Received: 30 November 2022 | Revised: 9 March 2023 | Accepted: 15 March 2023

DOI: 10.1002/bies.202200231

BioEssays

COMMENTARY

Targeting a novel apoptotic pathway in human disease

Francesca D'Addio[1,2] | Laura Montefusco[2] | Maria Elena Lunati[2] | Ida Pastore[2] | Emma Assi[1] | Adriana Petrazzuolo[1] | Virna Marin[3] | Chiara Bruckmann[3] | Paolo Fiorina[1,2,4]

[1]International Center for T1D, Pediatric Clinical Research Center Romeo ed Enrica Invernizzi, DIBIC, Università di Milano, Milan, Italy

[2]Division of Endocrinology, ASST Fatebenefratelli-Sacco, Milan, Italy

[3]Enthera s.r.l.

[4]Nephrology Division, Boston Children's Hospital and Transplantation Research Center, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence
Paolo Fiorina, Nephrology Division, Boston Children's Hospital and Transplantation Research Center, Brigham and Women's Hospital Harvard Medical School, 300 Longwood Ave. Enders Building 5th floor En511, Boston MA 02115, USA.
Email: paolo.fiorina@childrens.harvard.edu

Abstract

Apoptotic pathways have always been regarded as a key-player in preserving tissue and organ homeostasis. Excessive activation or resistance to activation of cell death signaling may indeed be responsible for several mechanisms of disease, including malignancy and chronic degenerative diseases. Therefore, targeting apoptotic factors gained more and more attention in the scientific community and novel strategies emerged aimed at selectively blocking or stimulating cell death signaling. This is also the case for the TMEM219 death receptor, which is activated by a circulating ligand, the Insulin-like growth factor binding protein 3 (IGFBP3) and induces a caspase-8-dependent apoptosis of the target cells. Interestingly, stimulation of the IGFBP3/TMEM219 axis exerts an anti-proliferative effect, while blockade of the TMEM219 deleterious signal protects TMEM219-expressing cells of the endocrine pancreas, lung, and intestine from damage and death. Here, we summarize the most updated reports on the role of the IGFBP3/TMEM219 apoptotic axis in disease conditions, including intestinal disorders and diabetes, and we describe the advancements in designing and testing novel TMEM219-based targeting approaches in emerging potential clinical applications.

KEYWORDS
apoptosis, IGFBP3, intestinal stem cells, pancreatic beta cells, TMEM219

INTRODUCTION

There is a growing body of evidence showing that targeting apoptotic pathways and cell death factors may be relevant in several disease conditions, with new cell death mechanisms (e.g., autophagy-dependent cell death, mitochondrial permeability transition pore-mediated necrosis, parthanatos, NETosis, and ferroptosis) being under extensive investigation.[1] Indeed, controlling the homeostasis of tissue specific cells and their lifecycle is highly desirable to prevent tissue degeneration and dysfunction. While it is clear that targeting cell death mechanisms has a significant impact in tumorigenesis and in cancer therapeutic applications,[2] it is now becoming evident that several immune-mediated diseases associated with the loss of functional specific functional cell subsets may benefit as well from the design of therapeutic strategies aimed at preventing cell death.[1] Biological systems work with redundant mechanisms, which tend to protect organs and tissues from the unwanted activation or inhibition of cell death pathways, particularly in healthy conditions.[1] Whether certain

Abbreviations: FADD, Fas associated death domain; IGFBP3, insulin-like growth factor binding protein 3; IGFBP3-R, insulin-like growth factor binding protein 3 receptor; IGF-I, insulin-like growth factor 1; ISCs, intestinal stem cells; T1D, type 1 diabetes; T2D, type 2 diabetes; TMEM219, transmembrane protein 219.

TABLE 1 Summary of the studies conducted on the IGFBP3/TMEM219 axis

Target molecule	Disease condition	Notes	Reference
IGFBP3-R	Cancer cells	Identification of IGFBP3-R as an apoptotic factor	[3]
IGFBP3-R	Asthma	Blockade of IGFBP3/IGFBP3-R signal is relevant in asthma	[9]
IGFBP3	Various disease	Novel ligands for IGFBP3	[4]
IGFBP3 and TMEM219	Diabetic entheropathy	Dysregulation of the IGFBP3/TMEM219 signal in intestinal disorders	[13]
TMEM219	Lung disease, melanoma	TMEM219 is an alternative binder of IL-13Ralpha2-Chi3l1 complex	[9]
IGFBP3 and IGFBP3-R	Pancreatic ductal adenocarcinoma	Downregulation of IGFBP3/IGFBP3-R in PDAC	[7]
TMEM219	Cancer	TMEM219 is an autophagy activator	[8]
IGFBP3 and IGFBP3-R	Cancer	Anti-tumor and anti-metastatic effect of IGFBP3/TMEM219 signal	[6]
IGFBP3 and TMEM219	Type 1 and type 2 Diabetes	Dysregulation of the IGFBP3/TMEM219 signal in diabetes	[14]

Abbreviations: IGFBP3-R, IGFBP3 receptor

disease conditions may disrupt these compensatory mechanisms and abnormally activate a single path, is a matter of investigation. Here we provide an example of a cell death pathway, the IGFBP3/TMEM219 signaling, which controls the homeostasis of TMEM219-expressing cells in endocrine pancreas and in the gastrointestinal tract, the dysregulation of which is associated with disease conditions such as intestinal diseases, diabetes, and tumorigenesis.

THE IGFBP3/TMEM219 AXIS MEDIATES CELL APOPTOSIS AND DEATH

In 2010 Ingermann and colleagues[3] first described a novel apoptotic pathway, in which the insulin-like growth factor binding protein 3 (IGFBP3) induced cell death in an in vitro model through the binding with a novel receptor, the IGFBP3-R, in an IGF-I independent manner (Table 1). The IGFBP3-R was next recognized as the death receptor TMEM219 (Table 1), which mediates cell apoptosis by activating the Caspase cascade, particularly acting on Caspase 8 and FADD as major downstream signaling partners.[3] The relevance of the IGFBP3 independent proapoptotic action was further explored in other in vitro models, and IGFBP3 stimulation was demonstrated to have a tumor suppressor effect.[4,5] This generated a potential novel therapeutic to block cell proliferation in disease processes.[6,7] Also, TMEM219 has been recently described as an autophagy activator in vitro, thereby acting as a potential regulator in balancing proliferation and cell death.[8] Moreover, TMEM219 was also studied as a potential interactor for the Chi3l1/IL-13Ralpha2 signaling pathway in lung disease (Table 1), in which silencing or absence of TMEM219 were associated with decreased tissue injury and oxidative-induced apoptosis.[9] However, the role of IGFBP3 as natural ligand of TMEM219 has not been explored in this setting, nor it was demonstrated whether triggering TMEM219 with IGFBP3 ligation had any effect in accelerating the disease condition in lung disease or prevented cell proliferation. More importantly, manipulation of abnormal signaling such as IGFBP3/TMEM219, represents an important therapeutic opportunity, regardless of whether it is to block or stimulate it, that merits further investigation.

THE IGFBP3/TMEM219 AXIS IN CANCER

The relevance of the IGFBP3/TMEM219 axis in cancer has been recently and extensively reviewed,[4,6] and it was emphasized that IGFBP3 may exert an anti-proliferative effect through the binding with TMEM219, which in turn may be responsible for an IGFBP3-mediated tumor suppressing action. However, expression of TMEM219 and IGFBP3 was found to be extremely variable in different types of cancer cells originated from various organs and tissues. In analyzing the gene expression pattern of TMEM219, this appeared upregulated in kidney and urinary cancer, in breast and thyroid carcinoma, while it was downregulated in lung and colorectal cancer.[6] This may also suggest that the IGFBP3/TMEM219 signal may represent a therapeutic target in some but not all types of tumors.

THE IGFBP3/TMEM219 AXIS IN INTESTINAL DISEASES

Intestinal diseases have been viewed as multifaceted disorders with multiple and complex etiology and pathogenesis. Abnormal proliferation of epithelial cells has been frequently associated with colorectal disease of malignant origin,[10] while degeneration of the intestinal mucosa has been regarded as major mechanism behind an heterogenous group of intestinal disorders, regardless of whether there is a toxic, immune, inflammatory, infectious cause, in which the integrity of the intestinal barrier appears highly compromised.[11] With the observation that an overlap exists in initial signs and symptoms, a novel physio-pathological perspective is now reconciling both conditions by focusing on the disruption of the intestinal crypts' homeostasis and of their regenerative potential, thus unveiling intestinal stem cells as novel players.[12] Interestingly, alterations in Intestinal Stem cells (ISCs) and in mucosa regeneration have been associated with intestinal diseases that include metabolic disorders, infectious pathologic conditions, and inflammatory mediated diseases.[12] In particular, our group came across a defect that ISCs exhibited in the large intestine of patients with long-standing type 1 diabetes who also suffered of severe gastrointestinal symptoms such as diarrhea, stypsis, abdominal

pain, and fetal incontinence.[13] As diabetes may target all organs and tissues, including vessels, nerves, kidneys, heart, skin and eyes, an effect on the gastrointestinal tract was expected. What was unexpected was the mechanisms whereby the intestinal mucosa was damaged, which relied primarily on a defect in the ISCs existence and function. While hyperglycemia was initially thought to be the major player, an unbiased proteome analysis revealed that IGFBP3 was elevated in the circulation in patients suffering of diabetic enteropathy, and that the IGFBP3 receptor TMEM219 was expressed on ISCs.[13] Indeed, when bound to its ligand a negative signaling was generated through TMEM219 into the target cells, which ultimately led to their apoptosis. This was associated with a loss of ISCs and their regenerative abilities, which in turn facilitated the disruption of the mucosa barrier integrity and then halted the crypts' turnover. As a proof-of-concept of the role that the IGFBP3/TMEM219 axis has in controlling ISCs homeostasis and function, we pharmacologically blocked the TMEM219-mediated signaling with a recombinant protein designed based on the TMEM219 extracellular domain and capable of quenching IGFBP3, and demonstrated a re-establishment of the self-renewal abilities of ISCs in vitro and in vivo.[13] These findings may open a novel path in the field of intestinal disorders, and we suggest to test the relevance of this pathway and its targeting also in other intestinal disease conditions.

THE IGFBP3/TMEM219 AXIS IN DIABETES

Numerous factors play a significant role in mediating beta cell injury and loss in diabetes and they are mainly immune-related when considering type 1 diabetes (T1D), or they are more often associated to metabolic and inflammatory processes in relation to type 2 diabetes (T2D). Despite this, a beta cell toxicity is evident in both T1D and T2D, which may suggest to look at a common path detectable in both types of disease. Our group recently published that the IGFBP3/TMEM219 signaling may be responsible for mediating beta cell loss and dysfunction in both T1D and T2D patients.[14] Indeed, we demonstrated that the signaling is dysregulated in both T1D and T2D conditions, with the circulating ligand IGFBP3 being elevated in pre-diabetes and diabetes, and the death receptor TMEM219 being expressed in human islets and in beta cells.[14] Moreover, blockade of the TMEM219 deleterious signaling within beta cells prevented beta cell death in vitro and restored insulin-related gene expression, while in vivo in murine models of T1D and T2D it was able to prevent diabetes onset and progression.[14] Overall, these findings demonstrate that the manipulation of the IGFBP3/TMEM219 axis aimed at blocking the negative signal delivered to the target cells by using the newly generated ecto-TMEM219 inhibitor, successfully protected the beta cell mass and reduced beta cell death, thereby opening a new therapeutic option in the field. These results also added some new insights to the diabetic community, as targeting apoptotic pathways involving beta cells is an emerging area of research and designing approaches aimed at promoting beta cell survival may also facilitate the development of beta cell replacement strategies.

CONCLUSION

Targeting cell death and apoptotic pathways is emerging as a novel field of application for disease modifying approaches and novel death factor and pathways are being explored. Due to the relevance that these pathways have in controlling tissue homeostasis and cell lifecycle, particular care must be taken when designing a stimulator or an inhibitor. Moreover, the cell target, whether it is an immune cell or a tissue-specific cell, needs to be as selective as possible, given the redundancies of these systems in biological functions and potential off-target effects. Whether the pathway is physiologically active, or it is triggered under certain stimulation/circumstances, including a disease condition, is also of great relevance to understand the result of manipulating such signaling. This ultimately confirms the biological relevance that all these cell death mechanisms have in controlling the regenerative potentials of tissues and organs, which is still a completely unexplored field of investigation in chronic degenerative diseases.

AUTHOR CONTRIBUTIONS

Francesca D'Addio, Laura Montefusco, Ida Pastore, Maria Elena Lunati, Emma Assi, Adriana Petrazzuolo, Virna Marin, and Chiara Bruckmann wrote the paper; Paolo Fiorina wrote and edited the paper.

ACKNOWLEDGMENTS

The authors thank the "Fondazione Romeo e Enrica Invernizzi" for extraordinary support.

Open Access Funding provided by Universita degli Studi di Milano within the CRUI-CARE Agreement.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose. Paolo Fiorina and Francesca D'Addio hold a patent on IGFBP3/TMEM219 axis. Paolo Fiorina and Francesca D'Addio hold equity in Enthera S.r.l. Virna Marin and Chiara Bruckmann are employees of Enthera S.r.l.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Paolo Fiorina https://orcid.org/0000-0002-1093-7724

REFERENCES

1. Christgen, S., Tweedell, R. E., & Kanneganti, T. D. (2022). Programming inflammatory cell death for therapy. *Pharmacology & Therapeutics*, *232*, 108010.
2. Petrazzuolo, A., Perez-Lanzon, M., Martins, I., Liu, P., Kepp, O., Minard-Colin, V., Maiuri, M. C., & Kroemer, G. (2021). Pharmacological inhibitors of anaplastic lymphoma kinase (ALK) induce immunogenic cell death through on-target effects. *Cell Death & Disease*, *12*(8), 713.
3. Ingermann, A. R., Yang, Y. F., Han, J., Mikami, A., Garza, A. E., Mohanraj, L., Fan, L., Idowu, M., Ware, J. L., Kim, H. S., Lee, D. Y., & Oh, Y. (2010). Identification of a novel cell death receptor mediating

IGFBP-3-induced anti-tumor effects in breast and prostate cancer. *Journal of Biological Chemistry*, *285*(39), 30233–30246.
4. Baxter, R. C. (2001). Signalling pathways involved in antiproliferative effects of IGFBP-3: A review. *Molecular Pathology*, *54*(3), 145–148.
5. Oh, Y., Gucev, Z., Ng, L., Muller, H. L., & Rosenfeld, R. G. (1995). Antiproliferative actions of insulin-like growth factor binding protein (IGFBP)-3 in human breast cancer cells. *Progress in Growth Factor Research*, *6*(2-4), 503–512.
6. Cai, Q., Dozmorov, M., & Oh, Y. (2020). IGFBP-3/IGFBP-3 receptor system as an anti-tumor and anti-metastatic signaling in Cancer *Cells*, *9*(5), 1261.
7. Gheysarzadeh, A., Bakhtiari, H., Ansari, A., Emami Razavi, A., Emami, M. H., & Mofid, M. R. (2019). The insulin-like growth factor binding protein-3 and its death receptor in pancreatic ductal adenocarcinoma poor prognosis. *Journal of Cellular Physiology*, *234*(12), 23537–23546.
8. Joyce, S., & Nour, A. M. (2019). Blocking transmembrane219 protein signaling inhibits autophagy and restores normal cell death. *PLoS ONE*, *14*(6), e0218091.
9. Lee, C. M., He, C. H., Nour, A. M., Zhou, Y., Ma, B., Park, J. W., Kim, K. H., Dela Cruz, C., Sharma, L., Nasr, M. L., Modis, Y., Lee, C. G., & Elias, J. A. (2016). IL-13Ralpha2 uses TMEM219 in chitinase 3-like-1-induced signalling and effector responses. *Nature Communications*, *7*, 12752.
10. Kanth, P., & Inadomi, J. M. (2021). Screening and prevention of colorectal cancer. *British Medical Journal*, *374*, n1855.
11. Aziz, I., & Simren, M. (2021). The overlap between irritable bowel syndrome and organic gastrointestinal diseases. *Lancet Gastroenterol Hepatol*, *6*(2), 139–148.
12. D'Addio, F., & Fiorina, P. (2016). Type 1 diabetes and dysfunctional intestinal homeostasis. *Trends in Endocrinology and Metabolism*, *27*(7), 493–503.
13. D'Addio, F., La Rosa, S., Maestroni, A., Jung, P., Orsenigo, E., Ben Nasr, M., Tezza, S., Bassi, R., Finzi, G., Marando, A., Vergani, A., Frego, R., Albarello, L., Andolfo, A., Manuguerra, R., Viale, E., Staudacher, C., Corradi, D., Batlle, E., … Fiorina, P. (2015). Circulating IGF-I and IGFBP3 levels control human colonic stem cell function and are disrupted in diabetic enteropathy. *Cell Stem Cell*, *17*(4), 486–498.
14. D'Addio, F., Maestroni, A., Assi, E., Ben Nasr, M., Amabile, G., Usuelli, V., Loretelli, C., Bertuzzi, F., Antonioli, B., Cardarelli, F., El Essawy, B., Solini, A., Gerling, I. C., Bianchi, C., Becchi, G., Mazzucchelli, S., Corradi, D., Fadini, G. P., Foschi, D., … Fiorina, P. (2022). The IGFBP3/TMEM219 pathway regulates beta cell homeostasis. *Nature Communications*, *13*(1), 684.

How to cite this article: D'Addio, F., Montefusco, L., Lunati, M. E., Pastore, I., Assi, E., Petrazzuolo, A., Marin, V., Bruckmann, C., & Fiorina, P. (2023). Targeting a novel apoptotic pathway in human disease. *BioEssays*, *45*, e2200231. https://doi.org/10.1002/bies.202200231