Cancer Treatment

Conventional and Modern Approaches

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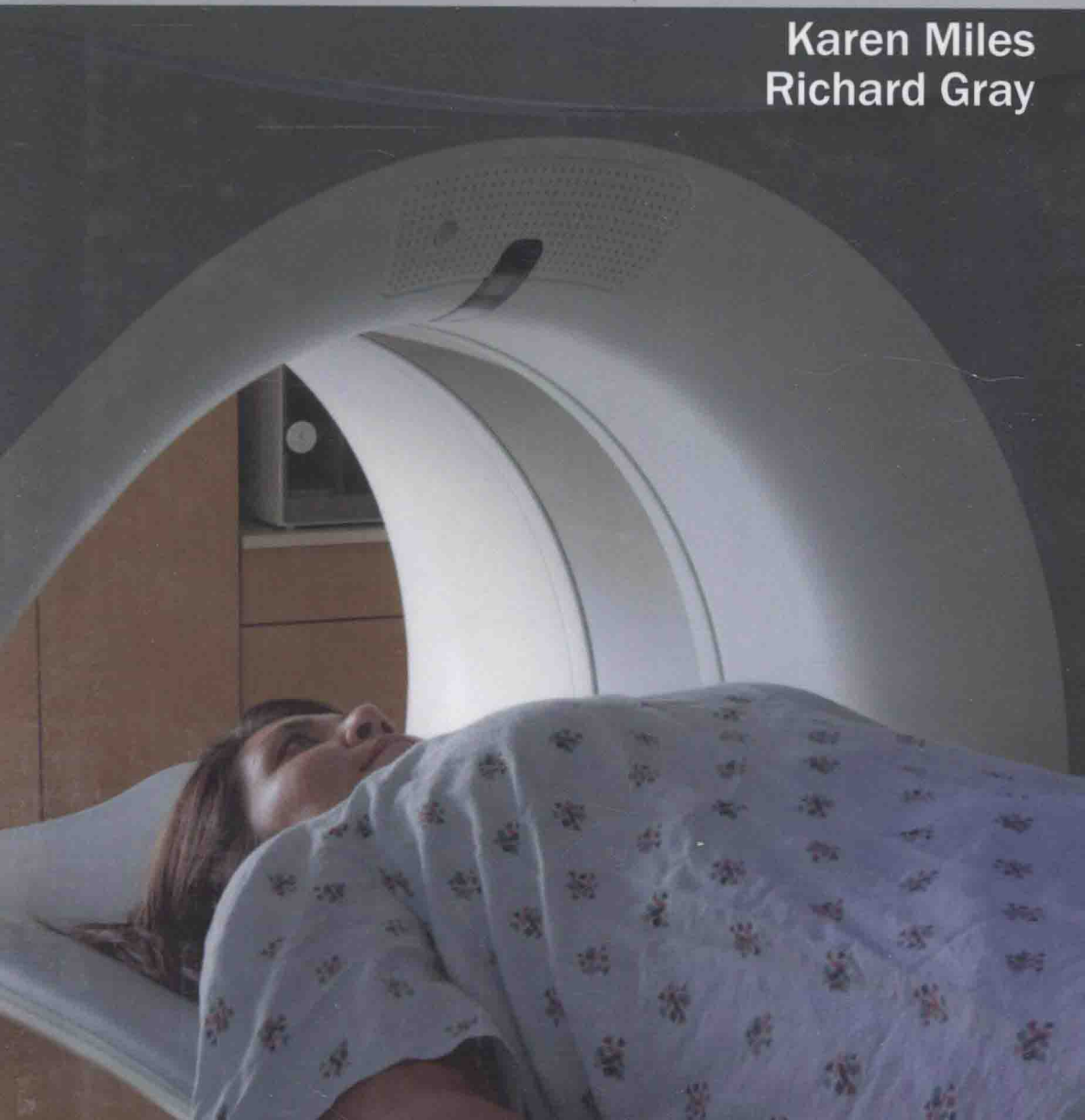
Richard Gray



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hayle medical
New York

Published by Hayle Medical,
30 West, 37th Street, Suite 612,
New York, NY 10018, USA
www.haylemedical.com

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International Standard Book Number: 978-1-63241-073-3 (Hardback)

Printed in the United States of America.

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Preface

Currently, there is an increase in the use of modern techniques and mechanisms for cancer treatment. However, an ultimate treatment has not yet been found, and it needs more time and research to develop more effective methods for cancer treatment. This book will serve not just physicians but also patients with an overview on new research and developments in this area. This book is a comprehensive and valuable account discussing various therapeutic methods in cancer treatment comprising of new modalities of cancer therapy like xenovaccinotherapy for cancer; antiangiogenic treatment concepts in gynecologic oncology; NKG2D-based cancer immunotherapy; photodynamic therapy in combination with antiangiogenic approaches; and electrotherapy on cancer experiment and mathematical modeling.

All of the data presented henceforth, was collaborated in the wake of recent advancements in the field. The aim of this book is to present the diversified developments from across the globe in a comprehensible manner. The opinions expressed in each chapter belong solely to the contributing authors. Their interpretations of the topics are the integral part of this book, which I have carefully compiled for a better understanding of the readers.

At the end, I would like to thank all those who dedicated their time and efforts for the successful completion of this book. I also wish to convey my gratitude towards my friends and family who supported me at every step.

Editor

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New Modalities of Cancer Therapy

1

Harnessing the Immune System to Fight Cancer: The Promise of Genetic Cancer Vaccines

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1. Introduction

In spite of significant progress in recent years towards the development of new targeted therapies Cancer is still a largely unmet medical need and the leading cause of death in industrialized countries (Globocan Project, 2008). Cancer is continuously increasing and is associated with a variety of factors, including genetic predisposition, infectious agents, exposure to mutagens, as well as lifestyle factors (Minamoto et al, 1999). Cancer is linked to the occurrence of genetic and epigenetic changes (Heng et al, 2010) and indeed tumour cells harbor hundreds of these modifications as also witnessed by the recent results of genome wide analyses of cancer genomes (Sastre, 2011). This feature of cancer cells implies that they can be recognized as foreign entities and eliminated by our immune system, and is at the basis of the theory of immunosurveillance (Dunn et al, 2004).

Several studies have shown that it is possible to establish clear correlates between the nature, density and location of immune cells within distinct tumour regions and the risk of disease relapse (reviewed in Mleknic et al, 2011). Compelling data have recently led to propose that an immune classification of patients, based on the density and the immune location within the tumour may have a prognostic value even superior to the standard TNM classification (Bindea et al, 2011; Fridman et al, 2011). In recent years a better knowledge of the immune system has led to an evolution of the initial concept of immunosurveillance into a more articulated theory of immunoediting (Schreiber et al, 2011). Cancer immunoediting acts as an intrinsic tumour suppressor mechanism that engages after cellular transformation has occurred and intrinsic tumour suppressor mechanisms have failed. One can envisage the existence of three sequential steps during clinical tumour evolution: elimination, equilibrium, and escape. In the first step, innate and adaptive immunity are capable of destroying transformed cells before they give rise to tumour masses. If this process is maximally efficient, then the host remains tumour free. If, however, cancer cell variants are not destroyed, they can enter into an equilibrium phase, in which their outgrowth is held in check by immunological mechanisms, which are principally due to the activation of IL-12/IFNγ-dependent adaptive immunity, mainly driven by antigen-specific $CD8^+$ and $CD4^+$

T cells. Equilibrium may still represent the end stage of the process and may restrain outgrowth of occult cancers for the lifetime of the host. However, as a consequence of constant immune selection pressure placed by the host on genetically/epigenetically unstable tumour cells, cancerous cells that are no longer recognized by adaptive immunity may emerge, become insensitive to immune effectors mechanisms and in addition they can induce the creation of an immunosuppressive environment. When tumour cells enter the escape phase in which their growth is no longer blocked by immunity, equilibrium is lost and disease becomes apparent. Re-establishing this equilibrium is the realistic goal of cancer immunotherapy.

In spite of being the object of intensive efforts over the past decades, Cancer Immunotherapy has seen many more clinical failures than successes. However, very recently major breakthroughs have been achieved, and these have led us to believe that this approach may become an established platform for the therapy of cancer within the next decade. One can envisage three distinct avenues for Cancer immunotherapy: a) Adoptive Cell Therapy (ACT); b) systemic immune-modulators; c) therapeutic cancer vaccines. ACT is based upon the possibility to isolate, *in vitro* expand and re-inject in immunodepleted hosts, tumour-specific T cells. This approach has seen its best demonstration in the treatment of patients with advanced metastatic melanoma. Superb clinical results have been obtained with objective response rates of up to 49-72% and disease control in some cases lasting several years (Rosenberg and Dudley, 2009). Although evolution of this approach such as genetic modification of T cells to redirect their effector cell specificity may open up to broader applications (Morgan et al, 2010), this strategy has several limitations that currently limit its wide applicability: it is patient specific, very expensive, requires hospitalization and can only be executed in highly specialized clinical centers. In contrast, systemic immunomodulators such as monoclonal antibodies against CTLA-4 or PD-1/PD-L1, do not suffer the manufacturing and delivery problems shown by ACT. On March 2011, FDA approved Ipilimumab (Yervoy® - BMS) (Culver et al, 2011), a human monoclonal antibody against CTLA-4 for the treatment of metastatic melanoma, based on the results of a randomized, controlled Phase III, where Ipilimumab showed statistically increased overall survival compared with controls (Hodi et al, 2010). Although the clinical development of anti PD-1 antibodies is at an earlier stage as compared to anti CTLA-4, results are highly promising both for efficacy and tolerability (Kline and Gajewski, 2010). Finally cancer vaccines recently gained increased visibility due to the demonstration that Sipuleucel-T, a immune cell vaccine for the treatment of hormone refractory prostate cancer, is capable of increasing overall survival of cancer patients (Kantoff et al, 2010). These results led to FDA approval as Provenge® (Dendreon) in year 2010 (Cheever and Higano, 2011). This recent approval has acted as a strong injection of enthusiasm in an area that has long suffered major setbacks.

In this review we will focus mainly on recent developments for therapeutic cancer vaccines and will not discuss in detail ACT and systemic immunomodulators (Klebanoff et al, 2011). Major emphasis will be given to aspects that are critical to increase vaccine immunogenicity and probability of success in the clinic. We believe these are mainly: a) efficient vaccine delivery systems, b) development of response biomarkers, c) modified clinical endpoints and d) combinatorial treatments with chemotherapy or other agents. In analyzing vaccine delivery systems a greater attention will be given to genetic vaccines which we believe represent the most promising methods to elicit immune responses against a wide variety of tumour antigens

especially when administered in combined immunization protocols (heterologous prime/boost). We invite the reader to other recent excellent reviews for aspects of tumour immunology and cancer immunotherapy that we may have missed in our work (Steer et al, 2010; Klebanoff et al, 2011; Palucka et al, 2011; Vergati et al, 2010; Aldrich et al, 2010) .

2. Tumour immunology

Our immune system has the intrinsic capability of recognizing tumour cells as foreign entities and to mount responses capable of impacting upon disease evolution. In this section of the chapter we review the main evidences for this spontaneous response, what are the targets of this response, which are the principal components of the immune system involved and what is curtailing this response leading to tumour escape and lack of control of the immune system over cancer.

2.1 Immunosurveillance and Immunoediting

The key studies that unequivocally demonstrated the role of the immune system in the control of cancer development date back to about a decade ago when mouse models of immunodeficiency on pure genetic backgrounds became available. These studies showed that interferon-γ (IFN-γ) is a key factor responsible for the immunological rejection of transplanted tumour cells (Dighe et al, 1994). Furthermore, mice lacking IFN-γ response (either as a consequence of IFN-γ receptor or STAT1 inactivating mutations) or adaptive immunity as a whole (RAG2 -/- deficient mice) are more susceptible to carcinogen induced or spontaneous tumours (Kaplan et al, 1998; Shankaran et al, 2001, Street et al, 2002). These evidences collectively demonstrated that the immune system can function as an extrinsic tumour suppressor. However, as mentioned in the introduction section, a new emerging concept in cancer immunology is that the immune system is not simply a component that protects the host against tumour development, but rather an agent that shapes tumour quality. In other words, tumours that develop in an immunocompetent organism are the resultant of a selection process imposed by the host and by the type of immune response that the host immune system is capable to mount. This concept was originated by pivotal studies that demonstrated that tumours developing in immunocompetent mice have a different molecular profile, are less immunogenic than tumours developing in immunodeficient hosts and progress more rapidly when implanted into naïve wt recipient mice (Dunn et al 2002).

Although both natural and acquired immunity are required to fully exert this control mechanism, the principal contribution comes from adaptive immunity and in particular from the development of tumour-antigen-specific T cells, mainly $CD8^+$, but also $CD4^+$. Indeed the fundamental principle of cancer immunology is that tumour cells express antigens (TAAs - tumour associated antigens) that differentiate them from normal cells. The existence of tumour antigens has been abundantly demonstrated both in mouse and human studies (Novellino et al, 2005). In the case of human cancers, identification of tumour antigens was made possible *via* the development of methods that used as probes antibodies and $CD8^+$ T cells derived from patients and capable of reacting with the autologous tumours (Sahin et al, 1997; Coulie et al, 1997). In the next section we will describe in more detail the types and nature of TAAs under study.

What is happening in the tumour cells that makes them "invisible" or "poorly visible" to the immune system? Certainly the most common mechanism is believed to be loss of tumour

antigen expression, which can occur in at least three possible ways: a) downmodulation of tumour antigen gene expression consequent mainly to epigenetic changes; b) downregulation of MHC class I protein expression and antigen presentation to the cell surface; c) alterations in tumour cells of the machinery responsible for antigen processing and peptide loading onto MHC molecules. In addition to this, it has to be taken into account that tumour cells develop mechanisms of resistance to apoptosis and to the cytotoxic effects of immunity through, for instance, upregulation of anti-apoptotic BCL-2 proteins or activation of transcription factors such as STAT-3. All these processes are strongly favoured by the genetic/epigenetic instability intrinsic of tumour cells, which in the presence of a continuous selection favors the emergence of "immune stealth" clones.

If we analyze in detail the three phases of immunosurveillance/immunoediting, namely Elimination, Equilibrium and Escape, the phase where we have more direct proof of the activity of the immune system is the Equilibrium phase. This phase can represent a type of tumour dormancy where growth of tumour cells is kept at bay for a long period of time, even for the entire life of an organism. Strong evidence for this phenomenon first came when immunocompetent mice treated with low dose carcinogens such as methylcolantrene, were shown to harbor occult cancers for an extended period of time (Koebel et al, 2007). Intriguingly, when these mice were subjected to treatments that selectively affected adaptive immunity, but not innate immunity, tumours rapidly developed, thus demonstrating that equilibrium is established only when a Tumour Antigen Specific $CD8^+$ and $CD4^+$ response has occurred. This may explain the clinical findings of aggressive tumour arising in organs from a donor apparently cured from cancer, when transplanted into a patient (MacKie et al, 2003).

Although studies of tumour development in mice served as the main driver for the formulation of the cancer immunosurveillance/immunoediting hypothesis, strong demonstration has also been obtained in humans by three different types of evidence. As mentioned before, the first is the demonstration that cancer patients develop detectable levels of antibodies and T cell responses to tumour antigens (Dougan and Dranoff, 2009). The second one is that patients affected by immunodeficiencies are at higher risk of developing cancers (Dunn et al, 2002). The third and strongest one is that intratumoural infiltration by cells of the immune system correlates with disease evolution. In this respect several studies have shown that the quantity, quality, and spatial distribution of tumour infiltrating lymphocytes correlate with patients survival. In fact, tumour infiltration by IFN-γ producing $CD8^+$ and $CD4^+$ T cells has been associated with an improved prognosis for patients with several different cancer types, including melanoma (Clemente et al, 1996; van Houdt, 1998), colorectal cancer (Chiba et al, 2004) and ovarian cancer (Nelson, 2008). More recent studies in colorectal cancer have extended these findings and have shown, through a global analysis of the tumour microenvironment from both a morphological standpoint and from a system biology approach, that the nature, functional orientation, density and location of cells of the adaptive immune system within distinct tumour regions influence the risk of relapse (Mlecnik et al, 2011). The same authors have come to the conclusion that the density and the immune cell location within the tumour may have a prognostic value superior to the standard TNM classification, and that tumour spread is statistically dependent upon the extent of the host-immune reaction (Bindea et al, 2011).

2.2 Tumour associated antigens (TAAs)

In the past years, several TAAs have been identified having unique expression patterns or being overexpressed by cancer cells. These antigens, under appropriate conditions, can be

recognized by components of the immune system (Campi et al, 2003; Frenoy et al, 1987; Kawashima et al, 1998). Therefore, many current vaccination strategies are designed to induce antibody as well as cell-mediated immune responses against the antigen of interest. A high number of TAAs has been discovered and evaluated in pre-clinical and clinical studies with different results. A list of well-known TAAs subdivided in four main categories is provided in Table 1. Among the most studied and validated TAAs, vaccinations against CEA (Marshall et al, 2003), HER-2/*neu* (Shumway et al, 2009), TERT (Vonderheide, 2008), EpCAM (Chaudry et al., 2007), survivin (Andersen and Thor, 2002), prostate-specific antigens (Doehn et al., 2008) provided good immunologic results. In light of the increasing interest and potential for cancer immunotherapy, the National Cancer Institute recently conducted an interesting pilot project to prioritize cancer antigens and to develop a priority-ranked list of cancer vaccine target antigens based on predefined and pre-weighted objective criteria (Cheever et al., 2009). **Shared TAAs**

Among the shared TAAs, the following three main groups can be identified: (1) cancer-testis (CT) antigens, (2) differentiation antigens, and (3) widely occurring overexpressed antigens. Among shared tumour-specific antigens, *cancer-testis (CT)* antigens are expressed in histologically different human tumours and, among normal tissues, in spermatocytes/spermatogonia of the testis and occasionally in placenta. CT antigens result from the reactivation of genes which are normally silent in adult tissues but are transcriptionally activated in different tumour histotypes (De Smet et al., 1999). Many CT antigens have been identified and used in clinical trials, although little is known about their specific functions, especially with regard to malignant transformation. This group of TAAs includes MAGE-A1 (Chaux et al., 1999) and NY-ESO-1 (Jager et al., 1998). *Differentiation antigens* are shared between tumours and the normal tissue of origin and found mostly in melanomas and normal melanocytes (Gp100, Melan-A/Mart-1, and Tyrosinase), although they are also found in epithelial tissues and tumours such as prostate tumours (prostate-specific antigen [PSA]). To variable extent, normal tissues can be target of the elicited immunity against shared TAAs. An example is the vitiligo developing as a consequence of the immune response in melanoma patients undergoing immunotherapy. Vaccine-induced T cells recognizing gp100 and tyrosinase are present at the *vitiligo* lesions and normal melanocytes are eliminated by the immune system (Jacobs et al., 2009). Importantly, this effect has been associated to a clinical response. Additionally, expression of several oncofetal antigens appears to be increased in many adult cancer tissues, including carcinoembryonic antigen (CEA), which is highly expressed in colon cancer (Tsang et al., 1995).

TAAs from this group, despite representing self-antigens, have been and still are commonly used in current cancer vaccination trials, often together with CT antigens. Widely occurring, overexpressed TAAs have been detected in different types of tumours as well as in many normal tissues, and their overexpression in tumour cells can reach the threshold for T cell recognition, breaking the immunological tolerance and triggering an anticancer response. Among the most interesting TAAs of this group are the antiapoptotic proteins (survivin) (Schmidt et al., 2003), hTERT (Vonderheide et al., 2008), and tumour suppressor proteins (e.g., p53) (Umano et al, 2001).

Unique tumour antigens. Unique TAAs are products of random somatic point mutations induced by physical or chemical carcinogens and therefore expressed uniquely by individual tumours and not by any normal tissue, representing the only true tumour-specific antigens (Ags) (reviewed in Parmiani et al., 2007). Such Ags characterize each single

neoplasm and were shown to be diverse between tumours induced in the same animal or even in different tissue fragments from the same tumour nodule. A relevant feature of unique Ags is their potential resistance to immunoselection if the mutated protein is crucial to the oncogenic process and thus indispensable for maintaining the neoplastic state. As a consequence, unique Ags should elicit an immune response clinically more effective than that of shared Ags. However, identification of unique tumour antigens for solid human tumours requires sequencing of the whole genome of each individual tumour in order to identify mutated genes and select peptides whose motifs are predicted to be presented by the patient's HLA alleles. Moreover, each tumour bears highly heterogeneous sets of defects in different genes which need to be further verified for their substantial contribution to the tumour development and progression and, consequently, for their relevance as vaccine targets (Fox et al., 2009). An interesting class of potential TAAs is associated with fusions between different proteins. Best example is the Bcr-Abl fusion protein, the driving force in chronic myelogenous leukemia (CML) (Daley et al., 1990). By establishing a causal link between a specific chromosomal lesion and a specific malignancy, BCR-ABL also pioneered cancer therapy: the TK inhibitor, imatinib (Gleevec), was introduced as the first widely used targeted therapeutic (Druker et al., 2001). Similar discoveries led to the characterization of causative fusions in other hematological malignancies. A variety of prostate cancer gene fusions have been identified so far (reviewed in Shah and Chinnaiyan, 2009), characterized by 5'-genomic regulatory elements, most notably the androgen-controlled prostate specific gene, transmembrane protease serine 2 (TMPRSS2), fused to members of the erythroblastosis virus E26 transforming sequence family of transcription factors, most notably ERG, leading to the overexpression of oncogenic transcription factors. This class of potential TAAs is matter of extensive studies and holds promise for personalized vaccine applications.

Viral Antigens. Some viruses, such as human papillomavirus (HPV) and hepatitis B virus (HBV) can induce cancer. As a matter of fact, HBV vaccination in newborns has eradicated hepatocellular carcinoma (HCC) in populations at high risk (McMahon et al, 2011; Blumberg et al., 2010). The high-risk HPV types (e.g., HPV16) are causally related to the development of anogenital lesions, including vulvar intraepithelial neoplasia (VIN), and their subsequent progression to invasive squamous cell carcinoma. The expression of viral antigens (hence non-self proteins) such as HPV E6 and E7 proteins by cancer cells can represent the mechanism through which the tumour becomes visible to the immune system. Recently, promising results have been obtained by vaccination of patients with HPV16 E6/E7 synthetic long-peptide vaccine (Van der Burg and Melief, 2011), providing an important proof of concept for the development of therapeutic cancer vaccines against cervical and anal cancers.

Stromal Antigens. During transformation, reciprocal interactions occur between neoplastic and adjacent normal cells, i.e. fibroblasts, endothelial, and immunocompetent cells. In general, stroma cells contribute 20–50% to the tumour mass, but the stromal compartment may account for up to 90% in several carcinomas. In contrast to cancer cells, tumour stroma cells are genetically more stable so that at least some immune evasion mechanisms of tumours do not apply to these cells. Nevertheless, stroma cells differ from their normal counterparts by upregulation or induction of various antigens (reviewed in Hofmeister et al., 2006). Some of the tumour stroma-associated antigens (TSAAs) are highly selective for the tumour microenvironment. TSAAs are also expressed by a broad spectrum of solid tumours, thus