32.226
pcDNA-LMP1	100	304.30	119.322	11.932
pcDNA	100	271.52	95.827	9.583

Received on February 5, 2021 and accepted on April 7, 2021

1) Centre for Pre-University Studies, Universiti Malaysia Sarawak
94300 Kota Samarahan, Malaysia
2) Department of Paraclinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak
94300 Kota Samarahan, Malaysia
Correspondence to: Sai-Peng Sim
(e-mail: spsim@unimas.my)