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More attention should be paid on the interpretation of gene expression data

Eric Verna

Eric Verna, Department of Molecular Diagnostics, Onasgen Inc., 34562 Galway, Ireland
Author contributions: Verna E wrote this letter.
Correspondence to: Eric Verna, PhD, Department of Molecular Diagnostics, Onasgen Inc., 34562 Galway, Ireland. ericverna@ovi.com
Telephone: +353-46-2345312 Fax: +353-44-5432345
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Peer reviewer: Luca VC Valenti, MD, Department of Internal Medicine, University of Milan, IRCCS Ospedale Maggiore Policlinico, Hall Granelli, Via F Sforza 35, 20122 Milano, Italy

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Abstract

Molecular profiling of gene expression is important for determining signatures in cancer progression and diagnosis. For this purpose, polymerase chain reaction-based techniques are preferentially used as a feasible and sensitive approach. Nevertheless, when relative quantitative analyses are performed on gene expression, the interpretation of mathematical equations must be carefully done. This letter to the editor is focused on recently published gene expression data in *World Journal of Gastroenterology* by Ozmen *et al* demonstrating increased levels of *LYVE-1*, *VEGFR-3* and *CD44* genes in gastric cancer samples compared to non-neoplastic gastric tissues. However, there are major concerns about misinterpretation of the gene expression data obtained with the $2^{-\Delta\Delta Ct}$ relative quantitative method. In the study, $2^{-\Delta\Delta Ct}$ values calculated for many samples were smaller than 1 ($2^{-\Delta\Delta Ct} < 1$) which indicate decreased levels of *LYVE-1*, *VEGFR-3* and *CD44* gene expression in the gastric cancer tissues. This unfortunate mistake is an important example showing how a simple error in the interpretation of relative-quantitative gene expression data may result in misleading scientific conclusions. In this letter, a brief explanation of the $2^{-\Delta\Delta Ct}$ method is given. In addition, the importance of technical quality and interpretation in gene expression studies is discussed.

LETTER TO THE EDITOR

I read with great interest the article "relationship between *LYVE-1*, *VEGFR-3* and *CD44* gene expressions and lymphatic metastasis in gastric cancer" by Ozmen *et al*[1] in *World Journal of Gastroenterology* published in July 21, 2011. The expression of three genes playing important roles in lymphangiogenesis was studied in a large group of gastric cancer patients. In their study, a relative quantitative method was applied to assess the levels of gene expression. In addition, it was a good approach to compare the gene expression of the gastric tumors' data with that of the surgically-resected non-neoplastic (so-called "normal tissues" by the authors) samples. However, I am concerned about the misinterpretation of the gene expression data calculated by using "$2^{-\Delta\Delta Ct}$ method". Rather than being increased as presented in the manuscript, the gene expression values calculated with $2^{-\Delta\Delta Ct}$ indicate decrement ($2^{-\Delta\Delta Ct} < 1$) for many samples.

According to $2^{-\Delta\Delta Ct}$ method, the polymerase chain reaction (PCR) threshold cycle (Ct) values of a specific gene and of a house keeping gene are obtained both from the case and control groups (tumor and non-neoplastic tissue samples, respectively in the manuscript by Ozmen *et al*[1])[2,3]. The difference between the Ct values of the specific gene and the house keeping gene is calculated (which is ΔCt) for individual cases and controls[2,3].

This provides an internal normalization for each sample. Then, ΔCt value of the control sample is subtracted from the ΔCt of the case sample (which is ΔΔCt, giving raw information about the difference in gene expression levels). Since DNA is amplified by the power of two for each PCR cycle, ΔΔCt is also presented as power of "2"[2,3]. Simply, if there is no difference between the case and control samples, ΔΔCt value will be "0" and two to the zero power is "1". Therefore, in the $2^{-\Delta\Delta Ct}$ method, if the gene expression level of the case is higher than that of the control sample, the $2^{-\Delta\Delta Ct}$ value is "> 1"; but, if it is lower than the control sample $2^{-\Delta\Delta Ct}$ value is between "0" and "1"[2,3].

In the manuscript of Ozmen *et al*[1], although $2^{-\Delta\Delta Ct}$ values are calculated correctly, they presumed that the values higher than zero give an indication of increment in the expression levels of the genes studied. When their data is distributed according to the $2^{-\Delta\Delta Ct}$ with an appropriate interpretation, two groups appear with high and low gene expression patterns, especially for *LYVE-1* and *VEGFR-3* genes (Figure 2 in the manuscript by Ozmen *et al*[1]). Additionally, to obtain a true Ct value, real-time PCR conditions should be finely optimized and final product should be devoid of non-specific products (extra bands) or primer dimers. However, there are several non-specific amplicons in the PCR products of *VEGFR-3* gene (Figure 1 in the manuscript by Ozmen *et al*[1]). This basic technical problem hampers the reliability of quantitative gene expression results. I believe the correction of this misinterpretation would provide additional value to their study.

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