**Reviewer's report**

**Title:** Over-expression of COX-2 mRNA in colorectal cancer

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**Reviewer:** Karen W. Makar

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The authors present a study in which they examined the expression of PTGS2 (the gene encoding the Cox-2 enzyme) in 60 tumor and normal tissue pairs via RT-PCR. Expression data was compared using three different normalization strategies- tissue weight and two individual housekeeping genes (B2M and GAPDH).

Their main finding is that PTGS2 mRNA is higher in tumor than normal colorectal mucosa, an observation that is not particularly novel. They also observed that the extent to which PTGS2 mRNA is upregulated in tumor over normal tissue depends on the standardization method used, also a known phenomenon.

Problems with using individual housekeeping genes, in particular GAPDH, are well known, as the author notes in the Discussion. While using tissue weight could be an attractive alternative normalization strategy in some situations, the data presented are inadequate to reach the conclusion that this method is somehow superior.

**Major Compulsory Revisions**

1. The authors argue that tissue weight is a superior choice over individual housekeeping genes as a normalization strategy. This appears to be based on the fact that this normalization method yielded the highest percentage of tumors with upregulated PTGS2 expression, rather than a careful comparison of the precision and accuracy of the different normalization strategies. Without such data, it is impossible to say whether the tissue weight is a superior normalization strategy and thus such a conclusion is not supported by the data offered.

2. The authors also argue that tissue weight is a simpler normalization strategy compared to B2M or GAPDH, due to the variable expression of these two genes. However, the authors do not provide any information on the variability of the weight measurements and how that may impact their findings.

3. No true replicate samples were analyzed, and no %CV was reported for any assay. Thus the accuracy and precision of the measurements generated are not known as the error associated with the weighing procedure, the reverse-transcriptase procedure, and inter-assay variability are not captured for any method.

4. The author only looked at two housekeeping genes, and did not look at the
combination of multiple reference genes, using algorithms such as geNorm (http://medgen.ugent.be/~jvdesomp/genorm/).

5. One alternative to using housekeeping genes that has been suggested in the literature is to measure cDNA input through the use of fluorescein-labeled oligonucleotides such as OliGreen (Invitrogen), and use that value as a normalizer (Rhin H, Scherman D, Escriou V (2008) One-step quantification of single-stranded DNA in the presence of RNA using Oligreen in a real-time polymerase chain reaction thermocycler. Anal Biochem 372: 116–118.) This would have been a useful and potentially more objective measurement.

Minor Essential Revisions

1. No discussion in the methods section of the type of balance used to weigh the tissues, or mention of the accuracy/precision of such weight measurements is included.

2. No discussion of the limitation of tissue weight as a normalization strategy are presented. For instance, this is not a useful strategy for formalin fixed tissue, and thus irrelevant for large epidemiological studies using clinical blocks.

3. The authors should use the accepted HUGO gene symbol- PTGS2 when they are discussing mRNA expression of COX-2.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have no competing interests