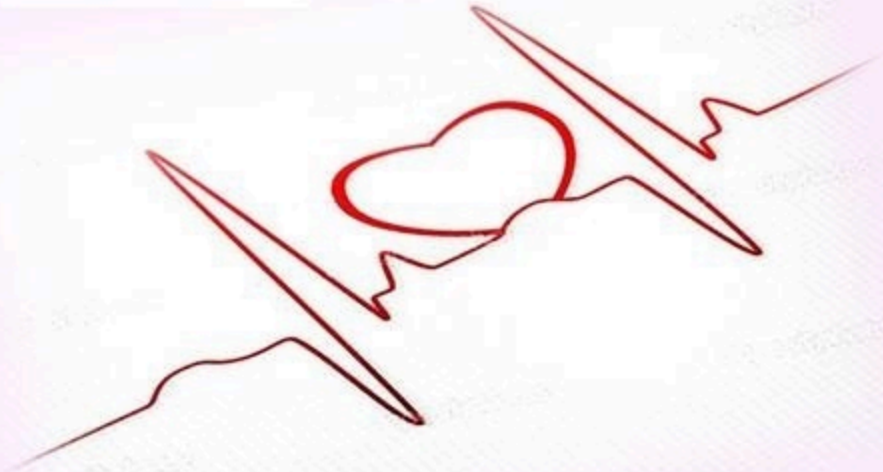
PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE



Lecture Number: 18

Doctor: Mazen Al-Salhi

DONE BY: Ali Khresat

Designed By: Majida Al-foqaraa'

The molecular basis of cancer

-This lecture is not easy I am not going to lie to you like Hasan Hammo .

Overview

The main cause of carcinogenesis is genetic damage which is caused normally by acquired environmental factors ,chemicals , viruses ,radiation affect the normal cell and at normal conditions you can repair this DNA damage so as a result the cell will stay normal .

If the DNA damage was too much for the cell to handle or if you have inherited mutations in genes responsible of DNA repair **(p53)**, you will have failure in DNA repair and if that failure of DNA repair results in favorable mutation for the tumor cells, it makes it gain an extra property from its neighboring cells as a result it will reproduce and survive (Darwinian selection) ,and this will cause the following :

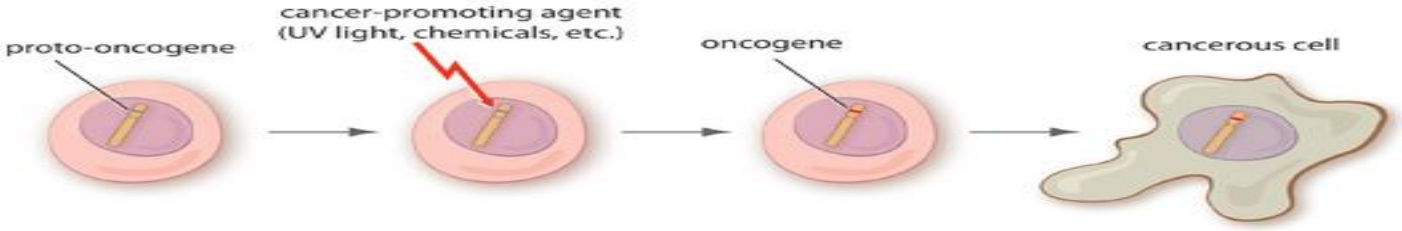
- Activation of Oncogenes .
- Inactivation of tumor suppressor genes.
- Inactivation of genes that regulate apoptosis .
  -So there is four groups of genes that we talked about so far: those that affect repair , apoptosis, Oncogenes and tumor suppressor genes.
  As a result of that you are activating cellular proliferation and inhibiting apoptosis which will give rise to **neoplasm** (tumor).

Remember that neoplasm growth is a clonal expansion (arising from the proliferation of a single mutated cell) for a clonal expansion to turn into malignant neoplasm this is called Tumor progression.

During Tumor progression these tumor cells will gain new specific function called Hallmarks of cancer which make these tumor cells more invasive ,more able to invade the immunity system and make them gain the ability to **metastasize** .

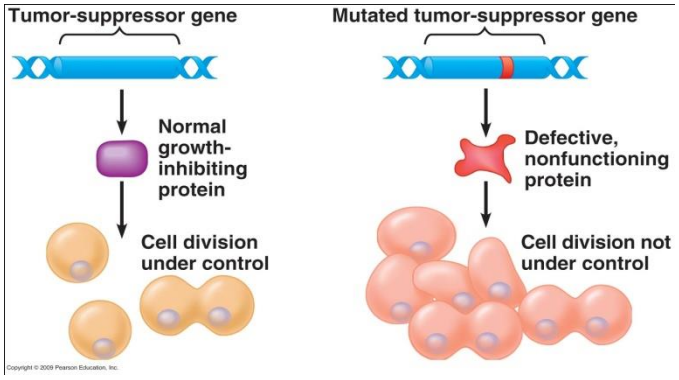
As you can see one event is not enough to create a cancer cell , carcinogenesis is a multi-step process .There is many steps in the pathway of creating a tumor ,rather than the first initial insult .

Definitions in carcinogenesis

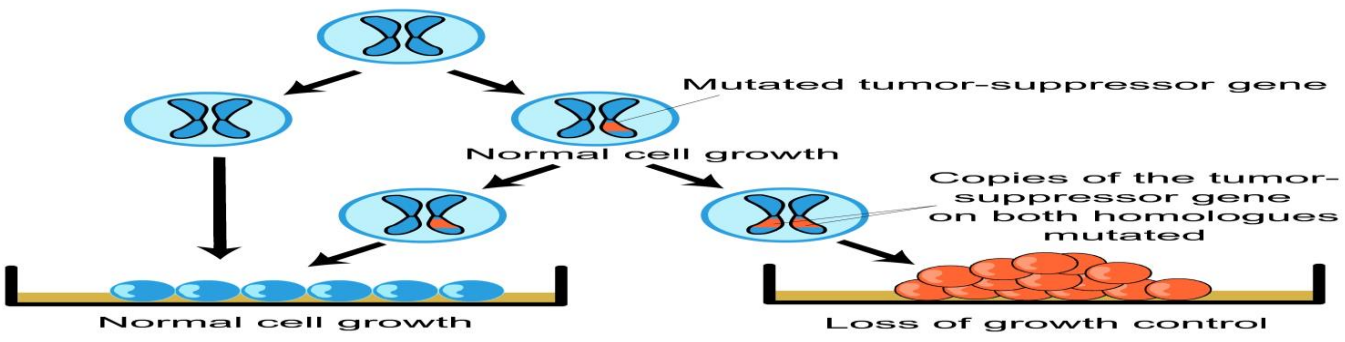


- Oncogenes are genes that has the potential to cause cancer , they are mutated or over-expressed proto-oncogenes .
- Proto-oncogenes are normal genes that codes for proteins which help to regulate cell growth , differentiation , survival, cell-matrix interaction and cell-cell interaction .Upon receiving a cancer promoting agent these proto-oncogenes will get mutated or over-expressed and then transform to an oncogene (cancerous gene) ,These Oncogenes allow the cell that is supposed to undergo apoptosis , to survive and proliferate instead , they are considered dominant which means mutation in one allele in the gene is enough to transform the proto-oncogene to an oncogene .

- Tumor suppressor genes are genes that prevent uncontrolled growth of the cell by synthesizing growth inhibiting proteins ,when these genes are mutated that will result in uncontrolled growth and transformation .



- Unlike oncogenes, tumor suppressor genes act in a <u>typically recessive fashion</u> which means the mutation must include both alleles of the gene for the effect to be manifested because if only one allele is mutated the other can still produce the correct protein .

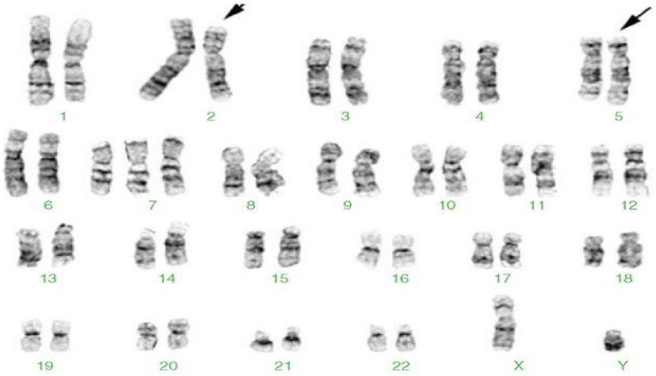


Recent work has shown that in some cases , loss of a single allele of a tumor suppressor gene can promote transformation and this is called **haploinsufficiency** and in this case one allele is mutated and the other is functional but its function is not enough to create a functional growth inhibiting protein (So not all Tumor suppressor genes require both alleles to be mutated).i.e.; you have a range (0-100%),if you lose half of the function (not enough protein) the remaining half is not enough for complete function this is called haploinsufficiency ,there is more haploinsufficiency when even small reduction of function can promote transformation

- Alleles are either Homozygous (same alleles) or Heterozygous(different alleles, Heterozygousity in this case means that there are one normal copy and one damaged copy) , when a point mutation (could be inherited or acquired) involve one allele of a gene then Heterozygousity will arise, loss Of Heterozygousity (LLH) happens when both alleles are mutated .

Karyotypic changes in Tumors

We are going to study the chromosomal mutations that give rise to neoplasia, this picture for example shows Karyotypic changes in a patient with enlarged anaplastic Lymphoma this is just an example to show how important it is to see Karyotypic changes in cancer at the molecular level .



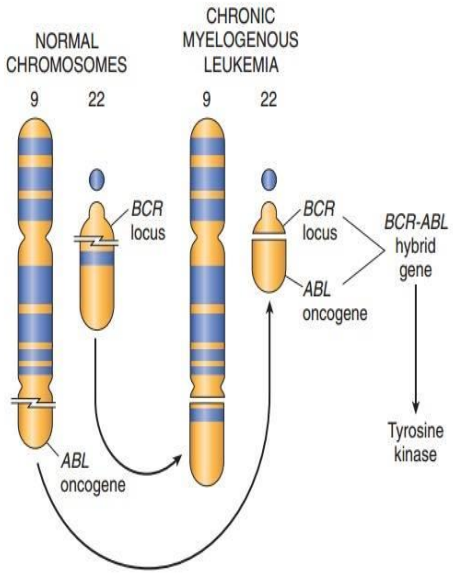
Balanced Translocation

A translocation is an exchange of genetic material , balanced translocation is an equal exchange of genetic material which means there is no gain or loss of a new genetic material this Translocation is important in specific kinds of hematopoietic and mesenchymal neoplasm . Reciprocal translocation means a translocation that will happen in two directions .Ex: Philadelphia genes because two genes(chromosomes) are involved

Balanced Translocation can activate proto-oncogene in 2 ways :-

1-over-expression of certain proteins exemplified by translocation between chromosomes 8 and 14, which leads to over-expression of MYC gene which codes for a transcription factor that turns on cellular proliferation , on chromosome 14 there is a gene that codes for the regulation of immunoglobulin heavy chain production and on chromosome 8 there is the MYC gene. so in this translocation(this is very common in lymphoid origin cancer) you get the MYC gene responsible for transcription near a promoter gene responsible for immunoglobulin production(normally not over expressed) this will lead to over-production of immunoglobulins that's why this translocation is associated with 90% of Butkitt lymphoma .

Another example about this point is a translocation between chromosome 14 and chromosome 18 which leads to over-expression of BCL-2 gene found on chromosome 18 and we remember that BCL-2 is an anti-apoptotic protein which inhibits apoptosis so if you induce it you inhibit the death of the cell that is supposed to die and this will cause cancer(accumulation of more cells) this translocation is associated with follicular B cell Lymphoma .



2-The creation of new fusion proteins that didn't exist in the past and have gained a function that didn't present in the past . Which is exemplified by Philadelphia chromosome translocation in 90% of cases of Chronic myelogenous leukemia. More than 90% of this translocation have this BCR karyotypic kind of translocation, the rest of Chronic myelogenous leukemia have a non-karyotypic translocation which is a translocation that we can't detect microscopically but still produce this BCR-ABL fusion protein .This translocation occurs between chromosome 9 and chromosome 22 , in which ABL gene on chromosome 9 that encodes for Tyrosine Kinase activity becomes fused with BCR gene on chromosome 22. So we gain Tyrosine kinase activity when no tyrosin kinase activity was present in the past. Now ABL is taking control of BCR products applying tyrosine kinase activity. Normally ABL has a regulatory elements that controls the BCR kinase activity now there is no control; because there is this fusion product. Because we understand BCR-ABL fusion gene we can make antibodies against it which makes chronic myelogenous leukemia highly treatable.

An explanation about Philadelphia chromosome translocation from a useful video on youtube : ABL gene on chromosome 9 encodes for Tyrosine Kinase activity , normal Tyrosine kinase acts on cellular proliferation upon receiving an external signal , when Philadelphia chromosome translocation occurs a new fusion protein will rise called ABL-BCR gene that codes for a new Tyrosine kinase called ABL-BCR tyrosine kinase this new enzyme initiate cellular proliferation without the need of an external stimuli so uncontrolled proliferation will occur (cancer) .

The antibodies that are produced by lymphoid cells can recognize millions (unlimited number) of antigens, though we know that every antibody has a specific gene and there is a limited number of genes in the cell. That happens by breaking the DNA and rearrange the genes constantly to produce different antibodies for different antigens. This also happens in myeloid cells and carcinoma but we don't know why.

As we noticed Lymphoid cells are most commonly the target of genes translocation because these cells make double stranded DNA breaks all the time and rearrange the genes responsible for the synthesis of the antibodies receptors .This happens for the recombination process when they want to memorize new antigens that enters the body which make them highly susceptible for these genetic mutations .And that's why these translocations are common in lymphoid cells.

and sarcomas, also frequently possess recurrent translocations, such as the t(11;22)(q24;12) translocation in Ewing sarcoma(lymphoid cells or myeloid cells) that results in fusion of the EWS transcription factor with Fli-1,so this transcription factor will be more active. The cause of the DNA breaks that lead to chromosomal translocations in myeloid neoplasms and sarcomas is unknown.

Another example about fusion genes is what happens in Prostate cancer which is characterized by a fusion between ETS gene which is a transcription factor And TMPRSS gene which codes for transmembrane serine protease this gene is over-expressed in response to testosterone which make it androgically responsive whereas ETS is not androginically responsive for testosterone ,what happens in this translocation is that a fusion gene will be created between TMPRSS and ETS gene making ETS gene androgically responsive(which is normally not responsive) and over-expressed in response to testosterone and as we know ETS gene is a transcription factor for the growth of the cell when it's over-expressed cancer will occur .

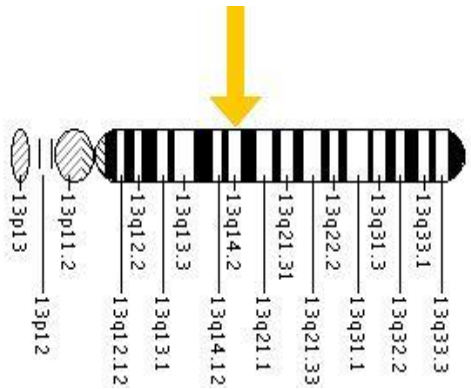
- Prostate cancer can happen because of translocation of chromosome 21 or from deletion of part of chromosome 21 because there is another ETS family transcription factor on chromosome 21,so the region between TMPRSS and ETS gene will be deleted and there will be a fusion product .
- TMPRSS 2 on chromosome 21 and ERG a non ETS family transcription factor gene ,normally you can detect karyotypically by attaching fluorescence material to ERG upstream(5',five prime) and downstream (3',three prime),if this area is deleted ,this particular fluorescence material can't bind and you find one of the chromosome rather than having green and red ,have only a red color and this indicates that this region has been deleted, creating a new fusion product that is androgenic responsive in case of prostate cancer.

-Till now we have typically talked about translocations in cancers of mesenchymal origin because we can easily study their translocations but in carcinomas we haven't been able to detect these mutations because there are A LOT of genetic mutations(abnormalities) .

Chromosomal Deletion

Deletion of a particular regions of chromosomes may result in a loss in a particular tumor suppressor gene. Its common in non-hematopoietic solid tumors.Deletion can happen in non solid tumor(hematopoietic) but we haven't been able to detect it karyotypicaly as we have to make a sequence of genes to detect it.

An example of deletion is13q14, we must know that p represent the short arm and q represent the long arm of the chromosome, so 13q14 means we have a deletion in the long arm of chromosome 13 at gene 14 which is the RB gene that is tumor suppressor gene and this deletion is common in retinoblastoma (tumor in the Retina of the eye)



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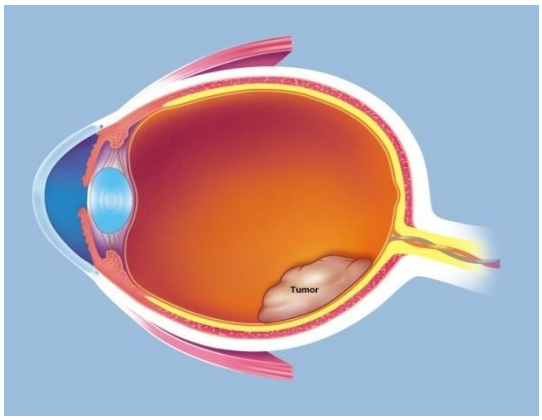
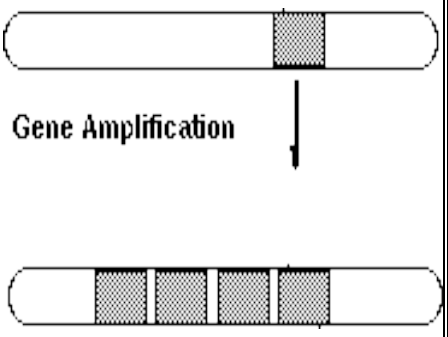


17p : deletion on the short arm of chromosome 17 is associated with the deletion of **p53** gene and that result in inhibition of DNA repair after DNA damage and cancer will arise .

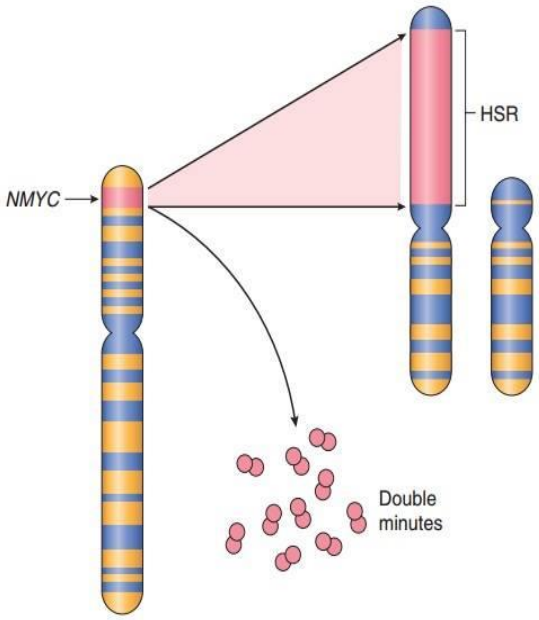
Gene Amplification :-



It means that a particular gene on the chromosome is being copied and copied and copied all over again inappropriately ,proto-oncogens can be converted to oncogenes by this amplification followed by over-expression .such amplifications will produce hundreds of copies of the proto-oncogenes in the tumor cell.

Patterns of Gene amplification :

1-double minutes :extra chromosomal fragments of the DNA swimming in the nucleus, increase in their amounts leads to over expression of that particular gene they carry (could be proto-oncogene)



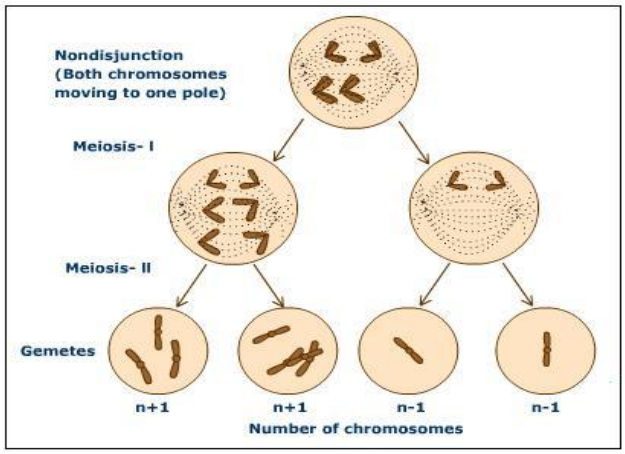
2-Ampilification of the gene in the same Chromosome and that will give rise to a homogenously staining region (region that contain the same gene)

Example about Gene amplification :-
1- Amplification of NMYC gene(present in neurons specifically) which is a transcription factor is associated with 25-30 % of the cases of neuroblastomas and its associated with poor prognosis(because we don't have the proper

molecular techniques to diagnose it, but in the next 5-10 years this can happen)…

2- ERBB2 gene also known HER2\NEU (the second in the group of four tyrosine kinase receptors) amplification is found in almost 20 % of the cases of breast cancer. In the past it was poorly diagnosed but now it's better diagnosed because we have antibodies against this receptor.

Aneuploidy



It's a condition at which the number of chromosomes in the nucleus of the cell is not a multiple of 23(abnormal) , the haploid number of chromosomes in the sperm or ovum is 23, so the diploid number of chromosome in a somatic cell nucleus is a multiple = 46. This does not occur in **Aneuploidy** .

Causes of Aneuploidy is abnormalities in the mitotic check points , at normal conditions we need to grab each sister chromatid of each chromosome and separate one for each daughter cell if there is a defect in the mechanism that is responsible for lining the chromosomes and for the separation process, then one cell will get both chromatids, and the other cell will get none and so on … and this will give rise to Aneuploidy , it happens most common in solid tumors particularly carcinomas .whether this is a cause or effect of the original insult, we don't know, you can think of it as mitotic checkpoint failure leads to Aneuploidy , or Aneuploidy leads to mitotic checkpoint failure and over expression because now there is an abnormal number of genes in the cell(these cells will be selected ;they will not die and will continue to make mutation) and that will lead to more Aneuploidy or we have mutation that led to transformation of these cells and genetic instability and those abnormalities led to Aneuploidy.

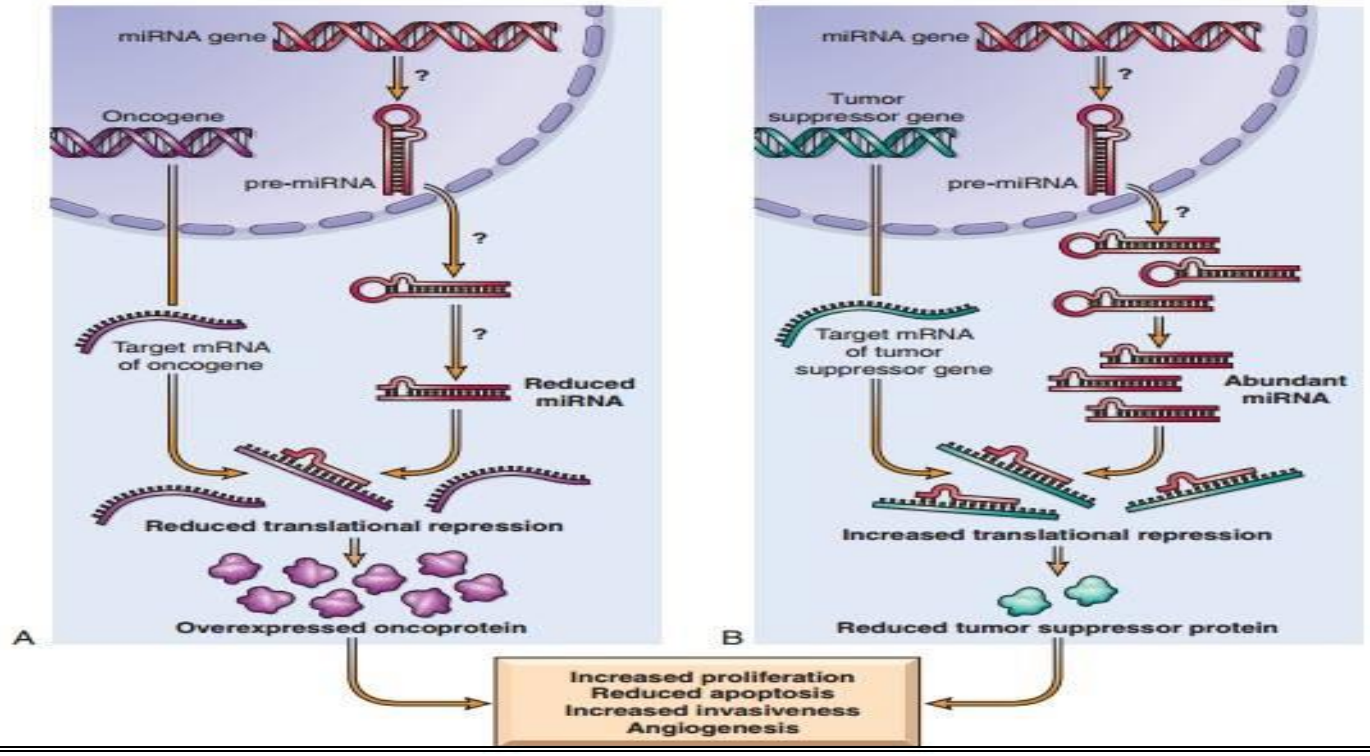
The mechanism in which Aneuploidy causes cancer is not well established but the doctor said that its probably caused by over-expression of proto-oncogenes because when a cell have an extra chromosome it will have an extra amount of proto-oncogenes however the mechanism is still not known .

MicroRNAs and cancer

miRNA is a single stranded RNA that is approximately 22 nucleotides in length it function as a negative regulator for genes expression and it inhibits translation of m-RNA .mRNA could have a complementary sequence for this mRNA coded elsewhere in the genome this is called miRNA ,after it is produced it will bind to a protein complex, this identifies a specific mRNA, either blocks translation or completely degrades the mRNA(controls expression of proteins from genes as you can control the transcription of DNA by transcription factors or control the mRNA translation)

If under certain conditions the amount of miRNA was reduced this will lead to over-expression of proto-oncogenes; because there is no miRNA to stop the translation process and cancer will occur .

On the other hand if there is a miRNA that specifically binds to the m-RNA of a tumor suppressor gene and you over-express that miRNA this will lead to decrease in the amount of tumor suppressor gene which will cause cancer .



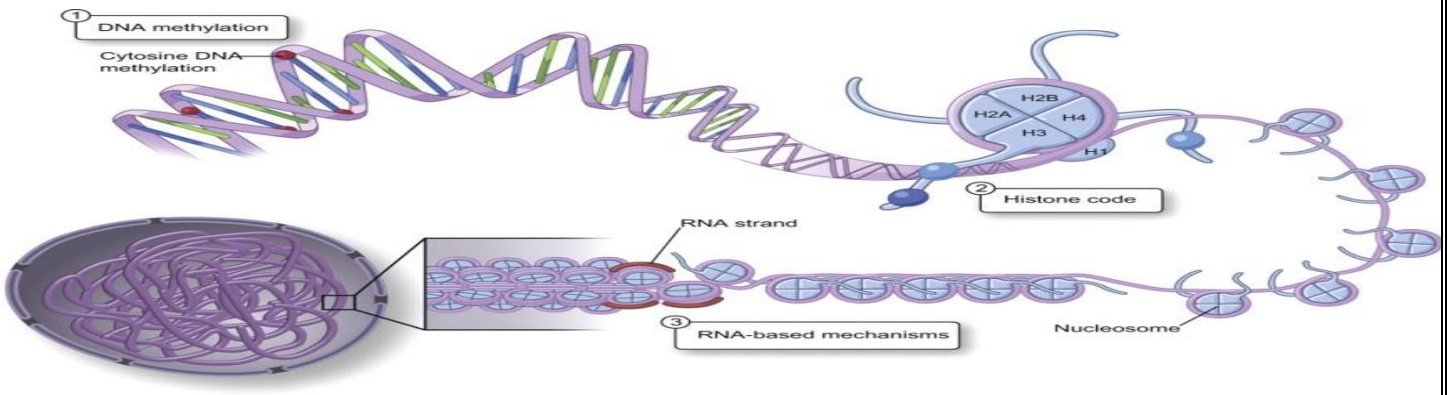
Examples on the role of miRNA in carcinogenesis

1-downregulation of the amount of miRNA has been associated with leukemias and lymphomas, because of the increased expression of BCL-2 gene(the anti-apoptotic gene) .

2-upregulation of the amount of miRNA of RAS(called RAS because it was found in a Rat sarcoma) and MYC oncogenes (miRNA for MYC is the exact opposite for BCL-2 ,MYC is a transcription factor that reduces proliferation) has also been associated with lung tumors and B-cell leukemia .

Very important note : the doctor said that tumors that arise from over-expression of BCL-2 gene are typically slow growing tumors because you are actually not increasing the rate of cellular proliferation , you are allowing more cells to accumulate instead of going through apoptosis .

Epigenetic Modifications and cancer



Epigenetics are reversible ,heritable, non-mutated genes, every cell have all the genetic material required for its life but if you look at each cell alone you will notice that each cell has different areas which are dense or loose what makes certain areas dense(closed, transcription factor can't get to them) or loose(open, accessible) are epigenetic this is done by 2 ways :

1-DNA Methylation : when you methylate a certain gene on the DNA you turn it off (inactivate it) .

2- Changing the tail Histone modification(by acetylation and methylation) because DNA is wrapped helically around Histones and then these histones will cluster together so if you affect Histone conformation epigenetics will rise

-So if there are certain mutations that affect DNA methylation or histone modification ,you will be able to turn on or off certain genes.

DNA methylation

Cancer cells in relation to epigenetics can rise by either whole DNA hypomethylation or selective and localized DNA Hypermetheylation

In the case of whole DNA hypomethylation (turning on a lot of genes) this will cause over-activation of proto-oncogenes. On the other hand localized DNA(promoter) hypermethylation could lead to the inactivation of tumor suppressor genes which in both cases lead to cancer and make the cancer cells gain "stem-cell-ness" property .

Example about cancer epigenetics CDKN2A (Cycline Dependent Kinase Inhibitor 2A) it expresses 2 different genes by combining 2 different exons p14/ARF and P16/INKA both of these genes are responsible for retinoblastoma and p53 activity .In the case on inactivating p14/ARF gene by hypermethylation it's associated with colon and gastric cancers .This is an example of turning off this particular gene, this locus because it produces two different genes, these genes are responsible for retinoblastoma and p53 activity ,hypermethylating it's promoter takes out two important steps :our ability to sense DNA damage and our ability to stop G1→S transition. P16 inhibits Cycline Dependent Kinase and prevents the phosphorylation of retinoblastoma. so if you inhibit the inhibitor, activation without control will occur.

p14ARF (also called ARF tumor suppressor, ARF, p14ARF)

ARF(inhibits mdm2, thus promoting p53) affects p53and inhibits the ubiquinlation(degradation) of p53.so if ARF is inhibited ,p53 will be

ubiquinlated and when you ubiquinlate a protein one of the things that happens is we degrade it. So if you inhibit the production of ARF you are sending p53 for degradation .So with one inactivation of one locus you have allowed the cell to go through the G1\S phase without control and you have destroyed P53 so the cell will not be able to sense the DNA damage.

Epigenetic context it means the epigenetic state of particular cell type that's why for example a cell found in the neurons responds to growth factors differently than the cells of the skin .That depends on which genes are open and which genes are closed.

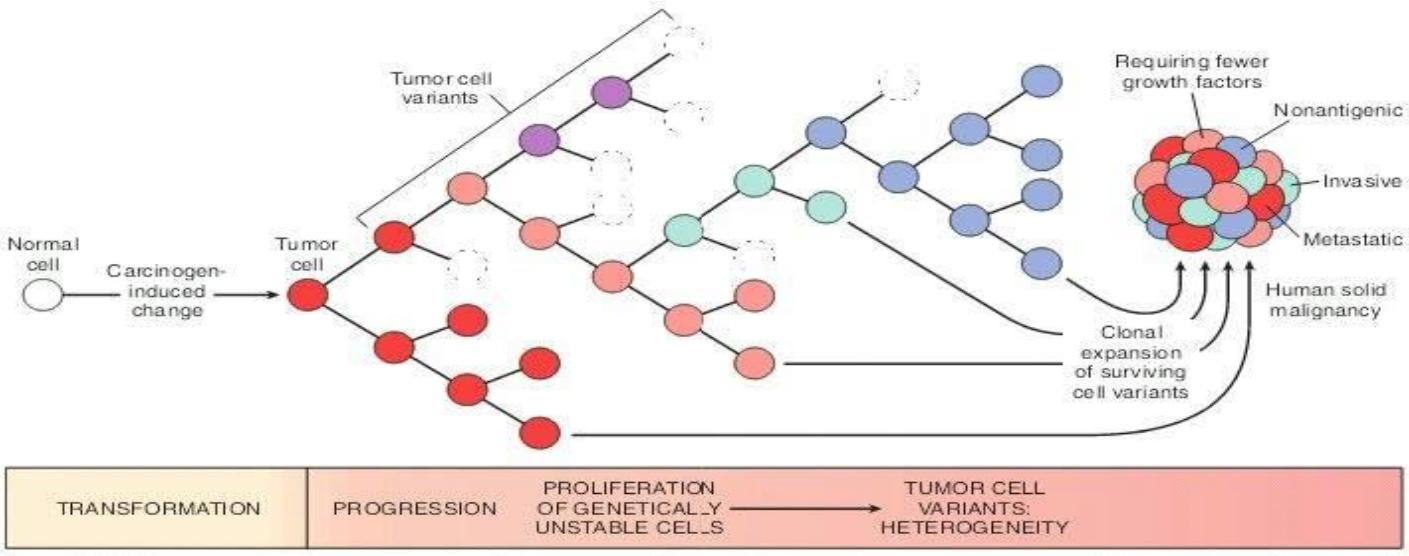
Epigenetic context in cancer : there is a pathway called NOTCH signaling pathway , when NOTCH signals are received ,it turns on the transcription of NOTCH target genes which are different in different cells and depends on which genes are open and which genes are closed .NOTCH1 gene has an oncogenic role in T cell leukemia(one of the target gene is MYC,which is open in this case) and has a protective role against cancer in Keratinocytes (one of the target gene is P21;inhibitor of the cyclin dependent kinase which are responsible for moving along the cell cycle) (different response to the same signal which explains epigenetic context) when NOTCH binds to the receptor of the T-cell leukemia it activate the expression of MYC gene and causes leukemia but when it binds to the surface of keratenocytes it activates a tumor suppressor genes like p21 protecting the cell from becoming cancerous

Carcinogenesis

Cancers are clonal they originate and replicate from a single mutated cell along the way certain cells gain certain functions that give them an advantage for example some cancer cells that carry a lot of antigens on their surface will be identified by the killer T-cell and get eliminated by our immunity system while other cancer cells that don't have these antigens on their surface can escape the immunity system , some cells proliferate even in the absence of

oxygen and nutrients they can turn on anaerobic glycolysis and they will be selected especially when there is no enough oxygen and nutrients meaning that they will replicate and survive while other cancer cells will die ,this is the whole evolution of cancer(natural selection) .

Even though most malignant tumors are monoclonal in origin by the progression of carcinogenesis the tumor cells will be extremely Heterogeneous because some cells will not require growth factors others will be invasive ,metastatic , Non-antigenic etc ....



HALLMARKS OF CANCER

Are things that tumor cells find beneficial to them (not for the body) .ex:

- Self-sufficiency in growth signals.(don't need external growth factors)
- Insensitivity to growth inhibitory signals
- Evasion of cell death(apoptosis),so if there is something wrong in the cell there will be no apoptosis
- Limitless replicative potential, somatic cells have limited number of replications but cancer cells replicate indefinitely like stem cells .
- Development of sustained angiogenesis (producing new blood vessels because cancer cells need oxygen and nutrients to survive ,any cell that don't have this ability will die)

• Ability to invade and metastasize. Remember that the difference between a malignant tumor and benign tumor is metastasize and invasion.

To this list may be added two "emerging" hallmarks of cancer, reprogramming of energy metabolism(cellular energetic) and evasion of the immune system (to survive in hostile environment), and two enabling characteristics, genomic instability (if the DNA is unstable or if you have a problem in DNA repairing mechanism) it will be mutated more easily and that is favorable for the cancer) and tumor-promoting inflammation(one of the risk factors of cancer is chronic inflammation which will cause continuous damage for cells and mutations. ex ;ulcerative colitis is a form of inflammatory bowel disease ,which tend to progress to colon cancer)

Review test

1-All of the followings are examples of benign tumors except :

A . Fibroma

B. Lymphoma

C. Nevus

D. Bronchial adenoma

2. All of the followings are Characteristics of carcinoma in situ except :

A. pleomorphism

B. Hyperchromatic granules

C.crab- like edges infiltrating in neighbouring tissues

D. Mitotic figures at all tissue levels in stratified epithelial tissues

3-Sarcoma is most likely to be diagnosed in which of the following patients :

A . 35-year old female with left breast mass and enlarged axillary Lymph nodes

B. 55-year old female with massive ascites and multiple peritoneal masses

C.15-year old male with a mass in his left femur and lung metastases

D.25-year old male with enlarged left testes

4-One of the following Histopathologic findings are the best indicator that the tumor is malignant

A.Pleomorphism

B. Atypia (atypical cells)

C. increased nuclear/Cytoplasm ratio

D. invasion

5- which of the following has the LEAST risk for subsequent malignant neoplasm

A . 50-year old male with chronic ulcerative colitis

B. 55-year old male with prostate glandular Hyperplasia

C.65 year old male showing oral Atypia and pleomorphism

D. 35-year old female showing Cervical Dysplasia

6- the most common initial pathway of spread for squamous cell carcinoma in lung via :

A . Lymphatics

B. Bloodstream

C. Pleural cavity

D. Bronchi

7.The most common genetic alteration that is found in human carcinoma that leads to loss in tumor suppression :

A .BCL-2

B. P53

C-MYC

D. 9:22 translocation

8. 47 Year old male has peripheral blood WBC count of 167.500/Mili liter with mature and immature neutrophilic cells predominantly , cytogenetic analysis of cells obtained through bone marrow reveals (9:22) translocation and this caused potent tyrosine kinase activity which of the following genes translocates from chromosome 9 :

A . P53

B. RAS

C. RB

D. ABL

9. deletion of both alleles on which of the following genes would result in retinoblastoma :

A . RB

B . MYC

C. ABL

D. ERBB2

10 . 38-Year old female present with abdominal distention and a CT-scan demonstrates bowel obstruction with a 6-cm mass in the jejunum . A burkitt lymphoma of the small bowel is resected and sent to the lab where they caught a lot of cells in the S-phase ,mutational activation of which of the following oncogens has led to the presence of this case ?

A . p53

B. RAS

C. MYC

D. APC

1	2	3	4	5	6	7	8	9	10
B	C	C	D	B	A	B	D	A	C

Explanations : 2. Carcinoma in situ is a pre-invasive step of cancer and it does involve metastasis (crab like edges in neighbouring tissues)

3 . Sarcoma is a malignant neoplasm from mesenchymal origin and prefer to metastasize in hematogenous spread that's Why A is not the answer because it less likely to metastasize in lymph , B is not the answer as well because masses in the peritoneum are in the mesothelium which is an epithelium so it's not a sarcoma

5. A is not the answer because chronic inflammation can progress to cancer , C and D are not the answers because these are the signs of a pre-invasive cancer , B is the answer because hyperplasia is a normal adaptive response and it's not cancerous

7. Carcinoma are solid tumors and ubnormal P53 is found in many solid tumors

it's going to get more complicated each time , don't start crying.

-Dr.mazen Al-salhi

تحية لمهند حدادين على دعمه النفسي لي اثناء كتابة هذه الشيت
وتحية لمحمد نوايسة على تدقيقها ☺