

PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 76311

Title: Oxidized low-density lipoprotein stimulates CD206 positive macrophages upregulating CD44 and CD133 expression in colorectal cancer with high-fat diet

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03560845

Position: Peer Reviewer

Academic degree: DSc, MD, PhD

Professional title: Chairman, Professor, Senior Research Fellow

Reviewer's Country/Territory: Russia

Author's Country/Territory: China

Manuscript submission date: 2022-03-14

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-03-14 11:47

Reviewer performed review: 2022-03-21 08:03

Review time: 6 Days and 20 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous
	Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No

SPECIFIC COMMENTS TO AUTHORS

This is a very interesting study, performed at a high methodological level, and therefore it will be of interest to researchers in the field of oncology of the tumor microenvironment. A number of questions arose during the defacement of the manuscript.

1. The method for enumeration of ox-LDL expression in colorectal cancer tissues is unclear. Due to the pronounced background staining in Figure 1, it is necessary to clarify the tissue localization of ox-LDL, either within cells or in the intercellular substance.
2. The authors used the CD206 marker to detect M2 macrophages. In this regard, there are several comments. The authors show an increase in the number of CD206. Is this a consequence of an increase in the total number of macrophages in the tumor, or is it the result of a change in the macrophage phenotype. In this regard, usually along with the markers of the functional state of macrophages, some general macrophage marker is used, for example, F4/80.
3. M1/M2 nomenclature is convenient for describing the obtained data. However, the M1/M2 paradigm has now been revised (<https://pubmed.ncbi.nlm.nih.gov/25035950/>). It is believed that clearly distinguishable M1 and M2 macrophages are absent, and between them there is a continuous series of transitional forms. In this regard, the markers of the corresponding states of macrophages have also been revised. Surface markers, including CD 206, have been almost completely removed from the list of nomenclature. In this regard, I think it is necessary to abandon the term M2-macrophages in the article, since only the marker CD 206 is used, and macrophages are still called CD 206-positive macrophages.

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Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04627955

Position: Peer Reviewer

Academic degree: MD, PharmD, PhD

Professional title: Academic Research, Lecturer

Reviewer's Country/Territory: Spain

Author's Country/Territory: China

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Reviewer chosen by: AI Technique

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Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

Peer-reviewer statements	Peer-Review: [<input checked="" type="radio"/>] Anonymous [<input type="radio"/>] Onymous Conflicts-of-Interest: [<input type="radio"/>] Yes [<input checked="" type="radio"/>] No
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SPECIFIC COMMENTS TO AUTHORS

The article presented by Shimin Zheng and collaborates, entitled “ox-LDL stimulates M2 polarization of macrophages to upregulate CD44 expression in colorectal cancer associated with a high-fat diet”, is an original article that aimed to investigate the role of ox-LDL in colorectal cancer associated with a high-fat diet. Specifically, they analyze the expression of CD206, iNOS and CD44 in the macrophage cell lines THP1 and RAW treated with ox-LDL or in mice subjected to a high-fat diet. On the human side, the article does not provide much novelty as the literature has described increases in CD206 and ox-LDL expression and the staining does not seem to be very specific. It would improve the work somewhat to demonstrate the co-localisation of both molecules in human tissue, and at least a correlation. Major revision: 1. Ox-LDL staining is not specific, no interstitial cell staining is seen in the picture used in the figure 1, only a non-specific brown shading that is usually given by secondary antibody staining. The authors should show a specific staining of interstitial cells where brown staining is seen surrounding a nucleus, differentiating the staining of the interstitium from the cell cytoplasm. Photos should be taken at a minimum of 40x. Authors should choose the same type of cut in the tissues shown in figure 1, in the control the crypts are cut transversely and the CA longitudinally. 2. A co-localization of CD206/oxLDL or some kind of correlation in CA and HFD mice is mandatory. Minor revision 1. The objective in the abstract is not specific. The objective does not contain the word macrophage which is an important part of the whole work. 2. The authors should specify the origin of the healthy tissue, i.e. the pathology of the patients undergoing colonoscopy. 3. Grammatical errors: 3µm-thick, 10ug/ml, 1h, 10µg, 1%BSA, 50µg/ mL, 800rpm, 3min, 50µg/ mL, cell

line(RAW 264.7) ... go separately; interstitium, in vitro are in italics; GAPDH is
GAPDH 4. There is no table with primary antibodies used, dilutions used for HI, IF and
WB. 5. Molecular weight in WB of CD44 and GAPDH

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Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03485912

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: China

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Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
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Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No
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SPECIFIC COMMENTS TO AUTHORS

This is an interesting study. Elevated ox-LDL expression and increased number of M2 macrophages were detected in colorectal tissue from CRC patients and mice with HFD, and ox-LDL can lead to increased expression of CD206 and CD44 in monocytes, to demonstrate the important role of ox-LDL in CRC. Although the purpose of the study is clear, there are still some flaws in the experimental design, which are shown below: 1. The clinical part lacks the analysis of the correlation between ox-LDL and patient progression and survival; in vitro experiments, it is necessary to knock out the ox-LDL gene in macrophages to observe whether it can inhibit the growth and metastasis of colorectal cancer. These data serve to demonstrate that ox-LDL is a predictor and prognostic biomarker in CRC. 2. Results 2 and 4 only show the expression of CD206 (marker of M2-type polarized macrophages), which should be compared with M1-type macrophage polarization markers at the same time. 3. To understand the effects of ox-LDL on M2 polarization, specific cellular and molecular pathways associated with polarized cells need to be linked to their specific functions, rather than purely quantitative. M2 macrophages are closely related to Th2 cytokines, such as IL-10, IL-4 and IL-13 or transforming growth factor- β , so the expression of related cytokines or chemokines should be detected. 4. The conclusion of the study mentioned that ox-LDL induces M2 polarization to promote the increase of CD44 levels in colorectal cancer cells. Whether the verification process is too simple, it is necessary to further explore the signaling pathway regulated by M2 macrophages. Is there a causal relationship between the two? In addition to CD44, does it also affect other analysis expressions?

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Professional title: Chairman, Professor, Senior Research Fellow

Reviewer's Country/Territory: Russia

Author's Country/Territory: China

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Reviewer chosen by: Jing-Jie Wang

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Scientific quality	<input checked="" type="radio"/> Grade A: Excellent <input type="radio"/> Grade B: Very good <input type="radio"/> Grade C: Good <input type="radio"/> Grade D: Fair <input type="radio"/> Grade E: Do not publish
Language quality	<input checked="" type="radio"/> Grade A: Priority publishing <input type="radio"/> Grade B: Minor language polishing <input type="radio"/> Grade C: A great deal of language polishing <input type="radio"/> Grade D: Rejection
Conclusion	<input type="radio"/> Accept (High priority) <input checked="" type="radio"/> Accept (General priority) <input type="radio"/> Minor revision <input type="radio"/> Major revision <input type="radio"/> Rejection
Peer-reviewer	Peer-Review: <input checked="" type="radio"/> Anonymous <input type="radio"/> Onymous



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statements

Conflicts-of-Interest: [] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

I consider that all the answers received are satisfactory; therefore, the revised manuscript can be accepted for publication in general priority.