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Anticancer metal drugs and immunogenic cell death☆

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ABSTRACT

Conventional chemotherapeutics, but also innovative precision anticancer compounds, are commonly perceived to target primarily the cancer cell compartment. However, recently it was discovered that some of these compounds can also exert immunomodulatory activities which might be exploited to synergistically enhance their anticancer effects. One specific phenomenon of the interplay between chemotherapy and the anticancer immune response is the so-called "immunogenic cell death" (ICD). ICD was discovered based on a vaccination effect exerted by cancer cells dying from pretreatment with certain chemotherapeutics, termed ICD inducers, in syngeneic transplantation mouse models. Interestingly, only a minority of drugs is able to trigger ICD without a clear-cut relation to chemical structures or their primary modes-of-action. Nevertheless, generation of reactive oxygen species (ROS) and induction of endoplasmic reticulum (ER) stress are clearly linked to ICD. With regard to metal drugs, oxaliplatin but not cisplatin is considered a bona fide ICD inducer. Taken into account that several experimental metal compounds are efficient ROS and ER stress mediators, presence of potent ICD inducers within the plethora of novel metal complexes seems feasible and has occasionally been reported. In the light of recent successes in cancer immunotherapy, here we review existing literature regarding anticancer metal drugs and ICD induction. We recommend a more profound investigation of the immunogenic features of experimental anticancer metal drugs.

1. Introduction on metal drugs and immune aspects

Conventional therapeutic approaches for cancer are primarily aimed at eradicating neoplastic cells by cytostatic and cytotoxic effects. Based on this assumption, classical anticancer agents, including anthracyclines, antimetabolites, and platinum drugs, were mainly developed based on their ability to kill preferentially cancer cells due to their elevated proliferation rate [1]. However, facilitated by the breathtaking developments in high-throughput sequencing methods, the knowledge on genetic drivers of malignant phenotypes has also changed the landscape of systemic cancer therapy. In 2001, the approval of the ABL kinase inhibitor imatinib (also known as Gleevec/Glivec; Novartis) for the treatment of Philadelphia chromosome- and, hence, BCR-ABL kinase translocation-positive chronic myelogenous leukemia (CML) [2] paved the way for the so-called "targeted" anticancer therapy. Since then, multiple novel anticancer compounds directly targeting malignant driver alterations in cancer have been clinically approved including respective inhibitors of BRAF-mutated melanoma and EGFR-mutated lung cancer [3,4]. Although impressive responses have been achieved, acquired therapy resistance of malignant cell subclones based on tumor heterogeneity and genomic instability is still a major hurdle for successful cancer cure also in times of precision oncology [5,6].

Importantly, during the past two decades, there was a change in perspective regarding cancer biology and, hence, oncological therapy toward a more integrative view considering also aspects of the cancer microenvironment. Accordingly, the famous six hallmarks of cancer (limitless proliferative potential; self-sufficiency in growth signals; insensitivity to antigrowth signals; evasion of apoptosis; sustained angiogenesis; tissue invasion and metastasis), postulated by Hanahan and Weinberg in their millennium review 2000 [1], were recently "updated" by the same authors with four additional features: i) altered metabolism, ii) escape from immuno-surveillance, iii) chromosomal defects and genetic instability and iv) inflammation [7]. On top of that, the important contributions of the cancer microenvironment to both malignant transformation and progression have been elucidated including soluble factors, components of the extracellular matrix, as well as stromal, endothelial and immune cells [8]. In terms of therapy, targeting

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the cellular components of the cancer microenvironment including endothelial cells (neoangiogenesis), tumor-associated fibroblasts or immune cells, is highly attractive, especially as these cells are – though deregulated in response to paracrine signals from the tumor – still believed to be genomically widely stable.

The increased likelihood of neoplasia in patients harboring immunodeficiency, based either on immune diseases or as a consequence of pharmacological immunosuppression, suggests that early (pre)malignant cells are well recognized by the immune system and readily eradicated (immunological surveillance) [9]. This implicates that cancer at the time of diagnosis has already passed the phase of "immune elimination", developed various strategies for "immune escape" and eventually might even be driven by immunological processes like inflammation [10]. The underlying molecular mechanisms include multiple alterations on the side of the malignant or the immune cells and consequent changes in the cancer microenvironment. Strong evidence has been delivered that cancer cells might either hide from being recognized by the immune detection machinery ("immunoevasion") or directly suppress immune cell functions to avoid immune-mediated cell death ("immunosubversion") [10]. Accordingly, the application of immune checkpoint-inhibiting antibodies is currently revolutionizing systemic cancer therapy in several if not all cancer types [11,12].

While at earlier days, based on the well-known chemotherapy-induced lymphopenia, it was taken for granted that chemotherapy would lead to immune suppression, nowadays multiple lines of evidence suggest even the opposite. Hence, it was shown that chemotherapy-induced short-term lymphodepletion might preferentially eradicate immunosuppressive compartments like myeloid-derived suppressor cells (MDSC) and regulatory T cells (T_{reg}) [13,14]. Additionally, numerous classical anticancer agents have the capability to directly stimulate certain aspects of the innate and acquired immune system [15]. Accordingly, the apoptotic cell death mediated by anticancer drugs was originally assumed to be non-inflammatory and non-immunogenic. However, this must be revised for tumors that grow in immunocompetent hosts where distinct apoptotic programs may also involve immune responses [8,16–18]. Hence, chemotherapy might reverse important aspects of immunoevasion and immunosubversion. Moreover, these findings demonstrate that the clear-cut categorization into chemotherapy, targeted therapy and immunotherapy might be far too narrow.

While the interactions between chemotherapy and anticancer immune responses have been extensively reviewed [8,19,20], a focused overview on the immune impacts of the diverse clinically used and experimental anticancer metal drugs is so far missing. Nevertheless, several findings implicate a complex interplay between classical (cisplatin, oxaliplatin, arsenic trioxide) but also novel metal complexes and the anticancer immune response. These effects may involve direct activating/inactivating impacts on immune effectors (natural killer cells, cytotoxic T cells, T_{reg}, dendritic cells), cancer cell immune recognition (MHC class I expression, immune checkpoint molecules) as well as sensitivity against immune cell-mediated cell death (mannose-6-phosphate receptor, FAS, caspases) [21,22]. One important aspect of reversion of immunoevasion is the re-induction of immune recognition of cancer cells by exposure of "find me" and "eat me" signals to phagocytic cells of the immune system, leading to the so-called immunogenic cell death (ICD). In this short review we focus on the role of this specific chemo-immunogenic form of cell death in the anticancer activity of metal compounds.

2. Immunogenic Cell Death, ICD

Physiological cell death and the related "programmed cell removal" occur as a continuous byproduct of cellular turnover. Under healthy conditions this process is immunologically "silent" and involves engulfment of the apoptotic cells by phagocytes to avoid release of intracellular components and consequent activation of inflammation and autoimmune reactions [23]. In contrast, "immunogenic cell death" in cancer therapy refers to an immunostimulatory cell death modality which involves 1) changes in the tumor cell surface promoting immune reactions, 2) release of soluble mediators into the tumor microenvironment which operate on receptors expressed by professional antigen-presenting cells (APC), and 3) in turn presentation of tumor-associated antigens, leading to T cell activation and proliferation eventually culminating in eradication of the tumor [24]. This model was first proposed in 2005 by Kroemer and his collaborators in the context of anticancer chemotherapy, based on the evidence that murine colon cancer cells dying from in vitro treatment with the anthracycline doxorubicin were able to elicit an effective antitumor vaccination response that suppressed the growth of inoculated tumors or led to the regression of established neoplasia [25].

Molecularly, ICD is characterized by induction of at least four distinct damage-associated molecular patterns (DAMP): i) exposure of calreticulin (CRT) on the outer surface of the plasma membrane [26], ii) secretion of adenosine-5′-triphosphate (ATP) [27], iii) the release of the non-histone chromatin-binding protein high-mobility group box 1 (HMGB1) [16] and iv) the production of type I and II interferons (IFN) by malignant cells or immune effectors [24,28,29] (Fig. 1). These essential features might be supported and augmented by other molecular processes like exposure of additional endoplasmic reticulum (ER) chaperons on the cancer cell surface, including heat-shock protein (HSP)70 and HSP90, as well as secretion of several immunostimulatory cytokines like interleukin (IL)-1β and IL-17 by distinct immune cell types involved.

In more detail, ICD-mediating compounds induce, as a consequence of unfolded protein response (UPR), ER stress at the pre-apoptotic stage with activation of the ER-resident kinase PERK and phosphorylation of the translation initiation factor eIF2α to impede global protein translation [30]. This leads, via a complex mechanism involving partial caspase 8 activation, to relocation of the ER-resident chaperon protein CRT and eventually other chaperons to the outer cell membrane ("eat me" signal) by an exocytotic process [31]. CRT binds to its transmembrane receptor CD91 (also known as LRP1) on immature DC and macrophages, essential APC of innate immunity. Additionally, during the process of apoptosis there occurs an autophagy-dependent release of ATP ("find me" signal) binding to the P2RX7 receptor on DC, hence inducing an inflammasome-mediated secretion of IL-1β [30]. Finally, at the later stage of cell destruction, also HMGB1 protein is released into the extracellular space ("danger" signal) as a consequence of membrane permeabilization. Its binding to toll-like receptor 4 (TLR4) activates myeloid differentiation primary response gene 88 (MyD88) which is essential for efficient processing of the phagocytic cargo for antigen presentation [17,32]. Together, these factors drive a stepwise process of DC recruitment to tumor cells, phagocytosis, antigen processing, maturation, and antigen presentation to T cells. Finally, the cascade results in an IFN-γ-mediated immune response involving γδ T cells and cytotoxic CD8 + T lymphocytes (CTL) [24,28]. Additionally, an autocrine TLR3-dependent IFN type I signaling circuit in cancer cells leading to excretion of CXCL10 was recently identified as an essential player in ICD [29].

Interestingly, besides external CRT also external phosphatidylserine (PS) represents a strong "eat me" signal, with the difference that PS exposure is delayed as compared to CRT and induces the removal of apoptotic corpses without activating any immune response or acting even as tolerogenic factor [26]. Additionally, many cancers can avoid the ICD-specific DAMP response via evasion of phagocytosis through up-regulation of the surface protein CD47, which serves as a "don't eat me" signal, counteracting exposed CRT. CD47 inhibits phagocytosis through binding to its receptor signal regulatory protein α (SIRPα) on phagocytic cells [33]. The balance between CRT and CD47 seems of pivotal importance in determining the chemo-immune response of a tumor and should be taken into account when considering ICD as the major mode-of-action of an experimental anticancer drug.

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