氏　　名	Chuluun-Erdene Ariunbold（チョロンーエルデネ アリウンボルド）
学位の種類	博士 (医学)
学位記番号	甲第 689 号
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学位授与の要件	自治医科大学学位規定第 4 条第 2 項該当
学位論文名	**マクロファージのエフェロサイトシース過程における細胞内 ATP 動態に関する研究**
論文審査委員	(委員長)　教授　今井　靖
	(委　員)　准教授　海老原　健　　講師　唐澤直義

論文内容の要旨

1　研究目的

Macrophages accumulate to hypoxic areas of inflammatory tissues and play central roles in clearing dead cells through efferocytosis. However, the intracellular energetic flow during efferocytosis under hypoxia remains largely unknown. My study aims to reveal spatio-temporal dynamics of intracellular energy currency, adenosine triphosphate (ATP), during efferocytosis in hypoxic macrophages.

2　研究方法

I adopted a genetically encoded sensor, GO-ATeam, to fluorescently visualize intracellular ATP dynamics in macrophages under microscopic observation. Bone marrow-derived macrophages (BMDMs) were harvested from mice ubiquitously expressing GO-ATeam, and were co-cultured with apoptotic cells to assess intracellular ATP dynamics and cell clearance in efferocytosis under various conditions.

3　研究成果

I established the GO-ATeam imaging system to visualize cytosolic ATP dynamics in BMDMs. GO-ATeam imaging revealed that the cytosolic ATP level was locally elevated at a site of apoptotic cell engulfment in BMDMs under hypoxic conditions. Also, I found that efferocytosis was activated under the hypoxic conditions in a hypoxia-inducible factor 1 alpha (Hif-1α) dependent manner. Immunofluorescent analysis showed that pyruvate kinase isozyme M2 (PKM2), a Hif-1α target enzyme, recruited at a site of apoptotic cell engulfment in BMDMs. Furthermore, efferocytosis was suppressed in BMDMs treated with a glycolysis inhibitor.

4　考察

I obtained potent evidences that link intracellular ATP dynamics and glycolytic metabolism during efferocytosis in hypoxic macrophages. Engulfing apoptotic cells in efferocytosis

requires drastic cytoskeleton remodeling, locally consuming a high amount of ATP. In this setting, glycolysis is beneficial to local ATP production and on-demand supply for its needs. This mechanism of local ATP production and supply might underpin efferocytosis activation under hypoxia where glycolytic metabolism is enhanced through several proteins including PKM2.

5　結論

I studied intracellular ATP dynamics and the metabolic status during macrophage efferocytosis under hypoxia. The collective data suggested that glycolytic metabolism is indispensable to activate efferocytosis programs in macrophages, boosting the clearance of apoptotic and dead cells under hypoxic conditions. My study would provide new insights to explain how macrophages efficiently conduct efferocytosis in hypoxic disease settings where oxygen lacks for the efficient production of ATP in mitochondria.

論文審査の結果の要旨

In this thesis he focused on the function of macrophages and evaluated the spatio-temporal dynamics of intracellular ATP in macrophage efferocytosis. He adopted a genetically encoded sensor, GO-ATeam, to fluorescently visualize intracellular ATP dynamics in macrophages under microscopic observation. Bone marrow-derived macrophages (BMDMs) were harvested from mice ubiquitously expressing GO-ATeam, and were co-cultured with apoptotic cells to assess intracellular ATP dynamics and cell clearance in efferocytosis under various conditions. In these settings he established the GO-ATeam imaging system to visualize cytosolic ATP dynamics in BMDMs. GO-ATeam imaging revealed that the cytosolic ATP level was locally elevated at a site of apoptotic cell engulfment in BMDMs under hypoxic conditions. Also, I found that efferocytosis was activated under the hypoxic conditions in a hypoxia-inducible factor 1 alpha (Hif-1α) manner. Immunofluorescent analysis showed that pyruvate kinase isozyme M2 (PKM2), a Hif-1α target enzyme, recruited at a site of apoptotic cell engulfment in BMDMs. Furthermore, efferocytosis was suppressed in BMDMs treated with a glycolysis inhibitor.

His study would provide new insights to explain how macrophages efficiently conduct efferocytosis in hypoxic disease settings where oxygen lacks for the efficient production of ATP in mitochondria.

The manuscript is well written and contains novel scientific evidence and perspectives, although we pointed out several concerns which should be addressed. The detail was shown as follows;

1 The morphological evaluation of macrophage efferocytosis and its effects under hypoxia and HIF-1α knockout have been discussed in detail. However, if you have data on ATP concentration or the amount of HIF-1α (that has not been degraded), please add them. If you

do not have such data, please comment on them in your discussion.

2 Macrophages are classified as M1 and M2 macrophages. This study is mainly concerned with macrophages, but please add to the discussion which macrophages belong to which category in this study and the differences between M1 and M2 macrophages.

In addition, we added one more recommendation to make his thesis better.

3. The authors added discussion about M1 and M2 macrophages, but there were no additional comments about ATP and HIF1a.

In the latter half of dissertation there was no data about ATP in the various conditions, i.e. hypoxia, HIF-1a knockout, and 3PO administration.

Therefore, the authors should describe that the aforementioned issues which were not assessed yet and add the future direction in the Limitation Part. In addition, please make some change in the Conclusion Part.

Finally he revised sufficiently according to the comments and advices of the reviewers and we decided now that his paper is now acceptable as the thesis for the Graduate School of Jichi Medical University.

最終試験の結果の要旨

In this thesis he focused on the function of macrophages and evaluated the spatio-temporal dynamics of intracellular ATP in macrophage efferocytosis.

He demonstrated his research in 30 minites' presentation and the detail was shown as follows;

To clarify the spatio-temporal dynamics of intracellular ATP in macrophage efferocytosis, he adopted a genetically encoded sensor, GO-ATeam, to fluorescently visualize intracellular ATP dynamics in macrophages under microscopic observation. Bone marrow-derived macrophages (BMDMs) were harvested from mice ubiquitously expressing GO-ATeam, and were co-cultured with apoptotic cells to assess intracellular ATP dynamics and cell clearance in efferocytosis under various conditions. In these settings he established the GO-ATeam imaging system to visualize cytosolic ATP dynamics in BMDMs. GO-ATeam imaging revealed that the cytosolic ATP level was locally elevated at a site of apoptotic cell engulfment in BMDMs under hypoxic conditions. Also, I found that efferocytosis was activated under the hypoxic conditions in a hypoxia-inducible factor 1 alpha (Hif-1α) manner. Immunofluorescent analysis showed that pyruvate kinase isozyme M2 (PKM2), a Hif-1α target enzyme, recruited at a site of apoptotic cell engulfment in BMDMs. Furthermore, efferocytosis was suppressed in BMDMs treated with a glycolysis inhibitor.

His presentation was clear and straight-forward and he responded satisfactorily to all the questions from the three reviewers.

We decided that he passed the final examination of the Graduate School of Jichi Medical University.