IRONic Cell Death: Unveiling the Immunogenicity and Efferocytosis of Ferroptosis in Cancer

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IULIIA EFIMOVA

Promotor:

Prof. Dr. Dmitri Krysko, MD, PhD
Ghent University, Belgium

Co-promotor:

Prof. Dr. Andre Skirtach, PhD
Ghent University, Belgium

Examination committee:

Prof. Dr. Anne Vral, PhD (chair)
Ghent University, Belgium

Prof. Dr. Karine Breckpot, PhD
Vrije Universiteit Brussel, Belgium

Em. Prof. Dr. Katharina D'Herde, MD,PhD
Ghent University, Belgium

Prof. Dr. Olivier De Wever, PhD
Ghent University, Belgium

Prof. Dr. Abhishek Garg, PhD
Katholieke Universiteit Leuven,Belgium

Prof. Dr. Oliver Kepp, PhD
the National Institute of Health and Medical Research, France

Dr. Ine Lentacker, PhD
Ghent University, Belgium

Enhancing and educating our body's immune system is undoubtedly one of the paramount objectives in the design of efficient cancer treatments. Cancer immunotherapy stands as a revolutionary approach in combating cancer, leveraging the body's immune system to target and eliminate malignant cells. Central to this strategy is the concept of immunogenic cell death (ICD), where cancer cells succumb to treatment in a manner that triggers an immune response against the cancer. ICD operates through a dual mechanism: firstly, cancer cells are eliminated by various therapeutic modalities, and then, the dying cancer cells release immunostimulatory signals such as damage-associated molecular patterns (DAMPs) and, in combination with tumor association antigens, induce a strong anti-cancer immunity leading to eradication of the cancer and generation of long-lasting immunological memory preventing the cancer relapse. The immunogenic potential of various therapeutic modalities has been demonstrated in tumor therapeutic and vaccination models in mice *in vivo*. Most importantly, ICD-inducing agents and therapies have been already admitted into the clinics and are used for the treatment of cancer in patients.
There is a persistent challenge in cancer therapy being the development of drug resistance and immune evasion mechanisms, allowing cancer cells to withstand various forms of anti-cancer therapy and targeted therapies. While many agents triggering immunogenic apoptosis or necroptosis in cancer cells can stimulate anti-cancer immune responses, yet resistance to these forms of cell death is frequently encountered. As a result, there is a pressing need for alternative therapeutic modalities capable of

overcoming the cell death resistance displayed by cancer cells.

Ferroptosis, among the emerging types of ICD, distinct in both morphology and biochemistry from apoptosis and necroptosis. Ferroptosis leads to lipid peroxidation via abnormal iron metabolism, which can overcome previously mentioned cell death resistance and more effectively induce anti-cancer immunity leading to more potent cancer eradication. At the beginning of the PhD thesis, it was not known whether ferroptotic cancer cells are immunogenic.

In the initial part of this study, our objective was to assess the immunogenic properties of cancer cells undergoing ferroptotic cell death. Our findings reveal that only early (1h and 3h cell death induction) and not late (24h cell death induction) ferroptotic MCA205 cells release DAMPs (ATP and HMGB1) and activate bone marrow derived dendritic cells ($CD11c^+CD86^+$, $CD11c^+CD80^+$, $CD11c^+MHCII^+$, IL-6) *in vitro*. Upon immunization of mice with early ferroptotic cancer cells, we observed efficient anti-tumor protection against the challenge with viable cancer cells in immune-competent mice but not in Rag-$2^{-/-}$ mice suggesting that the mechanism of immunogenicity is tightly regulated by the adaptive immune system and is time dependent. This work demonstrated that cancer cells in the early time-points of ferroptosis are immunogenic *in vitro* and *in vivo*.

In the second part of the PhD thesis, we focused on potential strategies to modulate the interaction of late ferroptotic cancer cells with the macrophages. An approach based on the coating of late ferroptotic cancer cells with biopolymers by the Layer-by-Layer (LbL) was selected method. As a first step, changes in mechanobiological properties of late ferroptotic cancer cells after the coating were measured by atomic force microscopy (AFM) and, in the next step, utilized to gain further understanding of the interaction between ferroptotic cancer cells and macrophages. This work revealed that enhancing the elasticity of late ferroptotic cancer cells through LbL coatings led to increased uptake by macrophages (*i.e.*, efferocytosis). The findings presented in this study highlight the significance of mechanobiological characteristics of dying cancer cells in the process of efferocytosis. Furthermore, these results offer promising prospects for nanomaterial-based therapeutic applications to increase the efferocytosis of dying cancer cells and thereby improve their adjuvanticity and anti-cancer immunity.

Overall, our work opens new avenues for creating cancer treatment modalities that focus on triggering ferroptosis. Moreover, the work presented in the second part of the PhD thesis highlights the importance of the mechanobiology of ferroptotic cancer cells in control of their efferocytosis by macrophages This insight not only expands our understanding of ICD but can be leveraged to create new therapeutic approaches for conditions where altering efferocytosis could be advantageous, as well as in the development of drug delivery mechanisms for cancer treatment.

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IULIIA EFIMOVA

2018-2024 PhD candidate, UGent (Belgium)

2016-2018 Master in Cellular and Developmental Biology, Charles University (Prague, Czechia)

2013-2016 Bachelor in Molecular Biology and Biochemistry Organisms, Charles University (Prague, Czechia)

Department of Human Structure and Repair

Research unit of Anatomy and Embryology

Research laboratory of Cell Death Investigation and Therapy

Publications:
Peer-reviewed:13 First author: 3
https://biblio.ugent.be/person/D7982FEC-D0C7-11E8-97EF-09125707D3EF

iuliia.efimova@ugent.be
T +3293320383

Image: a fragment from the 'Garden of Earthly Delights' by Hieronymus Bosch

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