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Review

Immunogenic cell death, DAMPs and anticancer therapeutics: An emerging amalgamation

Abhishek D. Garg [a], Dominika Nowis [b], Jakub Golab [b], Peter Vandenabeele [c,d], Dmitri V. Krysko [c,d,*], Patrizia Agostinis [a,*]

[a] Department of Molecular Cell Biology, Catholic University of Leuven, Belgium
[b] Department of Immunology, Center of Biostructure, Medical University of Warsaw, Poland
[c] Molecular Signalling and Cell Death Unit, Department for Molecular Biomedical Research, VIB, Belgium
[d] Department of Molecular Biology, Ghent University, Belgium

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ABSTRACT

Immunogenic profile of certain cancer cell death mechanisms has been transmuted by research published over a period of last few years and this change has been so drastic that a new (sub)class of apoptotic cancer cell death, redefined as 'immunogenic apoptosis' has started taking shape. In fact, it has been shown that this chemotherapeutic agent-specific immunogenic cancer cell death modality has the capabilities to induce 'anticancer vaccine effect', *in vivo*. These new trends have given an opportunity to combine tumour cell kill and antitumour immunity within a single paradigm, a sort of 'holy grail' of anticancer therapeutics. At the molecular level, it has been shown that the immunological silhouette of these cell death pathways is defined by a set of molecules called 'damage-associated molecular patterns (DAMPs)'. Various intracellular molecules like calreticulin (CRT), heat-shock proteins (HSPs), high-mobility group box-1 (HMGB1) protein, have been shown to be DAMPs exposed/secreted in a stress agent/factor-and cell death-specific manner. These discoveries have motivated further research into discovery of new DAMPs, new pathways for their exposure/secretion, search for new agents capable of inducing immunogenic cell death and urge to solve currently present problems with this paradigm. We anticipate that this emerging amalgamation of DAMPs, immunogenic cell death and anticancer therapeutics may be the key towards squelching cancer-related mortalities, in near future.

Contents

Abbreviations: APCs, Antigen-presenting Cells; ATP, Adenosine Triphosphate; CD, Cluster of Differentiation; CRT, Calreticulin; DAMP, Damage-associated Molecular Patterns; DC/DCs, Dendritic cell(s); DT-EGF, Epidermal Growth Factor Receptor-targeted Diphtheria Toxin; eIF2α, Eukaryotic Initiation Factor 2α; ER, Endoplasmic Reticulum; HMGB1, High-Mobility Group Box-1; HSP, Heat Shock Protein(s); IFN, Interferon; IL, Interleukin; LPS, Lipopolysaccharide; MAPK, Mitogen-activated Protein Kinase; MHC, Major Histocompatibility Complex; NFκB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; NK cells, Natural Killer Cells; PAMP, Pathogen-associated Molecular Patterns; PDT, Photodynamic Therapy; PERK, PKR-like ER kinase; PKR, Protein kinase R; PS, Phosphatidylserine; ROS, Reactive Oxygen Species; TAA, Tumour-associated Antigen(s); TGF, Transforming Growth Factor; TLR, Toll-like Receptor(s); TNF, Tumour Necrosis Factor; UPR, Unfolded Protein Response; UV, Ultra-violet (rays)

* Corresponding authors. P. Agostinis is to be contacted at Department of Molecular Cell Biology, Faculty of Medicine, Catholic University of Leuven, Campus Gasthuisberg O&N1, Herestraat 49, B-3000 Leuven, Belgium. Tel.: +32 16 345715. D.V. Krysko, Molecular Signalling and Cell Death Unit, Department for Molecular Biomedical Research (DMBR), VIB, Ghent University, Technologiepark 927, B-9052 Ghent (Zwijnaarde), Belgium. Tel.: +32 9 331 37 64.
E-mail addresses: dmitri.krysko@ugent.be (D.V. Krysko), patrizia.agostinis@med.kuleuven.be (P. Agostinis).

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1. Immunogenic cell death in anticancer therapy

Resistance to cell death and the ability to prevaricate immunological surveillance are two of the most malicious strategies within the defence arsenal of tumour cells [8,9]. Thus, over a long period of time, the strategies that have been anticipated to be capable of inflicting maximal damage upon the cancer/tumour cells have consisted of either increasing the cancer cell's susceptibility towards death or increasing/refurbishing of the immunological recognition of poorly immunogenic cancer cells [10]. A further more attractive ploy that holds the highest therapeutic value is the one that combines both of these above as it would not only ensure complete or extensive obliteration of cancerous cells but also provide a potentially firm barrier to cancer's recurrence habits. Paradigms based upon this ploy have the potential to become practically applicable if therapeutic strategies capable of inducing immunogenic cell death are explored.

In recent times, the concept of immunogenic cell death has been addressed by many extensive studies [2,12–14] and the paradigms based upon this concept have come closer to applicability in real-time than it was previously anticipated. These latest trends have not only changed 'classical' immunological profiles of certain cell death pathways but have also revealed that a subset of DAMPs are responsible for immunogenicity of these cell death modalities. These recent developments have made DAMPs an important set of molecules to focus upon as far as immunogenic cancer cell death is concerned.

Box 1. Cells of the innate and adaptive immune system

Development of an effective antitumour immune response depends on coordinated interactions between various populations of innate and adaptive immune cells [1] briefly described below.

1. **Innate immunity** mechanisms involved in tumour elimination are orchestrated by a vast array of immune cells, of which, natural killer (NK) cells, NKT cells, γδ-T cells, macrophages, granulocytes and dendritic cells (DCs) seem to be the most important ones.

1.1. **Antigen-presenting cells (APCs)**—APCs like DCs, macrophages and B cells play a crucial role in antigen presentation [7]. Their maturation after encounter of various "danger signals" initiates a series of processes leading to activation of antigen-specific T cell response.

1.2. **NK cells**—Activation of these cells is usually triggered by disappearance of class I MHC molecules from the surface of tumour cells or by exposure to antigens such as MHC class-I related molecules, MIC A and MIC B, which are NKG2D ligands. Their expression is triggered by DNA damage in tumour cells [16].

1.2.1. **NKT cells**—These cells express an invariant T cell receptor alpha chain that recognizes glycolipid antigens (e.g. gangliosides) presented by CD1d molecules on the surface of tumour cells following which, NKT cells exert their antitumour effects primarily through secretion of interferon γ and direct cytotoxicity [16–18].

1.3. **γδ-T cells**—These cells are considered to be the most important early source of IFN-γ and they mainly recognize heat-shock proteins (HSPs), MIC A and MIC B or phosphoantigens and kill tumour cells through direct cytotoxicity [16,19].

1.4. **Macrophages and neutrophils**—These are the most important subsets of phagocytes and might be activated directly by stress products of cancer cells through TLR (Toll-like receptor) signalling. Here, secretion of pro-inflammatory cytokines and extensive production of reactive oxygen as well as nitrogen species, might serve in the protective mechanisms.

2. **Adaptive antitumour effector mechanisms** include the activity of several populations of T cells and antibody production by B cells. Here, antitumour antibodies execute cancer cell death either through direct interplay with vital intracellular signalling pathways or through induction of complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). The latter (i.e. ADCC) involves cytotoxic activity of NK cells, macrophages, neutrophils and eosinophils, triggered by activation of receptors for the constant region of the antibody (FcR).

2.1. **Cytotoxic T cells (CTLs) or $CD8^+$ T cells**—CTLs are very potent professional killers; a single activated CTL can eliminate hundreds of target tumour cells. Once activated by mature DCs-based antigen presentation, they recognize and eliminate tumour cells bearing the particular antigen via secretion of monomeric perforin (when polymerizes, forms tiny holes in the plasma membrane allowing free water and ions flow), granzyme B (a serine protease that activates both intrinsic and extrinsic pathways of apoptosis induction) and activation of membrane death receptors (Fas/CD95, TNF-RI, DR4 and DR5 via FasL, TNF and TRAIL, respectively).

2.2. **Helper T cells or $CD4^+$ T cells**—They are the major conductors and orchestrators of the adaptive immune response. Through cytokine secretion they stimulate proliferation and enhance activation of $CD8^+$ T cells and contribute to DCs maturation. Moreover, $CD4^+$ cells are also indispensable for B cell activation and antibodies class switching [9,19].

In the present review, the 'classical' as well as the 'emerging' immunological silhouette of necrosis, immunogenic apoptosis and 'autophagic' cell death will be discussed along with specific attention paid to certain particular DAMPs (confirmed or proposed to be) associated with them. Also, the various resistance tactics employed by cancer cells along with a discussion on envisaged trends in the development of anticancer therapy based on 'immunogenic cancer cell death' concept, will be discussed.

1.1. Cell death pathways: 'classical' and 'new' immunological profiles

In 'classical' terms, the immunological profile of at least apoptotic and necrotic cell death mechanisms is considered to be largely 'straight-forward'.