Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2023 24(11):1053-1056
www.jzus.zju.edu.cn; www.springer.com/journal/11585
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https://doi.org/10.1631/jzus.B2300241

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HPCAL1 is a novel driver of autophagy-dependent ferroptosis

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Autophagy is a highly conserved physiological process in cells that degrades excess or damaged organelles, large protein aggregates, and pathogens via the lysosomal system (Li et al., 2021). Autophagy generally increases the self-protection mechanism of cells, which plays an important role in the maintenance of cell homeostasis as well as the synthesis, degradation, and recycling of cell products. However, in certain circumstances, activation of autophagy or excessive autophagy can lead to cell death, a phenomenon called "autophagy-dependent cell death" (Liu et al., 2020). Recent research has found that, under certain conditions, autophagy activation or excessive autophagy can regulate ferroptosis through corresponding mechanisms (Xie et al., 2023).

Ferroptosis is a form of iron-dependent programmed cell necrosis caused by lipid peroxidation (Qin et al., 2021; Ru et al., 2023). It is also a cell-death process, which can be regulated by autophagy. Autophagy can promote ferroptosis by increasing iron accumulation. Hou et al. (2016) indicated that nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy is linked to ferroptosis. NCOA4 can promote degradation of ferritin, which enhances iron accumulation and gives rise to ferroptosis. Moreover, autophagy can promote ferroptosis by accelerating lipid peroxidation. Bai et al. (2019) revealed that Ras-associated protein Rab-7a (RAB7A)-mediated lipophagy has contact with ferroptosis. RAB7A promotes degradation of lipid droplets (LDs), which induces lipid peroxidation and brings about ferroptosis. Yang et al. (2019) reported that sequestosome 1 (SQSTM1)-mediated clockophagy is also associated with ferroptosis. SQSTM1 can promote degradation of aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL1), which enhances lipid peroxidation and leads to ferroptosis. Wu et al. (2019) found that heat shock protein 90 (HSP90)-mediated chaperone-mediated autophagy (CMA) is relevant to ferroptosis as well. HSP90 can accelerate degradation of glutathione peroxidase 4 (GPX4), which increases lipid peroxidation and results in ferroptosis.

Hippocalcin-like protein 1 (HPCAL1), which expresses in the plasma membrane and is also known as a visinin-like protein-3 (VILIP-3), is a member of the neuron-specific calcium-binding protein family. The VILIP superfamily is a highly homologous subfamily of neuronal calcium sensor proteins, including VILIP-1, VILIP-2, VILIP-3 (HPCAL1), hippocalcin (HPCA), and neurocalcin δ (NCALD). HPCAL1 can be expressed in a variety of organs (such as the brain, pancreas, and kidney), as well as tissues (for instance epithelial and neural tissues) and cells (for example cardiac muscle and smooth muscle cells), and is most abundant in the cerebellum (https://www.proteinatlas.org) (Tang et al., 2012). HPCAL1 contains four EF-hand motifs and an attached N-terminal myristoyl group (Fig. 1a). It is highly homologous among different species (97.41% identity, 99.74% similarity) and conserved protein, suggesting that it may play an important role in physiological and pathophysiological processes (Fig. 1b).

Recently, Chen et al. (2023) reported that HPCAL1 is a novel driver of autophagy-dependent ferroptosis. HPCAL1, as a specific autophagy receptor, can selectively degrade *N*-cadherin (CDH2), which as a typical mechanotransduction receptor on the cell

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Received Apr. 10, 2023; Revision accepted June 18, 2023;
Crosschecked Oct. 16, 2023

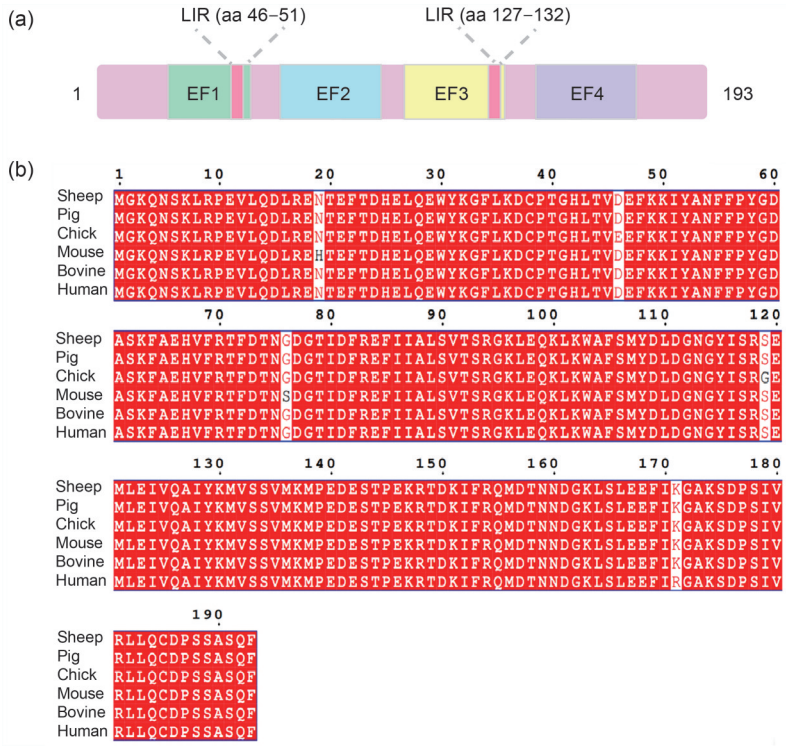

Fig. 1 Scheme of hippocalcin-like protein 1 (HPCAL1) domain and comparison of HPCAL1 amino acid sequences between different species. (a) Schematic diagram of HPCAL1 domain organization. EF: EF-hand; LIR: microtubule-associated protein light chain 3 (LC3)-interacting region; LIR motif (aa 46–51): DEFFKKI; LIR motif (aa 127–132): AIYKMV; (b) Comparison of HPCAL1 amino acid sequences between *Ovis aries* (sheep), *Sus scrofa* (pig), *Gallus gallus* (chick), *Mus musculus* (mouse), *Bos taurus* (bovine), and *Homo sapiens* (human). Amino acid sequence alignment of HPCAL1 in different species was analyzed by ESPript software. The identity of HPCAL1 amino acid sequence alignment is 97.41%, and the similarity of HPCAL1 among different species is 99.74%.

membrane is involved in regulating cell-membrane tension. Degradation of CDH2 can promote ferroptosis by damaging membrane tension, reducing mechanotransduction, and enhancing lipid peroxidation.

In order to explore the detailed mechanisms, Chen et al. (2023) first induced ferroptosis with ferroptosis activator Ras-selective lethal small molecule 3 (RSL3) in human fibrosarcoma (HT-1080) and human non-small-cell lung cancer (Calu-1), and found that HPCAL1 small interfering RNA (siRNA) inhibited RSL3-induced ferroptosis. They further demonstrated that RSL3 induced HPCAL1-mediated ferroptosis by promoting lipid peroxidation. Next, they looked into how RSL3 activates HPCAL1 to cause cell ferroptosis. They found that the ferroptosis activators Erastin and RSL3 promoted phosphorylation of calcium-independent protein kinase C theta (PRKCQ) at Thr538, and that activation of PRKCQ further promoted phosphorylation of HPCAL1 at Thr149, ultimately inducing ferroptosis.

HPCAL1, although a member of the calcium-binding protein family, is involved in HPCAL1-mediated ferroptosis not by calcium ions pathway but by inducing the degradation of CDH2. In addition, Chen et al. (2023) observed enhancement of autophagy flow during ferroptosis and found that HPCAL1 selectively degraded CDH2. The protein sequences of autophagy receptors contain microtubule-associated protein light chain 3 (LC3)-interacting regions (LIRs), which are able to bind to LC3 proteins located on autophagosomes to transport cytoplasmic contents into them. To further verify the hypothesis that HPCAL1 is an autophagy receptor for CDH2 degradation, they investigated the LIR motif of HPCAL1 and found two segments: DEFFKKI (aa 46–51) and AIYKMV (aa 127–132). Further investigation showed that HPCAL1 could bind LC3-II and CDH2 on phagocytic vesicles and autophagosomes and ultimately degrade CDH2. CDH2 is a typical mechanotransduction receptor on cell membrane and is involved in regulating cell-membrane

tension. By changing the shape and tension of cell membranes, cells are able to participate in cellular and subcellular physiological functions such as mechanotransduction, migration, and other physiological functions. Therefore, under conditions of CDH2 degradation, the fluorescent membrane tension probe Flipper-TR was used to detect the changes in membrane tension, and the membrane tension was reduced. The decrease in cell-membrane tension can also affect mechanotransduction, promote lipid peroxidation, and enhance ferroptosis.

In addition, Chen et al. (2023) identified a novel inhibitor of HPCAL1 called iHPCAL1. They then found that iHPCAL1 significantly inhibited ferroptosis in tumor cells and pancreatitis in a mouse model. In summary, phosphorylation of PRKCQ at Thr538 promotes phosphorylation of HPCAL1 at Thr149. Phosphorylated HPCAL1 contains two segments of the LIR motif, which are able to bind to LC3 proteins located on autophagosomes to transport CDH2 into the autophagosome. Degradation of CDH2 can decrease membrane tension, reduce mechanotransduction, further accelerate the lipid peroxidation process, and ultimately promote ferroptosis. However, the specific mechanism by which mechanotransduction promotes lipid peroxidation mechanotransduction is unclear, and thus requires further study.

Chen et al. (2023) predicted two LIR motifs on HPCAL1: DEFFKKI (aa 46–51) and AIYKMV (aa 127–132). An HPCAL1 mutation experiment showed that AIYKMV (aa 127–132) deletion did not affect binding of HPCAL1 to LC3, while DEFFKKI (aa 46–51) deletion inhibited it. Therefore, it is possible to explore new drugs which can target AIYKMV (aa 127–132) site and inhibit HPCAL1 binding to the LC3-II, thus inhibiting CDH2 degradation and ultimately inhibiting ferroptosis. In addition, HPCAL1 acts as a selective autophagy receptor, suggesting that targeting the autophagy pathway may help curb excessive ferroptosis.

Both CDH1 and CDH2 are members of the E-cadherin family. However, Chen et al. (2023) did not explore whether CDH1 can interact with HPCAL1 to mediate ferroptosis. Bao et al. (2021) have shown that overexpression of CDH1 can reduce intracellular iron content and lower lipid peroxidation, thus inhibiting ferroptosis. Therefore, it is necessary to further explore whether HPCAL1 can interact with CDH1 to increase the intracellular iron concentration and promote ferroptosis, or whether HPCAL1 can simultaneously regulate CDH1 and CDH2 to make cells more sensitive to ferroptosis.

In this study, Chen et al. (2023) found that HPCAL1-induced ferroptosis suppressed tumors. However, some studies show that HPCAL1 regulation of tumor growth produces inconsistent results (Zhang et al., 2019; Wang et al., 2022). On the one hand, HPCAL1 can promote the growth of non-small-cell lung carcinoma, activate the Wnt/β-catenin signaling pathway, and promote proliferation of glioblastoma (Zhang et al., 2019; Wang et al., 2022). On the other hand, it suppresses hepatocellular carcinoma (HCC) by activating the extracellular signal-regulated kinases 1 and 2 (ERK1/2)-mitogen-activated protein kinase (MAPK) pathway, promoting p21 (Waf/Cip1) stabilization, or directly targeting the RuvB-like protein 1-mechanistic target of rapamycin (RUVBL1-mTOR) signaling pathway to blunt liver lipid metabolism (Zhang et al., 2016). HPCAL1 is widely distributed in human tissues, and expresses in a variety of tumor tissues. Therefore, the mechanisms and function of HPCAL1 in regulating tumors need to be further investigated.

Chen et al. (2021) reported the existence of a small molecular compound named iHPCAL1, which can degrade HPCAL1 in a highly specific manner by ubiquitin-proteasome. In addition, HPCAL1-induced ferroptosis can promote acute pancreatitis. iHPCAL1 can improve ferroptosis-induced acute pancreatitis by degrading HPCAL1. These results indicate that HPCAL1-induced ferroptosis plays an important role in regulating inflammatory response. HPCAL1 is widely distributed and may cause inflammation in various tissues or organs. Therefore, it could become a new clinical therapeutic target for ferroptosis-induced inflammatory diseases.

Data availability statement

The basis of data is obtained from The Human Protein Atlas database (https://www.proteinatlas.org).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81970431) and the Hunan Provincial Natural Science Foundation of China (No. 2023JJ50136).

Author contributions

Conceptualization: Liwen WANG and Li QIN. Resources: Lanfang LI. Data curation: Liwen WANG and Li QIN. Software: Li QIN. Formal analysis: Liwen WANG and Li QIN. Supervision: Lanfang LI and Huimei LIU. Validation: Liwen WANG. Writing – original draft: Liwen WANG and Li QIN. Project administration: Lanfang LI. Writing – review & editing: Liwen WANG. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Liwen WANG, Li QIN, Huimei LIU, and Lanfang LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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