Tumor Ablation
Tumor Ablation

Effects on Systemic and Local Anti-Tumor Immunity and on Other Tumor-Microenvironment Interactions
Preface

In recent years more data emerged on the role of intratumoral immune components and of other functions of the tumor microenvironment in the development or destruction of tumors.

Tumor immunology and anti-tumor immunity started with the discovery that microbial agents can trigger immune responses, which will cause tumor regression. Over 120 years ago it was recognized that the immune response is involved in the development of cancer. Cancer immunotherapy research started with general immunostimulation (microbial products), before the role of danger signals was understood. An example for the power of microbial products such as BCG to induce anti tumor effects is the treatment of TCC by BCG. The discovery of tumor-associated antigens (TAA) (1950) boosted the hope that efficient anti-tumor vaccines will be developed, yet, in spite of the knowledge about TAA the identity of many such molecules is still obscure.

Today we have a more profound understanding of the function and intercellular interactions of immune cells. Anti tumor immunity requires the presence of peptidic TAA, presented by the MHC class I and II glycoproteins, the involvement of antigen presenting cells and cross presentation mechanisms to trigger helper and cytotoxic T lymphocytes, and danger signals for proper activation of APC, and expression of costimulatory molecules. We also understand better the role of suppressor cells such as T regulatory and myeloid derived suppressor cells (MDSC) in anti tumor immunity.

Thus, it is required to properly expose TAA to the cell-mediated immune response and boost the response with strong adjuvants, which facilitate the recognition of TAA, and stimulate cytokine production by antigen presenting cells and T helper cells. It is also imperative that the tumor specific antigens will be processed and presented on the tumor cells otherwise the CTL we induce will not attack these cells. In spite of our immunological knowledge no better tumor vaccines are available today. Although durable clinical remissions have been observed with various immunotherapeutic strategies, the percentage of patients who benefited from these interventions has remained too small to justify the general use of such strategies. An exception to the failure of immunotherapy is the use of passive transfer monoclonal antibodies, mainly against haematological malignancies, and the use of IL-2 to propagate lymphocytes,
naturally exposed to the tumor antigens, for adoptive cell transfer, as used in the treatment of melanoma.

In view of the lack of efficient tumor vaccines due either to unknown TAA or weak responses, it is the aim of this book to present an alternative to the conventional approach of developing injected tumor vaccines to activate anti-tumor immunity, which will fight primary and metastatic cancer.

It is argued that *in situ* tumor ablation (destruction) can involve tumor antigen release, cross presentation and the release of DAMPS and make the tumor its own cellular vaccine.

Tumor tissue destruction by *in situ* ablation may stimulate antigen-specific cellular immunity engendered by an inflammatory milieu. Dendritic cells (DCs) attracted to this microenvironment, will undergo maturation after internalizing apoptotic and necrotic cellular debris. Mature DCs can mediate antigen-specific cellular immunity via presentation of processed antigens to T cells. Different therapeutic modalities including chemotherapy, radiotherapy, and surgery, release tumour-associated antigens in the context of damage associated molecular pattern (DAMP) and boost cross-presentation and the manifestation of anti-tumor immunity.

Tumor ablation by thermal, chemical and radiological sources has received substantial attention for the treatment of many localized malignancies. The primary goal of most ablation procedures is to eradicate all viable malignant cells within a designated target volume through the application of energy or chemicals. Methods such as chemical ablation, radiotherapy, photodynamic therapy, cryoablation, high-temperature ablation (radiofrequency, microwave, laser, and ultrasound), and electric-based ablation have been developed for focal malignancies. Chemotherapy using cytotoxic drugs is mostly used as a systemic treatment but the nature of its action is *in situ* ablation of tumor cells at the primary tumor site and of distant metastatic foci.

In this book experts in their field will describe the various ablation modalities and review and discuss the literature pertinent to their effects on the tumor cells, the tumor microenvironment and the stimulation of specific anti-tumor immunity.

**We trust that this book will:**

1. Familiarize the readers with various methods of *in situ* tumor ablation.
2. Review the literature and stimulate comparisons on the efficacy of different ablation methods for the treatment of tumors of different histotypes.
3. Review the literature on the effects of various ablation methods on systemic and local anti tumor immunity and on other manifestations of the interactions of tumors with their microenvironment.
4. Stimulate comparative studies on the immunostimulatory effects of different ablation modalities.

Cytotoxic chemotherapy remains the principal treatment modality for advanced cancer, and an important component of multimodality therapy for earlier disease. Drs. Meniawy, Nowak and Lake review in Chap. 1 the evidence that certain conventional chemotherapeutic drugs cause cell death that can elicit a specific antitumor immune response, and discuss the characteristics of chemotherapy—induced cell death and
the properties which determine immunogenicity and the triggering of ‘danger signals’. Chemotherapy can also exert other immune modulatory effects on a number of immune cells. They argue, “The assumption that anti-cancer therapies that lead to tumor cell death are always immunosuppressive, or even a null event, is no longer valid”. The authors emphasize that understanding of the interactions between cytotoxic therapies and the immune system and the tumor microenvironment is crucial for the rational development of combination treatments of immunotherapy with conventional or targeted therapies to achieve a synergistic antitumor effect and improved treatment outcomes.

Elevated temperatures produce a wide range of effects in tumor bearing hosts and have been used in cancer therapy. In hyperthermia treatment of cancer, temperatures are raised artificially either in the whole body or locally in tumors using heated chambers, hot water or wax bath heating of tumors or input of energy by microwaves, radiofrequency heating or ultrasound. Hyperthermia treatment of cancer at 42–47 °C (Hyperthermia range) results in cell death mainly due to protein denaturation, and may be counteracted by cellular repair mechanisms mediated by heat shock proteins. At temperatures above 50 °C cell necrosis and tissue coagulation are observed (Ablation range). Data from animal models and human patients, which is summarized by Dr. Calderwood in Chap. 2, indicate that whole body and locoregional hyperthermia exerts many biological and therapeutic effects on immune competent cells and cytokines, and the immune effects may depend on the type of treatment. Hyperthermia range heating may lead to profound levels of apoptosis and its role in immunity is somewhat ambiguous. In broad terms, apoptotic cell death is tolerogenic and absorption of apoptotic cell bodies by immune cells inhibits immunity. However, in the ablation range, cancer cell necrosis dominates and tumor specific immunity is observed, an effect that may play an important role in the outcome of treatment.

Radio-Frequency Ablation (RFA) is a minimally invasive technique, which uses electromagnetic energy sources to generate heat. RFA is used as standard local therapy of primary and metastatic liver tumors. Electrode probes are placed within tumors deliver electromagnetic waves that cause ionic agitation and friction that locally generates heat. Tissue injury following this procedure can be subdivided in two distinct phenomena, resulting in direct and indirect effects. RFA destroys tumoral tissue generating a local necrosis followed by marked inflammatory response with a dense T-cell infiltrate. Drs. Nierkens, den Brok, Ruers, and Adema indicate in Chap. 3 that the release of tumor antigens by ablation and the inflammatory environment may contribute to stimulate innate and adaptive anti-tumor immunity. The released antigens can be processed and presented by dendritic cells for cytotoxic T lymphocyte activation. Immune stimulatory approaches that increase antigen presentation and induction of anti-tumor T cell reactivity should be engaged to prevent local recurrences and to induce long-term systemic protection against residual disease.

High intensity focused ultrasound (HIFU) is another emerging non-invasive treatment modality for localized treatment of cancers with high temperatures. Dr. Wu in Chap. 4 summarizes the history of HIFU, its effect on the tumor microenvironment and preclinical and clinical evidence suggesting the activation of anti tumor immunity. The author also discusses potential mechanisms and roles of the immune response in terms of local recurrence and metastasis control after HIFU treatment.
Cryoablation involves the use of freezing temperatures to kill cells and destroy tissue. Dr. Sabel reviews in Chap. 5 the history of cryosurgery for the treatment of cancer, details the mechanisms by which cryoablation leads to cancer cell death, and how this can be altered by variations in cryosurgical technique. Observations indicated that distant, un-treated sites of disease began to regress after cryoablation; yet, several studies reported immunosuppression following cryoablation. The author discusses the relationship between different cryoablation settings, cryoablation-induced cell death and the induction of both stimulatory and suppressive immune responses.

The delivery of external beam radiation therapy (RT) for cancer with intent to cure has been optimized and standardized over the last 80 years. Drs. McBride and Schaue review in Chap. 6 the radiobiological principles that underlie the success of classical RT treatments, which mostly aim to preserve normal tissue function while curing cancer. The development of more precise dose delivery techniques, accompanied by improved computing and imaging capabilities, offer certain improvements to what can be achieved with conventional RT, and enable the delivery of very high doses given in a small number of fractions.

Ionizing radiation exhibits immunomodulatory properties, which could portend a future collaboration of cancer immunotherapy with radiation therapy. Radiation has been utilized to create inflammation, and upregulate expression of immunomodulatory surface molecules and secretory molecules in the tumor, and its microenvironment. The authors discuss the advantages high doses or high dose fractions may have over conventional RT. They postulate that higher than conventional dose fractions might promote more tumor microvasculature damage and pro-inflammatory and pro-oxidant responses that may enhance “danger” signaling in tissues and promote RT-induced anti-tumor immune responses. These issues are still open for investigation but the authors predict that optimization of RT within these contexts could enhance the effects of RT both in terms of local control and in control of distant micrometastatic disease.

Photodynamic therapy (PDT) uses non-toxic photosensitizers and light in combination with oxygen to produce cytotoxic reactive oxygen species that kill malignant cells, and damage the tumor microvasculature and create rapid dramatic changes in tumor microenvironment. PDT destroys the structure of a tumor, thereby enabling direct interaction between immune cells and tumor cells, which can initiate a systemic anti-tumor immune response. Dr. Korbelik describes in Chap. 7 the PDT induced inflammation following cell death, debris elimination and resolution of the inflammation. Even more importantly for therapy outcome, the presence of an overwhelming number of dead cancer cells can overcome the capacity of sequestered professional phagocytes to remove cellular corpses fast enough to avoid breaking immune tolerance and can lead to the development of adaptive immune response against PDT-treated tumor.

Electric-based cancer ablation was developed for in situ ablation of solid tumors. The electrical parameters used for treatment range from several volts per cm delivered for a long time period, to very high electric fields (up to 300 kV/cm). The treatment can be delivered as a continuous treatment or pulses. These treatments are either based on electro-stimulation alone or in conjunction with chemotherapeutic drugs.
In Chap. 8 Drs. Keisari and Korenstein summarize data on the effect of various types of electric ablation of cancer, in various metastatic tumors in mice, and in clinical trials. They discuss the role of electric ablation in changes that occur in the tumor microenvironment, infiltration of immune cells into the tumor and induction of anti-tumor immunity. Special focus is given to the role of these responses in the elimination of residual metastatic cells, and the possible enforcement of such anti-tumor reactions by various immunostimulators.

I deeply thank and express my appreciation to the contributing authors who took upon themselves the complexed task of writing a professional review for both experts in cancer ablation and for experts involved in tumor microenvironment and immunology research.

Yona Keisari
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Chapter 1
Effect of Chemotherapy on the Tumor Microenvironment and Anti-tumor Immunity

Tarek M. Meniawy, Anna K. Nowak and Richard A. Lake

Abstract An accumulating body of evidence demonstrates that conventional chemotherapy and targeted therapies result in cell death that can elicit an antitumor immune response. A number of distinct biochemical properties of chemotherapy-induced cell death have an important role in determining its immunogenicity by triggering ‘danger signals’ that can elicit a specific antitumor immune response. Chemotherapy can also exert other immune modulatory effects on a number of immune cells including dendritic cells, myeloid-derived suppressor cells, CD8⁺ T cells, and regulatory T cells. An understanding of the interactions between cytotoxic therapies and the immune system and the tumor microenvironment is crucial for the rational development of combination treatments of immunotherapy with conventional or targeted therapies to achieve a synergistic antitumor effect and improved treatment outcomes.

Keywords Antigens · Antigen-presenting cells · Antineoplastic agents · Antitumor immunity · Cancer vaccines · CD8⁺ T-lymphocytes · Cell death · Chemoimmunotherapy · Chemotherapy · Cross-priming · Dendritic cells · Immunotherapy · Neoplasms · Drug therapy · Targeted therapies · Danger signals · Apoptosis · Necrosis · Autophagy · Immunogenicity · Immunosuppression · DAMPs · Danger associated molecular patterns

T. M. Meniawy (✉) · A. K. Nowak · R. A. Lake
School of Medicine and Pharmacology, University of Western Australia
Sir Charles Gairdner Hospital, 4th Floor G Block, Nedlands WA 6009, Australia
e-mail: meniat01@student.uwa.edu.au; anna.nowak@uwa.edu.au; richard.lake@uwa.edu.au

T. M. Meniawy · A. K. Nowak
Department of Medical Oncology, Sir Charles Gairdner Hospital,
Perth, WA, Australia

T. M. Meniawy · A. K. Nowak · R. A. Lake
National Centre for Asbestos Related Diseases, Perth, WA, Australia

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1.1 Introduction

Cytotoxic chemotherapy remains the principal treatment modality for advanced cancer, and an important component of multimodality therapy for earlier disease. However, despite significant advances in the past decade, it remains palliative rather than curative for the majority of patients with metastatic solid tumors.

Targeted therapies are a rapidly growing class of anti-cancer treatments that employ a more selective activity against one or several receptors or pathways that cancers may be dependent upon for development, proliferation and survival. This has led to significant advances in the treatment of several malignancies, especially those where a specific oncogene or biomarker has been identified, allowing a personalized treatment approach that matches a particular therapy to patients with disease which is more likely to respond.

Whilst chemotherapy and targeted therapies are usually palliative, with resistance emerging with ongoing use, immunotherapy is a therapeutic strategy which can produce durable remissions, albeit rarely. Nevertheless, most patients do not respond to single-agent immunotherapy, or respond to a modest degree only. Increasingly, investigators are seeking to combine immunotherapy with other standard therapies, such as cytotoxic chemotherapy and targeted therapies. Whilst there is now substantial pre-clinical and emerging clinical evidence to support such combination, many gaps remain in our understanding of the biological events involved, and the optimal clinical use of immunotherapy combinations. This chapter will address our current understanding of how chemotherapy and targeted therapies interact with the immune response and the tumor microenvironment.

1.2 Mechanisms of Cell Death Induced by Anti-cancer Therapy

The development and progression of cancer is not only a result of a series of genetic alterations within individual tumor cells, but is also dependent on the ability of malignant cells to escape physiological barriers to tumorigenesis. The complex tumor microenvironment is a result of significant changes to the host stromal, inflammatory, endothelial and immune cells that allow a tumor cell to escape death pathways and immune destruction [1, 2].

The ability to evade apoptosis is one of the hallmarks of cancer [3], but other non-apoptotic pathways have been described including necrosis, senescence, autophagy and mitotic catastrophe [4]. The mechanism of cell death and associated biochemical events caused by anti-cancer therapies has important sequelae for the interaction of these dying cells with the local tumor environment and with the immune system, and may set the tone of the subsequent immune response.

1.2.1 Apoptosis

The most common form of cell death resulting from anti-cancer therapy is apoptosis, a form of programmed cell death or ‘cell suicide’ with morphological features first
described in 1972 by Kerr et al. [5]. Billions of cells undergo this form of physiological, ‘scheduled’ cell death daily as part of a sophisticated mechanism to remove damaged cells or cells that are no longer needed. Apoptosis is typically mediated by the caspase family of cysteine proteases including initiator caspases (such as caspase-8 and caspase-9), with subsequent activation of effector caspases (such as caspase-3, -6 and 7) [6]. The result is a series of morphological changes that include chromatin condensation, nuclear fragmentation, blebbing of the cell membrane and finally fragmentation of the cytoplasm to form apoptotic bodies [4].

The term ‘extrinsic apoptosis’ describes a process whereby apoptotic cell death is initiated by extracellular stress signals via specific transmembrane receptors, such as the death receptor FAS (also called Apo-1 or CD95) and other members of the tumor necrosis factor receptor (TNFR) superfamily [7, 8]. FAS ligation is a prototypic signalling pathway of extrinsic apoptosis, which results in the formation of a supermolecular complex, the ‘death-inducing signalling complex’ (DISC), a platform that recruits caspase-8 and promotes caspase activation [9].

‘Intrinsic apoptosis’, on the other hand, can be triggered by a large number of intracellular stimuli such as DNA damage and oxidative stress. Although the initiating stimuli can be highly heterogeneous, they all lead to a mitochondrion-centered control mechanism [10]. The cell death process is mediated by mitochondrial outer membrane permeabilization (MOMP), and can be caspase-dependent or caspase-independent [10, 11].

There is evidence that both intrinsic and extrinsic pathways may be involved in apoptosis triggered by cancer therapies. Treatment with a number of anticancer drugs can lead to an increase in FAS ligand (CD95L) expression, which subsequently binds its receptor CD95 and initiates the apoptotic cascade [12–15]. Drugs implicated in using the extrinsic pathway include doxorubicin, cisplatin, etoposide, and bleomycin. However, the intrinsic pathway is also utilized for a number of chemotherapy agents through multiple mechanisms, including direct permeabilization of the outer mitochondrial membrane [16], upregulation of pro-apoptotic proteins in response to DNA [17], or microtubule damage [18]. Relevant examples are shown in Table 1.1.

1.2.2 Non-apoptotic Cell Death

Anti-cancer therapies can also result in non-apoptotic cell death, including necrosis, autophagy and mitotic catastrophe.

Necrosis was historically considered a ‘messy’ form of cell destruction, morphologically characterized by random degradation of nuclear DNA, organelle degeneration and swelling and rupture of the cell membrane with release of intracellular components [4]. Recent work, however, suggests that necrosis can occur in a regulated manner, which is dependent on specific signalling modules such as RIP1 activation and can be triggered by a number of processes including alkylating DNA damage and death receptor ligation [35–38]. Zong et al. [39] first demonstrated that in vitro cell death caused by the alkylating agents nitrogen mustard and MNNG
Table 1.1 Examples of drugs, which kill by apoptosis

| Drug class                | Drug          | Pathway   | Reference          |
|---------------------------|---------------|-----------|--------------------|
| Platinum compounds        | Cisplatin     | Extrinsic | [15, 19]           |
|                           | Oxaliplatin*  | Intrinsic | [20–22]            |
| Antimetabolites           | 5-fluorouracil| Extrinsic | [23]               |
|                           | Gemcitabine*  | Intrinsic | [24, 25]           |
|                           | Methotrexate  | Extrinsic | [26]               |
| Anthracyclines            | Doxorubicin*  | Both      | [15, 17, 27, 28]   |
|                           | Idarubicin*   | Intrinsic | [28, 29]           |
|                           | Mitoxantrone* | Intrinsic | [27, 28, 30]       |
| Alkylating agents         | Cyclophosphamide| Intrinsic | [31, 32]         |
| Spindle poisons           | Paclitaxel    | Intrinsic | [16, 33]           |
|                           | Vinorelbine   | Intrinsic | [34]               |
| Topoisomerase poisons     | Etoposide     | Extrinsic | [15, 26, 28]       |

*Drugs shown to cause immunogenic cell death

(N-methyl-N-nitro-N-nitrosoguanidine) occurred independently of apoptotic factors, but required activation of the DNA repair protein poly (ADP-ribose) polymerase 1 (PARP1), suggesting a necrotic form of programmed cell death. More recently, the alkylating agent cyclophosphamide was shown to cause tumor regression in a xenograft mouse tumor system in both apoptosis-competent and apoptosis-deficient tumor cells, with the observation of sporadic necrosis in both groups, as identified using cell morphology, high mobility box group 1 (HMBG1) extracellular release and activation of innate immune cells [31]. In an effort to define the molecular signalling network that regulates necrotic pathways, Hitomi et al. [36] carried out a genome-wide siRNA screen and identified 432 genes that regulate necroptosis of which 32 acted downstream of regulators of RIP, 32 genes were required for death-receptor-mediated apoptosis and 7 genes that were involved in both pathways. Together, these data suggests that necrosis can occur in a regulated manner that is independent of apoptosis and may play a role in several physiological and pathological settings, but its role in tumorigenesis, in chemotherapy-induced cell death, or in the antitumor immune response is yet to be fully elucidated.

Autophagy, or ‘autophagic cell death’, is a process that leads to cytoplasmic vacuolization without features of apoptosis. It often constitutes a cytoprotective response activated by dying cells as a defense against acute stress, and inhibiting autophagy can actually mediate death rather than prevent it [40–42]. It is therefore unclear whether autophagy can be responsible for cell death, or rather is a process that can accompany cell death, without participating in the lethal catabolic cascade [11].

A third death mechanism, ‘mitotic catastrophe’, refers to cell death triggered by aberrant mitosis and occurring during mitosis or in subsequent interphase [43]. A number of chemotherapeutic agents, namely spindle poisons or microtubule inhibitors (taxanes and vinca alkaloids) interfere with the function of microtubules during mitosis, resulting in activation of the spindle assembly checkpoint (SAC) and causing mitotic arrest. The result is caspase-mediated cell death, but the molecular mechanism connecting initial SAC activation to caspase activation has not been