Peer Review Report

Review Report on The modulation of Hexosamine Biosynthetic Pathway impacts the localization of CD36 in macrophages

EVALUATION

Q 1　Please summarize the main findings of the study.

Overall, while the idea regarding CD36 and its role in recognizing oxidized lipids in macrophages offers valuable insights, it's essential to critically evaluate its limitations and complexities to advance our understanding of CD36 biology and its implications in disease. While targeting CD36 holds promise for therapeutic intervention in diseases like atherosclerosis, there are challenges associated with modulating its activity. CD36 is involved in a variety of physiological processes beyond lipid recognition, and interfering with its function may have unintended consequences.

The study suggests that the availability of UDP-GlcNAc may play a role in mediating endocytic traffic, as evidenced by the redistribution of Rab-5 between the cytoplasm and cell surface. This effect was observed following treatment with Thiamet G as an inductor and inhibitors such as OSMI-1 and Azaserine. These findings highlight the potential involvement of UDP-GlcNAc in modulating endocytic processes mediated by CD36, shedding light on potential mechanisms underlying its regulatory role in macrophage function.

Q 2　Please highlight the limitations and strengths.

Strengths:

The study provides novel insights into the role of glycosylations in modulating the structure and function of the CD36 receptor, particularly in the context of vesicular traffic regulation and metabolic conditions. By elucidating the impact of glycosylations on macrophage functions related to CD36, the study contributes to a deeper understanding of cellular physiology and pathophysiology.

The use of various experimental approaches, including the manipulation of UDP-GlcNAc availability and the use of inhibitors such as Thiamet G, OSMI-1, and Azaserine, adds depth to the investigation. These tools allow for the exploration of specific pathways and mechanisms underlying the observed effects on CD36-mediated endocytic traffic.
Given the central role of CD36 in processes such as lipid uptake and inflammation, the findings of the study have potential implications for understanding and potentially targeting CD36-related diseases, such as atherosclerosis and metabolic disorders.

Limitations:

The study likely utilized in vitro cell culture models to investigate the role of glycosylations in CD36 function. While these models offer valuable insights, they may not fully recapitulate the complexity of cellular interactions and signaling pathways present in vivo.

Glycosylation is a complex and dynamic process influenced by various factors, including cellular metabolism, environmental cues, and substrate availability. The study may not fully capture the intricacies of glycosylation patterns and their functional consequences on CD36-mediated processes. Further investigation into the specific glycosylation sites and modifications involved in CD36 regulation could enhance our understanding of its biological effects.

The Western blots have very strong signal, making it challenging to accurately measure the signal.

The authors did not specify how they determined the concentrations of TMG, OSMI, and Aza used in the study.

Q 3   **Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns**

1. Figure 2 – this Western blot image exhibits a very strong signal, making it challenging to accurately measure the signal. This could indeed indicate that the antibody concentration is too high.
2. Considering that it's a brief review, I'd suggest shortening the introduction.
3. Since these are preliminary studies, it's essential to acknowledge their limitations.
4. Please write a paragraph with the aim of the study.
5. Please provide information on how the Authors determined the concentrations of TMG, OSMI, and Aza used in the study.

Check List

Q 4   **Is the English language of sufficient quality?**

Yes.

Q 5   **Is the quality of the figures and tables satisfactory?**

No.

Q 6   **Does the reference list cover the relevant literature adequately and in an unbiased manner?**

Yes.

Q 7   **Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)**

Yes.

Q 8   **Are the methods sufficiently documented to allow replication studies?**

Yes.

Q 9   **Are the results presented correctly and interpreted in light of previous knowledge?**

Yes.

Do the discussion and conclusion address the research questions or hypothesis posed in the introduction?

Yes.

Q 11 Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication.)

Yes.

Q 12 Does the study adhere to ethical standards including ethics committee approval and consent procedure?

Yes.

Q 13 Have standard biosecurity and institutional safety procedures been adhered to?

Yes.

Q 14 Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List):

The recent article shedding light on CD36, a type 2 cell surface scavenger receptor, and its recognition of oxidized lipids in macrophages presents a compelling narrative in the landscape of cellular biology, particularly in the context of lipid metabolism and atherosclerosis. It aims to delve deeper into the implications of the findings presented in the article, elucidating the significance of CD36 in physiological and pathological processes and highlighting potential

The article highlights the intricate posttranslational modifications governing CD36's function, particularly N-glycosylation and O-GlcNAc modification. These modifications not only ensure the structural integrity of CD36 but also dictate its subcellular localization and activity. Notably, N-linked glycosylation plays a crucial role in the proper folding and trafficking of CD36 to the plasma membrane – a prerequisite for its ox-LDL sensing capacity. Moreover, the dynamic interplay between CD36 and cellular metabolism, as influenced by the availability of UDP-GlcNAc, unveils a sophisticated regulatory mechanism that integrates metabolic cues into CD36-mediated cellular responses.

The article provides valuable insights into the multifaceted role of CD36 in cellular physiology and pathology, particularly in the context of lipid metabolism and atherosclerosis. By unraveling the intricacies of CD36-mediated ox-LDL recognition and its regulatory mechanisms, researchers pave the way for the development of targeted therapies aimed at modulating CD36 activity in disease states. Moreover, the broader implications of CD36 in lipid metabolism and inflammation underscore its significance as a central player in health and disease, offering promising avenues for future research endeavors aimed at deciphering the complexities of cellular biology.

Comments:
Overall, while the idea regarding CD36 and its role in recognizing oxidized lipids in macrophages offers valuable insights, it's essential to critically evaluate its limitations and complexities to advance our understanding of CD36 biology and its implications in disease. While targeting CD36 holds promise for

therapeutic intervention in diseases like atherosclerosis, there are challenges associated with modulating its activity. CD36 is involved in a variety of physiological processes beyond lipid recognition, and interfering with its function may have unintended consequences.

The study suggests that the availability of UDP-GlcNAc may play a role in mediating endocytic traffic, as evidenced by the redistribution of Rab-5 between the cytoplasm and cell surface. This effect was observed following treatment with Thiamet G as an inductor and inhibitors such as OSMI-1 and Azaserine. These findings highlight the potential involvement of UDP-GlcNAc in modulating endocytic processes mediated by CD36, shedding light on potential mechanisms underlying its regulatory role in macrophage function.

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QUALITY ASSESSMENT

Q 15	Originality
Q 16	Rigor
Q 17	Significance to the field
Q 18	Interest to a general audience
Q 19	Quality of the writing
Q 20	Overall quality of the study