Feature Review

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Immune-mediated mechanisms influencing the efficacy of anticancer therapies

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Conventional anticancer therapies, such as chemotherapy, radiotherapy, and targeted therapy, are designed to kill cancer cells. However, the efficacy of anticancer therapies is not only determined by their direct effects on cancer cells but also by off-target effects within the host immune system. Cytotoxic treatment regimens elicit several changes in immune-related parameters including the composition, phenotype, and function of immune cells. Here we discuss the impact of innate and adaptive immune cells on the success of anticancer therapy. In this context we examine the opportunities to exploit host immune responses to boost tumor clearing, and highlight the challenges facing the treatment of advanced metastatic disease.

Then and now: the link between the immune system and anticancer therapies

The relationship between anticancer therapies and the immune system is as old as the invention of anticancer therapies themselves. After the use of mustard gas in the trenches of World War I, a seminal observation was made that some exposed soldiers displayed severe loss of bone marrow and lymph-node cells [1]. This observation then spurred the idea that the antiproliferative capacity of mustard gas may also slow the growth of cancer cells. Experiments carried out in mice transplanted with lymphoid tumors were convincing enough to treat a lymphoma patient [2], and these events initiated the standardized treatment of cancer patients with chemotherapy [3,4].

Fast-forward 100 years. The influence of immune cells on tumor progression and metastasis is well established [5], and an appreciation of the impact of the immune system during conventional anticancer therapy treatment is growing. Recent seminal advances indicate that immune cells can shape the outcome of various anticancer therapies. As such, immune cells and their molecular mediators have evolved into *bona fide* targets of therapeutic manipulation in cancer patients. The recent breakthrough of immunotherapeutics that inhibit negative immune regulatory pathways, such as anti-CTLA4 (cytotoxic T lymphocyte-associated protein 4) and anti-PD1, has initiated a new era in the treatment of cancer [6]. In parallel, immunomodulatory strategies aimed at dampening protumor functions of immune cells are currently being tested in cancer patients [7]. Immune cells also function as reliable biomarkers because their abundance or activation status often predicts how well patients respond to a particular treatment regimen. We review these novel experimental and clinical insights, highlighting potential implications for the development of synergistic therapies designed to combat primary tumors and, more importantly, metastatic disease.

The pros and cons of experimental mouse models

Research questions aimed at understanding the role of immune cells during anticancer therapy require models that mirror the complex interactions between the immune system and diverse forms of human cancers. Transplantable cancer cell line models and carcinogen-induced cancer models are the most frequently used for these purposes. However, studies are gaining ground in genetically engineered mouse models (GEMMs; see Glossary) that spontaneously develop specific cancer types as a consequence of germline or somatic mutations in discrete cell types. There are key differences between cancer cell line inoculation models and GEMMs of cancer (Box 1). In GEMMs, normal cells are transformed *in situ* resulting in the development of spontaneous tumors that faithfully recapitulate each stage of cancer progression – from tumor initiation to advanced disease, and in some models also metastasis. These spontaneous tumors develop in their natural microenvironment, and share the genetic heterogeneity and histopathology of human tumors. In stark contrast, transplantable models rely on the inoculation of large numbers of selected, homogenous cancer cells grown in 2D. The tissue of tumor origin and location of injection are often disparate in transplantable models, with subcutaneous injection being the most common site of implantation. Moreover, these tumor cell line inoculation models do not mimic the multistep progression of *de novo* tumors, and the speed of tumor outgrowth is very fast. Upon inoculation, a large proportion of the cancer cells die, which can prime antitumor immune responses in an

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Glossary

Alkylating agents: a class of chemotherapy drugs that directly damage DNA by substituting alkyl groups for hydrogen atoms on DNA, causing the formation of crosslinks within DNA chains and thereby resulting in cell death. Examples of alkylating agents are cyclophosphamide and melphalan.

Anthracyclines: a class of chemotherapy drugs that are widely used to treat many different types of cancer. Anthracyclines prevent cell division by disrupting the structure of the DNA via several mechanisms. Examples of anthracyclines are doxorubicin and daunorubicin.

***Braf*^{V600E}*;Tyr::CreERT2* or *Braf*^{V600E}*;Pten*$^{F/F}$*;Tyr::CreERT2* mouse tumor models:** a conditional GEMM of melanoma driven by an activated form of BRAF and loss of PTEN under the control of the tyrosinase (*Tyr*) promoter. Tumors are induced by topical administration of tamoxifen to the skin, and therefore the timing of tumor development can be initiated as desired.

***C3(1)-Tag* mouse tumor model:** a GEMM model in which SV40 large T antigen (Tag) expression under the control of the 5′ flanking region of the C3(1) component of the rat prostate steroid-binding protein drives tumor development. In females, mammary ductal epithelium is transformed leading to invasive mammary tumors that resemble human ductal carcinoma *in situ* (DCIS). Male mice develop phenotypic changes in the prostate that progress into invasive carcinoma.

Genetically engineered mouse models (GEMMs) for cancer: in GEMMs for cancer, normal cells are transformed *in situ* as a consequence of germline or somatic mutations in specific cell types, resulting in the development of spontaneous tumors that faithfully recapitulate each stage of cancer progression – from tumor initiation to advanced disease and in some models also metastasis.

***K14-HPV16* mouse tumor model:** a GEMM for *de novo* squamous carcinogenesis of the skin. These mice transgenically express the early region genes of the human papilloma-virus type 16 (HPV16) under control of the human keratin 14 promoter/enhancer. Cervical tumors can also be induced in these mice by administration of low-dose estrogen, hence K14-HPV16/E_2.

***K14cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ mouse tumor model:** a conditional GEMM for invasive lobular breast cancer. These mice transgenically express Cre recombinase under the control of the human keratin 14 promoter. In these mice, the alleles encoding E-cadherin and p53 are homozygously floxed. As a consequence, mammary and skin epithelial cells stochastically lose E-cadherin and p53, which induces the formation of tumors in these tissues.

***Kit*$^{V558/+}$ mouse tumor model:** these mice carry a gain-of-function point mutation on one allele of the *Kit* receptor gene predisposing them to spontaneous gastrointestinal stromal tumor (GIST) development.

Metastatic cascade: cancer dissemination is a multistep process, consisting of the following steps: local invasion at the primary tumor site, intravasation and survival into the circulation, extravasation and survival at distant sites, adaptation to a foreign microenvironment, and outgrowth of a metastasis. During every step of the metastatic cascade, cancer cells encounter normal host cells, such as immune cells. Interactions between disseminated cancer cells and normal host cells largely dictate the success of metastasis formation.

***MMTV-Neu* mouse tumor model:** a GEMM for HER2$^+$ breast cancer in which wild type rat ERBB2 expression is driven by the mouse mammary tumor virus (MMTV) promoter, which is only active in the mammary gland. These mice develop multifocal tumors in all 10 mammary glands, as well as spontaneous lung metastases in most mice. They are maintained on the FVB/n background.

***MMTV-NeuT* mouse tumor model:** similar to *MMTV-Neu* mice, this GEMM represents another model for HER2$^+$ breast cancer. However, a mutated form of the rat proto-oncogene, ERBB2, is expressed under control of the MMTV promoter in this case. Multifocal tumors also arise in these mice from all five pairs of mammary glands and they develop spontaneous lung metastases. These mice are usually maintained on the BALB/c background.

***MMTV-PyMT* mouse tumor model:** a GEMM for mammary tumorigenesis. These mice transgenically express the polyomavirus middle T antigen (PyMT) oncogene under the control of the MMTV promoter. These mice develop multifocal tumors in all 10 mammary glands, as well as spontaneous lung metastases.

Patient-derived xenograft (PDX) tumor models: fresh tumor tissue from patients undergoing surgery is implanted into immunodeficient mice (usually NOD/SCID/*Il2rg*, otherwise known as NSG, mice) directly or following enzymatic digestion. Tumors can be grafted subcutaneously or orthotopically. PDX tumors are serially passaged in additional mice.

***Probasin-Cre4;Pten*$^{F/F}$ mouse tumor model:** a conditional GEMM for *Pten*-deficient prostate cancer, where loss of *Pten* expression is driven by the probasin promoter. These mice develop prostatic intraepithelial neoplasia (PIN) lesions that progress to invasive adenocarcinomas.

Platinum compounds: a class of platinum-containing chemotherapy drugs that bind to and crosslink DNA, resulting in apoptosis. Examples of platinum compounds are cisplatin, carboplatin, and oxaliplatin.

***RIP1-Tag5* mouse tumor model:** a conditional GEMM of pancreatic cancer, in which the rat insulin gene promoter drives sporadic expression of SV40 large T antigen (Tag) in a subset of pancreatic β cells. Unlike *RIP-Tag2* mice that are systemically tolerant to SV40 large T antigen, these mice develop an autoimmune response against the oncogene-expressing beta cells.

Taxanes: a class of chemotherapy drugs that disrupt microtubule function, and thus inhibit mitosis. Taxanes were first derived from plants of the yew tree. Examples of taxanes are paclitaxel and docetaxel.

Tumor microenvironment: in addition to cancer cells, many 'normal' cells are recruited to and activated in tumors. The tumor microenvironment is composed of many different types of immune cells, fibroblasts (referred to as cancer-associated fibroblasts), endothelial cells and other cells that normally reside in the organ afflicted by the tumor (e.g., adipocytes in breast cancer), soluble mediators, and the extracellular matrix (ECM). Throughout cancer progression there is extensive crosstalk between normal cells, soluble mediators, and cancer cells. These interactions largely dictate tumor behavior and therapy response. Each tumor type and each tumor stage is characterized by a unique tumor microenvironment.

unphysiological manner. Importantly, comparative studies have shown that immune cell behavior and tumor response to anticancer therapies differs between transplantable cell lines derived from GEMMs and the original GEMM [8–10]. Similarly, other studies indicate that GEMMs used in preclinical studies may be better predictors of clinical trials than transplantable models [11]. Xenografted human cancer cells established from cell lines or fresh patient material (patient-derived xenograft, PDX) in immunocompromised mice are other frequently used models. While it may be argued that PDX models are the best representation of human disease from a cancer genetics or drug response point-of-view, these models exclude the participation of the adaptive immune system in cancer progression and anticancer therapy response. Therefore, they cannot predict the full breadth of drug response in immunocompetent humans. These issues, as well as other advantages and disadvantages, various strategies to refine these models, and their suitability for preclinical studies, have been extensively discussed elsewhere [12–16].

The influence of the immune system on chemotherapeutic efficacy

Various types of chemotherapy drugs exist which kill cancer cells via different mechanisms (Figure 1). Cytotoxic drugs can eliminate cancer cells by inhibition of DNA replication, chemical damaging of DNA, inhibition of the function of crucial enzymes required for DNA synthesis, or prevention of mitosis. Drug-induced cancer cell death, as well as off-target effects of chemotherapy, elicits several systemic and intratumoral changes in the host immune system. In turn, the efficacy of chemotherapeutic drugs is influenced by the interplay between tumor and immune components. These mechanisms are outlined below for both innate and adaptive immune cells.

Innate immune cells

The microenvironment of solid tumors consists of multiple cell types, including many immune cell populations that participate in and regulate tumorigenesis and metastasis [17,18] (Box 2). Tumor-associated macrophages (TAMs) represent one of the most extensively studied innate immune cell populations in chemotherapy response. Research spanning over the past three decades has shown that TAMs interfere with or augment the therapeutic activity of several types of chemotherapy, and their role in these processes has been reviewed recently [19,20]. One of the first studies addressing the impact of macrophages on chemo-responsiveness showed that doxorubicin