PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE

Lecture Number: 24

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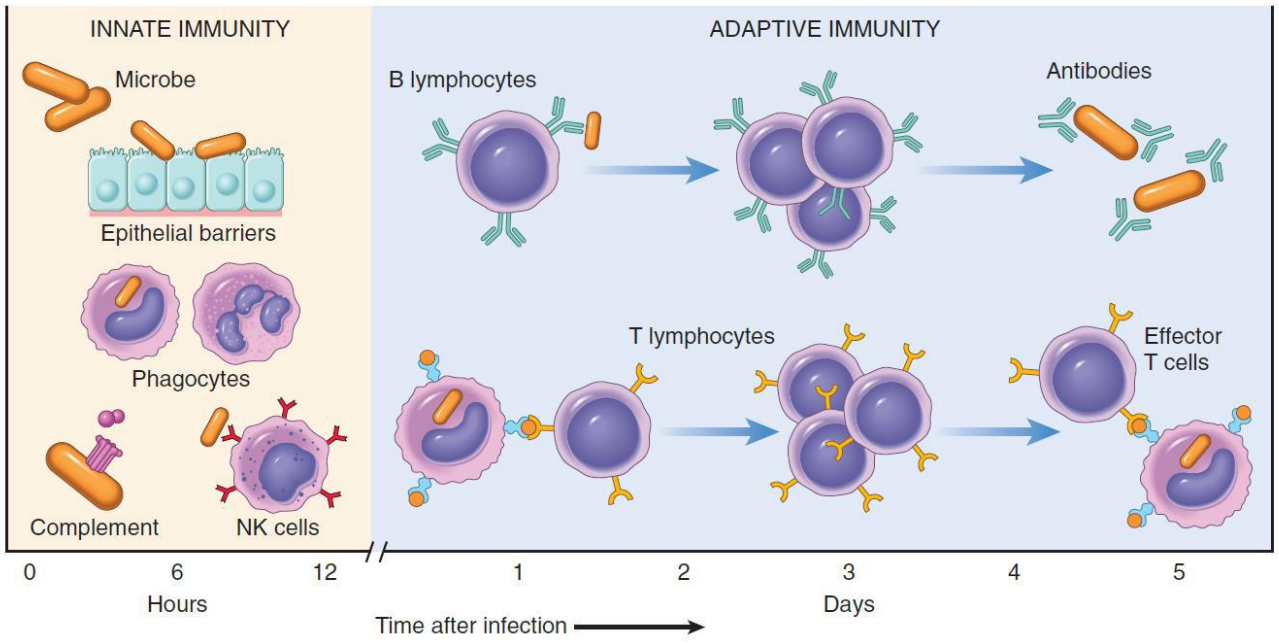
Tumor Immunity

Note: Many details and examples in the book were not mentioned in the lecture. Most of these details are included in this sheet, for those of you who don't have time to go over the book. Molecular Techniques, which the Dr. told us to study from the book (pages 211-213), have also been summarized. Good luck ☺

Before discussing tumor immunity, we need to mention some basic points in Immunology, since tumor immunity is largely based on Immunology.

As we all know, our immunity is divided into Innate and Adaptive immunity:



- Innate immunity is already present in all of our bodies; there is no need to produce it when the foreign antigen comes in. Complement phagocytes and Natural Killer cells, which are already reactive to foreign antigens, are what take care of these antigens.

Written by Mohamad Hindi

- Adaptive immunity means that there has to be an adaptation to this antigen, such as the maturation of antibodies in B-cells, or recognition by the T-cell receptor of this new antigen that wasn't known before. This is known as a delayed response; it is not immediate since the immune system must adapt to it. Adaptive immunity is further divided into Humoral (antibody based), and Cell Mediated (as in Cytotoxic T-lymphocytes).

- Cytotoxic T-cells, when used in the context of tumor immunity, are basically cells that kill tumor cells. They are also known as CD8 positive T-cells. CD8 and CD4 are surface markers that differentiate our two major T-cell populations.

- CD4+ T-cells (also known as T-helper cells), when presented with an antigen by the Major Histocompatibility Complexes (MHC's) of antigen presenting cells, produce cytokines, which attract other inflammatory cells to induce tissue injury. In other words, CD4+ cells mediate CD8+ cell response to antigens. When the antigen is detected by CD8+ cells, the cell responsible for presenting this antigen is immediately killed off. In the case of EBV viral infection, where Latent Membrane Protein-1 is produced, Cytotoxic T-cells are capable of detecting this antigen and killing the infected cell.

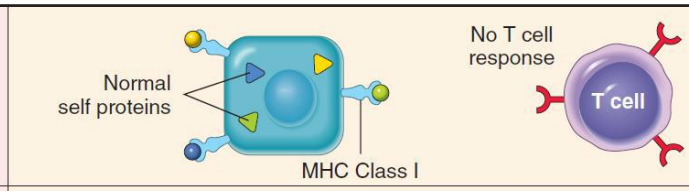
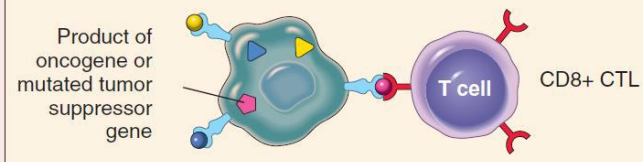
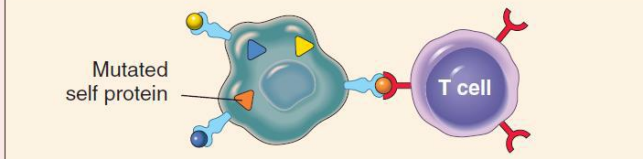
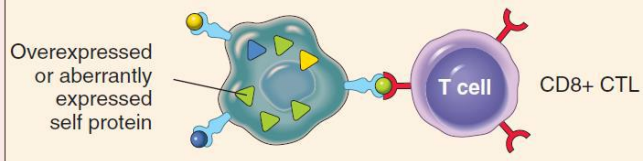
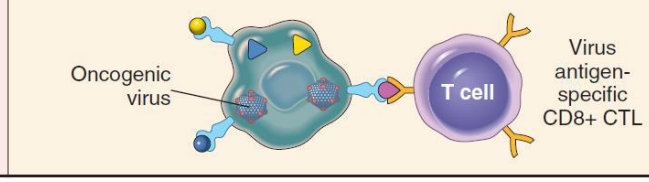
- There are activating and inhibitory molecules that can affect the recognition of the T-cells to the antigens presented by the MHC molecules, thus controlling immune response (fine tuning response).

- Natural Killer (NK) cells are part of our innate immunity. They recognize foreign antigens through MHC's and other activating ligands. As mentioned previously in immune system evasion, some tumor cells can down-regulate their MHC-1 molecules, evading detection by cytotoxic T-cells. A stimulatory mechanism for NK cells is expressed by cells who have lost their MHC molecules. So while cancer cells can evade cytotoxic T-cells, they may still activate NK cells through various activating ligands. Some cells are also capable of expressing stress factors when they are under duress (as in viral infection and DNA damage), which can activate receptors, such as NKG2D on NK cells. So although the cell is not presenting antigens through MHC's,

the stress signals can activate NK cells, killing cells at risk of neoplastic transformation.

- Macrophages: Activated T-cells and NK cells produce Interferon-Gamma (IFN γ), which pushes Macrophages down the M1 pathway. M1 Macrophages produce reactive oxygen species, lysosomal enzymes, and Tumor Necrosis Factor (TNF) to kill microbes. This same mechanism is used by Macrophages to kill tumor cells.

For the most part, immunity against tumor cells is mainly mediated by **cytotoxic T-cells**, which recognize various Tumor Antigens, such as:

Normal host cell displaying multiple MHC-associated self antigens		EXAMPLES
Tumor cells expressing different types of tumor antigens		Oncogene products: mutated RAS, BCR/ABL fusion proteins Tumor suppressor gene products: mutated p53 protein
		Various mutant proteins in carcinogen- or radiation-induced animal tumors; various mutated proteins in melanomas
		Overexpressed: tyrosinase, gp100, MART in melanomas Aberrantly expressed: cancer-testis antigens (MAGE, BAGE)
		Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV-induced lymphoma

1. Mutated oncogene (mutated RAS, BCR/ABL, etc.) products, and mutated tumor suppressor genes (mutated p53). Because the mutant genes are present only in tumors, their peptides are expressed in tumor cells only. Pieces of the peptide can be expressed on the surface of the tumor cell, and that particular cell is killed off by cytotoxic T-cells. Non-mutated oncogenes may also be overexpressed; the

primary example of this is the overexpression of the estrogen receptor HER2/NEU in breast cancers. Anti-HER2/NEU antibodies have been proven useful in breast cancer treatment.

2. Mutated self-protein caused by indiscriminant mutations in the genome, mutating genes other than oncogenes and tumor suppressors (like mutated non-coding areas of the genome, or mutated proteins that have nothing to do with carcinogenesis). These mutated proteins can also be detected by cytotoxic T-cells.

3. Overexpressed self-proteins (proteins such as gp100, tyrosinase, and MART in melanomas):

    - These proteins are normally expressed at low levels within cells, if they are overexpressed, or expressed on an abnormal location due to lost cell polarity, they can be detected by cytotoxic T-cells.

    - (from the book): Cancer-testis antigens are proteins expressed by genes that are silent in all normal tissues except the testis. Although they are produced in the testis, they are not expressed on the cell surface, because sperm does not express MHC molecules. Therefore, expression of these proteins by various tissues can be an indication for cancer. MAGE (Melanoma Antigen GEne) are prototypical of this group of proteins, and are expressed in various carcinomas (mainly melanomas).

    - Differentiation antigens (antigens specific to a specific type of cells) may also be detected.

    - Most of these self-proteins do not usually elicit an immune response, why? – because they are self-antigens. When these antigens are commonly expressed in our bodies, and sees common contact with our T-cells, T-cells will eventually develop an indifference towards them, so they will stop reacting to them, i.e. they develop a tolerance towards them.

4. Oncogenic viruses: Cells with viral infections (HPV, EBV) produce proteins which can be detected by cytotoxic T-cells, as mentioned earlier in EBV virus which

produces latent membrane protein-1. Vaccines against viruses such as HPV have proved useful in preventing cervical cancer in women.

As you can see, our immune system has various methods to deal with tumors. As a result, immunocompetent people will generally develop less tumors than people with compromised immunity (immunocompromised patients are incapable of producing a full immune response to foreign antigens, as in organ transplant patients on immunosuppressant drugs. Some tumors can suppress immunity by secreting different factors such as **TGF-β**).

5. (from the book): Oncofetal antigens, such as carcino-embryonic antigen (CEA) and alpha-fetoprotein, are expressed during embryogenesis, but not in normal adult tissues. Various colon and liver carcinomas may induce their re-expression in tumor cells, which can be detected by antibodies.

We mentioned in past lectures that tumor cells express glycocalyx, glycoproteins, and glycolipids (such as gangliosides and mucins) more than normal cells. Mucins such as CA-125 and CA-19-9 are found in ovarian carcinomas, and MUC-1 is found in breast carcinomas. Unfortunately, these molecules can be used in **Antigen Masking** (Block access of immune cells to antigen presenting cells). Tumor cells can also evade immunity through down-regulation of co-stimulatory molecules. These molecules strengthen T-cell response to MHC activation. If a tumor inhibits or down-regulates co-stimulatory molecules, T-cell response will be diminished.

Clinical Aspects of Neoplasia

Tumor effects on host: (Examples aren't for memorization)

1. **Location**: Depending on its location, a tumor may have a wide range of effects. Examples:

    - Benign or malignant tumors that grow beyond a certain size may obstruct the pituitary gland, cutting off its blood supply, and causing **hypopituitarism**.
    - Tumors in the lungs can restrict the exchange of CO2 and O2, compromising our ability to breathe.
    - Benign or malignant tumors in the brain can increase the intracranial pressure, causing **herniation**.

2. **Function:**

    - Lung tumors, as mentioned above.
    - A tumor in the liver may obstruct the biliary duct, causing **jaundice** (yellow pigmentation of skin).
    - Tumors can compress various nerves (In Acoustic Neuroma, a tumor compresses the auditory nerve and causes **hearing loss**)
    - Tumors that produce hormones can have drastic effects (As in ACTH in the pituitary gland, or T3 and T4 in the thyroid gland). Tumors can also produce hormones not normally secreted in our bodies, such as parathyroid-like hormone, which causes **hypercalcaemia**, eventually leading to metastatic calcification, causing functional loss.

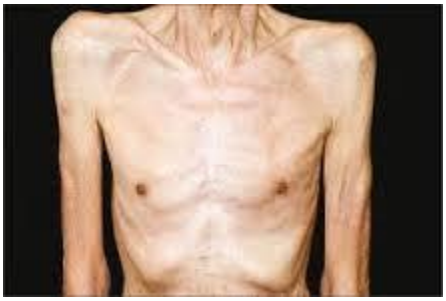
3. **Bleeding / Ulceration / Infection:**

    - Skin tumors can cause ulcerations, leading to bleeding and infection, which causes inflammation.
    - Tumors that ulcerate the intestines can cause intestinal perforation
    - Chronic bleeding will lead to Anemia and other complications.

4. Cancer Cachexia

- Cachexia is the progressive loss of muscle mass and body fat associated with cancer (see picture). It is related to the size and aggressiveness of the tumor, and characterized by weakness, anemia, and anorexia.

- It is NOT caused by the increased nutritional demands of the tumor.



- Essentially, Cachexia is caused by cytokines (mainly TNF) produced by the host cells in response to the tumor, or by the tumor cells themselves. TNF induces loss of appetite, which decreases caloric intake, while the metabolic demand of the body (BMR) remains the same. TNF also increases lipolysis (fat degradation) and proteolysis, which induces ubiquitin-proteosomal degradation of skeletal muscle.

- There is no real treatment for Cachexia except **removal** of the tumor.

Paraneoplastic Syndromes:

- Paraneoplastic syndromes are syndrome complexes that occur in patients with cancer that cannot be readily explained by the primary tumor or its metastases. A tumor can induce several paraneoplastic syndromes concurrently.

Note: You have to memorize the table :p

Clinical Syndrome	Major Forms of Neoplasia	Causal Mechanism(s)/Agent(s)
Endocrinopathies		
Cushing syndrome	Small cell carcinoma of lung Pancreatic carcinoma Neural tumors	ACTH or ACTH-like substance
Syndrome of inappropriate antidiuretic hormone secretion	Small cell carcinoma of lung; intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung Breast carcinoma Renal carcinoma Adult T cell leukemia/lymphoma Ovarian carcinoma	Parathyroid hormone–related protein, TGF-α, TNF, IL-1
Hypoglycemia	Fibrosarcoma Other mesenchymal sarcomas Hepatocellular carcinoma	Insulin or insulin-like substance
Carcinoid syndrome	Bronchial adenoma (carcinoid) Pancreatic carcinoma Gastric carcinoma	Serotonin, bradykinin
Polycythemia	Renal carcinoma Cerebellar hemangioma Hepatocellular carcinoma	Erythropoietin
Nerve and Muscle Syndrome		
Myasthenia	Bronchogenic carcinoma, thymoma	Immunologic
Disorders of the central and peripheral nervous systems	Breast carcinoma, teratoma	
Dermatologic Disorders		
Acanthosis nigricans	Gastric carcinoma Lung carcinoma Uterine carcinoma	Immunologic; secretion of epidermal growth factor
Dermatomyositis	Bronchogenic and breast carcinoma	Immunologic
Osseous, Articular, and Soft Tissue Changes		
Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma	Unknown
Vascular and Hematologic Changes		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma Bronchogenic carcinoma Other cancers	Tumor products (mucins that activate clotting)
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Anemia	Thymoma	Immunologic
Others		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

- There are three common and important paraneoplastic syndromes we should mention:

1. Hypercalcaemia: Other than the causes mentioned in the table, hypercalcaemia can result from extensive metastasis to the bones. However, in this case, it **isn't** considered to be a paraneoplastic syndrome, since it was directly caused by the tumor's metastasis (increased turnover of the bone leads to increased release of calcium from the bones into the bloodstream).

2. Hypercoagulability can cause **nonbacterial thrombotic endocarditis** in cancer patients, due to clots formed from hypercoagulability. So these patients are at a higher risk to develop blood clots.

3. Cushing's syndrome: Characterized by increased ACTH production. (characterized by the 'Buffalo's hump')

Grading and Staging of Cancer:

- Grading is based on the cytological differentiation of the tissue and the number of mitoses. The cancer is classified as grade I-IV. This varies widely depending on the cancer type. Grading will be discussed further in systems, since each system has specific tumors and each tumor has specific grading.

- Generally, if it looks and behaves like the original tissue, the grade is low. (Grade I)

- If it looks and behaves completely unlike the original tissue, the grade is higher (grade IV). (Grading increases with increasing Anaplasia).

- Staging is based on the size and spread of the tumor. There are two methods of staging used:

    1. The TNM system:

    Tumor (T1-T4; depends on size and depth of the tumor).
    Node (N0-N3; depends on how many and how far the affected nodes are).
    Metastases (M0 or M1; has the tumor metastasized or not).

    2. AJC (American Joint Committee) classification combines the TNM classification into four stages. (don't memorize the table)

Stage*	Tumor-Node-Metastasis (TNM) Criteria			5-Year Survival (%)
	T	N	M	
I	T1, T2	N0	M0	74
II				
IIA	T3	N0	M0	67
IIB	T4	N0	M0	59
III				
IIIA	T1, T2	N1	M0	73
IIIB	T3, T4	N1	M0	46
IIIC	Any T	N2	M0	28
IV	Any T	Any N	M1	6

- When compared with grading, staging generally has a **higher clinical value**.
- As you can see from the table, mortality varies widely from stage I to stage IV of cancer. The earlier a cancer is diagnosed, the higher the 5 year survival rate.

Note: the doctor showed an example in intestinal cancer, check the slide for it.

Lab Diagnosis of Cancer:

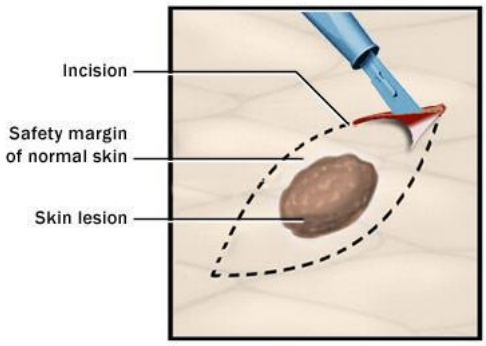
- While we depend heavily on lab diagnosis in cancer, you should not underestimate your own diagnosis.

- Clinical history and physical examinations are a crucial part of lab diagnosis. If we were to give a pathologist a tumor sample, they would not be able to give a diagnosis unless we specify who and where we took the sample from.

- Additionally, when providing a sample, you should make sure the sample is well preserved and representative of the tumor. If you take the sample from the middle of a proliferating tumor, the pathologist would see blood and necrotic tissue (useless for diagnosis). If we take the sample from the periphery, we could miss the tumor altogether. It's very important to know what kind of tumor you're dealing with and how to sample it. The laboratory evaluation of a specimen can only be as good as the specimen itself.

So, how do we take a sample?

- Excisional Biopsy: In the case of small tumors, we excise (take out) the whole tumor and give it to the pathologist. The pathologist will examine it to determine what kind of tumor it is.

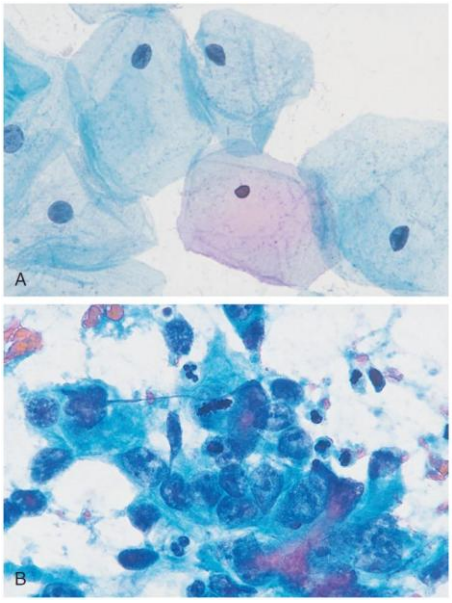


- We can't always do excisional biopsies because the tumor are either too large or because we need to be extra-careful with the tumor (as in breast tumors where we need to have **clean margins**). In this case, we take part of the tumor as a sample (Incisional Biopsy). During operations, we can send frozen samples from the tumor periphery or frozen lymph nodes to check whether these samples are

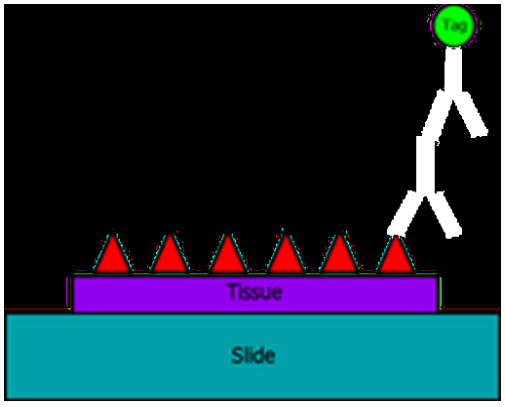
involved in the cancer or whether there is a clean margin or not. These tests are usually done within minutes (**frozen sectioning**). However, frozen sections are not always ideal, sometimes it is better to wait and use proper staining of the sample (takes longer) so you don't end up preforming inappropriate surgery (too much or too little).

- In Fine Needle Aspiration, a small number of cells are taken from the tumor and tested for a wide range of things (different stains could be used, whether the cells are differentiated or not, etc.). Fine needle aspirations are generally taken from organs you can see and reach, like the thyroid and breast. However, with radiology, you can take fine needle samples from deep organs, such as the liver and lungs, without being invasive, as in other biopsies (radiological assistance guides you where to go without injuring the neighbouring tissues). Fine needle aspiration usually requires an experienced hand to be useful. It is very important to know the ideal way to sample different tumors.

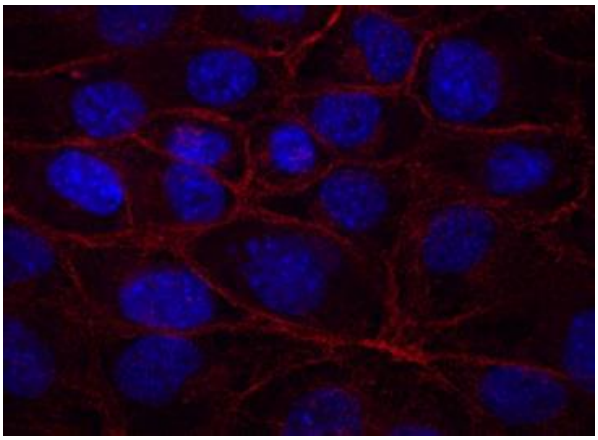
- Cytological Smears (Pap smears), where cells are put directly on a slide and examined under a microscope. As you can see in the picture, malignancy is easily recognized by the presence of hyper-chromatic nuclei, mitotic figures, and loss of normal shape. Tumor cells are generally less organized than normal cells. Cervical smears are not the only cytological smears done, cytological smearing is also used in bronchoscopies, in which lavage samples (samples taken from the lungs using a bronchoscope that passes through the mouth or nose) are taken. It can also be utilized in the bladder or GI tract.

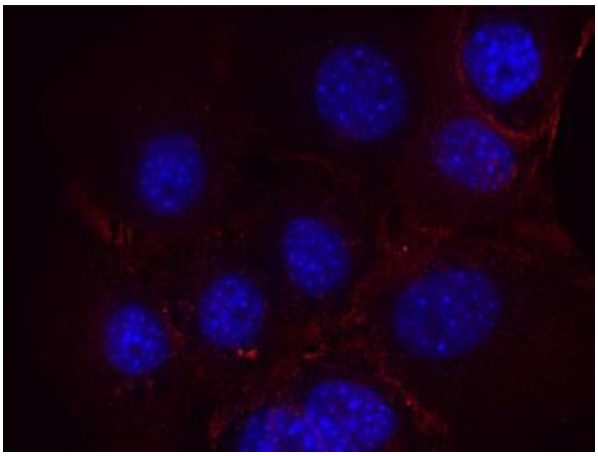


- Immunocytochemistry and Immunohistochemistry: We all know that proteins expressed on the surface of cells can be detected by antibodies. We can use this method to detect antigens on a slide using artificial antibodies (acquired by injecting the antigens in a rabbit or goat and purifying the antibodies produced by the animal). Another antibody with a fluorescent tag detects the antigen/antibody complex and tells us whether the antigen presents in the sample or not. This is the primary method used in microscope-based examination in labs.

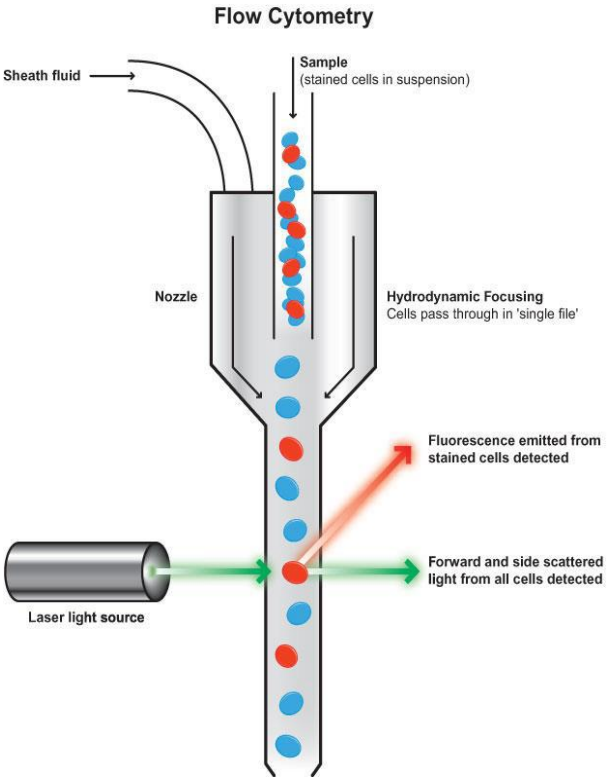


- As you can see in the top picture, the epithelial cells are cohesive and normal. The antibody used to stain this sample was an antibody against **e-cadherin** (represented by the **red lines**). When we added TGF-β (induces epithelial to mesenchymal transition), the cells lose e-cadherin, which is evident by the disappearance of the red lines in the bottom picture. Although these methods are useful in research, they can also be utilized clinically. For example, when we receive a patient with a tumor, and we are unsure whether it is the primary tumor or a metastatic locus (we don't know what the primary tumor is), we can take a sample from the tumor and perform immunocytochemistry for a wide range of antibodies for the tumors we suspect, until we find a particular antigen. For example, if the patient has a secondary metastasis (the primary tumor may be small and hasn't caused major symptoms) with prostate specific antigen (PSA), this immediately tells us that the primary tumor is in the prostate. We call PSA a Tumor Marker.





- Specific for lymphomas and leukemias, since they are separate cells and not solid tumors, we use Flow Cytometry, in which the sample is placed in an aqueous solution appropriate for the sample, and antibodies against specific differentiation antigens are added to specify the type of lymphoma or leukemia. A single cell line of the sample is created (see picture) the cell line goes through a laser detector. We can then specify the type of lymphoma or leukemia. Depending on the type, we can decide the treatment.



Molecular Techniques (not mentioned in lecture):

The molecular methods mentioned in the book (PCR (Polymerization Chain Reaction), FISH (Fluorescence In-Situ Hybridization) have been mentioned in previous lectures. How can these methods be utilized in diagnosis?

- Diagnosis of malignancy: Because each T-cell and B-cell exhibits a unique rearrangement of its antigen receptor genes, PCR based detection of these genes (antigen receptors in T-cells, immunoglobulins in B-cells) allows distinction between monoclonal and polyclonal proliferations. FISH or PCR detection of gene translocation can be used to diagnose several leukemias and lymphomas, as well as some solid tumors (such as Ewing sarcoma, a type of bone tumor). For example, PCR detection of BCR-ABL translocations provides the molecular diagnosis of chronic myeloid leukemia.

- Prognosis and behavior: Some genetic alterations are associated with poor prognosis (the cancer is harder to control and treat). For example, FISH and PCR methods can be used to detect amplifications of oncogenes, such as HER2/NEU in breast cancers and NMYC in neuroblastomas, providing us with prognostic and therapeutic information.

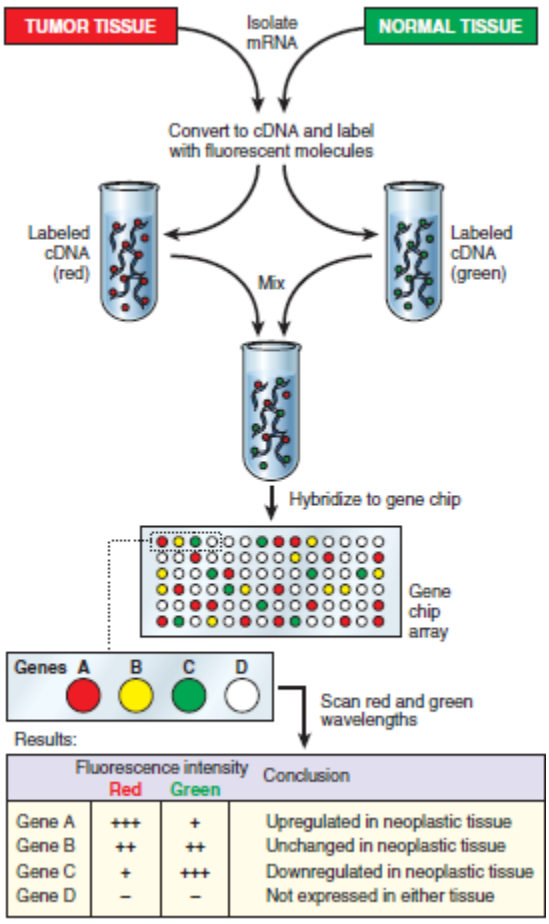
- Detection of minimal residual disease: PCR methods can be utilized to measure residual disease (cancer cells that remain after treatment), as in detection of BCR-ABL transcripts in patients that have been treated for myeloid leukemia. This can be a sign of relapse.

- Detection of hereditary disposition to cancer: Inherited mutation of several tumor suppressor genes (such as BRCA1) increases the patient's risk of developing certain cancers. Detection of these mutated alleles using molecular methods allows the patient and physician to devise an aggressive screening protocol, as well as an opportunity for prophylactic surgery. It also allows genetic counseling for relatives at risk.

- Therapeutic decision making: Therapies that directly target specific mutations are being increasingly developed, which means detection of these mutations in a tumor can guide the therapy used. It is now evident that certain targetable mutations can be found in several types of tumors. For example, mutations of ALK kinase, which was originally identified in T-cell lymphomas, can be found in other cancers like neuroblastomas. Clinical trials have shown that lung cancers with ALK mutations respond well to ALK inhibitors, leading to FDA approval of ALK inhibitors for treatment of ALK-mutated lung cancers. This has also been noticed in melanomas, in which tumors with a valine for glutamine substitution in amino acid 600 (V600E) of serine/threonine kinase (BRAF) respond well to BRAF inhibition. This V600E BRAF mutation has also been noticed in colon cancers, thyroid cancers, and hairy cell leukemia (discussed later).

Molecular Profiling of tumors (also not mentioned):

- Expression profiling: This technique allows the measurement of the expression levels (activity) of several thousand genes. This method is also called gene-chip technology. This process begins by extraction of mRNA from any two sources (normal and malignant, normal and preneoplastic, or two tumors). Complementary DNA (cDNA) copies of the mRNA are synthesized in-vitro with fluorescently labeled nucleotides. The fluorescence-labeled cDNA strands are hybridized to sequence-specific DNA probes linked to a silicon chip. A chip can contain thousands of probes arranged in an array of columns and rows. After hybridization, high-resolution laser scanning detects fluorescent signals from each of the spots. The fluorescence intensity of each spot is proportional to the level of expression of the original mRNA used to synthesize the cDNA hybridized to that spot. For each sample, therefore, the expression levels of thousands of genes is obtained, and relative levels of gene expression in different samples can be compared. Such analysis has revealed that phenotypically identical B-cell lymphomas from different patients are varied in regards to their gene expressions and survival rates.



	Fluorescence intensity		Conclusion
	Red	Green	
Gene A	+++	+	Upregulated in neoplastic tissue
Gene B	++	++	Unchanged in neoplastic tissue
Gene C	+	+++	Downregulated in neoplastic tissue
Gene D	–	–	Not expressed in either tissue

- Whole genome sequencing: The progression and development of sequencing technologies promises even more in-depth analysis of tumors. Sequencing an entire genome, which just a couple years ago would have taken months and millions of dollars, now takes days and costs a few thousand dollars. Sequences of the entire tumor genomes, when compared to the normal genome from the same patient, can reveal all the genetic alterations in a tumor.

- Recent results from genomic analysis of tumors have revealed that individual tumors can contain from only a few somatic mutations (as in childhood leukemias) to tens of thousands of mutations, with the highest mutational burden being found

in cancers associated with mutagen exposure, such as lung cancer and skin cancer. Among these are two types of mutations:

1. Mutations that alter normal control of cell proliferation, differentiation, and homeostasis. They are referred to as Driver Mutations, because they drive the neoplastic process and could be therapeutic targets.

2. Mutations that have no effect on cell phenotype. These mutations are often much more numerous than driver mutations, and occur in the non-coding areas of the genome, or have a neutral effect on growth, not causing advantages or disadvantages. These mutations are called Passenger Mutations, because they result from the instability of cancer cells and are simply "along for the ride".

- In general, driver mutations are recurrent and present in a substantial percentage of patients with a particular cancer. For example, BCR-ABL fusion genes are present in all cases of chronic myelogenous leukemia, making them an excellent drug target. However, driver tumors may be preset in only a small percentage of particular tumors, as in non-small cell lung cancers, in which 4% of patients harbor an EML4-ALK tyrosine kinase fusion gene. An additional complication is that some passenger mutations have important roles in drug resistance. For example, mutations in BCR-ABL that confer resistance to Imatinib (Gleevec, anti-cancer drug mentioned before) in chronic myelogenous leukemia are present as passenger mutations in rare clones before therapy begins. Because they possess a powerful selective advantage, these mutations are converted from passengers to drivers in the face of drug therapy. It is suspected that drug resistance is caused by the genomic instability of tumor cells through similar mechanisms to the one mentioned above.

A Final Note:

Why is it important to talk about molecular diagnosis? It is possible that tumors will eventually be classified based on the **molecular pathway** that is abnormal in that tumor. As you can see in the picture below, all the listed tumors have the same **BRAF mutation** (discussed earlier). These cancers also have in common the course of treatment which inhibits this oncogene. So these tumors could be classified as "**BRAFomas**" in the future. In conclusion, it is important to understand the molecular techniques, since they guide *diagnosis*, *prognosis*, and *treatment*.