Modification of Relative Gene Expression Ratio Obtained from Real Time qPCR with Whole Carotid Body by Using Mathematical Equations

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Abstract  Quantitative real time PCR (qPCR) is a common tool used to compare the relative gene expression between treated/untreated cells, different types of tissues, or immature/mature organs. When homogeneous cells are used for qPCR, the Ct number of a tested gene solely represents the quantity of gene expression in cells. However, when a heterogeneous tissue is used for qPCR, the Ct number of a tested gene should be modified depending on several factors: the percentage of each cell type in the sample tissue, the cell type where the target gene is expressed, and the cell type in which the target gene is regulated. The carotid body (CB) is mainly composed of three types of cells: type I (chemoreceptor) cells, type II cells, and other types of cells. Therefore, the relative gene expression ratio obtained from qPCR data using whole CB could be modified by applying one of the following 19 different cases: (1) the target gene is expressed in only one type of cell (3 cases), (2) the gene is expressed in two types of cells and increased in only one or both cell types (9 cases), and (3) the gene is expressed in all three types of cells and increased in only one, two, or all three cell types (7 cases). For example, in the case that the target gene is expressed in all three types of cells and the gene is increased in only a cell comprising 10% of whole CB, the gene expression ratio in that cell will be 9 times as that derived from whole CB. Thus, once the percentage of each cell type in whole CB is observed, the cell type of interest gene (E-gene) expression is identified, and the cell type that regulates E-gene expression by treatment is identified. Thus, the corresponding mathematical equation out of 19 cases could be applied to modify the gene expression ratios measured by qPCR.

Keywords  Mathematical equation · qRT-PCR · Gene expression · Ratio · CB · Type I cell · Type II cell · Cell type · Percentage · Modification

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1 Introduction

Quantitative real time reverse transcription PCR (qRT-PCR) is the most suitable and sensitive method for the detection and quantification of gene expression levels, in particular for low abundance mRNA, from limited tissue samples, and to elucidate small changes in mRNA expression levels (Bustin 2000, Pfaffl and Hageleit 2001). The relative expression is based on the expression ratio of the target gene versus a reference gene and is adequate for most purposes to investigate physiological changes in gene expression levels (Pfaffl et al. 2002). However, when the heterogeneous tissues are used for qRT-PCR, the relative gene expression ratio could be considered to re-evaluate depending on several factors: the percentage of each cell type which sample tissue consists of, the cell type expressing target genes, and the cell type in which the target gene is regulated.

For example, when whole rat carotid body (CB) is used for the gene expression studies, the relative gene expression ratio of qRT-PCR could be re-evaluated. Carotid body is a small organ sensing blood O$_2$, CO$_2$, and pH level. CB consists of four principle components: cell clusters, blood vessels, connective tissue, and nerve fibers (Izal-Azcarate et al. 2008) and is mainly composed with chemoreceptor glomus cells (type I cells) and sustentacular cells (type II cells) at $\sim$60% of total CB volume (Gonzalez et al. 1995, Lopez-Barneo et al. 2001). When we assume that CB consists of three types of cells: type I cells, type II cells, and other types of cell, we find 19 different cases, as shown in methods. We try to generate mathematical equations to modify the relative gene expression ratio of qRT-PCR for all possible cases. In cases which the target gene is only expressed in one type of cell, the ratio won’t be changed, as shown in case 1–3. However, for other cases the relative gene expression ratios for qRT-PCR could be modified with the proper mathematical equations generated for 16 cases (case 4–19). Therefore, if we know what percentage each cell type is occupied, which cell types express target genes, and which cell types in which the target gene is regulated, the relative gene expression ratio of qRT-PCR can be easily re-calculated by applying the corresponding mathematical equations.

2 Methods

In order to study the developmental gene expression ratio of postnatal 1 day and 14 days old CB having three different cell types, the following notations will be used;

\[N_{c_1}, d_1 \text{ or } N_{c_1}, d_{14} : \text{number of E-gene in cell 1 (type I cell) at day 1 or 14}\]
\[N_{c_2}, d_1 \text{ or } N_{c_2}, d_{14} : \text{number of E-gene in cell 2 (type II cell) at day 1 or 14}\]
\[N_{c_3}, d_1 \text{ or } N_{c_3}, d_{14} : \text{number of E-gene in cell 3 (other type) at day 1 or 14}\]
\[N_{d_1} \text{ or } N_{d_{14}} : \text{total number of E-gene in whole CB cells at day 1 or 14}\]
\[P_{c_1}, d_1 \text{ or } P_{c_1}, d_{14} : \text{the percentage of cell 1 in whole CB cells at day 1 or 14}\]
\[P_{c_2}, d_1 \text{ or } P_{c_2}, d_{14} : \text{the percentage of cell 2 in whole CB cells at day 1 or 14}\]