Reviewer’s report

Title: Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR

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Reviewer: Olaf J.C. Hellwinkel

Reviewer’s report:

The paper from Rho et al. is dedicated to the analysis and discussion of several housekeeping genes for application as internal controls in quantitative RT-PCRs on stomach cancer. As the authors state, the choice of suitable reference transcripts is critical, as it depends on the tissue type analysed; in deed, several studies demonstrated, that transcription activity of various housekeeping genes varies considerably from cell type to cell type. For the normalisation of gene activities in stomach cancer measured by quantitative RT-PCRs, so far no methodical analysis has been published.

Here, the authors test six housekeeping genes selected earlier by a medline-analysis for their applicability as internal controls in stomach cancer and cell lines by partially computer automated comparisons of their Cp-values (determined by real-time RT-PCRs). As a consequence of their study, RPL29 and RPL29-B2M are assigned to be the best reference genes for RT-qPCR on stomach tissues, while B2M and B2M-GAPDH are recommended for stomach cancer cell lines.

The increasing number of molecular analyses on tumours and their potential applicability in clinical diagnostics emphasize the importance of proper internal standardisation of tumour-relevant gene activities. Taking this in mind, it is clear that the findings described by the authors just represent first line results which should be further tested on larger stomach cancer collectives. However, this compact but well designed study applies appropriate analytical tools and thus provides substantial and interesting hypotheses on reference genes putatively appropriate for quantification purposes on stomach tissues and cell lines.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

'I declare that I have no competing interests’