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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastrointestinal Oncology
ESPS manuscript NO: 24824
Title: MicroRNA-320 family is downregulated in colorectal adenoma and affects tumor proliferation by targeting CDK6
Reviewer's code: 00068256
Reviewer's country: China
Science editor: Ze-Mao Gong
Date sent for review: 2016-02-17 15:59
Date reviewed: 2016-02-28 10:23

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The manuscript by Tadano T et al investigated the expression of miRNA in colorectal adenoma and submucosal invasive carcinoma and revealed miR-320 family affects colorectal tumor proliferation by targeting CDK6. This article is overall interesting and gives new insight in the field of dysregulation of miRNAs and colorectal cancer. Major comments: 1. MicroRNA-320a is a well known tumour suppressive miRNA in colorectal cancer, many papers have reported its roles, especially anti-metastasis, in colorectal cancer, including several publications as followed (in addition to the papers that had been cited in the manuscript). 1) miR-320a suppresses colorectal cancer progression by targeting Rac1. Carcinogenesis. 2014 Apr;35(4):886-95. doi: 10.1093/carcin/bgt378. Epub 2013 Nov 21. 2) Differential expression of microRNA-320a, -145, and -192 along the continuum of normal mucosa to high-grade dysplastic adenomas of the colorectum. Am J Surg. 2014 May;207(5):717-22; discussion 722. 3) miR-320 enhances the sensitivity of human colon cancer cells to chemoradiotherapy in vitro by targeting FOXM1. Biochem Biophys Res Commun. 2015 Feb 6;457(2):125-32. 4) Identification of a metastasis-specific MicroRNA signature in human colorectal

cancer. J Natl Cancer Inst. 2015 Feb 6;107(3). 2. A luciferase reporter assay should be performed using wild-type and mutant-type of CDK6 3′UTR to confirm the direct regulation of miR-320s on CDK6 in a sequence-specific manner. 3. A western blotting analysis also need to be performed to detect the CDK6 protein expression in miR-320s-ovexpressed and/or miR-320s-silenced CRC cells.

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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastrointestinal Oncology
ESPS manuscript NO: 24824
Title: MicroRNA-320 family is downregulated in colorectal adenoma and affects tumor proliferation by targeting CDK6
Reviewer's code: 03003235
Reviewer's country: China
Science editor: Ze-Mao Gong
Date sent for review: 2016-02-17 15:59
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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The study by Tadano T et al. follows up the observation that miR-320 family affects colorectal tumor proliferation and plays an important role in the growth of colorectal tumors. Here it is reported that CDK6 were identified as the target gene of the miR-320 family. Overexpressed miR-320 repressed CDK6 expression and finally led to SW480 proliferation inhibition. Finally, the authors found that increased CDK6 expression from non-neoplstic mucosa with inverse correlation to the miR-320 expression. Although these are interesting observations, a lot of efforts need to be done on this manuscript to pretend to a publication in WJG. Detailed comments: 1.The authors concluded that that miR-320 repress SW480 proliferation. As a target gene of miR-320, the expression of CDK6 mRNA and protein are both downregulated by miR-320. Could these data indicate that miR-320 inhibits SW480 proliferation by targeting CDK6? To make this point, the authors should observe whether deficiency of CDK6 similarly inhibit SW480 proliferation and perform a rescue experiment in which ectopic expression of CDK6 in miR-320 overexpressed cells restore the cells proliferation. Besides SW480, how about other colorectal cancer cell lines? 2. In Figure 4, according to the results

of miRNA binding-site prediction analyses, the authors indicated that CDK6 has a putative miR-320 family's binding site that is mapped to the 3'UTR. The authors should further performed dual-Luciferase reporter assay to test the specific regulation through the predicted binding sites. 3. The tissue sample size is not enough in this study.

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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastrointestinal Oncology
ESPS manuscript NO: 24824
Title: MicroRNA-320 family is downregulated in colorectal adenoma and affects tumor proliferation by targeting CDK6
Reviewer's code: 01588319
Reviewer's country: Taiwan
Science editor: Ze-Mao Gong
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Date reviewed: 2016-02-29 14:00

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
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		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

1. In the "Results" of the "Abstract" section, this study identified miR-320 family, including miR-320a, miR-320b, miR-320c, miR-320d, and miR-320e, were differentially expressed in adenoma and submucosal invasive carcinoma. However, the miR-320a did not shown in "Core tip", please explain it. 2. In Table 1, the expression level was present as the "mean" without standard deviation/error, does it make sense? Especially, this experiment was only performed in 3 paired samples. 3. Several items should be justified and consistent in the beginning of this study. Such as LSTs and protruded tumors ; adjacent non-neoplastic mucosa,adenoma and submucosal invasive carcinoma. Please clarify which groups are the specific compared targets of this study. 4. In the " Gene expression analysis and miRNA target prediction " of the "Materials and Methods" section, why the authors only performed the transfection experiment with a mimic control or miR-320a mimics? How about other so-called significant miR-320 family? 5. In the " Statistical analysis", the authors claimed that "The difference between two groups was analyzed by the Student's t-test.", is it appropriate for the comparison of the expression levels derived from paired samples, especially for 3 paired tissues only.

6. On comparing adenomas and carcinomas, expression levels of the miR-320 family, except for 320d, in carcinomas were lower than that in adenomas; however, differences were not statistically significant. Based on this finding, the interpretation of " These results indicated that the expression of the miR-320 family progressively decreased from the early stages of the adenoma–carcinoma sequence." is not appropriate. " 7. Regarding the " miR-320a targets CDK6", the authors stated that.....CDK6 expression was shown to be associated with prognosis in patients with CRC;, please identify where are the results from Tables? or Figures? 8. In the "Discussion" section, the contents should be more concise , for example: "The most commonly used approach to find the target genes of miRNA is through..... ; however, recent reports have provided evidence that miRNAs may downregulate a greater number of transcripts than previously appreciated [22]." and " Finally, we narrowed down the candidate target genes of the miR-320 family using two bioinformatics algorithms and our results of the mRNA array; we selected seven genes as common to all of these analyses."