Advances in Biotechnology

Chapter 1

Advances in Cancer Immunity, A Formidable Army

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1. Introduction

Cancer is a group of diseases involving abnormal cell division in an uncontrolled manner. Cancer affects almost all tissues; probably there are more than 200-250 types of cancer including breast cancer, ovarian cancer, skin cancer, leukemia etc. Different cell types in our body that can undergo the changes associated with cancer result in one or the other type of cancer [20].

Cancer cells acquire alteration in their genes which allows these cells to proliferate abnormally and make more copies of itself than a normal cell can and forms a compact mass of cells. These cells might look identical to its neighbors, but there are too many changes like over growth of tissue, a process called hyperplasia. Within this collection of cells, the cells divides more rapidly and abnormally, pile up on one another and there is a loss of contact inhibition. This is the development of the early stage of the tumor, a benign tumor or Adenoma. Within this mass of cells, further alterations may take place. Tumor cells accruing blood vessels into the tumor to nourish it and bring the growth factors to tumor that are required for their survival. In addition, these cells degrade the extracellular fluid also acquire the ability to move away from the initiation site and leave the primary site to disseminate throughout the body, creating a secondary tumor. This happens when cells access the blood vessels and take up residence in the secondary site, this is called metastasis.

2. Genetics of Cancer

In normal cell cycle, the cell goes from mitosis to G1 [gap1] phase, here cell increases in size and prepares itself to copy its DNA. The replication of DNA in the next phase is termed 'S' phase. Once the chromosomes replicate, the cell enters second gap phase G2, it is a period of protein synthesis and rapid cell growth where cell prepares itself for mitosis or M phase. In M phase cell growth stops and cell is ready to complete cell division and produce its two daughters. The new daughters immediately ensue into G1 phase and the cycle continues. It is a well regulated process.

There are basically two types of mutations that occur in the genes of tumor transformed cells.

1. Acquired mutation: Acquired mutation is not hereditary. It cannot pass from generation to generation. In this type of mutation, genes are damaged by ultra-violet rays, tobacco smoke and other factors like age, virus, dietary carcinogens and environmental carcinogens.

2. Germ line mutation: In Germ line mutation, mutation occurs in the reproductive cell. So it transmits from parents to a child.

There are different types of genes linked to cancer. E.g.: protooncogenes, oncogenes, tumor suppressor genes etc. protooncogenes are those which helps in normal cell proliferation and growth. The protooncogenes code for the protein which stimulates the cell division. Normally these genes encode proteins when growth factor is available. Mutation in these protooncogenes results in conversion into oncogenes and cell grows uncontrollably. For instance, a mutation in Ras protein makes it oncogenic, resulting in stimulation of cell division even when no growth factor around.

Tumor suppressor genes regulate the cell growth or slow down the cell division, repair DNA mismatch and apoptosis. Mutation in tumor suppressor genes results in loss of trait that is their ability to limit cell growth. Eg: p53 genes, pRB genes (protein retinoblastoma genes), p21 genes. pRB gene and p53 genes act as checkpoints between G1 and S phase. pRB genes block the transition from G1 phase to S phase in its active form. It can be inactivated by phosphorylation through kinases which is stimulated by growth promoting signals. p53 plays a crucial role in cellular response to DNA damage. It binds to damaged DNA and trigger cell growth arrest or apoptosis. However, in cancer cell defective in p53, it is unable to binds the damaged DNA and results in damaged DNA passes on [5,8].

Normal cell division is regulated by stop signals. Cancer cells have defects in these classes of stop signals. Because of alteration in Ras (proto-oncogenes), cancer cells are more capable of dividing because Ras protein plays an important role in controlled cell growth,

proliferation and migratio. Moreover, Mutation in tumor suppressor genes like pRB genes and p53 genes the stop signal is misprocesses or completely lost and cell continue to divide.

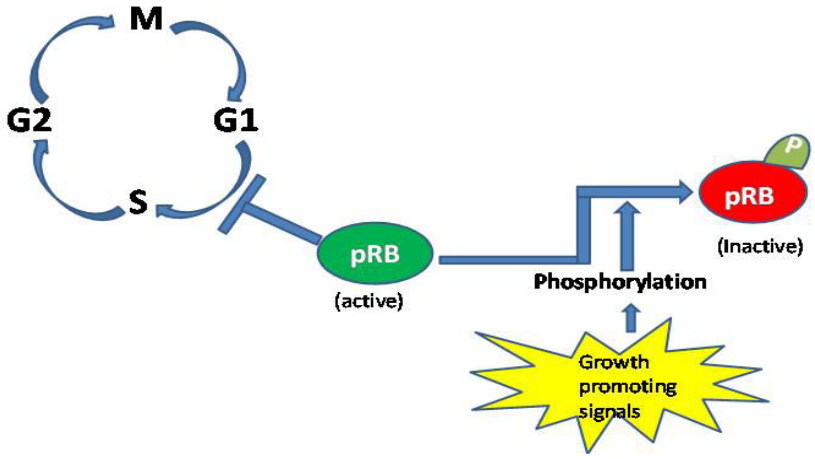

Figure1: Growth promoting signals phosphorylate pRB and inactivate it.

3. Immune Cells in Tumor Microenvironment

Our immune system is the body's defense against the infected and tumor transformed cells. Thus it is imperative to understand the role of our immune system and its responses in tumor formation and development and also the suppression of tumors.

Traditional immune responses like immune surveillance and immunoediting are crucial for preventing and inhibiting tumor development, for example, CD8+ and CD4+ Th1 cells along with cytokine IFN-γ are major anti-tumor immune effector cells. On the other hand, more current research show that incomplete responses can, in fact, promote growth and progression of cancer, particularly responses like inflammation. The convoluted nature of the tumor microenvironment is delineated by the elucidation of different subsets of immune effectors and regulatory cells. Tumor-induced effects on the differentiation and functioning of cells make this unique environment even more variable [2].

3.1. Dendritic cells

Dendritic cells are the most potent and well-known antigen presenting cells (APCs). Dendritic cells identify, process and present different tumor antigens to specific T-cells. They also maintain both, innate and adaptive immune responses by networking with myeloid and lymphoid cells. Immunohistochemistry shows that infiltration of DCs into primary tumor lesions has been associated with reduced incidents of metastasis, delayed tumor progression and lengthened patent survival.

Additional data support that the maturation state of DCs has diagnostic relevance. For instance, IHC analysis of the density of CD1a expressing DCs and the maturation marker DC-LAMP in cutaneous malignant melanoma shows that CD1a+ DCs were detected both in infiltrating melanoma cell nests and in the surrounding stroma, while DC-LAMP+ mature

DCs were mostly confined to the peritumoral areas. The degree of infiltration by CD1a+ and DC-LAMP+ DCs was inversely proportional to the thickness of melanomas and the high peritumoral density of mature DCs was associated with prolonged survival, simultaneously, the density of CD1a+ cells had a prognostic impact [11].

Programmed cell death in DCs plays an essential role in the regulation of immune responses and elimination of DCs from the tumor microenvironment significantly impacts the efficacy of anti-tumor immunity and facilitates tumor escape from immune recognition. Research has shown that a multitude of cancers, for instance, the apoptotic rate of TIDCs in endometrioid adenocarcinoma has been reported to be particularly higher than in normal endometrium. Many tumor-derived factors, including gangliosides (GM3 and GD3), neuropeptides, and other molecules are prominent inhibitors of DC function and known to induce apoptosis of DCs. DCs in tumors lose (or have limited) their ability to present tumor-inducing cells and induce the proliferation of tumor-specific CD4+ and CD8+ T cells. Studies also reveal abnormalities in the form of reduced production of IL-12, suppressed endocytic activity, inhibited antigen-processing machinery, abnormal motility, etc [11].

On the other hand, several molecules and signaling pathways, including the production of IL-10 and TGF-β, expression of IDO, iNOS and arginase, or expression of inhibitory B7-related molecules, play a role in immunosuppression by regulatory DCs. For instance, the interaction of B7-H1 with PD-1 on tumor-infiltrating T cells is a widely cited theory of immune suppression involving B7-H1 in ovarian cancer, PD-1+B7-H1+ DCs have a classical DC phenotype, but are immature, suppressive and respond poorly to danger signals. T cell suppressor function of these DCs appeared to be mediated by T cell-associated PD-1

Thus, the tumor milieu, controls functional polarization of DC differentiation and activity, as well as their ability to interact with other immune cells. Simultaneously, the network of immunosuppressive DCs are a critical part of supporting tumor progression and restricting the success of different therapeutic modals in the cancer patient.

3.1.1. NK Cells

NK cells are lymphocytes found to origin in the bone marrow. They are a part of the innate immune system with the capability to kill tumor cells upon activation. Once activated, they can follow two effector paths, first, they exocytose various cytotoxic granules containing perforins and granzymes also. These granules permeate the target cell and induce apoptosis. Alongside this, NK cells initiate the death receptor cascade. The NK cells interact with the TNF receptor superfamily, (FAS, TRAIL-R1, TNFR1, etc) by secreting their ligands. The second effector mechanism involves their ability to release a myriad of cytokines and chemokines, including INF-g, TNF, GM-CSF, MIP-1α and RANTES.

Extensive studies done in mice models show that those lacking NK cells have more aggressive tumor growth and metastasis. In human, clinical studies (in leukemia patients) have produced evidence for the benefits of NKCs. Implementation of these cells for anti-tumor strategy is done by the proper activation or inhibition. In humans, inhibitory NK receptors are members of the KIR family and in mice, of the C-type lectin-like Ly49 receptors, both sensing the expression of various allelic variants of classical MHC class I molecules. Other inhibitory NK receptors that engage non-MHC-encoded self-surface molecules for example, NKC-encoded CTLR KLRG1 (human and mouse), NKR-P1A (human) and Nkrp1d (mouse). While NCR NKp30, NKp44 and NKp46, and the CTLR NKG2D are prominent activating receptors [18,19,20].

3.1.2. Tumor -associated Macrophages

Macrophages, phagocytosing immune cells which are distributed in all tissues, macrophages are well recognized for their roles in homeostasis, tissue repair and development. One area of research on macrophages is of particular interest, tumor associated macrophages (TAMs).TAMs are myeloid derived suppressor cells (MDSC), which play a critical role in tumor progression in the tumor environment [10]. They augment cell proliferation, invasion, and metastasis; promote angiogenesis and hamper anti-tumor immune response. At initial stages, M1 cells infiltrate, activate and release pro-inflammatory cytokines and chemokines (CXCL19 and CXCL10) which in turn attract Th1, Th17 and NK cells. However, in advance tumors TAMs are polarized to form M2 cells, which release CCL17, CCL22 and CCL24, they encourage Th2 and Treg cell recruitment and differentiation. Thus TAMs can serve as tumor inhibitory as well as promote tumor development and immunoregulation.

Other MDSCs produce high levels of IL-6. Whereas MDSC would normally differentiate after migration, the factors within the tumor microenvironment prevent differentiation and instead promote expansion and activation of the immature myeloid cell population that may result in the suppression of tumor immunity [20].

3.1.3. T cell response in tumor environment

- **CD4+ and CD8+:** T-cells are commonly known as tumor infiltrating Lymphocytes. Research elucidate that infiltration by these cells is considered a positive prognostic factor for initial stages of cancer. CD4+ and CD8+ cells release IFN-γ which has an important role in inhibiting tumor growth and killing tumor cells [4,20].

- **Treg Cells:** Regulatory T cells comprise a subset of immunosuppressive cells that aid in maintaining immune homeostasis and self-tolerance, thus promoting immune evasion and tumor progression.For example, in pancreatic and breast cancer patients, the prevalence of CD25+ regulatory T cells is visualized in the blood at much higher rates than that found

in normal donors. In cervical and cancer patients, functioning CD25+ regulatory T cells have been identified within the tumor draining lymph nodes.

Tregs infiltrate cancerous lesions or tumor-draining lymph nodes (TDLN) and get activated by TAAs and tumor-derived factors. These activated Tregs downregulate the anti-tumor activity of, NK cells and DCs, and secrete immunosuppressive molecules. Tregs are also known to promote and establish sites for metastasis. Thus Tregs are an intensively researched sites for immune targeting [1,20].

- **Th17 cells:** Th17 cells (T helper cells) and their effector cytokines (IL-17A, IL-17F, IL-21, and IL-22) maintain host defensive mechanisms against various infections and pathogens, especially extracellular bacterial infections, and are involved in the pathogenesis of many autoimmune diseases. TGF-β and IL-6, through activation of Stat3, signal the differentiation of CD4+ cells into Th17 cells and IL-23 (a pro-carcinogenic cytokine) maintains and propagates the inflammatory cell population. Thus Th17 cells act as an antagonist of IFN-γ, restricting their differentiation and tumor suppressing function [14,20].

3.1.4. Regulatory Cytokines

- **IL-6:** IL-6 is an integral cytokine which is known to encourage cancer cell proliferation and simultaneously inhibiting their apoptosis by activation of transcription 3 (Stat3). It also influences differentiation, in the presence of other cytokines as discussed above. It has shown to play an important role in carcinogen propagated liver cancer development. It has been implicated in many of the processes that involve TNF which itself plays an essential role in several cancers.

- **IL- 10:** IL-10 also plays a significant role in growth and maintenance of cancer cells, especially those derived from CD25+ Tregs. It downregulates inflammation and can also inhibit activation of NF-kB, however, it can activate Stat3 also, hence playing a key part in cell proliferation and survival.

- **TGF –β:** TGF-β is widely recognized as an immunosuppressive cytokine, usually inhibiting immune responses anti-cancer. Moreover, TGF-β plays an important role in initiating generation and functioning of CD4+ CD25+ Tregs under particular conditions. For instance, TGF-β suppresses IFN-γ production along with promoting the generation of Foxp3+ Tregs and the differentiation of Th17 cells, which together, favor the growth and proliferation of cancer cells. In conjunction, TGF-β is also a strong inhibitor of macrophage activation and reduces their signaling of inflammatory cytokines such as IL-6, TNF and IL-1b, which are aimed at impeding inflammation-associated cancer [20].

3.1.5. Inflammation

The inflammation acts to protect and isolate an infected or damaged area. However if the inflammation does not subside, evidence indicates that it can lead to tumor formation, growth and angiogenesis. Inflammatory cells produce Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) cause mutations in neighboring epithelial cells. Furthermore, inflammatory cells release cytokines which elevate the intracellular ROS and RNI in pre malignant cells. This inflammation can lead to epigenetic changes that encourage tumor initiation. Cytokines synthesized activate NF-κB or STAT3 pathways in pre-malignant cells which promotes various pro-tumorigenic processes like survival, proliferation, growth, angiogenesis, and invasion. This forms a vicious circle as NF-κB and STAT3 further induce production of cytokines and chemokines that lure supplemental immune cells to support tumor-associated inflammation [3].

4. Therapeutics

The development and survival of cancer cell include several factors like a mutation in genes, many physiological alterations. The changes include loss of functions like alteration of tumor suppressor gene, mutation in oncogenes leading to rapid proliferation and immortality.

Mortality in cancer is fastidiously associated with metastasis of the tumor. Even though the primary tumor can be removed surgically there are always chances of remission due to metastatic tumor growth which might be lethal if unchecked or associated with vital organs such as brain or lungs. As conventional surgical treatment of tumor is not effective especially in the case of distant metastasis, there is a greater need for therapies involving the immune system of the body due to its specific nature and widespread reach.

The Anticancer drug can primarily be divided into four categories based on their mode of action. Chemotherapy is the first category as it involved a drug that induces cell lysis by either interfering with the synthesis of nucleic acid or negatively impacting the process of cell division. The second category is targeted molecule therapy which involves the use of molecules with high specificity for the target of choice. The third category consists of using a new kind of antibody with kinase inhibitors to block cellular signaling pathway important for cell proliferation by hormonal therapy. Lastly, immunotherapy involves the starting and aggravating anti-tumor immune response [16].

4.1. Immunotherapies

4.1.1. Vaccines

The concept of utilizing immune related cells was serendipitously discovered by William Coley in 1893, when he discovered that Streptococcus pyrogens infection led to spontaneous

remission in a patient suffering from sarcoma. This led to a series of experiments resulting in the discovery of Coley's toxins. The pair of toxins had high efficacy, even comparable to modern therapies.

Cancer vaccines work just like conventional vaccines, eliciting a lasting immune response. Just like early vaccines for infectious diseases, the old cancer vaccines made use of inactivated or killed cancer cells in the form of lysates and irradiated cells. These vaccines required no detailed knowledge about the intricate system of antigens. However, most resulted in quick relapse or low efficacy, therefore, this line of inquiry was not pursued further.

Most of the modern cancer vaccines utilize specific immunogen to elicit an immune response. Viral vectors are generally used for the vaccination due to their ease of disarmament and ability to induce strong cytotoxic T cell response. The second approach makes use of dendritic cells as they are prominent antigen presenting cells. The dendritic cells based vaccines skip the step from the transfer of antigenic peptide from the vector to the antigen presenting cell and directly present the antigen for detection by lymphocytes.

Both the vaccine types have been successfully tested for prostate cancer. However, the only FDA approved vaccine in the market is Sipuleucel-T manufactured by Dendreon for prostate cancer [17]. It contains monocytes collected ex vivo from patients and cytokines (GM-CSF) is used as an adjuvant.

4.1.2. Monoclonal Antibodies

Hybridoma technology enables the production high quantities of antibodies specific for a single antigen called a monoclonal antibody. In cancer therapy, monoclonal antibodies are used as a primary blocker of important antigens and ligands but can also be used as immune modulators.

Epidermal Growth Factor Receptor (EGFR) plays an important role in signal transduction for proliferation, migration and invasion utilizing the MAPK/KRAS signal transduction pathway. Several monoclonal antibodies such as cetuximab prevent the binding of the activator molecule (EGF) for EGFR, blocking the signal pathway. In a similar vein, human epidermal growth factor receptor 2 (HER2), is overexpressed in a quarter of cases of breast cancer and can be targeted by using Herceptin®. Both of the antibodies are FDA approved and in commercial use [17].

Despite the high efficacy of monoclonal antibody treatment, there have been cases of development of resistance against these antibodies as the cancer cells that do utilize the specific transduction pathway get aggressively selected.To circumvent the problem, a new strategy has emerged, wherein the inhibitors of CD-8 T cells, such as Cytotoxic T lymphocyte antigen 4

(CTLA-4) and Programmed Cell Death Protein 1 (PD-1), are being targeted for blocking by the monoclonal antibodies [12]. T cells are the major antitumor factor in immune response, thus increasing their population can increase the efficiency of the immune response. Other such drugs are also in development and seem promising

4.2. Chemokine Therapies

The introduction of immune stimulatory compounds in the vicinity of tumor cells leads to an increase in the potency of anti-tumor activity in the host. Due to such an immune stimulatory nature and their chemo attractant behavior of chemokines upon the white blood cell populations, a large number of studies are in effect to determine the extent of the impact of chemokine therapy in oncology. In monotherapy (using a single chemokine) studies it was determined that chemokines such as CCL -1, 2, 3, 5, 10, 16, 19 and 20, can mediate regression as well as increase immunity against future challenge.

Chemokines by themselves show little anti-tumor efficacy. However, when used in conjunction with other immune stimulatory chemo attractants IL-2 (T and NK cell activator) and XCL-1 (T and NK cell attractant), the efficacy is increased. The effects are primarily based upon the natural immune reactions involving $CD\text{-}4^{+}$ and $CD\text{-}8^{+}$ cells [7].

Another strategy involves fusing chemokine and tumor antigen using its immunooglobulin variable region for the fusion. It is predicted that the vaccine using the hybrid protein would generate enhanced protection against cancer [13].

Thus, chemokines alone and in conjunction with other molecules possess the capability of acting as immune cell attractant and decrease the tumor forming capability of malignant cancer cells. The combination is also a tumor suppressing agent. Lastly, chemokines might be used as adjuvants in cancer vaccines.

4.3. Adoptive Cell Therapy

Adoptive cell therapy is distinct from in vivo methods as a large number of T cells with desired epitopes can be generated in a short period of time in vitro that can later be selected for efficacy and specificity to effectively mediate cancer regression. In vivo growth allows for the production of T cells free from inhibitory factors that are a part of in vivo production.

Rather, it is a living therapy as the cells can proliferate after administration to continue on with their functions. However, one disadvantage is the identification of a cell that selectively targets the cancer cells while not affecting normal cells. ACT has mediated dramatic regressions in a variety of cancer histologies, like melanoma, cervical cancer, lymphoma, leukemia, bile duct cancer, and neuro- blastoma [15].

The current therapies make use of cells that are –

1. Native host cells with preexisting anti-tumor properties

2. Modified host cells made to express

i. Antitumor T cell receptors (TCR)

ii. Chimeric Antigen Receptors (CAR)

During the 60's little was known about T cells and their functions. A big leap in ACT development was the detailed description of IL-2 in 1976, which enabled the ex vivo culture of lymphocytes without a loss in function. Later in 1988, studies showed that adoptive transfer of tumor infiltrating lymphocytes (TILs) of autologous nature could help mediate regression in melanoma patients. This provided the first evidence that T Cells played a significant role in cancer immunotherapy for humans. However, the transferred cells had a short lifespan and disappeared within a few days of administration. The solution came in 2002, when it was demonstrated that non myeloablative chemotherapy, done before administration of autologous lymphocytes, not only increased the regression of cancer but also lead to a persistent proliferation of the cells within the host. However, melanoma appears to be the most effective TIL producer amongst cancer histologies, reliably giving rise to T Cells capable of expressing anti-tumor receptors. The continued interest in ACT leads to the development of genetically engineered lymphocytes modified to express anti-tumor receptors. In 2010, it was demonstrated that the genetically engineered lymphocytes expressing chimeric antigen receptors against the CD19 antigen of B Cell could mediate regression of B Cell lymphoma [16]. The above findings using natural and genetically engineered T cells has led to the widespread interest in using adoptive cell therapy in the treatment of human cancers.

The typical method for developing tumor infiltrating lymphocytes involves excising sections of tumor and either dissolving them into single cell suspensions or dividing the excised fragments to be grown in presence of IL-2. This leads to supported proliferation of lymphocytes that kill of the remaining cancer cells in the suspension, and within 2-3 weeks, a pure culture of lymphocytes is obtained [9]. The pure cultures are rigorously tested for anti-tumor activities in assays. Selected cultures are then rapidly proliferated in the presence of irradiated feeder lymphocytes. Within 5-6 weeks of tumor excision, a high concentration of lymphocytes may be obtained for administration into the patients.

An increase in the effectiveness of the therapy is observed when the patients undergo a lympho-depleting routine before the introduction of TILs into the patients. The routine maybe modified for the duration and intensity according to the physician and the patient. In humans, the lymphodepletion regimen induces the release of IL-15 which serves to enhance

the proliferation rate of the infused cell due to a lack of competing endogenous lymphocytes. Lymphodepletion also serves to increase the effect of the infused cell by inducing Toll-Like Receptor (TLR) mediated antigen presentation in APCs [15].

The observation that TILs can mediate regression in melanoma has helped raise interest in the use of the therapy for other tumor histologies.

It has been hypothesized that mutations in cancer cells might be the site of recognition for the TILs as several studies have shown that the target for TILs might be the various nonsynonymous peptides coded by the mutations in melanoma cells. However, the lymphocytes are not capable of recognizing all the mutations as the peptide produced must be able to be excised into sequences of approximately 9 amino acids so that it may be presented by the Major Histocompatibility Complex I while it may be a longer to be used by Major Histocompatibility Complex II. Such peptides maybe identified by the study of peptides with 20-25 amino acids, containing a mutation in the middle flanked by non-mutated residues. Using bioinformatics tools, the binding affinity of these peptides with MHC was tested and those with highest binding affinity may be synthesized and tested under laboratory conditions. Another method involves designing short DNA sequences that may be capable of producing the likes of above mentioned amino acids. The DNA sequence is cloned and transcribed into an RNA sequence which is then electroporated into the antigen presenting cells that might express the peptide. The APC is then tested for binding affinity with MHCs. The lack of an autoimmune response in the case of TIL might too be explained by the mutation target theory [6].

To increase the reach of ACT, genetic engineering has been used to introduce desired T cell receptors in host T Cells as their selectivity can be modified at will. Chimeric antigen receptors (CARs) can be produced by linking the variable regions of light and heavy chains of an antibody with intracellular signaling molecule. Linkage of additional sequences to the CAR might be done to enhance its immune stimulation capabilities. The chimeric sequences are usually transferred into the host cells using lentivirus and gamma retrovirus, however newer techniques such as transposon systems and CRISPR Cas9 are also being experimented with [16]. The selection of appropriate T cell subpopulation and the antigenic targets of the modified cell is of prime importance. Therefore, CARs are artificial receptors that recognize specific antigen present on the surface of the tumor cell and is thus independent of MHC presentation.

On the other hand, the TCR receptors introduced in the cells is composed of an alpha and a beta chain which help recognize the antigen presented by the MHC of the patient.

The acceptance of ACT in the mainstream therapy of cancer depends upon the identification of suitable target molecules for immunologic action. The hunt for monoclonal antibodies for targets expressed only by tumor cells and not normal cells has been going on

for a few decades, but rather unsuccessfully. Several studies to increase the potency of the T cells used in the therapy are underway such as in vitro proliferation of undifferentiated T Cells, improvement in lymphodepletion routine and improvement in vectors, which are likely to improve the clinical viability of ACT in the near future. Adoptive cell therapy, being one of the more risky and expensive therapy has been under public scrutiny, especially as an option for widespread healthcare option, as the personalized nature of the medicine does not suit the mass production tendencies of most pharmaceutical companies. For the introduction into widespread usage, the effectiveness of the procedure must overtake the tedious nature of the therapy.

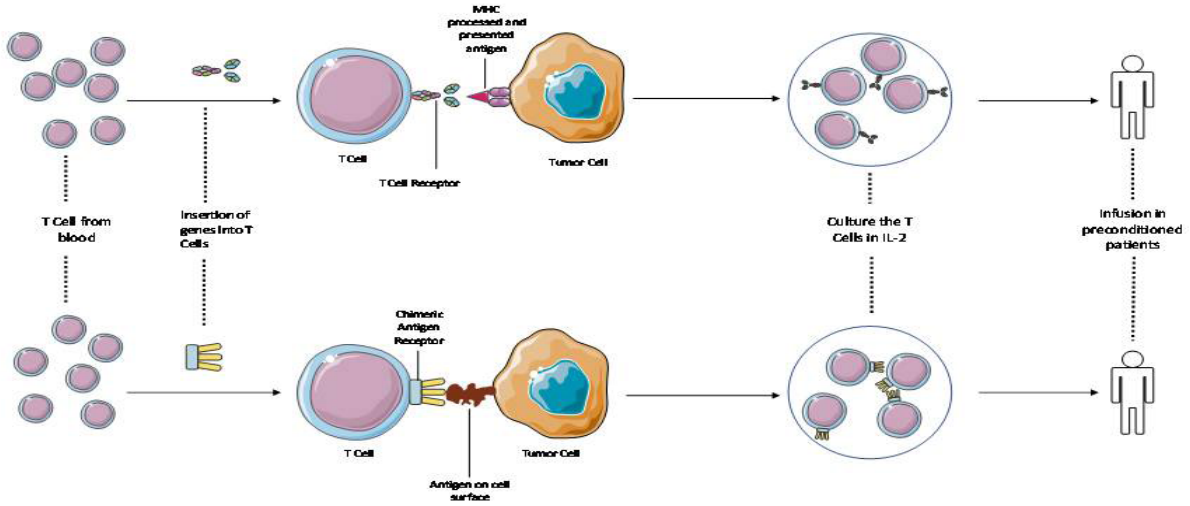

Figure2: Gene modification of lymphocyte

Because of the dual nature of critical factors in the tumor microenvironment, it becomes imperative to study them in even more depth than is currently being done so that we can utilize them as targets for immune therapy. Immunotherapy is a promising approach for the development of integrative therapies for cancer. In combination with strategies such as surgery, chemotherapy and radiation therapy, immunotherapy can provide a tool to efficiently attack residual disease and provide prolonged tumor-specific survival.

5. References

1. Chaudhary B, Elkord E. Regulatory T Cells in the Tumor Microenvironment and Cancer Progression: Role and Therapeutic Targeting. Vaccines (Basel). 2016; 4(3): 28.

2. Frederick S. Varn, Yue Wang, David W. Mullins, Steven Fiering and Chao Cheng. Systematic Pan-Cancer Analysis Reveals Immune Cell Interactions in the Tumor Microenvironment. Cancer Research. 2017; 77(6).

3. Grivennikov S I, Florian R. Greten, and Karin M. Immunity, Inflammation and Cancer. Cell. 2010; 140(6): 883-899.

4. Hadrup S., Donia M., and Straten P. Effector CD4 and CD8 T Cells and Their Role in the Tumor Microenvironment. Cancer Microenviron. 2013; 6(2): 123-133.

5. Hickman ES, Moroni MC and Helin K. The role of p53 and pRB in apoptosis and cancer. Curr Opin Genet Dev. 2002;12(1): 60-6.

6. Hinrichs C. S. & Rosenberg S. A. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol.

Rev. 2014; 257(1): 56–71.

7. Homey B., Müller A. & Zlotnik A. Chemokines: Agent for the immunotherapy of cancer? Nat Rev Immunol. 2002; 2(3); 175- 84.

8. Huun J, Lønning PE and Knappskog S. Effects of concomitant inactivation of p53 and pRb on response to doxorubicin treatment in breast cancer cell lines. Cell Death Discov. 2017; 3: 17026.

9. June CH. Adoptive T cell therapy for cancer in the clinic. J. Clin. Invest. 2007; 117(6): 1466–1476.

10. Liu Y & Cao C. The origin and function of tumor-associated macrophages. Cellular and molecular immunology. 2015; 12: 1-4.

11. Ma Y, Galina V. Shurin, Zhu Peiyuan, and Michael R. Shurin. Dendritic Cells in the Cancer Microenvironment. J Cancer. 2013; 4(1): 36–44.

12. Miller JFAP & Sadelain M. The journey from discoveries in fundamental immunology to cancer immunotherapy. Cancer Cell. 2015; 27(4): 439–449.

13. Nagarsheth N., Wicha M. S. & Zou, W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat. Rev. Immunol. 2017; 17(9): 559–572.

14. Ouyang W., Kolls JK and Zheng Y. The Biological Functions of T Helper 17 Cell Effector Cytokines in Inflammation. Immunity. 2008; 28(4): 454–467.

15. Restifo NP., Dudley ME. & Rosenberg S. A. Adoptive immunotherapy for cancer: Harnessing the T cell response. Nat. Rev. Immunol. 2012; 12(4): 269–281.

16. Rosenberg SA., Restifo NP., Yang JC., Morga, RA. & Dudley ME. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. Nat. Rev. Cancer. 2008; 8(4): 299–308.

17. Snook AE and Waldman SA. Discov Med. 2013; 15 (81):120–25.

18. Waldhauer I. and Steinle A. NK cells and cancer immunosurveillance. Oncogene. 2008; 27: 5932–5943.

19. Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, Cocco L and Vitale M. NK Cells and Cancer J Immunol. 2007; 178 (7): 4011-4016.

20. Zamarron B F and Chen W. Dual roles of immune cells and their factors in cancer development and progression. Int J Bio Sci. 2011; 7(5): 651-8.