Malays. Appl. Biol. (2018) 47(1): 173–179

VALIDATION OF ENDOGENOUS REFERENCE GENES FOR RELATIVE QUANTITATION STUDIES OF GENE EXPRESSION IN NASOPHARYNGEAL CARCINOMA

ASHLEY EDWARD ROY SOOSAY[1*], ISMAIL MAAMAR MAKHZOUM ALHWIJ[1],
PARVATHEE PONNIAH[1], ALAN SOO-BENG KHOO[2] and CHUN-YIING WONG[3]

[1]*Faculty of Medicine & Health Sciences, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak*
[2]*Institute for Medical Research, Kuala Lumpur*
[3]*Sarawak General Hospital, Kuching, Sarawak*
[*]*E-mail: sashley@unimas.my*

Accepted 27 February 2018, Published online 31 March 2018

ABSTRACT

Reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) is a useful molecular contraption in translational biomedical research and clinical settings. RT-qPCR requires normalization. Housekeeping gene (HKG) as reference gene (RG) is commonly used for the relative quantification of the target gene (TG) in gene profiling assays. Normalization requires stably expressed endogenous RG. Recently, RGs were found to be regulated in a various experimental milieu in different tissues. Therefore, it is pertinent to identify HKGs that are stably expressed and are independent of factors influencing the cell. To validate 4 endogenous RGs for the relative quantification of TGs in gene expression analysis performed via RT-qPCR in nasopharyngeal carcinoma. The qbase+ software utilizing geNorm analysis identified GAPDH, TBP and YARS as stably expressed HKGs. ACTB was the least stable RG in this study. The most suitable set of RG for NPC gene expression studies include GAPDH, TBP and YARS. No single gene was identified as the best RG for expression study. The RG that can be utilized during RT-qPCR on normal and malignant nasopharyngeal tissue samples is a collection of 3 genes (GAPDH, TBP and YARS) used as an average.

Key words: RT-qPCR, nasopharyngeal carcinoma, normalization, gene expression

INTRODUCTION

Reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR), is a benchmark for gene expression profiling. It is very rapid, precise and sensitive. RT-qPCR used to quantitate the expression of prognostic and predictive molecular markers in oncology study. A target gene can be analyzed with ease and accuracy by correlating to a stable independent parameter such as expression of a stable housekeeping gene (HKG) which can be a reference gene (RG) (Lyng *et al.*, 2008). This is referred to as relative quantification in gene expression studies. In order to quantitate gene expression in tumors, a gene with unchanged expression is needed to correct for basic sample differences, including cellular input, RNA quality, and RT-qPCR efficiency (Gao *et al.*, 2008). This method of using HKGs as a stable independent parameter is referred to as 'normalization' (Huggett *et al.*, 2005). The HKGs/RGs acting as endogenous controls are the best direct indicators for the quantity and quality of the samples being used in the gene expression experiments. Most commonly used RGs are β-actin (ACTB) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) but the expression of these two RGs are regulated during cellular processes such as differentiation, cancer progression, malignant transformation, and hypoxia. GAPDH has been reported to be regulated by oestradiol in MCF-7 cells (Revillion *et al.*, 2000). Microarray data suggests that ACTB expression varied greatly between parts of the tumor (Popovici *et al.*, 2009; Khoshnoud *et al.*, 2010). Although these genes are regulated, they are still commonly used as RGs. It is suggested that the use of two HKGs for RT-qPCR normalization can compensate for slight differences in HKG gene expression (Gao *et al.*, 2008).

* To whom correspondence should be addressed.