（博士課程医学領域）　　（別記様式第５号）
（課程・論文博士共通）

論　文　内　容　要　旨

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論文題目	Connexin43 Contributes to Inflammasome Activation and Lipopolysaccharide-Initiated Acute Renal Injury *via* Modulation of Intracellular Oxidative Status（インフルマソーム活性化およびリポ多糖による炎症性腎障害におけるConnexin43の役割）		

論文内容要旨

[Purpose]

Gap junctions (Gjs), formed by the specific protein termed connexins (Cxs), regulate many cell responses, including those induced by oxidative and inflammatory insults. Currently, the mechanisms involved are still poorly understood. Given that NLRP3 inflammasome activation is a pivotal cellular event contributing to inflammatory cell responses and that ROS is the central mechanism underlying inflammasome activation, we tested whether and how Gjs regulated inflammasome activation in cultured macrophages and explored the potential *in vivo* implication.

[Methods]

Primarily cultured peritoneal macrophages (PMs) and bone marrow-derived macrophages were cultured and exposed to inflammasome activator LPS plus ATP. The changes in NLRP3 inflammasome markers, such as IL-1β, caspase-1 and NLRP3, were detected with Elisa kits or Western Blot analysis. The intracellular redox status was evaluated through measurement of the levels of ROS, NADPH oxidases (NOXs), protein carbonylation and redox-sensitive kinases. The effects of Gjs/Cx43 were determined using chemical inhibitors, siRNA or through comparison of the differences between $Cx43^{+/-}$ and $Cx43^{+/+}$ cells. *In vivo* roles of Cx43 and inflammasome in inflammatory renal injury were assessed through a comparison of LPS-stimulated Cx43 wild-type (WT, $Cx43^{+/+}$) and heterozygous ($Cx43^{+/-}$) mice.

[Results]

(1) Exposure of PMs to LPS plus ATP caused NLRP3 inflammasome activation, which was associated with a marked increase in Cx43.

(2) Inhibition of Cx43 channels with chemical inhibitors or downregulation of Cx43 with siRNA blunted inflammasome activation. Consistently, PMs from $Cx43^{+/-}$ mouse exhibited a weak inflammasome activation, in comparison with those from $Cx43^{+/+}$ mouse.

(3) Further analysis revealed that inflammasome activation was associated with an early increase in

備　考

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intracellular ROS, NADPH oxidase 2 (NOX2), protein carbonylation and MAPK activation. Suppression of ROS with antioxidants or inhibition of NOX or MAPK with inhibitors or siRNA blocked Cx43 elevation, and inflammasome activation as well.

(4) Suppression of Cx43 with inhibitors or siRNA blunted NOX2 expression, reduced protein carbonylation, and decreased P38 phosphorylation. Accordingly, $Cx43^{+/-}$ PMs had a lower level of ROS, NOX2, and MAPK activation, as compared with $Cx43^{+/+}$ PMs.

(5) In an *in vivo* model of acute renal injury induced by LPS, $Cx43^{+/-}$ mouse exhibited a significantly lower level of blood IL-1β, blood urea nitrogen (BUN), and urinary protein, together with a milder renal pathological change and renal expression of NLRP3, NOX4 and cleaved Caspase 3, as compared with $Cx43^{+/+}$ mouse.

(6) Moreover, inhibition of Cx channels suppressed IL-1β- and TNF-α-induced expression of NOX4 in cultured glomerular podocytes and tubular epithelial cells.

[Discussion]

In this study, we demonstrated a pivotal role of Cx43 in the control of NLRP3 inflammasome activation and the development of acute renal injury through regulation on intracellular redox status. Given that inflammasome activation has been implicated in many inflammatory processes, our findings could have important clinical and basic implications.

In our study, we found that inflammasome activation was associated with an elevated Cx43. Genetic or pharmacological inhibition of Cx43 blunted inflammasome activation, indicating involvement of Cx43 in inflammasome activation. In line with previous reports, we demonstrated a mediating role of NADPH oxidase, ROS, and oxidative-sensitive MAPK in inflammasome activation. Intriguingly, the changes of the cellular redox status induced by inflammasome activators, as evidenced by NOX2 expression, ROS generation, protein carbonylation and kinase activation, were all affected by Cx43. These observations indicate that Cx43 regulated inflammasome activation through modulating intracellular redox status.

In an *in vivo* model of AKI induced by LPS, Cx43 heterozygous mouse ($Cx43^{+/-}$) displayed less severe changes in renal function and structure, which was associated with a reduced level of serum IL-1β and lower level of NLRP3, NOX4 and cleaved Caspase 3 in renal tissue. These results indicate that Cx43 also promotes inflammasome activation *in vivo* and participates in the processes of inflammatory renal injury.

Our study could have significant clinical and basic implications. First, our study provides novel mechanistic insight into the regulatory actions of Cx43 on immune responses and inflammatory cell injury; second, our study indicate that Cx43 could be targeted for the treatment of certain inflammatory renal diseases; and third, our study provides additional evidence supporting a pivotal role of Cx43 in the control of the intracellular redox status and indicating that regulation of intracellular redox status could be an essential mechanism by which Cx43 exert its regulatory effects.

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[Conclusion]

Collectively, our study characterized Cx43 as a novel molecule controlling inflammasome activation and inflammatory cell injury. Furthermore, we revealed that this effect of Cx43 was mediated by its actions on the NOX-dependent redox signaling pathway. Our study thus provides novel mechanistic insight into the regulatory effects of Cx43 on inflammatory processes and suggests that targeting Cx43 could be developed for the treatment of certain inflammatory diseases.

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