Genome Editing and Engineering

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Module - 10
Engineered immune cells for Cancer therapy (I)
Lecture - 02
Cancer therapy (I) -Part A

Welcome to my course on genome editing and engineering. Today we are going to discuss about engineered immune cells for cancer therapy. According to World Health Organization, cancer or neoplasm or malignant tumor is a large group of diseases that can start in almost any organ or tissue of the body when abnormal cell grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body, and/or spread to other organs. The process of spreading to other organs is called metastatizing and is a major cause of death from cancer. Cancer immunotherapy exploits the body's own immune system to fight against the disease. It was designated as an annual scientific breakthrough in 2013 by Science magazine and has exhibited promising anti-tumor efficacy in recent years.

Cancer immunotherapies are categorized as immune checkpoint inhibitors, adopted cell therapies and tumor vaccines. Numerous patients with advanced tumors have benefited from cancer immunotherapy and some have achieved complete remission. The essential cellular and non-cellular components of the tumor microenvironment is composed of numerous cellular and non-cellular components. As you can see over here, under the cellular components we have T lymphocytes, B lymphocytes, macrophages, adipocytes, neutrophils, cancer associated fibroblasts, cancer cells, NK cells and so on.

While under non-cellular components, we have ECM, the extracellular matrices, IFN gamma, TNF, then various growth factors, then interleukins, chemokines, exosomes, apoptotic bodies and so on. This tumor microenvironment is very important to understand from the point of view of various cancer therapies. It has been established that evading immune destruction is one of the characteristics of cancer. The immune system try to

control the cancer and the cancer cells try to avoid that immune protection. Tumor cells can inhibit immune effector cells or cause immune tolerance through the secretion of extrinsic factors affecting the tumor microenvironment.

TME is composed of tumor cells, stromal cells and immune cells and the interaction among these cells affect tumor progression. We have shown you some of the components of these tumor microenvironment and also we will show you some interaction map in one of the slides later. In the tumor microenvironment, macrophages in T cells are the most distorted immune system components. Tumor-associated macrophages support tumorigenesis and metastasis and inhibit anti-tumor responses by releasing EGF, IL-6, TNF, MMPs, VEGFA, and so on. In addition, T cells' anti-tumor activity and metabolic state is disrupted by the immune modulatory cytokines and checkpoint inhibitors present in the tumor microenvironment.

Accordingly, a study by Chung et al. on 11 breast cancer cases revealed that the presence of M2 macrophages in the TME was correlated with T cell exhaustion. So, here are a detailed list of the different cell populations in the tumor microenvironment and listing the natural function and then their altered or new function in the tumor microenvironment. and the produce substances by these cells are shown here. So, helper T cells, if you consider for instance, the natural function is Th1 stimulates dendritic and NK cells attracts T lymphocytes in the tumor microenvironment. Th1 blocked by interleukin 4, 10, TGF beta, TRIAG, Th2, M2, Th2, inhibition of Th1, stimulation of M2 population of macrophages.

And the produced substance of anti-tumor activity, you can see here the TNF alpha, interleukin 12, 17, 18, 21, 27 and so on. Similarly, macrophages, their natural functions are destruction, phagocytosis of abnormal cells and inducing inflammation. In the tumor microenvironment, their functions alter to pro-tumor inhibition of the inflammatory processes. For a detailed discussion on these cell populations, their natural functions and the altered function or novel function in the tumor microenvironment, you can refer to Bojig et al. Biology 2022-11, page number 929 onwards.

So, this is an extended list of those various cells. You need to understand that the various cells which are involved in the tumor microenvironment already listed earlier have some natural functions when there is no any cancer in the patient. But, when cancer occurs, they may have altered or novel functions. And this is the network of the intracellular interactions I was referring to in our earlier slide. These various cells, they work in interaction with one another in the tumor microenvironment.

This interaction ultimately determines the progress of the disease or the arrest of the disease. If the immune system wins, the body can take care of the cancer to some extent on its own. And if the cancer cells are able to break this reaction outcome, which results out of the networked interactions, the disease would progress. So, T cell exhaustion and differentiation in tumor microenvironment is an important phenomena. T cell exhaustion is a hyporesponsive state of T cells in chronic environment with increased inhibitor receptors, decreased effect of cytokines and impaired cytotoxicity.

Most T cells in tumor microenvironment are exhausted leading to cancer immune evasion. Naive T-cells activate and differentiate into effector T-cells, as you can see here, in secondary lymphoid organs. When effector T-cells enter the tumor microenvironment, they are polarized into exhausted T-cells with decrease in effector cytokines and increase in inhibitory receptors. Subsequently, exhausted T cells may turn to be defective memory T cells or to be deleted physically. Stimulants present in a tumor microenvironment such as cytokines, chemokines and growth factors determine the polarization of macrophages and their differentiation towards M1 or M2 sub types.

M1 macrophages are able to, number one, release pro-inflammatory cytokines such as interleukin-12, IFN-gamma, IL-1, IL-23 and INOS. Secondly, re-educate the DC and the CD4+ T cells and thirdly, activate CD8+ T cells and as a result, promote an immune response against tumor and prevent tumor progression. In contrast, M2 macrophages and TAM (tumor-associated macrophages) increase angiogenesis and formation of tumor-associated fibroblasts. These cells attenuate immune responses in the TME and increase tumor progression. The state of TME strongly affects the patient's prognosis.

Identifying the mechanisms underlying the tumorigenic characteristics of interactions between immune suppressive cells and tumors can reveal novel therapeutic targets for developing antagonists, such as monoclonal antibodies and immunomodulatory drug intervention. Another approach is to fortify already existing immune responses or develop new ones through bypassing their dependence on the robust and intact immune system which becomes non-functional in the tumor microenvironment. Immunotherapy of cancer has recently proven its potential in numerous clinical trials. Let us now discuss about the functional properties and dynamic changes of the immune cells in the tumor microenvironment. So, here is the blood stream as you can see and this is the tumor microenvironment with various cellular components.

T cells in the peripheral blood infiltrate into tumors and undergo functional state transitions, possibly driven by the immunosuppressive microenvironment. Naive CD8 T cells or CD4TH (T helper) cells differentiate into traditional states and finally reach exhausted states while resting tracts regulatory T cells transit into suppressive states in the tumors as you can see in the figure. Such state transitions result in a reduction of affected T cells yet an accumulation of exhausted T cells and suppressive tracts both of which are proven to be proliferating and highly clonally expanded in the tumor microenvironment. Myeloid cells in blood are mainly monocytes including CD14+ and CD16+ subsets while these cells tend to differentiate into macrophages and dendritic cells in tumors. The TME is sculpts them to harbor immunosuppressive phenotypes resulting in an accumulation of suppressive TAMs (tumor-associated macrophages), and CDC2s, classical dendritic cells, but a reduction of CD16+ monocytes and CDC1s.

In addition, single cell integration facilitates the identification of novel subsets of CDCs and TAMs in the tumor microenvironment reveals that TAM subtypes tend to co-express M1 and M2 signatures thus inconsistent with the polarization models. NK cells exert cytotoxic functions with perforin and granzymes when activated by the integrated signals of activating and inhibitory receptors, yet they show reduced cell numbers, impaired cytotoxic function, and an impeded orchestrating effect for immune responses exemplified by the hampered Cdc1 classical dendritic cell recruitment in the TME. The functional defects of NK cells are possibly driven by tumor cells, through secreting

immunosuppressive factors and expressing ligands of inhibitor receptors, while decreasing the expression of ligands of activating receptors to hinder NK activation. B cells play important roles in anti-tumor immunity and immune checkpoint inhibitor treatment as B cells and tertiary lymphatic structures, TLSS, contiaining aggregates of immune cells including T cells, B cells and follicular dendritic cell are found to mediate improved responses to immune checkpoint inhibitors, the mechanism of which involves the activation of TFS and B cells.

The activated B cells can differentiate not only into plasma B cells to produce antibodies to clear cancer cells, but also into active T cell mediated immune responses by presenting antigens to CD4 T cells that could promote the activation of CD8 T cells.

Tumor immune evasion in hematological malignancies: As per tumor immuno-editing theory, the loss of equilibrium between tumor cell generation and immunity mediated elimination results in tumor development secondary to immune evasion. In hematological malignancies, targeted recognition on tumor cells by cytosoxic T lymphocytes is a central step necessary for effective T cell mediated immunity. Impairing targeted recognition of CTLs on the tumor cells is an important strategy for tumor immune evasion.

Immune evasion mechanisms mostly include defective co-stimulation, immune checkpoint blockade, increased suppressive immune cells, tumor-altered metabolism, regulated soluble factors, and impaired apoptosis-related pathways that are not directly related with targeted recognition of CTLs on tumor cells. In cellular immunity, APCs including dendritic cells, macrophages, and subsets of B cells phagocytose, and present tumor antigens on the cell surface in an HLA-dependent manner, providing co-stimulatory signals for priming the T cell response. Upon activation by APCs, CTLs can recognize tumor cells via HLA-dependent presentation of tumor antigens on the cell surface, resulting in CTL-mediated lysis or apoptosis. In hematological malignancies, this process can be impaired, contributing to the loss of recognition of CTLs to malignant cells. Immune-mediated elimination by cytotoxic T lymphocytes and tumor immune evasion strategies that are dependent on or independent of targeted recognition of CTLs on tumor cells in hematological malignancies.

Antigen presenting cells uptake and present tumor antigens on the cell surface in an HLA dependent manner, providing co-stimulatory signals for priming the T cell response. Upon activation by APCs, CTLs can recognize the tumor cells with the presentation of tumor antigens in the context of proper metabolism, for example, sufficient oxygen and glycose. Subsequently, CTLs kill tumor cells by releasing perforin and granzyme B or by expressing fast ligand on the surface, inducing cytolysis or apoptosis.

Mechanisms of immune evasion in hematological malignancies: The impaired targeted recognition of tumor cells by CTLs is primarily attributed to three mechanisms.

Let us discuss the first one: dysfunctional APCs. Generally dendritic cells can promote anti-tumor immunity via uptake and presentation of altered self-antigens or neoantigens from malignant cells. However, dendritic cells in hematological malignancies can be decreased in quantity and quality by tumor cells or other components of tumor microenvironment. Tumor progression related soluble factors like psychooxygenase 2, COX-2, prostaglandin E2, PGE2, transforming growth factors beta, TGF beta and vascular endothelial growth factor VEGF can deregulate dendritic cell functions to impair the presentation of tumor antigens interfering with activation of tumor-specific CTLs. This results in dysfunction of APCs that indirectly impedes activation of tumor-specific CTLs, inhibiting T cell-mediated de-intimidation by interfering with targeted recognition of CTLs on tumor cells.

The second one is the defective antigen presentation of tumor cells. Normally upon priming of APCs, The TCR of activated tumor-specific CTLs can recognize peptides derived from tumor antigens in the context of HLA molecules leading to targeted killing of tumor cells. However, in hematological malignancies, the expression of HLA on the surface of tumor cells are downregulated as a result of mutations or deletions in the HLA loci. B cells can present their own idiotypes in an HLA-dependent manner. However, structural loss of HLA class 1 and class 2 expression on mutations in HLA class 1 and class 2 loci can cause immune evasion of B cell lymphoma cells.

Alternatively, mutations and deletions in the beta-2 microglobulin gene of HLA have been observed in Hodgkin lymphoma. Further downregulation of genes associated with

antigen presentation machinery has been described in lymphoma. The third one is the low rate of mutational recognition. A third strategy to escape from targeted recognition of CTLs can be described by the low rates of mutational recognition in hematological malignancies. Short linear peptide epitopes of 9 to 10 and 15 to 18 amino acids long are presented by HLA class 1 and class 2 molecules respectively.

Some of these peptide residues mediate HLA binding while others bind to complementary determining region III of the TCR inducing T cell activation. Patient T cell activation is not started with cell peptides derived from cell proteins. However, both HLA binding and TCR interaction are sensitive to point mutations in tumor cells resulting in even single amino acid substitutions which illicit robust T cell response. The peptides generated as a result of the mutations are called neoantigens and they can elicit effective CTL response and likely play a key role in controlling tumor development.

Generation and recognition of neoantigenic peptides: generation and recognition of neoantigenic peptides after mutational or structural changes to somatic DNA, changes to coding nucleotide sequence can be generated by non-synonymous point mutations, insertions, deletions events leading to reading frameshifts or large-scale structural changes such as chromosomal translocation and gene fusion events as shown in this diagram.

When this changes to somatic DNA cause an alteration in amino acid sequence creating a peptide that can be processed and presented in the context of MSC 1 or 2 and induce TCR activation such a peptide is designated as a neo antigen. So, this is the TCR activation and T cell proliferation, this is the noble peptide and this is the normal peptide and there is no any TCR activation as a outcome.

Mutation types derived neoantigens: mutation derived neoantigens can be divided into two classes, type 1 neoantigens and type 2 neoantigens. The type 1 neoantigens can alter the amino acids in regions that make contact with the TCR anomaly. without changing the anchor residues in relation to HLA molecules.

These mutations do not change the binding affinity of the peptides to HLA molecules, but may make the peptides immunogenic. In contrast, type II neoantigens are created from

the mutations that can generate a new anchor residue promoting the binding of the mutated peptide onto HLA complexes. Upon presentation by tumor cells, both types of neoantigens can be recognized by specific T cells followed by CTL mediated killing of tumor cells. However, subdominant neoantigens that exist in hematologic malignancies cannot be efficiently presented, resulting in tumor immune evasion. Neoantigen presentations might be also a determinant factor for influencing tumor evasion, although the exact details of these mechanisms remain to be determined.

Genome instability is generally a hallmark of tumor cells and can lead to somatic mutations that are entirely absent from the normal human genome across the whole genome-wide sequence. In contrast with other tumors such as melanoma and lung cancers, hematological malignancies are 10 to 20 times lower in the frequency of mutations. Multiple myeloma contains about 3000 somatic mutations while acute myeloid leukemia, acute lymphocytic leukemia and chronic lymphocytic leukemia each contains about 1500 to 2000 mutations. The reduced mutational load in hematological malignancies resulting in reduced neoantigens likely relates to the inactive T cell responses in the context of tumor progression.

It was reported that only 0.3% to 1.3% of mutated peptides induced a CD8+ T cell response and only 0.5% of mutated peptides elicited a CD4+ T cell response. Mutational load was shown to positively correlated anti-tumor immunity in many cases.

Alternative strategies of immune evasion: Alternative strategies of immune evasion involves immune checkpoint and pathways, regulatory soluble factors, suppressive immune cells, and tumor-altered metabolism and factors promoting escape from immunity-mediated surveillance. Immune checkpoints, which refer to a number of inhibitory pathways, are critical for maintaining self-tolerance and modulating the immune response. Tumor cells in hematological malignancies such as multiple myeloma or non-Hodgkin lymphoma, Classic Hodgkin lymphoma and myelodysplastic syndrome can escape from the host immune system through immune checkpoint pathways such as cytotoxic T-lymphocyte-associated protein 4 and program date 1 pathways (PD-1). Suppressive immune cells including regulatory T-cells, tumor-associated macrophages, and myeloid-derived suppressor cells can form an inhibitory microenvironment

surrounding the tumor cells. These cells can inhibit the response of leukemia-specific CTLs to the malignant cells by secreting soluble factors including inhibitory cytokines such as IL-4, IL-10, and transforming growth factors B, as well as chemokines CCL-22, CCL-17, and CCL-12.

Additionally, tumor altered metabolisms can shape anti-tumor immunity. For example, in tumor genesis, the derivation of glucose and amino acids caused by tumor growth can impair the proliferation and effective functions of T cells, thereby promoting tumor cell evasion from the immune system. Metabolic enzymes such as indole amine 2,3-dioxygenase which can function to deprive arginine and tryptophan from the microenvironment are over expressed in tumor cells, MDSCs and APCs. Counteracting these critical pathways may be critical in the development of therapeutics for eliciting effective CTL response to tumors.

Let us now sum up the immune evasion landscape. CTL mediated immunity can be suppressed by targeted recognition dependent and targeted recognition independent mechanisms leading to immune evasion in hematological malignancies. Strategies including dysfunctional APCs, defective co-stimulation and impaired antigen presentation represent targeted, recognition-dependent immune evasion. In contrast, it is including immune checkpoint pathways, suppressive immune cells, tumor altered metabolism, upregulation, oxygen and glucose, Glycose deprivation and regulatory soluble factors represent a process independent of targeted recognition of CTLs on the tumor cells. Let us now discuss about the cancer immunotherapy. We have a fair understanding about the tumor microenvironment and how do we do immunotherapy with respect to cancer.

Immunotherapy aims to boost natural defenses to eliminate malignant cells. This is a monumental breakthrough for cancer treatment and has revolutionized the field of oncology. Although the idea of unleashing the host immune system to eradicate cancer could trace back to a century ago, significant advances have been achieved in recent years in terms of basic and clinical investigations. Multiple cancer types have shown sustained clinical responses to immunotherapy, although with limited response rates and unclear underlying mechanisms. Immune cells are the cellular basis of immunotherapy.

Thus, understanding the immune infiltrates in the TME is the key to improving responsive rates and developing new therapeutic strategies for cancer treatment with immunotherapy. Let us discuss about the historical development of cancer immunotherapy, the various advances made in the last decades. since its beginnings in 1890, when Dr. William Colley observed that some cancer patients with erysipelas, a superficial skin infection caused by Streptococcus pyrogens experienced better condition than those without infections. Later it was explained that the immune responses elicited by bacterial infections is responsible for improvement in the cancer patient.

So, it acts as a cross protection. Dr. William Cawley is often called the “father of cancer immunotherapy” for his acute observation. Extracts of heat, inactivated S. pyogenes and Serratia marcescens, termed Cawley's toxins, were used by Cawley to treat patients with cancer and achieved favorable responses in various cancers. In 1909, Paul Ehrlich hypothesized that the human body constantly generates neoplastic cells that are eradicated by the immune system. Lewis Thomas and Sir Frank McFarlane Barnett independently hypothesized that tumor-associated neoantigens are recognized and targeted by the immune system to prevent carcinogenesis in a manner similar to graft rejection, which is called cancer immunosurveillance hypothesis. Immunological assisted cancer therapy remained a controversial subject for decades until 1965, when leukemia cells regression of a patient was reported following bone marrow transplantation in response to adopted immune cell response against tumor cells.

The phrase “adopted immunotherapy” originated from that case. Later it was elucidated that T cells accompanied by natural killer cells had the principal role in that observed phenomena. What are the various immunotherapeutic approaches? The various immunotherapeutic approaches can be classified into two main categories. Indirect modification of T-cells regulatory elements or immunologically active proteins like interference. And secondly, direct ex vivo manipulation and restoration of T-cells or implanting engineered universal T-cells.

Initial cancer immunotherapy trials have been majorly performed by using some antibodies such as ipilimumab. Let us discuss about the various immunotherapeutic approaches against cancer. These approaches can be classified into two main categories.

The first one is the indirect modification of T cells regulatory elements or immunologically active proteins like interferons. The second falls into direct ex vivo manipulation and restoration of T cells or implanting engineered universal T cells.

Initial cancer immunotherapy trials have been majorly performed by using some antibodies such as ipilimumab, CTL4A targeting antibody, anti-program cell late 1 or anti-PD1, antiprogrammed death ligand 1, anti-PDL1, and anti-4-1BB, alongside with the administration of cancer vaccines, like trastuzumab, emtansine for advanced HER2+ breast cancer, NCS-DNA E7 vaccine against cervical cancer and Atezolizumab for non-small cell lung cancer. Afterwards, the development of novel combinatorial methods exhibited more reliable and efficient anti-tumor responses in comparison with their separate applications. Thank you for your patient hearing. We will continue this discussion in part B of this lecture.