Reviewer’s report

Title: Knockdown of anterior gradient 2 expression extenuates tumor-associated phenotypes of SNU-478 ampulla of Vater cancer cells

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Reviewer: Bogi Andersen

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AGR2 has been observed to be overexpressed in various human adenocarcinomas, most notably ER-positive breast cancer, and correlates with increased cell survival, invasion, and metastasis. Kim et al. examine the expression and activity of ARG2 in cell lines representative of biliary tract cancers. mRNA and protein expression was assessed in these cell lines, and AGR2 knockdown by shRNA decreased cell numbers and anchorage independent growth in vitro and reduced tumor initiation capacity when xenografted subcutaneously into nude mice. Overexpression of AGR2 enhanced cell numbers and invasiveness in vitro. These results add insights into the potential functions of AGR2 in human biliary tract cancers.

Strengths: The variation in expression of AGR2 and the insights into cellular functions that depend on AGR2 in biliary cancer cell lines are novel and provide potential avenues to pursue to uncover the mechanisms of AGR2 in these cells. The manuscript is generally well written, but would benefit from editing for grammar and spelling (for example, pancreatic is misspelled on line 371).

Weaknesses: The functional data rely heavily on in vitro studies and xenograft models. In future studies, these results will need to be confirmed in rodent models targeting Agr2 deletion or overexpression in the bile duct.

Specific comments:

1. The authors claim in the introduction (lines 118-121) that there have not been any studies regarding AGR2 expression in biliary tract cancers, but Lepreux et al. (Liver Int 2011, 31:322-328) describe AGR2 expression during morphogenesis and carcinogenesis of the biliary tree with human tissue specimens. The results of this study should be included in the introductory material. This is important because without the knowledge that AGR2 is indeed expressed in human biliary tree cancer these in vitro studies have limited relevance. (minor)

2. In Figure 1, do the authors know how the levels of AGR2 compare to those found in well described AGR2-expressing cells such as MCF-7 after E2 treatment? Without any kind of reference it is difficult to know whether these levels are very low or reasonably robust. (discretionary)

3. The authors describe an effect of AGR2 on cell number in both MTT and colony forming assays. This could be due to an effect on cell proliferation or cell death. The authors should determine whether AGR2 controls cell proliferation in
these cells. In the mammary gland and in breast cancer cells, AGR2 controls cell proliferation as shown by Verma et al. (Dev Biol. 2012, 369:249-260). (major)

4. Figure 2F would be improved with a “legend” inside the figure itself to describe what each bar represents. (minor)

5. The authors should explain the rationale for only using the SNU-478:KD2 cells for xenografts. Did the authors also inject SNU-478:KD1 and KD3 cells, and if so, what were the results of these xenografts? (minor)

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I declare that I have no competing interests