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Letter to the Editor

Choosing a stable housekeeping gene and protein is essential in generating valid gene and protein expression results

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Sir,

We have read and support the concerns of Caradec *et al* (2010) in the *British Journal of Cancer* regarding the stability of GAPDH as an internal control gene that was used in the recent study by Kontos *et al* (2010) to normalise L-DOPA decarboxylase mRNA as a prognostic marker in colorectal adenocarcinoma. We too have recently found variance in protein and gene expression of the commonly used housekeeping genes, *GAPDH* and ribosomal 18s, in the hearts of colon adenocarcinoma-bearing mice. Although differences in GAPDH mRNA were not statistically significant, results show some variation in GAPDH protein levels between murine adenocarcinoma (MAC) models of tumour-bearing cachectic (MAC16) and non-cachectic mice (MAC13; $P = 0.05$). We have also found significant variation in the gene expression of ribosomal 18s in the hearts of these two MAC cancer models ($P < 0.001$) and in each cancer model compared with non-tumour-bearing control mice ($P = 0.004$; $P < 0.001$). Further to this, variations in ribosomal 18s expression were observed at different stages of cancer development in the hearts of MAC13 tumour-bearing mice compared with control mice. Ribosomal 18s gene expression in the hearts of MAC13 tumour-bearing mice showed significant differences at days 12 ($P = 0.011$) and 21 ($P = 0.047$), when compared with day 29 after implantation.

Although still widely used as housekeeping genes, *GAPDH* and ribosomal 18s expression levels have been shown to vary under different experimental conditions (Mahoney *et al*, 2004; De Santis *et al*, 2010; Nelissen *et al*, 2010), and disease states (Mahoney *et al*, 2004; De Santis *et al*, 2010; Nelissen *et al*, 2010) showed GAPDH to be highly variable in cancerous compared with non-cancerous tissue. Nelissen *et al* (2010) recently described GAPDH and ribosomal 18s as the most unstable housekeeping genes under different experimental conditions in qPCR experiments with rat oligodendrocytes. The use of variable housekeeping genes as normalisers will lead to misinterpretation and irrelevant results.

Thoughtful consideration as well as evaluation of a valid housekeeping gene that is ubiquitously expressed and stable across sample groups is absolutely necessary to obtain reliable gene expression results and eliminate misinterpretations. Alternatively, fluorescein-labeled oligonucleotides such as OliGreen (Invitrogen, Mulgrave, Victoria, Australia), which measures cDNA content in each sample, may be a more reliable reference for normalising protein and gene expression results (Lundby *et al*, 2005; Rhinn *et al*, 2008).

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