News Release

Title

CD63 is Regulated by Iron via the IRE-IRP System and is Important for Ferritin Secretion by Extracellular Vesicles

Key Points

- **CD63 is involved in extracellular vesicle (EV) secretion from cells and is shown herein to be regulated by iron *via* the IRE-IRP system**
- **Iron-loading increased secretion of CD63 positive EVs containing iron-loaded ferritin**

Summary

Extracellular vesicles (EVs) transfer functional molecules between cells. CD63 is a widely recognized EV marker that contributes to EV secretion from cells. However, the regulation of its expression remains largely unknown. Ferritin is a cellular iron storage protein that can be also secreted by the exosome pathway, with serum ferritin levels classically reflecting body iron stores. Iron metabolism-associated proteins, such as ferritin, are intricately regulated by cellular iron levels via the iron responsive element (IRE)-iron regulatory protein (IRP) system. Herein, we present a novel mechanism demonstrating that the expression of the EV-associated protein, CD63, is under the regulation of the IRE-IRP system. We discovered a canonical IRE in the 5'-untranslated region (UTR) of CD63 mRNA responsible for regulating its expression in response to increased iron. Cellular iron-loading caused a marked increase in CD63 expression and the secretion from cells of CD63 positive (i.e., CD63(+)) EVs, which were shown to contain ferritin-H (FtH) and -L (FtL). Our results demonstrate that under iron-loading, intracellular ferritin is transferred via nuclear receptor coactivator 4 (NCOA4) to CD63(+) EVs that are then secreted. Such iron-regulated secretion of the major iron storage protein ferritin via CD63(+) EVs, poses significant impact for understanding the local cell-to-cell exchange of ferritin and iron.

Research Background

Extracellular vesicles (EVs) are released by cells and found in most biological fluids. CD63 is localized to intraluminal vesicles of late endosomes and multi-vesicular bodies and is enriched in EVs. Ferritin is the major intracellular iron storage protein and is also found extracellularly in EVs. The mechanism of regulation of ferritin secretion by cellular iron remains unknown.

Research Results

We used IMR90SV cells to reveal that CD63 expression is up-regulated by iron-loading. We then showed that iron-induced CD63 expression occurs by a different mechanism to CD63 induction by autophagy inhibition. Further, iron induces secretion of CD63 and ferritin in EVs.

Considering CD63 was up-regulated by iron-loading, studies then aimed to dissect the mechanism involved. To examine the possible role of the IRP-IRE mechanism in regulating CD63 by iron, we examined the presence of IREs in 5'- and 3'-UTR regions of CD63 mRNA by the IRE prediction software, SIREs Web Server 2.0. In the 5'-UTR region of CD63 mRNA, a 31-nucleotide long mRNA sequence was predicted as a consensus IRE. Then, we showed that IRP1 and IRP2 bind to the CD63 IRE and that NCOA4 associates with CD63(+) EVs and transfers ferritin into EVs. Finally, we demonstrated that FtH secretion through EVs under iron-loading is dependent on NCOA4 expression.

Research Summary and Future Perspective

CD63, which is involved in EV secretion, is under the regulation of the IRE-IRP system, with a canonical IRE in the 5'-UTR of CD63 mRNA regulating its expression. Iron-loading resulted in secretion of CD63(+) EVs from cells, which contained iron-loaded ferritin, with intracellular ferritin being demonstrated to be transferred via NCOA4 to CD63(+) EVs that are secreted. Such a mechanism provides new understanding regarding the regulation of iron metabolism, especially local, cell-to-cell exchange of ferritin and iron.

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Authors: Izumi Yanatori[a], Des R. Richardson[a,b], Herschel S. Dhekne[c], Shinya Toyokuni[a,d], Fumio Kishi[e]

[a] Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[b] Centre for Cancer Cell Biology and Drug Discovery, Griffith Institute for Drug Discovery, Griffith University, Nathan, Brisbane, Queensland, 4212, Australia
[c] Department of Biochemistry, Stanford University School of Medicine, Stanford, 94305, CA, United States
[d] Center for Low Temperature Plasma Sciences, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8603, Japan
[e] Kenjinkai Healthcare Corporation, Yamaguchi, 757-0001, Japan
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