INTRODUCTION TO MEDICAL

IMMUNOLOGY

SLIDE SHEET

NUMBER	21
DONE BY	Sondos Al-Najjar
CORRECTION	Karam Darwish
DOCTOR	Issa Abu-Deyyeh

Cancer and Immune System

This sheet will discuss the main idea of cancer, the immune surveillance against cancer, the tumor mechanisms to overcome the immune response, the targeted immunotherapy and finally cancer vaccines.

Cancer is one of the most common diseases worldwide, alongside with diabetes and obesity. It's estimated that 1 in 3 people get cancer throughout their lives as it's spread in both genders; males and females. You can find some types of cancers common in both genders such as certain lymphoma, leukemia and Lung cancer. The later (Lung cancer) is placed in the 2nd rank among all other types, at the same time you can find some gender-specific cancers, for example, breast cancer in females and prostate cancer in males.

**Breast and prostate cancers are the most common cancers in USA, 2014*
**these numbers and ranks might differ here in Jordan as we have high incidence of colorectal cancer, but lung cancer is still the 2nd rank here also.*

Cancers are very different, but all of them have problems with cell division which causes uncontrolled cell growth. The uncontrolled cell growth resulted from a mutation or a problem in **proto-oncogene** that promotes the growth of the cell and **tumor suppressor gene** that suppresses the growth. Both together will help regulate the cell cycle and maintain the cell growth within right levels and time. And for cancer to happen there must be a mutation that increases the activity of proto-oncogene followed by a second mutation that leads to the loss of tumor suppressor gene function.

One of the main examples of Tumor suppressor genes is p53. It was a great revolution when it was found that this gene is mutated in almost 50% of cancers; since it could be a good target for drugs. So (they thought that) we can use a single drug to correct and balance the mutation in p53 and that would be a drug for many types of cancers. Unfortunately, that is not the case; there are lots of individual differences between the each type of cancers and also between the tumors of the same kind. For example, in Lung cancer, small cell carcinoma is different from non-small cell carcinoma. Furthermore, even within the same small cell carcinoma in an X-patient, it behaves different than the small cell carcinoma in Y-patient; that's based on ethnic background, environmental factors, medication, geographic area...etc. So researches are directed towards the individualized therapy nowadays.

How did they know about the importance of p53?

1- Special tools to knock out any gene of interest. So, they found that mice lacking p53 gene will develop cancer within a month!
2- Techniques to induce mutations at certain sites such as CRISPR/Cas9 technology. It's a new, powerful and very specific one. It consists of protein-RNA complex found in the immune system of the bacteria. As the name implies, it's made of RNA sequence that is adjusted to complement any sequence of interest and cas9 which can cut the DNA. So if we want to make a mutation in certain gene in certain nucleotide, we bring its complementary sequence and add it to CRISPR/Cas9 and put it inside the organism. There it will bind to its complement (the gene) and cut it inducing a mutation.

** This technique is used in treating genetic disorders; especially those with single nucleotide change. By applying it to the 4 or 8 cell-stage-fetus, the baby will be born healthy.*

Cancer is a multistep disease, that's why it's a disease of aging. Although some cancers have genetic factors and happen at earlier ages such as certain types of lymphoma and leukemia but it's very uncommon for a cancer to be caused by a purely genetic factor. Retinoblastoma a pure genetic factor plays a role but it is very rare.
Always remember that the majority of cancers require 4-7 mutations to accumulate over a period of time to develop. The more we live, the more chances we have to accumulate those mutations the more chance we have to get cancer.

If a certain cancer requires 4 mutations to develop and someone was already born with a genetic mutation (one of these 4), it will be easier for him to acquire the other 3 mutations even at earlier age; that's exactly how genetic factors play a role in cancer development.

After the first mutation, the cell looks normal but it's now predisposed to proliferation. And by getting the 2nd mutation, the cell is still look normal under microscope (histological appearance) but it starts proliferation. As more and more mutations the cell acquires, it will look abnormal by structural changes development and fast proliferation.

Types of cancers:

1- Carcinomas: they are derived from epithelial cells
2- Sarcomas: they are derived from mesenchymal tissue; muscle, bone, cartilages, fat and CT
3- Hematopoietic (lymphoma, leukemia and myeloma)

Cancers could be spontaneous or virus-induced

1- **Spontaneous cancer**: It's the most common type and it happens as a result of factors in our lives that causes these mutations. These factors could be natural processes (ex. DNA replication), carcinogens (chemicals), smoking, fatty diet, life style and radiation.

*Natural processes such as the normal DNA replication could be a factor that aids in cancer development, as we are prone to errors every day. When the cell replicates its full genome it makes hundreds and thousands of mistakes that could be further reduced and corrected by corrective, proof reading enzymes such as p53.

***** Radiation can be in different forms; (1) UV radiation, from the sun over exposure and it is related to skin cancers and melanoma. (2) Ionizing radiation as X-ray and Gama rays. So you must consider protections for you and the patient when you are making an X-ray.

* Radiation effect is an accumulating effect, and after a certain dose it will be very dangerous. Don't forget this!

2- **Virus-induced cancer**: Certain viral infections can cause certain types of cancers. This is as a result of viral ability to incorporate their genome (randomly) into our genome. It could be with no significance if the viral genome was incorporated into an intron sequence. But imagine if a virus was able to incorporate its genome into tumor suppressor gene! Here it's with great significance.
Examples of these viruses:
(1) Human Papilloma Virus, HPV that causes cervical cancer
(2) Hepatitis C virus and to a lesser extent Hepatitis B virus that both cause Liver cancer.

** You'll be surprised that the human genome (especially the intron sequences) has so many genes coming from viral genome! In other words, our genome is a history work; it tells us about different viruses that enter our bodies throughout different stages of life.*

Immune Surveillance against Cancer

We've talked about the cancer itself and how it develops; now we'll mention how the immune system can fight cancer! It does this simply by mechanisms that we took throughout the course that all drain into the two main parts of the immune system; Innate and Adaptive. Almost all immune cells take part in fighting the tumor and each cell has its own role that will be discussed now.

MACROPHAGES

Macrophages are found in tissues, and that's an ideal location since solid tumors also arise there. So macrophages can recognize the tumor and secret certain **cytokines** such as TNF that will kill different tumors.
Also, macrophages can **recognize specific chemical molecules on the surface** of cells that are normally inner components and not found on the surface. For example, phosphatidylserine which is part of the inner components of plasma membrane in intact normal cell. But the transformation and the damage in the cancerous cell lead to its expression on the outer surface. Macrophages now can recognize it and attack the cancerous cell.
By using this concept, some of the localized bladder cancers are being treated with BCG injection as it activates macrophages and the innate immune system around the tumor which leads to its shrinkage. It's a curable treatment in some of the localized cases! **Bacilli Calmette-Guérin, (BCG) it's used in tuberculosis (TB) vaccination*

We have different subtypes of Macrophages like Macrophages-1 (M1) and Macrophages-2 (M2), and they play a role in certain conditions.
(The same concept as Th-1 and Th-2)

1- Th-1 secrets IFN-G that will activate certain subtype of macrophages which is **M1**. M1 produces an **anti-tumor response** by secreting TNF-alpha, ROS and IL-12. IL-12 in turn activates Th-1 again… it's a circle! This anti-tumor response/circle is needed in any regiment of cancer immune therapy.
2- On the other hand Th-2 secretes anti-inflammatory cytokines such as IL-4, IL-13 and IL-10, which activates **M2** cells that secrete IL-6, IL-8 and VEGF; being pro**-tumor macrophages**.

This can answer why some solid tumors, renal cell carcinoma as an example, have lots of immune cells (macrophages). It was discovered that most of these cells are M2 and they help the tumor to spread. They are called TAM (tumor associated macrophage).

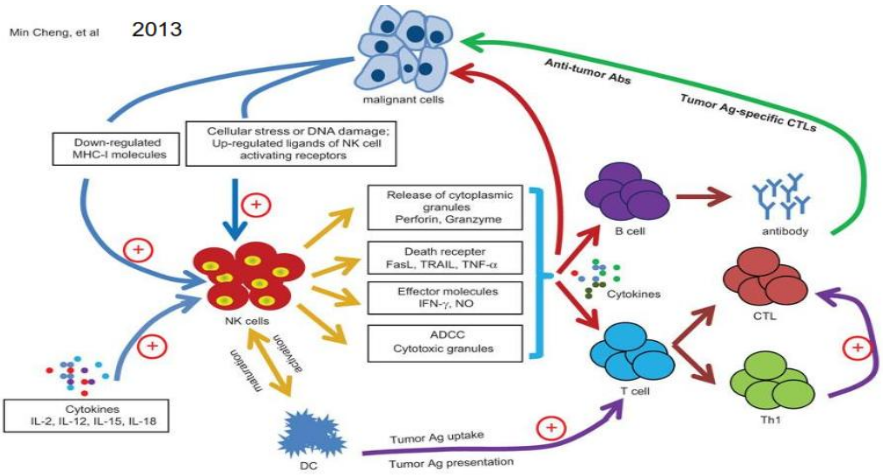
Natural Killers (NK)

NK cells can also fight the tumor by their known ways of killing. They kill through ADCC, Perforin-granzyme B and death receptors like Fas and Fas-L. Furthermore they produce cytokines such as IFN-gamma that in turn will activate Th-1. (Remember the first point in Macrophages, as Th-1 will induce the production of M1 that has anti tumor-response). So all in all, NK cells can attack the tumor in so many ways.

*Note1: NK cells are the only cells licensed to kill with no 2ry signal with the absence of MHC molecule.
*Note2: Another example for death receptors is TRAIL receptor that after binding its ligand will induce apoptosis.

Antigen Presenting Cells, T-cells and B-cells.

Antigen presenting cells like Macrophages and DC can represent the cancer antigens to the adaptive immune system which leads to its activation, mainly to Th-1 cells. Th-1 will produce cytokines and activate the cytotoxic T cells to kill the tumor directly. In addition to that, B cells are activated by T cells, and when they are activated they are able to produce antibodies that bind tumors and opsonize it to be killed by complement system (the classical pathway), phagocytosis through Fc receptor or by ADCC.



This picture above summarizes the role of immune system in fighting the tumor. And you can clearly see that there are LOTS of mechanisms to kill the tumor, so how the tumor managed to overcome those mechanisms and still not be killed?!

Tumors have their special mechanisms to escape the immune response; this is the tumor evasion. And here **problems of the immune system appear**.

1- Cytotoxic T cells and NKs are found in the circulation not the tissues, so they can't see and fight solid tumors.
2- Even if they managed to go to tissues, they need a co-stimulation signal for activation.
3- Immune cells at the cancer site further suppress these cytotoxic T cells.

(The mechanisms that protect us from excessive inflammation and autoimmunity are the exact same mechanisms that promote cancer. So there always must a balance!!)

Mechanisms of Cancer to overcome the immune response

- Not only immune cells, but tumor cells also can **produce cytokines** such as IL-6, IL-10 and VEGF. All these play a role in the suppression of the immune response by preventing the maturation and activation of DCs, macrophages, and so on.

- Tumor cells express certain **inhibitory ligands** on their surface, for example, PD-1 L and PD-2 L. When binding those ligands with their receptors on T-cells they will suppress their function. (PD-1 L binds to PD-1 receptor on a tumor specific T cell.)

- Tumor cells are able to force the generation and differentiation of new immune cells populations at the site of tumor that will help the tumor itself! One of the main cells here is **MDSC**, myeloid derived suppressor cell.
  When a myeloid cell -without any differentiation- travels to the site of the tumor, there it will receive certain signals and certain cell-to-cell surface contact that lead to its differentiation into MDSC. The mature MDSC starts producing arginase that will consume arginine to block T cell function. The end result will be blocking of the T cell function so production of immunosuppressive environment which is suitable for the cancer.

- The MDSC also converts the naïve monocytes -that came to the tissue in order to kill the tumor- into M2 which is an immune suppressive cell!

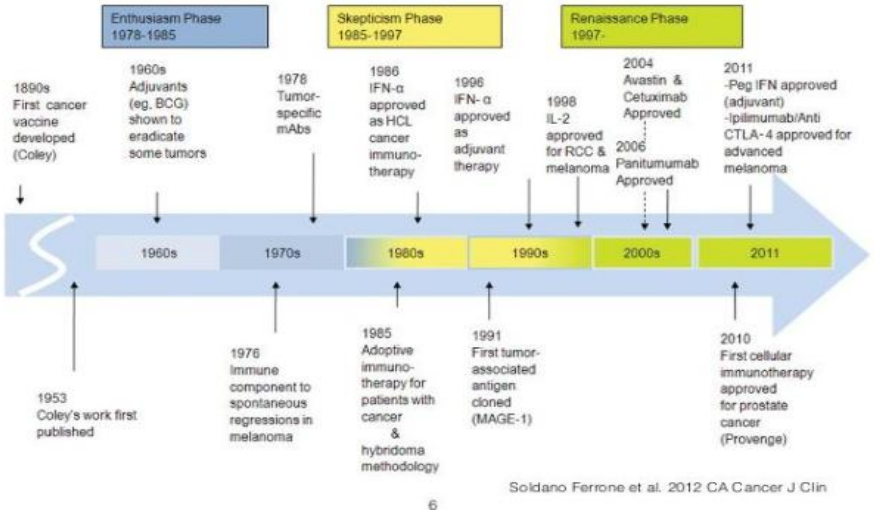
- Tumors are known to induce the proliferation of **T-regs** that suppress the immune system *(This is a good in autoimmunity!!)*

Targeted Immune Therapy

T cells have inhibitory receptors (CTLA-4 and PD1) to control their over proliferation. These receptors can be targeted by drugs, and by blocking the inhibitory receptor, the T-cells are allowed to be activated and kill the tumor.

PD1-L (produced by many tumor cells/not all of them) will bind to its receptor PD1 on the T-cell suppressing its function. New drugs are made to block either the receptor or the ligand and they won't give the inhibitory signal. So the Th-cells are activated and will activate cytotoxic T cells to kill the tumor directly.
→So we make antibodies against PD1, PD1-L or CTLA-4 to block inhibitory signals and thus activate T cells.

Immunotherapy has started long time ago, look at this timeline when you are reading the next paragraph.



(1) The first attempt was in 1890 in bone cancer patient by Coley
(2) Then the immunotherapy entered an enthusiasm phase between 1970's and 1980's where they started using BCG to stimulate the immune response against cancer.
(3) After that researches were done and skepticism phase appeared; however, they used INF-alpha and the adoptive immune therapy.
(4) In 1991, in the genetic phase, they started cloning the tumor antigens. That's because if they can purify the tumor antigens, they will be able to produce T-cells specific to these antigens, and then the patient can be injected with the T-cells as treatment or a person can be injected with the antigens for vaccination!

(5) The Renascence phase started in 1997.
(6) In 1998 IL-2 was approved to be injected in high doses for skin carcinomas and melanoma. (IL-2 induces the proliferation to T cells)
(7) Then the idea of monoclonal antibodies appeared which is the production of a clone cell that is able to produce large amounts of the same antibodies. In this era drugs like Avastin, Cetuximab and Panitumumab were made.

*** **Avastin** is a drug that targets VEGF blocking it, so it prevents angiogenesis and decreases metastasis.

***__Cetuximab and Panitumumab__ target EGFR. (EGFR leads to cell proliferation).

Panitumomab is now used in colon cancer and in lung cancer. Although it's expensive but it's highly effective, as it prevents the proliferation signaling pathway and the cell stop the division. Please note that, Panitumomab is highly effective unless we have another molecule downstream the pathways of EGFR also mutated such as K-RAS. So it's very important to test the EGFR mutation and K-RAS mutation before giving this drug to a colon or a lung cancer patient.
(To give the drug, EGFR must be mutated, while K-RAS must be a wild type-not mutated).

**Note: In the future, you won't find chemotherapy or radiotherapy anymore. Only customized therapy by detecting specific DNA mutations and treat them. This is the pharmacgentics field.*

Cancer vaccines

1- **Prevention vaccines**
those vaccines are given before the appearance of cancer in high susceptible groups. For example, HBV vaccine and HPV vaccine.

(a) **Hepatitis B vaccine** is given for a healthy person who is at risk "mainly in the medical field"

(b) **HPV vaccine** is first made by GSK company, they put the high risk strains in the vaccine which are HPV16 and HPV18; because these high risk strains are the ones associated with cancer while the other strains are associated more with warts. After that, Merck company developed

the same vaccine with an extra two low risk strains HPV6 and HPV11 (responsible for genital warts in both men and women) to encourage men to take the vaccine as men wouldn’t want to take the vaccine because they claimed that it's not useful for them. But don't forget that at the end they are reservoir for the virus so they will transmit it to their female partners, and the later will develop cancer.
So vaccination must be done for both genders at a young age because it was discovered that this vaccine is most effective when given in the first 10-12 years. At the end, HPV vaccine proved to be useful and powerful way to reduce the incidence of the cervical cancer; because almost all cases of cervical cancer are associated with this virus.

**Note: there is no vaccination for Hepatitis C "nowadays we have curable treatment for it*

2- Treatment vaccines

It's given for the cancer patients to boost their immune system using cancer antigens to attack tumor. FDA approved Sipuleucel-T in non-metastatic prostate cancer.
It's done by bringing a patient with prostate cancer and taking a sample from his blood. WBCs can be separated by luekopheresis, so we can have separate APCs from the patient. (DC and macrophages)
A specific gene* that encodes for a cancer antigen is brought from a prostate cancer cell and then introduced into the APC of the patient. After making sure that these cells started producing the tumor antigen (by flowcytometry), the patient is re-injected with them again "adoptive transport". Now these APC are able to present the tumor antigens to T cells (cytotoxic and helper) to initiate a potent immune response and fight the prostate cancer.

* The gene taken from the prostate cancer cell is Prostatic Alkaline Phosphatase, PAP and it's usually fused with GM-CSF gene.

"NEVER SAY ANYTHING ABOUT YOURSELF
YOU DON'T WANT TO COME TRUE"
GOODLUCK