Reviewer's report

Title: Comprehensive Expressional Analyses of Antisense Transcripts in Colon Cancer Tissues Using Artificial Antisense Probes

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Reviewer: eric adriaenssens

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In their report "Comprehensive expressional analyses of antisense transcripts in colon cancer tissues using artificial antisense probes", Saito et al, described identification of antisense transcripts in collection of cancer related genes. Methods employed are accurate. Results and discussion are consistent with data presented. Furthermore, some concerns exist and can be resolved to improve the manuscript.

° Authors exploit their previously established microarray platform and referred often to their previous works (ref 7 and 10) especially for control experiments (WB, RT-PCR to validate data of microarray). Some of these controls can be included because this report appears as "more of the same".

° For fig 1, authors identify genes in which expression of sense-antisense transcripts changes by 10% (between normal and cancer) for at least 3 among 6 patients. This threshold seems weak. The variations of expression of numerous genes between 2 cells or 2 patients are often superior in 10 %. In this line, what is the physiological relevance of this report?

° As indicated, most of the sense and antisense genes showing altered expression balances in cancer tissues were both protein-coding genes. So, a simple deregulation of gene expression (frequent in cancer) can cause this result. It is more interesting to examine the variations of expression of non-coding RNA leading perturbation of protein-coding gene expression.

° Authors compare 6 tumor samples with adjacent normal tissues. This number is very too small to give sound conclusions. Tissues adjacent to tumor can be quite different from normal ones (inflammation, change in cell types). By addition, cancer samples are often exclusively composed of epithelial cells, but normal tissue is composed of different types of cells (epithelial, fibroblast and others). Authors must evaluate the composition of their biopsies. In addition, no clinical data (stage, grade, ...) or molecular features (DCC, ret, APC genes loss, aneuploidy, ...) are presented but commonly searched and available in anatomopathological labs.

° As indicated by the authors, antisense transcripts may be “transcriptional noises”. And this feature can be increased in cancer cells in which transcriptional control are often loss. The antisense detected in this report could have biological functions, probably by acting on sense mRNA and protein realized. But, nothing in this report allows a characterization of this type of antisense even if this point
is well discussed. The fact that antisences are often non polyA and that their border are not defined are in favour of transcriptional noises.

° Several data shown possible antisense artefacts in transcriptome microarray experiments due to non desired priming in reverse transcription (Perrochi et al., 2007). Authors have been examined this possibility?

° Legend and presentation of the figures could be greatly ameliorated. By example, fig 1 described sense-antisense transcripts showing altered expression balances but what is a) and b)?? it is not indicated in the legend (idem for fig 3). It is impossible to compare the fig 1 with table 1. There is 68 sense-antisense transcripts with altered expression in table 1, but less in fig 1, Why ?

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

**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