Author’s response to reviews

Title: Expression of NDRG2 is down-regulated in high-risk adenomas and colorectal carcinoma

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Author’s response to reviews:

Dear Sir/Madam,

My co-authors and I respectfully submit our revised manuscript entitled "Expression of NDRG2 is down-regulated in high-risk adenomas and colorectal carcinoma" for consideration for publication in BMC Cancer. We have revised the manuscript according to the reviewers’ comments:

Reviewer Tamotsu Sugai

1. As suggested by the reviewer, we have clarified the ratio of tumor cells to nonneoplastic cells in the Methods section: "The vast majority of CRC samples had 75-80% tumor cells surrounded by stroma, as evaluated by hematoxylin and eosin staining by a pathologist." (page 4, 2nd line from bottom)

2. The reviewer raises an interesting point that genetic or epigenetic alterations may depend on the localization of the tumor. We have added a reference to this possibility in the Discussion section: "Recent studies have demonstrated that colorectal cancer is a heterogenous disease with distinct molecular components. Distinct genetic or epigenetic alterations have been identified which correlate with the location of the tumors [17]. Although it was not investigated in this study, it could be interesting to compare NDRG2 expression in tumors located in either the proximal or distal colon." (page 9, 3rd paragraph)

3. New data has now been included about the b-catenin status in the colorectal
carcinoma samples: "we have investigated a subset of the CRC samples (n=54) from the KAM study for b-catenin expression by immunohistochemistry. All of the tested CRC samples are positive for cytoplasmic b-catenin and 72% are b-catenin positive in all nuclei (data not shown). The remaining samples contain nuclear b-catenin in occasional nuclei. This suggests that c-Myc levels are likely to be elevated since c-Myc is known to be positively regulated by nuclear b-catenin [19]." (page 10, line 6)

4. Unfortunately, we do not have any data on NDRG2 mRNA expression in superficial vs. conventional polypoid tumors.

Reviewer Libo Yao

Table 4 shows that a reduction in NDRG2 mRNA levels is statistically significant in mild/moderate dysplasia (n=52) compared to normal tissue from the same individual. The lack of statistical significance in cases of severe dysplasia (n=20) does not mean that levels of NDRG2 mRNA are not reduced, just that we cannot conclude statistical significance. We base our suggestion that down-regulation of NDRG2 correlates with the progression of dysplastic tissue to carcinoma on the observation that "when compared to the control group of healthy individuals, only the affected tissue from individuals with colorectal carcinoma shows a statistically significant reduction in NDRG2 mRNA levels." This clarifying statement has now been added to the discussion. (page 10, middle)

We look forward to hearing from you about the suitability of our revised manuscript for publication in BMC Cancer.

Sincerely Yours

Cathy Mitchelmore, Ph.D.

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