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ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 10232
Title: Modulating effects of ACSL5-derived mitochondrial Wnt2B palmitoylation on intestinal Wnt activity
Reviewer code: 00289703
Science editor: Su-Xin Gou
Date sent for review: 2014-03-21 13:34
Date reviewed: 2014-03-29 00:45

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	
[ ] Grade D (Fair)		BPG Search:	[ ]Rejection
[ ] Grade E (Poor)	[ ] Grade D: rejected	[ ] Existed	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

The manuscript by Christina Klaus et al. titled "modification effects of ACSL5-derived mitochondrial Wnt2B palmitoylation on intestinal Wnt activity" describes that ACSL5 selectively adds a palmitoyl group to Wnt2B by S-palmitoylation. The authors found that the anti-proliferation function of ACSL5 is via blocking Wnt2B signaling. ACSL5 modifies Wnt2B by S-palmitoylation that retains Wnt2B in the cytoplasmic compartment thus abolishes Wnt2B nuclear translocation and gene transcription activation. The authors also observed a rudimentory interaction of ACSL5 and Wnt2B in intestinal cancer cells. The O-palmitoylation of Wnt has been reported, it is interesting and important to know a S-palmitoylation within Wnt and its function. However, a few issues need to be improved before publication. Since Wnt is S-palmitoylated, the S-palmitoylation site(s) should be screened within cysteine residue(s) of Wnt2B. Site directed mutagenesis may be used to replace the cysteine residues to observe the S-palmitoylation level. Minor comments: A. The abbreviations in the title should be avoided. B. The abstract is too wordy that needs to be clarified. C. A lot of typos should be corrected. For example, in Page 9 Paragraph 2, the sentence of "The resulting pellet after centrifugation for 3000g for 2 min. was treated with 10ml 320μM Biotin-BMCC and rotated for 2h at 4°C." There should be a space between the number and the unit; in addition, the dot after "min" is redundant.

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ESPS Peer-review Report
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[ ] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS
This is a very interesting paper aimed to characterize molecular mechanisms of antiproliferative ACSL5 activity, assumed to be a modifier of enterocytic maturation, on Wnt signaling. In cell culture systems, the authors found a strong relation of ACSL5 expression, Wnt2B palmitoylation, and degree of malignancy. The investigated molecular pathway was found of relevance for the homeostasis of the intestinal barrier in normal mucosa and the mechanism was only rudimentarily observed in intestinal neoplasias, possibly overwhelmed by other pathways. The results are based on a large set of experiments in cell lines, Apcmin/+ mouse model and normal and neoplastic human intestinal tissues. The paper is well written and complete from a technical point of view. Minor changes are suggested: - Introduction can be shortened, strictly attaining to the molecular mechanism that were investigated - Introduction, first paragraph: attaining to general differentiation of enterocytes the cited references, although important and in theme, could be enriched with some more recent such as Gerbel F, et al. JCB 2011, 192:767-780 and Solanas G, et al. Nat Cell Biol 2011;13:1100-1107. - Materials and methods: western blot: add antibody's dilution. - IHC: describe image capture and software acquisition and analysis.

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[ ] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

In this manuscript the Authors purchase evidence sustaining the modulatory effect of ACLS5 on Wnt signaling through palmoylation of Wnt2B. In particular, they show that the ACLS5-derived palmoylation of Wnt2B in the mitochondria, prevents its traslocation in the cytoplasm and nucleus and decreases Wnt signaling. They demonstrated that in cell lines of colon cancer potentially exhibiting increasing malignancy, palmitoylation of Wnt2B in mitochondria was downregulated, with increasing ACSL5 mRNA expression. In the ex-vivo analyses , the Authors recovered that in normal human intestinal mucosa, both ACSL5 and Wnt2B were expressed in an increasing gradient along the crypt-villus axis (CVA), whereas Wnt activity was inverse, as indicated by nuclear beta-catenin expression. The molecular association of ACSL5 and Wnt2B has been also partially confirmed in human adenomas and adenocarcinomas, but, differently to the in vitro results, they found that in human intestinal adenocarcinoma, expression of ACSL5 and Wnt2B, as well as palmitoylation of Wnt2B, were significantly increased compared to tumor-surrounding normal tissues. They conclude that the the ACLS5-driven modulation of Wnt signaling could be of relevance for the homeostasis of the intestinal barrier, but could be overwhelmed by other pathways in intestinal carcinogenesis. Nevertheless, due to the role ascribed to Wnt signaling activation in cancer insurgence, the inhibitory ACSL5 activity on Wnt signaling via palmitoylation of Wnt2B could have some significance in early carcinogenesis This is an interesting study in which the experiments are smart and well-performed, particularly in the in vitro studies, and the results obtained sustain the author conclusions. However, there are some points that must revised before

acceptance. The major and minor points are listed below. Major points: - Pg. 12, M&M, paragraph "Patients": "The staining intensity of normal mucosa was used as standard"; the use of a staining control performed by omitting the primary antibody should be more appropriate. - Figure 3: Wnt2B expression in Fig.3B seems quite negative: Authors should show a more contrasted image or should show the staining control (see my previous comment) for highlighting the positive results from Wnt2B specific staining - End of Pg. 16, Results: "In human sporadic adenocarcinoma of the colon, a significant upregulation of ACSL5 and Wnt2B in carcinoma was shown (figure 5C-F). Wnt2B was detectable both nuclearly and cytoplasmatically"; The images in panel 5D and (much more) in panel 5F are too bright and this could distort the results. The brightness of all panels must be the same for pointing out the difference in staining intensity (and, consequently, in expression of each marker). Moreover, details at higher magnification could help the reader to visualize the difference in nuclear or cytoplasmic localization Minor points: - Pg 6, lane 15-16: "Wnt13B exists in two forms, L-Wnt13B with an N-terminal mitochondrial target sequence and mitochondrially localized, S-Wnt13B nuclear": insert "and" before "S-Wnt13B nuclear" - Pg. 11, M&M, lane 3: "Citratbuffer" should be "Citrate buffer" - Pg. 14, Results, lane 6: "Wnt signaling was induced by adding recombinant Wnt3A and detected luminometric"; "by luminometry" instead of "luminometric " should be better. - Pg. 15, Results: "Figure 2D shows palmitoylation in relation to Wnt2B protein expression, depending on ACSL5": add "(Figure 2E)"... Figure 2E is not mentioned in the results. - Pg. 16, Results: "These results indicate decoupling of ACSL5-Wnt interaction in adenomas"; Speculative sentence based on descriptive data... "suggest" should be better than "indicate". - Pg. 16, Results, Lane 9: "In adenomas of the Apcmin/+ mouse model at 21 weeks of age, translocation of beta-catenin from cytoplasm into the nucleus was found (figure 5A,B)". Fig. 5A shows beta-catenin staining in human FAP and not in APC