PERSPECTIVES

National Science Open
1: 2021004, 2022
https://doi.org/10.1051/nso/2021004

Life Sciences and Medicine

Special topic: COVID-19: Virus, Immunity and Vaccines

Cell-in-cell: an Emerging Player in COVID-19 and Immune Disorders

Qiang Sun[1,2,*], and Wei Chen[1,2]

[1]*Beijing Institute of Biotechnology, Academy of Military Medical Sciences, 20 Dongda Street, Beijing 100071, China;* [2]*Research Unit of Cell Death Mechanism, Chinese Academy of Medical Science, 2020RU009, 20 Dongda Street, Beijing 100071, China*

*Corresponding author. E-mail: sunq@bmi.ac.cn

Received: 1 December 2021; accepted: 30 December 2021; published online: 10 March 2022

Abstract: Cell-in-cell is a unique phenomenon mostly documented in human cancer tissues. A recent study demonstrated that cell-in-cell might promote lymphopenia by internalizing and killing immune cells in COVID-19, which implicates cell-in-cell as an emerging player in a broader spectrum of pathological processes, such as immune dysregulation.

Keywords: cell-in-cell, cell death, COVID-19, lymphopenia, immune dysregulation.

1 MAIN TEXT

For a long time, scientists frequently observed a type of unique cellular structure in multiple types of human cancerous tissues, where one or more morphologically intact cells are present inside the cytoplasm of a cancer cell. These structures are now referred to as cell-in-cell (CIC) structures that were usually described in the early literature with alternative terms, sun as bird eyes, cell cannibalism, cytophagocytosis [1]. Not only in cancer tissues, CIC structures were also detected in non-cancerous tissues, particularly in the inflammatory tissues, which could be dated back to as early as the middle of the 19th century, when lymphocytes were found to be enclosed within intestinal epithelial cells [2]. It's now clear that CIC structures could be formed homotypically between cells of the same kinds, such as cancer cells, or heterotypically between different kinds of cells, such as lymphocytes and cancer cells. In fact, up to five subtypes of CIC structures had been detected in human cancer tissues [3-7]. Accordingly, different models were employed to investigate their formation mechanisms and functional implications, including entosis, emperitosis, cannibalism, phagoptosis, suicidal emperipolesis, and the like [8].

2 BIOMEDICAL IMPLICATIONS OF HOMOTYPIC CIC STRUCTURES

Entosis, taking place by the active invasion of the inner cells into their neighboring cell, is one of the most investigated models corresponding to the formation of homotypic CIC structures. The studies on entosis, and

other CIC models as well, promoted the conception that CIC formation may constitute a cell death program that kills the internalized cells in an unconventional way [9]. The formation of entotic CIC structures is tightly controlled by a set of molecular machinery that consists of three core elements (adherens junction, mechanical ring, and contractile actomyosin) and a group of regulatory factors [10-19], on which readers are referred to a recent review [20] for detail. Following CIC formation, the internalized cells were dead and cleared in an acidified huge lysosomal compartment [21], which was regulated by the unconventional autophagic signaling [22]. It was found that homotypic CIC structures were important players of multiple important biological processes, such as tumor evolution as a mechanism of cell competition [16,23], epithelial homeostasis as a mechanism of mitotic surveillance [24], embryonic development by eliminating unwanted cells [25,26], and genome instability by interfering cytokinesis [27,28]. Along with the studies on homotypic CIC structures by entosis, considerable progress was made on heterotypic CIC structures (Figure 1).

3 HETEROTYPIC CIC STRUCTURES IN COVID-19

Recently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread all over the world, leading to the pandemic of coronavirus disease 19 (COVID-19) [29]. A remarkable clinical feature of patients with COVID-19 is the reduced lymphocyte count, or lymphopenia, which was a critical factor associated with unfavorable prognosis. However, the underlying mechanisms are poorly understood. By examining a panel of post-mortem autopsies, we found that the multinucleated syncytia were readily detected in the lung tissues of patients with severe COVID-19 [30]. Remarkably, the majority of the syncytia contained $CD45^+$ lymphocytes that were mostly $CD8^+$ within their cytoplasm, morphologically resembling

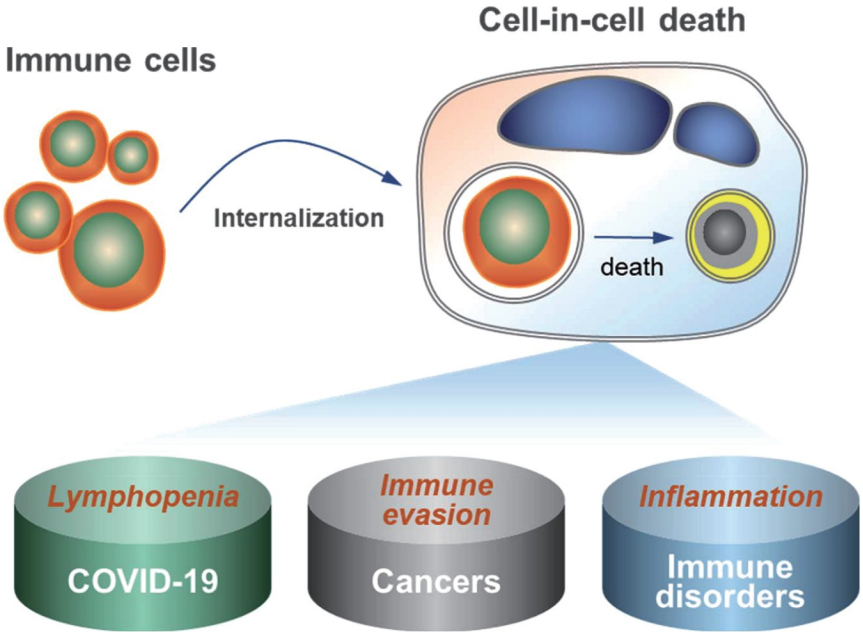

Figure 1 Biomedical implications of cell death mediated by heterotypic cell-in-cell structures.

heterotypic CIC structures. Moreover, the number of the heterotypic CIC structures in COVID-19 autopsies had a negative correlation with the number of lymphocytes in the periphery blood, suggesting a negative impact of CIC structures on circulating lymphocytes [30]. In agreement with this idea, subsequent co-culture experimentations showed that syncytia could effectively internalize periphery blood mononucleated cells (PBMC) to form heterotypic CIC structures, and promoted the death of PBMCs, in particular those that are $CD8^+$ [30]. These data fit well with a working model that the multinucleated syncytia, induced by SARS-CoV-2 infection, may serve as a disastrous unity to eliminate lymphocyte via CIC-mediated death [31], and targeting syncytia and/or CIC formation holds the promise to treat COVID-19 and syncytia-related diseases. It is noted that syncytia are common in various of virological diseases. For example, the infection of respiratory syncytial virus was known to cause syncytia formation of infected cells, the same was true for other pathogenic viruses, such as human immunodeficiency virus and highly contagious flu viruses H5N3, H7N1 and the like [30, 32]. The formation of syncytia was ascribed to the fusogenic glycoproteins expressing on the surface of viral particles that mediate membrane fusion and host entry of the viral genomes [32]. Interestingly, the victims of virus infection frequently developed lymphopenia. It therefore remains to be explored whether syncytia formation is correlated with lymphocyte loss in these patients, and to what extend syncytia formation may contribute to lymphopenia, and furthermore to what extends blocking the formation of syncytia and/or CIC structures may relieve the related virological diseases, including COVID-19 and the like.

4 HETEROTYPIC CIC STRUCTURES IN CANCERS

Similar to syncytia in virological diseases, multinucleated cells are common in human cancer, where immune edition was identified as one of the key drivers promoting tumor development and progression [33]. Multiple lines of evidence supported the idea that heterotypic CIC formation may contribute to immune evasion and cancer malignancy. First, the formation of heterotypic CIC structures with cancer cells internalizing immune cells was shown to be an independent prognostic factor that associated with shorter overall survival time (8 months vs 15 months) in two independent cohorts of patients with pancreatic ductal adenocarcinoma [4]; second, multinucleated cancer cells were demonstrated *in vitro* to internalize more immune cells and in a higher frequency as compare with mononucleate cancer cells [30]; third, experimental models of emperitosis or cell cannibalism showed that the formation of heterotypic CIC structures could result in the death of the internalized NK or $CD8^+$ T cells [34, 35], either apoptotically or non-apoptotically. Nevertheless, it remains to be confirmed further *in vivo* in animal models and in human cancer samples whether a physiological relevance and a causal link existed between heterotypic CIC formation and immune evasion. To this end, a systemic deciphering of the molecular mechanisms controlling heterotypic CIC formation in a context-dependent way would be helpful.

5 HETEROTYPIC CIC STRUCTURES IN IMMUNE DISORDERS

In addition to virological diseases and cancers, heterotypic CIC structures were also implicated in the

immune disorders related to inflammations. Benseler et al. reported that hepatocytes could internalize self-reactive $CD8^+$ T cells for destruction. This was believed to help maintain immune homeostasis to avoid autoimmune hepatitis. Consistent with this notion, blocking the formation of heterotypic CIC structures by Wortmannin, an inhibitor of myosin light chain kinase, led to an accumulation of autoreactive $CD8^+$ T cells in the liver and breach of tolerance, with the development of autoimmune hepatitis [36]. Conversely, there were studies that proposed that penetration of $CD8^+$ T cells into hepatocytes was positively associated with autoimmune hepatitis [37] and chronic hepatitis B [38]. Though these studies are descriptive and the quantification of CIC structures warrants calibration, the proposed conclusions suggest that heterotypic CIC formation may be either an effect secondary to inflammation, or a driver/promoter of inflammation, or a vicious circle with inflammation. A recent study actually touched this issue by claiming that CD44/p-ERM/F-actin pathway mediates the penetration of $CD8^+$ T cells, unfortunately, the conclusion was not solidly supported by the experimental data presented [39]. Hence, this issue remains to be explored for future heterotypic CIC studies. Other than T cells, NK cells were also reported to be internalized to form heterotypic CIC structures during the development of liver cirrhosis associated with chronic hepatitis B [40], whereas, the outer cells were not hepatocytes, but activated hepatic stellate cells. Under this context, TGF-β seemed to be a promoter that facilitates penetration of NK cells into hepatic stellate cells for apoptotic death, leading to enhanced liver fibrosis associated with chronic hepatitis B.

6 CONCLUSION REMARKS

Together, the existing data suggest complicated roles for heterotypic CIC structure in various contexts, including virological diseases, cancers, and inflammatory disorders, and the fourth (Figure 1). On top of these is the regulation of cellular immunity by targeting immune cells for internalization into different host cells, which was followed by altered cell fates or behaviors of all the cells, inner and outer, engaged in the CIC structures. Currently, the study on heterotypic CIC structures is still in its infancy with different models being developed. In the short future, much effort may be endeavored on deciphering the key molecules that control the CIC formation and the fates of cells involved, the progress on which may dictate the development of potential therapeutic strategies for different immune-related diseases.

DATA AVAILABILITY

All data generated during this study are included in this published article.

ACKNOWLEDGEMENTS

We thank Dr. Hongyan Huang, Mr. Zubiao Niu and Ms. Zhengrong Zhang for insightful discussion and careful edition of this manuscript. We sincerely apologize for not citing many excellent works and reviews on cell-in-cell study due to the limited space.

FUNDING

This work was supported by the National Natural Science Foundation of China (31970685).

AUTHOR CONTRIBUTIONS

QS and WC conceived and wrote the manuscript. All authors read and approve the submission of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

REFERENCES

1 Fais S, Overholtzer M, Cell-in-cell phenomena in cancer. *Nat Rev Cancer* 2018; **18**: 758-766.

2 Eberth J. About the finer construction of the intestinal mucosa. *Wurzb Naturwiss Zeitschr* 1864; 5.

3 Wang Y, Niu Z, Zhou L, *et al*. Subtype-based analysis of cell-in-cell structures in esophageal squamous cell carcinoma. *Front Oncol* 2021; **11**: 670051.

4 Huang H, He M, Zhang Y, *et al.* Identification and validation of heterotypic cell-in-cell structure as an adverse prognostic predictor for young patients of resectable pancreatic ductal adenocarcinoma. *Sig Transduct Target Ther* 2020; **5**: 246.

5 Huang H, Chen A, Wang T, *et al.* Detecting cell-in-cell structures in human tumor samples by E-cadherin/CD68/CD45 triple staining. *Oncotarget* 2015; **6**: 20278-20287.

6 Fan J, Fang Q, Yang Y, *et al.* Role of heterotypic neutrophil-in-tumor structure in the prognosis of patients with buccal mucosa squamous cell carcinoma. *Front Oncol* 2020; **10**: 541878.

7 Zhang X, Niu Z, Qin H, *et al.* Subtype-based prognostic analysis of cell-in-cell structures in early breast cancer. *Front Oncol* 2019; **9**: 895.

8 Wang X, Cell-in-cell phenomenon: A New Paradigm in Life Sciences.. *CMM* 2015; **15**: 810-818.

9 Galluzzi L, Vitale I, Aaronson SA, *et al.* Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* 2018; **25**: 486-541.

10 Wang M, Niu Z, Qin H, *et al.* Mechanical ring interfaces between adherens junction and contractile actomyosin to coordinate entotic cell-in-cell formation. *Cell Rep* 2020; **32**: 108071.

11 Wang C, Chen A, Ruan B, *et al.* PCDH7 inhibits the formation of homotypic cell-in-cell structure. *Front Cell Dev Biol* 2020; **8**: 329.

12 Ruan B, Zhang B, Chen A, *et al.* Cholesterol inhibits entotic cell-in-cell formation and actomyosin contraction. *Biochem Biophysl Res Commun* 2018; **495**: 1440-1446.

13 Ruan B, Wang C, Chen A, *et al.* Expression profiling identified IL-8 as a regulator of homotypic cell-in-cell formation. *BMB Rep* 2018; **51**: 412-417.

14 Liang J, Fan J, Wang M, *et al.* CDKN2A inhibits formation of homotypic cell-in-cell structures. *Oncogenesis* 2018; **7**: 50.

15 Ning X, Luo T, Chen Z, *et al.* The physics for the formation of cell-in-cell structures.. *CMM* 2015; **15**: 867-872.

16 Sun Q, Luo T, Ren Y, *et al.* Competition between human cells by entosis. *Cell Res* 2014; **24**: 1299-1310.

17 Sun Q, Cibas ES, Huang H, *et al.* Induction of entosis by epithelial cadherin expression. *Cell Res* 2014; **24**: 1288-1298.

18 Hinojosa LS, Holst M, Baarlink C, *et al.* MRTF transcription and Ezrin-dependent plasma membrane blebbing are required for entotic invasion. *J Cell Biol* 2017; **216**: 3087-3095.

19 Purvanov V, Holst M, Khan J, *et al.* G-protein-coupled receptor signaling and polarized actin dynamics drive cell-in-cell invasion. *eLife* 2014; **3**: 1.

20 Niu Z, He M, Sun Q, Molecular mechanisms underlying cell-in-cell formation: core machineries and beyond. *J Mol Cell Biol* 2021; **13**: 329-334.

21 Su Y, Ren H, Tang M, *et al.* Role and dynamics of vacuolar pH during cell-in-cell mediated death. *Cell Death Dis* 2021; **12**: 119.

22 Florey O, Kim SE, Sandoval CP, *et al.* Autophagy machinery mediates macroendocytic processing and entotic cell death

by targeting single membranes. *Nat Cell Biol* 2011; **13**: 1335-1343.

23 Huang H, Chen Z, Sun Q, Mammalian cell competitions, cell-in-cell phenomena and their biomedical implications.. *CMM* 2015; **15**: 852-860.

24 Liang J, Niu Z, Zhang B, *et al.* p53-dependent elimination of aneuploid mitotic offspring by entosis. *Cell Death Differ* 2021; **28**: 799-813.

25 Lee Y, Hamann JC, Pellegrino M, *et al.* Entosis controls a developmental cell clearance in *C. elegans*. *Cell Rep* 2019; **26**: 3212-3220.e4.

26 Li Y, Sun X, Dey SK, Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation. *Cell Rep* 2015; **11**: 358-365.

27 Mackay HL, Moore D, Hall C, *et al.* Genomic instability in mutant p53 cancer cells upon entotic engulfment. *Nat Commun* 2018; **9**: 3070.

28 Krajcovic M, Johnson NB, Sun Q, *et al.* A non-genetic route to aneuploidy in human cancers. *Nat Cell Biol* 2011; **13**: 324-330.

29 Huang H, Zhu Y, Niu Z, *et al.* SARS-CoV-2 N501Y variants of concern and their potential transmission by mouse. *Cell Death Differ* 2021; **28**: 2840-2842.

30 Zhang Z, Zheng Y, Niu Z, *et al.* SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination. *Cell Death Differ* 2021; **28**: 2765-2777.

31 Lin L, Li Q, Wang Y, *et al.* Syncytia formation during SARS-CoV-2 lung infection: a disastrous unity to eliminate lymphocytes. *Cell Death Differ* 2021; **28**: 2019-2021.

32 Zheng Y, Zhou LL, Su Y, *et al.* Cell fusion in the pathogenesis of COVID-19. *Military Med Res* 2021; **8**: 68.

33 Sun Q, Melino G, Amelio I, *et al.* Recent advances in cancer immunotherapy. *Discov Onc* 2021; **12**: 27.

34 Lugini L, Matarrese P, Tinari A, *et al.* Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. *Cancer Res* 2006; **66**: 3629-3638.

35 Wang S, He M, Chen Y, *et al.* Rapid reuptake of granzyme B leads to emperitosis: an apoptotic cell-in-cell death of immune killer cells inside tumor cells. *Cell Death Dis* 2013; **4**: e856.

36 Benseler V, Warren A, Vo M, *et al.* Hepatocyte entry leads to degradation of autoreactive CD8 T cells. *Proc Natl Acad Sci USA* 2011; **108**: 16735-16740.

37 Miao Q, Bian Z, Tang R, *et al.* Emperipolesis mediated by CD8 T cells is a characteristic histopathologic feature of autoimmune hepatitis. *Clinic Rev Allerg Immunol* 2015; **48**: 226-235.

38 Hu Y, Jiang L, Zhou G, *et al.* Emperipolesis is a potential histological hallmark associated with chronic hepatitis B.. *CMM* 2015; **15**: 873-881.

39 Liang JB, Chen Y, Chen RL, *et al.* $CD8^+$ T cells actively penetrate hepatocytes via the CD44/p-ERM/F-actin pathway in autoimmune hepatitis. *J Dig Dis* 2021; **22**: 351-362.

40 Shi J, Zhao J, Zhang X, *et al.* Activated hepatic stellate cells impair NK cell anti-fibrosis capacity through a TGF-β-dependent emperipolesis in HBV cirrhotic patients. *Sci Rep* 2017; **7**: 44544.