



Response letter regarding the interpretation of gene expression data

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Abstract

This is a response letter to Verna E's comments regarding our previous manuscript published last year in the *World Journal of Gastroenterology* entitled "Relationship between *LYVE-1*, *VEGFR-3* and *CD44* gene expressions and lymphatic metastasis in gastric cancer", which evaluated the relationship between these expression levels and clinicopathological parameters (Ozmen F *et al*, *World J Gastroenterology* 2011; 17: 3220-3228). The mean values for lymphatic vessel endothelial hyaluronan receptor-1, CD44 and vascular endothelial growth factor receptor-3 expression (represented as $2^{-\Delta\Delta Ct}$) were 1.13, 1.24 and 1.17, respectively, suggesting an increase in gene expression in tumor tissue compared to normal tissue. Despite the increase in gene expression in the cancer tissues ($2^{-\Delta\Delta Ct} > 1$), only some of the results reached statistical significance, which was thoroughly discussed in our paper. In the present letter, we report that his comments are flawed and result in confusion. Therefore, we herein provide more explanation regarding gene expression in gastric cancer. We hope that this letter will address Verna E's misunderstandings.

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Key words: Gastric cancer; Lymphatic metastasis; Lymphatic vessel endothelial hyaluronan receptor-1; Vascular endothelial growth factor receptor-3; CD44

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TO THE EDITOR

I recently noticed that a Letter to the Editor written by Verna E, regarding our paper (Ozmen F *et al*, *World J Gastroenterol* 2011; 17: 3220-3228), had been published in the June issue of the Journal this year (Verna E, *World J Gastroenterol* 2012; 18: 3181-3182)^[1,2].

Although I would like to thank Dr. Verna for his interest and for providing us with this opportunity to address his concerns, I have to stress that it is very important to understand the paper in its entirety before coming to certain conclusions: (1) Our study investigated the expression levels of the lymphatic vessel endothelial hyaluronan receptor-1 (*LYVE-1*), vascular endothelial growth factor receptor-3 (*VEGFR-3*), and *CD44* genes in human tissues with or without a tumor using real-time polymerase chain reaction (RT-PCR) and evaluated the relationship in gastric cancer between these expression levels and clinicopathological parameters that included tumor type, stage, differentiation, and the presence of lymph node metastasis, vascular invasion, and neural/perineural invasion^[1]; (2) Relative expression levels were calculated using the PCR cycle threshold (Ct) number for each tissue and control sample using the formula $2^{-(\Delta C_{\text{sample}} - \Delta C_{\text{control}})}$. ΔC_t represents the difference in Ct values between the target and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. RT-PCR was performed in duplicate for each sample, and average Ct values were calculated according to the previous papers from Pfaffl^[3] and Livak *et al*^[4], which are references he cited in his letter; (3) It is not clear why he required an explanation of the interpretation of the gene expression data because everyone involved in this type of study is familiar with

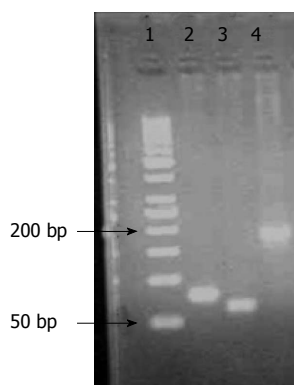


Figure 1 Polymerase chain reaction products of the *CD44*, vascular endothelial growth factor receptor-3, and lymphatic vessel endothelial hyaluronan receptor-1 genes. Lane 1: Ladder (50 bp); Lane 2: *CD44* (80 bp); Lane 3: Vascular endothelial growth factor receptor-3 (63 bp); Lane 4: Lymphatic vessel endothelial hyaluronan receptor-1 (184 bp).

the analysis. As previously explained by Livak *et al*^[4], the choice of the calibrator for the $2^{-\Delta\Delta C_t}$ method depends on the type of gene expression experiment that one has planned. The simplest design is to use the untreated control (in our study, the patient's own normal tissue) as the calibrator and GAPDH as an internal control. Using the $2^{-\Delta\Delta C_t}$ method, the data are presented as the fold change in gene expression normalized to an endogenous reference gene and relative to the normal control. For the control sample, $\Delta\Delta C_t$ equals zero, and 2^0 equals one; as a result, the fold change in gene expression relative to the untreated control equals one, by definition. For the treated samples (tumor tissue), an evaluation of $2^{-\Delta\Delta C_t}$ indicates the fold change in gene expression relative to the untreated control. The gene expression levels in tumor tissues represent the difference from normal controls in our study, and all values were shown in Fig. 2^[1]. There were some values over 1, representing increased expression, and there were also values less than 1, representing decreased expression; (4) The mean values for *LYVE1*, *CD44* and *VEGFR3* expression (represented as $2^{-\Delta\Delta C_t}$ and shown in Fig. 2) were 1.13, 1.24 and 1.17, respectively, suggest-

ing increased gene expression in tumor tissues compared to normal tissue. Therefore, we believe it is natural to conclude from these results that the expression levels were increased. Despite the increase in gene expression in the cancer tissues ($2^{-\Delta\Delta C_t} > 1$), only some of the results reached statistical significance, which was thoroughly discussed in our paper^[1]; (5) In our study, we did not only report the gene expression data, but also presented data obtained using immunohistochemistry, pathology, and other clinical features of the tumors. Although we are commenting on our results, we used all of these data to reach a logical conclusion. As shown in Fig. 3, 4 and 5, gene expression was increased ($2^{-\Delta\Delta C_t} > 1$) with increased T-stage, a PLN/TLN ratio > 0.4 and the presence of perineural invasion^[1]; and (6) In our manuscript, we used Figure 1 to show the PCR products of the genes. Dr. Verna is correct to note that there are some non-specific amplicons of the *VEGFR-3* gene in that Figure. However, we optimized both the conventional PCR and real-time PCR reactions for the *VEGFR-3* gene and also all others, as shown in the lower part of Figure 1. It is easy to note that no nonspecific band is present in this reaction for *VEGFR-3*.

Once again, I would like to thank Dr. Verna for his interest in our study and hope that this letter will resolve any misunderstandings.

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