Is GAPDH a relevant housekeeping gene for normalisation in colorectal cancer experiments?

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Sir,

We have read with great interest the study by Kontos et al (2010) in British Journal of Cancer about the use of L-DOPA decarboxylase as a prognostic marker for colorectal adenocarcinoma. To assess the expression level of L-DOPA decarboxylase transcripts, the authors have performed qRT-PCR using GAPDH as the dedicated housekeeping gene (HKG) to normalise their results. However, no results concerning the stability of GAPDH that has lead to its use as the best housekeeper in their model system were available.

We have recently shown on human cell lines that choosing an unstable internal control gene could generate dramatic misinterpretations (Caradec et al., 2010a). In our study, and in our conditions, GAPDH was one of the most variable HKG so far impairing its use as a relevant normaliser. As this gene is likely to be emblematical to normalise gene expression results, we have developed a specific qRT-PCR with calibration curve to specifically study GAPDH expression in different cell lines grown with various hypoxic conditions. Our results unambiguously showed that GAPDH expression varies according to oxygen tension. We have also analysed GAPDH variability comparing meta-analysis data from microarray experiments on human samples available online (https://www.oncomine.com/resource/login.html). Using Oncomine 4.3, a powerful tool allowing rapid gene expression comparison between healthy and/or tumour human samples, we report here that GAPDH variability differs largely from one study to another and more importantly may largely vary between patients in a given study (Figure 1). As, aside still unidentified factors, hypoxia can be considered as a major one to have a critical role in cancer development, especially in colorectal cancer (Baba et al., 2010), we would be interested in learning how Kontos et al have tested HKGs variability in their system and found GAPDH to be the most relevant.

Another unclear point concerns qRT-PCR Ct intervals between GAPDH and L-DOPA target gene amplification. Indeed, the study results of Kontos et al. showed a ΔCt equal to 13 (Ct (GAPDH) 15, Ct (L-DOPA) 28), signifying that GAPDH transcripts are likely to be expressed 213 (8200) times higher than those encoding L-DOPA. As we stressed this particular point very recently, discussing about the use of r18S as a normaliser (Caradec et al., 2010b), we would be very interested to know Kontos et al. opinion about the accuracy of such disproportion.

Definitely, the choice of a valid HKG set will determine the relevance of the results that will be further interpreted, and so it should be seriously considered.

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