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Systems Biology in Cancer Immunotherapy

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Systems Biology in Cancer Immunotherapy

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FOREWORD

I am honoured to be able to contribute to this exciting eBook about Systems Biology in Cancer Immunotherapy. The eBook has excellent credentials for describing important knowledge and approaches for the understanding of cancer cell metabolism and immunotherapy.

Dr. Mahbuba Rahman is one of my former PhD students, and she is among the top students in our laboratory in Kyushu Institute of Technology. She has long been involved in cancer research based on molecular biology as well as ^{13}C-metabolic flux analysis. She has published many articles in this area.

In summary, I am delighted to see such expertly produced and well referenced eBook which will contribute to molecular biology, biochemical science, and medical science.

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PREFACE

Robustness is an essential property of all biological systems. This property is maintained and controlled by a large number of protein and signalling molecules, enzymes and regulatory molecules, which form an intricate regulatory network to transfer signals inside and outside of the cell. Considering the complex regulatory network of a cell inside its own milieu or its surrounding environment, cells have been referred to as 'systems' for more than a century ago. These networks control several checkpoints that are associated with cell proliferation, differentiation and metabolic regulation. Interestingly, the robustness or plasticity is observed both in healthy cells and in diseased or transformed cells like cancer cells. However, in cancer cells, the checkpoints are either mutated or dysregulated. Therefore, an in-depth understanding of the systems of these cells is an essential requisite prior to developing therapy against cancer. This can be achieved by using robust approach, such as the systems biology approach.

The technological platforms for systems biology are rooted in molecular biology. Unlike the conventional molecular biology techniques, systems biology takes advantage of the high-throughput omics platforms. The main strength of this approach is that it integrates results carried out by researchers of broad disciplinary such as molecular biology, immunology, bioinformatics, physicians and even the R&D of pharmaceutical companies in different parts of the world. Thus the approach produces unbiased data which enables researchers to investigate the system of a cell.

Over the past several years, systems biology approaches have been applied in different areas of life sciences research including cancer. Researchers were able to understand the hallmarks of cancer cells such as abnormal cell growth, inflammation, dysregulated metabolic pathways, drug resistance properties, *etc.* It also enabled researchers to investigate specific fields of cancer immunology including identification of cancer related signature molecule on the immune cells, biomarker identification, effect of monotherapy and combination therapy in tissue culture and mouse model. More recently, systems biology approach has been applied to cancer immunotherapy. Several immunotherapy drugs received US FDA approval and are at phase III of clinical trials. However, many immunotherapy drugs that are tested in laboratory tissue culture and mouse model failed to show significant tumor regression in patients. Investigation of the underlying cause of therapy resistance at the genetic and phenotypic level requires the use of robust approach like systems biology. Although systems biology is still at its infancy in cancer immunotherapy research, considering the strength of the approach to dissect a robust system or cell, in this volume of eBook series, we discussed the scope of systems biology in cancer. However, during preparation of this volume, an important platform

of systems biology, metabolic flux analysis (MFA) was found to be less studied. This is a robust tool to understand the metabolic concentration of a cell resulting from specific cellular processes and from the function of a gene. Future research in cancer immunotherapy should consider implementing this method to understand and tie the diseased phenotype with the targeted cells.

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CHAPTER 1

Immune System in Cancer

Abstract: Our immune system is a dynamic environment that is orchestrated by a network of immune cells and signalling molecules and is differentially expressed and regulated at different stages of life. Interestingly, components of the immune system function differently under diseased or non-diseased state, healthy or immune-deficient state. Cancer is generally regarded as a genetic disease and caused from inflammation. Due to the complex nature of the disease, it is necessary to understand how the immune system responses in a tumor environment. A detailed understanding on the cancer immuno-biology will enable formulation of appropriate treatment strategies for cancer.

Keywords: Circulating tumor cells, Cytokines, Dendritic cells, Immuno-escape, Immune-surveillance, Immuno-system, Regulatory T cells, Tumor associated antigens, Tumor derived factors, Tumor specific antigens.

INTRODUCTION

Cancer is a multistage disease that progresses *via* prolonged accumulation of multiple genetic and/or epigenetic changes which control cell proliferation, survival, differentiation, migration and interactions with neighbouring cells and stroma. More than 200 different types of cancers have been reported. The classification of cancer is based on the tissue of origin or pathogenesis (Table **1**). Classification based on tissue of origin shows that cancer can originate from any tissues of the body and can be either localized or systemic. Whatever the origin of cancer is, our immune system, which is our body's defensive system, responds to the inflammations caused during cancer development [1, 2].

In general, our immune system performs two critical activities in response to exposure to foreign particles: 'recognition' where the cells identifies the harmful agent called 'antigen' and 'effector responses' by which specific receptors

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expressed on the surface of immune cells bind antigens and confer protection by cellular behaviors. In case of cancer, where the cancer cells originate from the host's body, cells of both the innate immune system and the adaptive immune system are often involved [3, 4].

Table 1. Classification of cancer based on tissue of origin and pathological point of view [5].

Type of cancer	Tissue of Origin	Special features
Carcinoma	Originate from epithelial cells in skin or tissues that line or cover internal organs. About 80% human cancers are of this type.	Localized, also known as solid tumor. The tumor is often confined by basement membrane. Until the tumor cells turn into invasive carcinomas where the basement membrane is disrupted and tumor grows into the surrounding tissues.
Sarcoma	Originate in bone, cartilage, fat, muscle, blood vessels and other connective or supportive tissues.	Also known as solid tumor
Leukemia	Originate in blood-forming tissues such as bone marrow and causes large number of abnormal blood cells to be produced and enter the bloodstream.	Systemic and also known as liquid tumor.
Lymphoma	Originate in the cells of the immune system.	Known as liquid tumor

BASICS OF THE HUMAN IMMUNO SYSTEM

Our immune system broadly comprises of two major subgroups: the innate/acquired immune system and the adaptive immune system. The primary function of the innate system is to provide a rapid non-specific response to foreign invaders such as virus, bacteria or foreign antigens, wound, inflammatory insult or newly initiated diseased cells. On the other hand, the adaptive immune system helps to provide a latent but highly specific response by producing antibodies against foreign or non-self antigens to generate immune memory against the antigens that cross the first line of the defensive system. Hence, the immune system plays crucial role to protect the body from infection. Each of the arms of the immune system consists of cellular and humoral (antibody) components which function in unique ways to combat against the infection. Despite the uniqueness, there is interplay between components of the systems that protects the body from

foreign invaders [6].

The major difference between the innate/acquired immunity and the adaptive immunity is that innate immune response are non-specific and occurs within minutes and lasts for a few days whereas, the adaptive immunity occurs over weeks to years and is more specific where the immunological memory invokes rapid eradication of subsequent infections. This mechanism of the adaptive system is used as the basis for immunization and vaccination in humans. Despite these differences, the main function of the immune system is self-nonself discrimination including the foreign invaders and modified or altered cells (*e.g.*, cancer or malignant cells) to protect the organism from foreign invaders or eliminate abnormal cells. To perform this, both the adaptive and innate immune system connects in some way with the help of the cells of the system and specific molecules to initiate acute inflammation followed by wound healing of the diseased cells [7].

Origin and Formation of the Immune System

Our immune system comprises of a varied collection of interconnected cells and tissues that are distributed throughout the body. The lymphoid organs that consist of the primary lymphoid organ (*e.g.*, bone marrow and thymus) and the secondary lymphoid organ (*e.g.*, regional lymph nodes and spleen) are connected to one another through two separate circulatory systems. These are the blood system and the lymphatic system. White blood cells are produced and differentiated in the primary lymphoid organs. The secondary lymphoid organs together with the circulatory systems outside of the primary lymphoid organs are collectively referred to as the "periphery." While the lymph nodes and spleen serve to filter and trap foreign molecules and cells that are delivered from the tissues *via* the lymph fluid or the blood, the secondary lymphoid organs provide organized tissues in which the white blood cells can encounter foreign antigen molecules and physically interact with other white blood cells to initiate an appropriate response [8].

The majority of the cells of the immune system are circulatory or migratory. All cells of the immune system originate from the bone-marrow hematopoietic stem

CHAPTER 2

Cancer Metabolism: A Perspective on the Involvement of the Immune System and Metabolic Pathways in Cancer Development

Abstract: Cancer cells show excessive need of nutrients and energy. Not only this, activated lymphocytes, in particular the T lymphocytes and myeloid derived cells also show differential expression in the metabolic pathway genes in tumor microenvironment. Therefore, understanding the metabolic changes in the context of cancer development will help to identify new therapeutic targets to treat cancer.

Keywords: Arginine metabolism, Autophagy pathway, Immune-metabolism, Immune-suppressors, Leptin, Reactive oxygen species, Tumor metabolism.

INTRODUCTION

The two most fundamental ways in which cells interact with their environment is through metabolism and through immune response. The former one involves obtaining nutrients to sustain life and the later one is to defend against pathogenic organisms. While the former one is generally known as metabolism, the later one is referred to as immune response. For a long time, immunology and metabolism have been addressed as two separate fields of study. However, both processes involve thousands of enzymes, proteins and regulatory molecules which indicate that these processes might be connected and closely interacted to maintain cellular homeostasis. In fact, in the recent years, the field of immune-metabolism has drawn much attention to understand the mechanism by which nutrients affect the immune system in the context of cancer cells. This has emerged as a new field for the development of therapies that can be used as chemotherapy or immunotherapy or a combination therapy [1, 2].

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Cancer cells show highly complex and dynamic environment in which tumor cells interact with stromal cells within a modified extracellular matrix (ECM). During tumor progression, pervasive stromal cells reprogramme and remodel, and transform a normal environment into tumorogenic microenvironment. Since cancer originates from inflammation, therefore, the tumorigenic micro-environment consists of a variety of mesenchymal immune and inflammatory cells and non-immune stromal cells or fibroblasts. However, fibroblasts, which represent the principal cellular component of tumor microenvironment, become cancer associated fibroblasts (CAF) or myofibroblasts once they are recruited, activated and accumulated to the tumor microenvironment. CAFS are a hetero-geneous cell group originating from various sources. The locally resident stromal cells are considered as a major source of CAFs and they have distinctly different morphological and functional features from their normal counterparts. Bone-marrow derived mesenchymal stem cells (MSCs) serve as another group of CAF. Several studies suggest that tumor epithelial cells induce aerobic glycolysis in neighboring fibroblast. CAFS then secrete lactic acid and pyruvic acid, which are taken up by tumor cells. In fact, the heterotypic interactions between stromal cells and tumor cells with genetic and epigenetic alterations generate numerous adaptive strategies for the tumor cells, giving it a distinct phenotype as compared to the surrounding tissues. The adaptive strategies in tumor microenvironment include plasticity, acquisition of stem-like properties, unfolded protein response, production of exosomes, autophagy, invasion, metabolic reprogramming, immunosuppression and therapeutic resistance. Among these strategies, metabolic and immune reprogramming has been highlighted recently since the immune cells in tumor environment also compete for the nutrient. Therefore, overcoming metabolic stress is a critical step for tumor cells to survive and metastasize [1].

While tumor cells show increased metabolic and energy demand, proliferating T lymphocytes also show similar type of metabolic profiles. Nonetheless, patients with cancer showed impaired T cell response which was found to decrease the potential therapeutic effect of cancer vaccines and other forms of immunotherapy. Therefore, it is important to understand the metabolic profiles of both the tumor cells and T lymphocytes as metabolic reprogramming has been shown to be associated with therapy resistance in cancer cells which is also a part of the

immune escape mechanism. In fact, nowadays it is well established that a variety of metabolites (*e.g.*, lactic acid, reactive oxygen species *etc.*) promote tumor progression and also intimate the immune cells for immune escape mechanism. Furthermore, different cytokines (*e.g.*, leptin), secreted by tumor infiltrating immune cells (TILs) also support tumor growth. Apparently, cancer-immune-metabolism is a complex process having potential importance to elucidate the underlying mechanism of therapeutic resistance and development of new drugs or therapies for cancer treatment. Keeping in view the importance of metabolism in supporting cancer development, in this section we will discuss the metabolic reprogramming of cancer cells followed by its importance in assisting tumor cells to adopt the stressful conditions [3, 4].

METABOLIC REPROGRAMMING OF THE IMMUNE CELLS IN TUMOR MICROENVIRONMENT

Interaction of the immune system with tumor complex is a dynamic process as several components of the anti-tumor immunity are involved in eradicating tumor cells. The anti-tumor immune response is mediated by tumor antigen-specific cytotoxic T (CTL) cells and T effector (Teff) cells together with antibody-producing B cells and antigen presenting dendritic cells (DCs). These responses elicit adaptive anti-tumor activity through directly recognizing and removing tumor cells or thorugh cross-talk between the adaptive and innate immune responses. In addition, macrophages, natural killer (NK) cells, and NK-T cells form an important layer of non-specific immunity to suppress tumor progression [5, 6].

A proper immune system must have appropriate and sufficient energy to perform their function in response to non-self-particles or antigens. Accordingly, metabolic regulation and cell signalling are tightly and ubiquitously linked with immune response. The distinct metabolic profiles of different lymphocytes are intimately linked to their status and function. For example, resting cells are quiescent and rely only on adenosine triphosphate (ATP) for basal cell functions. Naïve T lymphocytes rely mainly on fatty acid oxidation and glycolysis to fulfill their energy demand for survival. However, to provide protection against the pathogen, T cells must remain capable of rapid responses and effector function.

CHAPTER 3

Signal Transduction Molecules and Pathways in Cancer: Implication of the Immune System in Modulating Cancer Development and Progression

Abstract: Signal transduction pathways and associated molecules play important role in maintaining cell death and cell survival. However, in cancer cells, some of these molecules are mutated and lead to cancer progression. These molecules also interfere with the components of the humoral and the cell mediated immune systems in tumor environment. Therefore, identifying these can be potential targets for cancer treatment.

Keywords: Apoptosis, Autophagy, JAK-STAT, K-ras, Myc, p53, Signal transduction pathways.

INTRODUCTION

The tumor microenvironment consists of primary tumor and stromal cells. A variety of cell types populate the stromal compartments, which include smooth muscle cells, myofibroblasts, carcinoma associated fibroblasts, vascular cells and immune cells. Of these, vascular cells include lymph-endothelial, vascular-endothelial cells and pericytes whereas the immune cells include lymphocytes, tumor associated monocytes/macrophages (TAM) and dendritic cells. Once the tumor cells and the stromal cells interact inside the tumor microenvironment, this induces the secretion of growth factors and cytokines leading to neovasculation and modifications of the extracellular matrix (ECM), which greatly support the growth and survival of tumor cells. The onset of cancer metastasis or dissemination of the primary tumor to distant body sites is one of the most complicated processes and cancer metastasis is one of the major causes of cancer related death. Critical steps in cancer metastasis include: (i) detachment of the

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primary cancer cells followed by migration and invasion to adjacent tissues, (ii) penetration of the extracellular matrix (ECM) and blood vessels (intravasation), (iii) subsequent penetration out of vessels (extravasation) and (iv) proliferation in a secondary site [1].

Interestingly, not all epithelial cells are capable of expressing the metastatic phenotype. Only those that are able to escape the primary tumor and enter the circulatory system (blood or lymphatic) and break down the basement membrane allow invasion, which is critical for metastasis. The ability of the cancer cells to leave the tumor mass not only depends on losing cell-cell contact at an early stage, but it is also associated with a change in cell shape referred to as the epithelial-to-mesenchymal transition (EMT). EMT is associated with the loss of cell adhesion proteins such as E-cadherin. Once the cells are in the circulatory system, the isolated cancer cells must survive at a distant site and form a micro-colony. While the inflammatory responses within the microenvironment can be triggered by tumor hypoxia, necrosis and excessive tumor cell proliferation, the inflammatory cytokines including colony stimulating factors (CSF)-1, granulocyte- monocyte (GM-CSF), transforming growth factor (TGF)-beta, chemokines (CCL2, CCL7, CCL3, CCL4) , vascular endothelial growth factor (VEGF), IL-8, *etc.* play an important role in promoting and recruiting additional inflammatory cells such as mast cells and neutrophils to facilitate tumor progression [1, 2].

It is now well established that the association between cancer and inflammation leads to cancer development. Various pro-inflammatory mediators triggered by inflammation aid tumor progression by regulating cascades of cytokines, chemokines, adhesion and pro-angiogenic activities. Whereas chronic inflammation predisposes to cancer, neoplastic transformation predisposes to an intrinsic pro-inflammatory microenvironment which further promotes progression towards malignancy [2].

Stimuli that are associated with this transformation can be grouped into two types. The extrinsic stimuli are bacteria, viruses, non-healing wounds, irritants, *etc.* On the other hand, the intrinsic stimuli are oncogenes, protein kinases, *etc.* Both types of stimuli trigger inflammation in tumor microenvironment. For example, about

1.2 million cases of infection related malignancies are caused due to chronic inflammation induced by bacteria and virus. Viruses such as human papilloma virus, hepatitis C virus and hepatitis B virus not only inhibit tumor suppressor proteins but also cause malignancy through inflammation related mechanisms. Organs that are highly susceptible to tumor development following chronic inflammation are gastrointestinal tracts, lungs, bladder, liver, pancreas and oesophagus. On the other hand, intrinsic stimuli for inflammation are mostly the oncogenens, cytokines and even the transcription factors such as necrosis factor (NF-κB), HIF1α, STAT3, Ras-Raf signaling, MYC, *etc.* These intrinsic stimuli converge signals in the nucleus of the tumor cells and co-ordinate inflammatory transcriptional activity by activating various nuclear transcription factors mentioned above. The cross talk between these transcription factors results in a complex web of signaling processes that further promote inflammation, facilitate tumor progression, proliferation, survival and angiogenesis [2, 3].

Since the intrinsic signal transduction molecules play an important role in triggering pro-cancer inflammatory responses, in this chapter, we will discuss some of those molecules that are potential targets for cancer treatment. Signaling molecules can be single protein molecules that activate several pathways or can be transcription factors that regulate the expression of several proteins and transduce signals to the cells. However, a number of signaling pathways that play an important role in tumor regression may be involved in cancer development or progression [4].

SIGNALING MOLECULES ASSOCIATED WITH CANCER DEVELOPMENT

JAK-STAT

Cytokines like interferons (IFNs) exert their biological functions by activating genes such as Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, which are used by many other cytokines too. Once a cytokine binds to its cell-surface receptor, the receptor dimerizes and subsequently activates JAK tyrosine kinases, which are constitutively associated with the receptor. Specific tyrosine residues on the receptor are then phosphorylated by

CHAPTER 4

Cancer Immunotherapy

Abstract: Treatment of cancer does not depend on a single drug and the existing strategies are challenged by the the frequent development of drug resistance properties of cancer cells. Although chemotherapy drugs are still used as the first line of treatment modalities for cancer, immunotherapyis also used as a targeted treatment modality nowadays. However, major challenge with the treatment is that, patient recovery is very low. Therefore, combination therapies are being investigated and some of them are at the clinical stage with FDA approval. In this chapter, we will discuss currently available immunotherapy drugs and their synergistic effects in combination with chemotherapy drugs or two different immunotherapy drugs

Keywords: Checkpoint blockades Chemotherapy, Combination therapy, Immunotherapy, Monoclonal antibodies, Synergistic, Vaccines.

INTRODUCTION

Cancer is treated either locally or by applying drugs. The main difference between these two approaches is that, local treatments are performed by surgery and radiotherapy whereas drugs are used for systemic treatment. At present, four different categories of drugs are used to treat cancer. These include chemotherapy, hormonal therapy, targeted therapy and immunotherapy. Of these, chemotherapies involve a large group of cytotoxic drugs and they interfere with cell division and DNA synthesis. Hormonal therapies interfere with growth signaling through hormone receptors on cancer cells. Targeted therapies involve a group of antibodies and small molecule-kinase inhibitors that specifically target the growth signaling pathways in cancer cells. Immunotherapies induce or augment anti-cancer immune responses [1 - 3].

Of the different types of cancer drugs, the concept of immunotherapy dates back

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to the late nineteenth century during which tumor shrinkage was observed following administration of bacterial products in and around tumors. At that time, antibodies were recognized as "magic bullets" for cancer therapy. Since then many observations were documented including spontaneous remissions of cancer, higher incidences of cancer in immuno-suppressed patients and identification of tumor specific antigens and lymphocytes. In addition, considerable knowledge has been obtained by investigating the components that either induce or enhance the anti-tumor immune responses. Agents and products that enhance the immune system include non-specific inflammatory inducers such as bacterial lipo-polysaccharides (LPS), tumor associated antigens (TAA), cytokines and antibodies (Abs). Components of the immune system that are used as cancer therapy include cytokines, immune cells and monoclonal antibodies (mAbs) [1, 2].

Despite its discovery almost a century ago, several factors hampered the development of cancer immunotherapy. Two major obstacles are: (i) the presence of the immunosuppressive cells of the tumor microenvironment; and (ii) challenges of designing immunotherapy trials [1, 2].

We already know from the previous chapters that tumor microenvironment is a highly dynamic system and it is able to avoid and escape the immune system (ref: cancer immunotherapy- revisited). Thus, tumor evolution proceeds through induced immunosuppression and adaptation to immune recognition by altering the expression of surface markers. The presence of tumor associated macrophages (TAM), T regulatory cells (Treg) and myeloid derived suppressor cells (MDSCs) forms immune suppressive network in tumor microenvironment. The presence of these diverse suppressed populations in tumor environment is an obstacle in increasing effective immune response for tumor elimination [1, 2, 5, 6].

Obstacles associated with designing the clinical trials include difficulty to define the optimal dose and schedule for immunotherapies. Compared to classical chemotherapeutic agents, there is insufficient correlation between the maximal tolerated dose and the maximal effective dose. Another problem associated with immunotherapies is that the classical volumetric response criteria are inadequate for evaluating the efficacy of immunotherapy. Immunotherapy is favorable for

testing the drug in a low volume and or microscopic stage but less effective in patients with a large tumor burden [1, 2]. Another difficulty with immuno-therapies that hampered the clinical development of many immunotherapies is lack of patentability and lack of funding by pharmaceuticals [7]. Despite these difficulties and drawbacks, some recent pre-clinical and clinical findings have given a great boost to immunotherapy and several of them also received approval from the US Food and Drug Administration (FDA) [7].

Not only immunotherapy drugs, but other treatment modalities of cancer (*e.g.*, chemotherapy drugs) also induce immune response. Therefore, combination treatment modalities can be used to design synergistic treatment modalities. In this chapter, we will discuss different types of immunotherapy drugs and their effect on the immune systems, clinical phases and trials and effects of the combination therapies on the immune system.

CHEMOTHERAPY DRUGS AND THE IMMUNE SYSTEM

Chemotherapy drugs are used as the first line of treatment modalities for different types of cancer. Chemotherapies are used as drug or medicine and differ from other treatment strategies such as surgery and radiotherapy as these are localized treatments and chemotherapy is a systemic treatment. Currently, more than 100 different types of chemotherapies are known and these are broadly classified based on their chemical structure [3, 8]. Most of the chemotherapy drugs are cytotoxic to the cancer cells. Cell death following treatment with chemotherapy may or may not induce immunogenic response. The non- immunogenic death can be either apoptotic death or non-apoptotic death. The apoptotic death in response to anticancer drug is mediated by death-receptor-dependent and independent pathways. Anticancer drugs such as 5-FU increases the expression of death receptors FAS, tumor necrosis factor (TNF) and TNF related ligand receptors. Other anticancer drugs trigger apoptosis by inducing the release of cytochrome c from mitochondria. The non-apoptotic death includes necrosis and mitotic cell death and characterized by inhibition of cell cycle. However, the frequency of non-apoptotic death is less compared to apoptotic death [7, 9].

Chemotherapy drugs can induce immune response too. Immunogenic cell death

CHAPTER 5

Systems Biology Approach in Cancer Immunotherapy

Abstract: Systems biology is relatively a new field of study in cancer research. However, this approach has gained much attention as it can be used to understand the molecular level of a system under diseased or healthy condition and under dynamic or static condition. The approach allows understanding the interaction of DNA, RNA, protein and metabolite levels of a cell. Since, the cancer micro-environment consists of highly heterogeneous population of cells, systems biology is the robust tool that can be applied to understand this complex environment in the presence of perturbed condition using small molecules or targeted drugs like immunotherapy. Systems biology is already applied by drug design and discovery companies as well as by drug regulatory agencies to monitor safety and toxicity of the drug. The high throughput (HT) technological platforms generate un-biased datasets. However, data–mining is a problem for this approach. Despite this drawback, systems biology has been used in cancer immunotherapy to some extent. In this chapter, we discuss the known application of systems biology in cancer immunotherapy in particular to its application in biomarker identification, vaccine development, application in combination therapy, use in the development of validation models and future application in personalized medicine.

Keywords: Biomarkers, Drug discovery, Omics platforms, Personalized medicine, Systems biology.

INTRODUCTION

The field of immunotherapy is challenged by several factors. These include biomarker identification that correlates to the diseased state, target selection, animal model validation, decision on whether the drug should advance into phase III trial or not and placement of the drug in market by drug discovery and

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development company [1]. Overcoming these challenges requires the use of approaches which provides integrated information about different layers of a cell ranging from DNA to RNA to protein to metabolites. This eventually requires the involvement of broad group of professionals ranging from molecular biologists, immunologists, clinicians, bionformaticians, drug design and discovery companies and regulatory bodies. Systems biology is such an approach and over the last 25 years, it has been extensively used in different fields of life sciences research including drug design and discovery, biomarker identification of complex diseases such as cancer and autoimmune diseases, target validation and many other applications. The approach is also being considered for personalized medicine so that the treatment can be tailored to specific diseased group. The field of cancer immunotherapy belongs to drug design and discovery field [2]. As a result, many challenges faced by drug design and discovery company are common challenges for cancer immunotherapy field. In this chapter, we will discuss the challenges associated with immunotherapy development. Other challenges such as marketing, clinical trial and safety related issues will be discussed in chapter 6.

CHALLENGES IN CANCER IMMUNOTHERAPY

Challenges in Biomarker Identification

Detection of biomarkers in tumor environment may involve invasive repeated biopsies. Peripheral blood (PB) may be attractive to analyse some biomarkers. However, it may not reflect the local tumor microenvironment. In addition, potential biases may be introduced by the site of tumor collection. Samples of superficial cutaneous and lymph node metastases may be the easiest to collect, but their biology differs from the primary tumor and the metastatic sample. This also affects tumor staging. For example, PDL1 expression varies by melanoma location. Again, the location of metastases itself works as a prognostic marker. Visceral metastases are associated with a worse prognosis than non-visceral metastases [3, 4].

Another challenge is the assessment of tumor samples by flow cytometry (FC). FC is used to analyse functional information on specific immune cell types using cell suspensions. This is very difficult to carry out due to limited sample volume.

Accurate quantification can be carried out by using immunohistochemistry (IHC) and gene expression profiling technologies. However, incorporation of multi-colour tissue immunofluorescence can maximize the information obtained from limited biopsy specimens [3, 4].

Another technological challenge in biomarker identification is the application of serological analysis of recombinant cDNA expression libraries (SEREX) to identify cancer testis antigens, such as NY-ESO-1, and the MAGE, LAGE, GAGE families. SEREX involves the construction of cDNA expression libraries and protein expression followed by autologous serological screening for detecting reactivity of the proteins with antibodies present in the sera of the patient or subjects. Although this is a productive approach, it is a laborious technique and may not yield quantitative data [5].

Challenges with Combination Therapy

Two main types of combination regimens are under investigation. These include a combination of immunotherapies with standards of care and combination immunotherapies among themselves. However, combination therapy is challenged by several factors. Decision on the optimal immunotherapy combinations accurately requires the identification and validation of reliable surrogate biomarkers. In addition, confirmation on their clinical activity is also dependent on biomarker identification. Besides, new combinations into the clinic are to be informed by preclinical data in animal models and mechanistic evidence for pharmacodynamic interactions and selection of patients based on biomarkers primarily found in malignant tissue biopsies. However, only limited data are available on the information as which combination is the best for a given specific malignant indication or for a given patient and predictive models exist for clinicians. Therefore, scientists and clinicians need to work together actively in order to discover and develop new agents as partners for combination and take advantage of biotechnological advances, especially the systems biology approaches to produce improved, next-generation immuno-oncology agents. Consideration needs to be given to delivery routes of immunotherapies to maximize their bioavailability in tumors and tumor-draining lymph nodes, efficacy, specificity and toxicity that might be caused from the combination of the

CHAPTER 6

Perspectives

Abstract: Both system biology and cancer immunotherapy are emerging fields in life sciences research. Immunotherapy is considered as targeted treatment modalities for cancer. However, the complex nature of the tumor environment affects the efficacy and clinical outcome of this type of therapy. Combination therapy can be a solution. But this requires investigation at the cellular and molecular level of effect of the drugs. Systems biology is such a powerful tool, which allows the cellular level understanding and has been used in target discovery and validation in different fields of cancer research. Despite this advantage, challenges exist in large dataset mining. In this concluding chapter, we discuss some of the challenges and possible future directions that may help to overcome this problem.

Keywords: Animal models, Cancer, Inflammation, Software tools, Systems biology, Targets, Validation.

INTRODUCTION

Cancer is caused from inflammation. As a result, tumor cells grow in the presence of heterogeneous cell population such as epithelial cells, vascular and lymphatic vessels, cytokines, chemokines and infiltrating immune cells. This heterogeneous cell population is at different stages of growth, differentiation and metabolic state. Therefore, design and development of drugs as anticancer agent is a challenging task for researchers and pharmaceutical companies and requires extensive knowledge on its surrounding environment to get the systems level understanding of the diseased and treated cells. Systems biology approach offers a robust tool to study the molecular and cellular level of a cell. Therefore, the purpose of this volume was to discuss the application of this approach in possible areas of cancer immunotherapy.

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The presence of immune-surveillance system provides natural protection against cancer. However, few of the cancer cells carry mutation in genes of the anti-tumor immune response, which helps these cells in immune escape and immune evasion process (Chapter 1). Cancer cells are also known to hijack the body's normal cellular functions. A number of oncogenes and proto-oncogenes play important role in this process and aid in cancer progression involving different signalling molecules, transcription factors and cascades of protein molecule of the same signalling pathway (Chapter 2). Cancer cells also show deregulated metabolic pathways and these have been associated with drug resistance property. Interestingly, many cells of the anti-tumor immune response also show high proliferation and growth rate like the normal cells (Chapter 3). This creates non-specific effect on these antitumor cells when treated with anticancer drugs, especially chemotherapy drugs. Most of the conventional anticancer drugs are less specific and the side effects are enormous. Therefore, targeted therapies with less toxicity are of importance nowadays. Immunotherapy drugs are considered as targeted therapy and some of them received US FDA approval. However, immunotherapy drugs have also drawbacks as these are effective only on narrow range of cells. Therefore, combination therapies have been proposed at clinical and pre-clinical trials (Chapter 4).

Since its execution, systems biology and omics technologies have been widely used in the field of oncology and extensively used for characterizing the molecular complexity and heterogeneity of tumor not only for the development of targeted therapies but also for the identification of responder populations for such treatments (Chapter 5). In addition, the approach has been used for disease subtyping, disease progression and multi-gene diagnostic tests using gene expression profiling arrays [1 - 3]. Systems biology is also used in drug or target identification and validation and has been proposed for regulatory bodies to incorporate the modeling approach for drug approval policy. This process comprises of the complex set of experiments that aim to identify the key molecular drivers of disease and confirm the pharmacological modulation of the drug-in-development that leads to net clinical benefit. Thus, systems biology approaches can be used to enhance trial design, execution and analysis of drugs entering clinical studies.

However, challenges exist with large dataset mining of the omics platform. Several steps can be taken once the challenges are identified [4, 5]. In chapter 5, we discussed some of the challenges associated with the design of immunotherapy development. Here we extend our discussion on marketing of drugs and challenges associated with clinical trials.

CHALLENGES WITH MARKETING, CLINICAL TRIAL AND SAFETY ISSUE

Drug discovery and development companies are often need to make rapid and rational decision to place a product in the market in the face of incomplete clinical trial data. This was a significant concern when drug design and discovery groups were dependent on cell based reductionist approach and non-validated animal models on the human condition. Additional challenge is whether the drug should advance into phase III trial or not [6, 7].

The success rate of new drugs entering clinical studies is at historic low. The success rate of novel drugs also depends on the type of disease. Higher success rates are reported in infectious disease and cardiovascular disease whereas lower rates are reported for oncology and disease of the central nervous system. The most frequent cause of failure is lack of efficacy, particularly at the late stage of large scale pivotal studies. This significantly increased the cost for pharmaceutical companies leading to decreased rate of production of novel drugs. However, to enhance the efficacy and safety of drugs entering clinical trials, FDA approved model based drug development (MBDD) improved decision making and acquisition of knowledge from clinical studies This saved significant cost for pharmaceutical companies [4, 6, 8].

Challenges with novel drugs also exist during clinical trial and at several phases such as discovering the dose response in relation to the efficacy and toxicity of the drug, patient subgroups and validation stage. Model based data integration and analysis can improve our understanding of the clinical path and confirm processes. Since the systems approach integrates complex data on clinical studies such as disease process, time scale and clinical endpoints, previous information from other studies and heterogeneous clinical subjects, this improves quantitative

SUBJECT INDEX

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