

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 13161

Title: Decreased CDX2 expression is associated with poor prognosis in colorectal cancer patients

Reviewer code: 00227433

Science editor: Ya-Juan Ma

Date sent for review: 2014-08-11 15:36

Date reviewed: 2014-09-07 23:43

| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------|----------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input type="checkbox"/> High priority for publication |
| <input type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
| <input type="checkbox"/> Grade D: Fair | <input type="checkbox"/> Grade D: Rejected | BPG Search: | <input type="checkbox"/> Minor revision |
| <input type="checkbox"/> Grade E: Poor | | <input type="checkbox"/> Existing | <input type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

This is a good paper, representing extensive analysis of CDX2 expression, how it interacts with other molecular characteristics, and colorectal cancer prognosis in a large Korean study of 675 patients. The authors should be commended on a comprehensive study design, including a sample drawn from all 2006 cases from the respective hospital. Decreased CDX2 expression was shown to be associated with a poorer prognosis after colorectal cancer diagnosis, independently of CIMP/MSI status. I have some comments/suggestions for the authors: 1. Discussion: The structure of the discussion could be strengthened – is the main message that CDX2 is associated with survival, or that it is related to CIMP/MSI status? There are some results that are introduced for the first time (eg. stage-stratified analysis results in the penultimate paragraph), while very little emphasis has been placed on other key results, such as the shorter survival associated with CDX2 expression. Only stage is discussed as a potential explanation for the association between decreased CDX2 expression and poorer survival, however stage has been included as a confounder in analysis therefore other potential explanations/mechanisms should be discussed. Please ensure all results discussed have been described in the results section. A strengths and limitations section should also be included as a penultimate paragraph in the discussion, as should recommendations for future research. 2. Introduction/discussion: Since the authors submitted their paper to WJG, an excellent systematic review of CDX2 expression and colorectal cancer has been published – I would encourage the authors

to incorporate this into their paper, particularly to compare and contrast the current study findings in the context of the review findings into the discussion: Surg Oncol. 2014 Sep;23(3):167-176. doi: 10.1016/j.suronc.2014.07.003. Epub 2014 Jul 17. The clinical perspectives of CDX2 expression in colorectal cancer: A qualitative systematic review. Olsen J1, Espersen ML2, Jess P3, Kirkeby LT4, Troelsen JT5

3. Methods: are the authors able to comment on the representativeness of their study sample (n=675) compared with the remaining cases of the potentially eligible n=989 patients in terms of age, sex and overall survival?

4. Methods: further information is needed for the section 'Clinicopathologic analysis'. How was data extracted from electronic medical records (was this done using a standard electronic proforma, how many staff were involved, was there any QC or duplicate reviewing conducted)? Did a dedicated study pathologist evaluate CIMP, MSI, KRAS and BRAF status, or is analysis of these factors based on results from routine analysis of these factors?

5. Methods: can the authors reference their use of ROC curves for determining cut-offs for marker expression, rather than utilising a biologically relevant cut-off? The use of ROC curves limits the external validity of the study.

6. Methods/Results: please include the number of deaths that occurred, the censor date used for patients still alive, and the median survival according to CDX2 expression groups.

7. Results/Tables: Please clarify throughout if 'age' refers to age at diagnosis, or age at surgery?

8. Methods/Results/Table 1: Adjuvant chemo should be included in Table 1. Information is presented on luminal necrosis, tumor budding, tumor infiltrating lymphocytes, Crohn's like lymphoid reaction, luminal serration and mucin production. Please report if this information was collected as part of the electronic medical record review, and reconsider the relevance of presenting these data if they were not tested as potential confounders in survival analysis. If they were tested, this should be stated.

9. Results/Table 2: Please write MSI or CIMP-high/-low rather than MSI or CIMP-H/-L , for ease to the reader. Also include the cut-offs used for increased/decreased/retained for markers as a table footnote.

10. Table 3: The

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Reviewer code: 00027205

Science editor: Ya-Juan Ma

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Date reviewed: 2014-08-26 16:56

| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input checked="" type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input type="checkbox"/> High priority for publication |
| <input checked="" type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
| <input type="checkbox"/> Grade D: Fair | <input type="checkbox"/> Grade D: Rejected | BPG Search: | <input type="checkbox"/> Minor revision |
| <input type="checkbox"/> Grade E: Poor | | <input type="checkbox"/> Existing | <input checked="" type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

In the present study, authors have investigated the expression of CDX2 by immunohistochemistry (IHC) in 675 cases of colorectal cancer. As a result, negative expression (loss) of CDX2 was observed in 109 cases (16%), which was associated with location of the proximal colon, ulcerative gross type, advanced stage (T, N, overall), poor differentiation, increased number of tumor-infiltrating lymphocytes, and luminal serration. Loss of CDX2 was also associated with expression of CK7, negative expression of CK20, CpG island methylator phenotype (CIMP), microsatellite instability (MSI) and BRAF mutation. In addition, loss of CDX2 was an independent adverse prognostic factor for both overall and disease-free survivals, as determined by multivariate analyses using Cox-proportional hazard model. My criticisms for this paper are as follows. Major comments Generally, the present study is moderately interesting. However, the majority of the observations in this paper are very similar to those in previous publications (References 17, 18, 26). Authors should clarify what the novel findings are in the present study. Minor comments 1. "Decreased CDX2 expression": I think that the term "decreased" should be changed to "negative" or "loss of", because this study had investigated CDX2 protein expression by IHC, but not CDX2 RNA expression (Ref. 16), 2. Table 3 shows that adjuvant chemotherapy was an independent prognostic factor for both overall and disease-free survivals. However, authors do not state this finding in the text. Authors should provide the number of patients who underwent adjuvant chemotherapy. 3. Figure legends: all



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legends for figures are too short and insufficient. 4. Both Supplementary table 2 and Supplementary figure 1 demonstrate the percentages of positive expression for CK7, CK20 and CDX2 proteins stratified by CIMP and MSI status. However, each set of corresponding values seems not identical. Authors should clarify whether these values were express either “mean” or “median” values.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 13161

Title: Decreased CDX2 expression is associated with poor prognosis in colorectal cancer patients

Reviewer code: 00503405

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| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input checked="" type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input type="checkbox"/> High priority for publication |
| <input checked="" type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
| <input type="checkbox"/> Grade D: Fair | <input type="checkbox"/> Grade D: Rejected | BPG Search: | <input type="checkbox"/> Minor revision |
| <input type="checkbox"/> Grade E: Poor | | <input type="checkbox"/> Existing | <input checked="" type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

In the original article of Bae JM et al. (Decreased CDX2 expression is associated with poor prognosis in CRC patients) the authors aimed to reveal correlations among CDX2 protein expression and demographical/clinico-pathologic/molecular characteristics of CRC cases. The examined number of cases is quite large. The authors found association among the downregulated CDX2 protein expression and tumor location, tumor gross type, tumor progression, grade of differentiation, number of TILs, and expressions of other cytokeratins (7 and 20). Moreover they found association between poor CDX2 immunoreactivity and CIMP/MSI/BRAF mutation status. According to their results the loss of CDX2 was associated with poor OA and DF survivals. The study is well designed but moderately presented, there are some points that needs major revision. Some referred publications [Baba Y et al (2009); Lugli A et al. (2008); Zlobec I et al (2011)] have already described most of the current results. What are the major novel findings of the current study? Authors must clarify this. In case of an IHC study, at least two different antibody against different epitopes must be used to cross-validate the initial results. Or, IHC results must be validated by WB analysis. Are there any data regarding the expression level of other CDX2 antibody? In Fig. 1/C: CDX2 is a nuclear antigen. Why can cytoplasmic immunoreaction be seen on the photo? (What was used for positive control for CDX2 IHC?) In table 3. authors present the effect of adjuvant chemotherapy on DF and OA survivals. There's no discussion on it in the text. Means or medians are



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presented in supplementary table 2 and supplementary fig. 1.? It should be clarified. English language needs minor polishing. After major revision I suggest to reconsider a possible acceptance of the manuscript for publication in WJG.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 13161

Title: Decreased CDX2 expression is associated with poor prognosis in colorectal cancer patients

Reviewer code: 00058361

Science editor: Ya-Juan Ma

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| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------------|----------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input type="checkbox"/> High priority for publication |
| <input type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
| <input type="checkbox"/> Grade D: Fair | | BPG Search: | <input type="checkbox"/> Minor revision |
| <input checked="" type="checkbox"/> Grade E: Poor | <input checked="" type="checkbox"/> Grade D: Rejected | <input type="checkbox"/> Existing | <input type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

I have been reading similar work of the group. The similarities with three papers of the group are too large to consider this work for possible acceptance to WJG. I give one example: British Journal of Cancer (2013) 109, 1004–1012. doi:10.1038/bjc.2013.430 The abstract and intro is just a copy of the above mentioned work.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 13161

Title: Decreased CDX2 expression is associated with poor prognosis in colorectal cancer patients

Reviewer code: 00503549

Science editor: Ya-Juan Ma

Date sent for review: 2014-08-11 15:36

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| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------|-------------------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input checked="" type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input checked="" type="checkbox"/> High priority for publication |
| <input checked="" type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
| <input type="checkbox"/> Grade D: Fair | <input type="checkbox"/> Grade D: Rejected | BPG Search: | <input type="checkbox"/> Minor revision |
| <input type="checkbox"/> Grade E: Poor | | <input type="checkbox"/> Existing | <input type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

Bae J. M. et al. studied the clinicopathological and prognostic significance of decreased CDX2 expression in colorectal cancer. This work confirmed clinicopathological significance of decreased CDX2 expression and analyzed the association of CDX2 expression with molecular subtype of colorectal cancer. The number of cases is sufficient to conclude the results and methods for analysis are solid. Several minor comments are addressed.

1. When a term that is used in abbreviations, the term should be spelled out at first. This rule should be kept throughout the manuscript.
2. The part "to identify whether CIMP-H was .. but not significantly associated with MSI-H" in the first section of Discussion should be shown in Results.
3. In Table 3, a large number of parameters (n = 14) are listed for multivariate analysis for independent prognostic implication in overall survival and disease-free survival. Were all these parameters statistically significant as prognostic factor by univariate analysis? The authors should perform univariate analyses for all these 14 parameters, and using significant parameters only, they should perform multivariate analysis. They should confirm the procedure, and mention about that in text. It should be clearly mentioned that 114 stage IV cases were excluded from the analysis of disease-free survival.
4. In Figure 2, Supplementary Figures 3 and 4, the authors should describe the curves were different or not different significantly statistically with p values in the legends and text.
5. It is of interest whether CDX2 status is the same or different between primary sites and lymph node metastasis sites.
6. Typographic errors should



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be amended, e.g., molecu^lr, in the section of Tissue samples.

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 13161

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Reviewer code: 00070916

Science editor: Ya-Juan Ma

Date sent for review: 2014-08-11 15:36

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| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---------------------------------------------|----------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Grade A: Excellent | <input type="checkbox"/> Grade A: Priority publishing | Google Search: | <input type="checkbox"/> Accept |
| <input type="checkbox"/> Grade B: Very good | <input type="checkbox"/> Grade B: Minor language polishing | <input type="checkbox"/> Existing | <input type="checkbox"/> High priority for publication |
| <input type="checkbox"/> Grade C: Good | <input type="checkbox"/> Grade C: A great deal of language polishing | <input type="checkbox"/> No records | <input type="checkbox"/> Rejection |
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| <input type="checkbox"/> Grade E: Poor | | <input type="checkbox"/> Existing | <input type="checkbox"/> Major revision |
| | | <input type="checkbox"/> No records | |

COMMENTS TO AUTHORS

In their manuscript "Decreased CDX2 expression is associated with poor prognosis in colorectal cancer patients", Bae et al. analyze a large cohort of Korean CRC patient' tumors concerning expression of CDX2, CK7 and CK20 using immunohistochemistry. In addition, they correlate the protein expressions with clinico-pathological parameters with a special focus on the molecular subtype. Despite the fact that this is clearly no novel finding, the study is overall well designed and performed, the conclusions are relatively clear and the authors do not overstate their findings. However, a few points have to be addressed to improve the manuscript to justify publication: The Core tip should include a little bit more information. Introduction: "CIMP-high (CIMP-H)/MSI-high (MSI-H), CIMP-H/MSS, MSI-low (MSI-L), CIMP-0, L/MSI-H and CIMP-0, L/MSS, MSI-L" is confusing - as it was already in the original publication. As a simple solution, a table may be really helpful here. Alternatively, it may help to first introduce the abbreviations and then name the four molecular classes: CIMP-H/MSI-H; CIMP-H/MSS, MSI-L; CIMP-0, L/MSI-H and CIMP-0, L/MSS, MSI-L. Manuscript should be re-checked for spelling and grammar errors. Were the mono/dinucleotide markers judged "MSS/MSI" in direct comparison to matching normal tissue-derived gDNA? Please comment and include into the manuscript. More specifically, which difference in the mono-nucleotide length was judged MSI? Finally, the molecular classification by Ogino and Goel is not the first and only one. Please comment on that.