Sheet# 16

Carcinogenesis

The molecular basis of cancer.

- What is carcinogenesis?

A multistep process at both the phenotypic and genetic levels, resulting from the accumulation of multiple mutations.

- What is the main cause of carcinogenesis? Genetic damage which is caused normally by acquired environmental factors (chemicals, viruses & radiation) which affect the normal cell and in normal conditions you can repair this DNA damage so as a result the cell stays normal.

- For a normal cell that gets DNA damage you normally have DNA repair machineries that can fix that damage, copy the undamaged strands and cells will become fine again. However, if there is a failure in DNA repair you get mutations in the somatic cells.

These mutations could be:

1- Activation of oncogenes (growth promoters).

2- Inactivation of tumor suppressor genes (inhibition of the inhibitor).

3- Affecting genes that regulate apoptosis (inactivation).

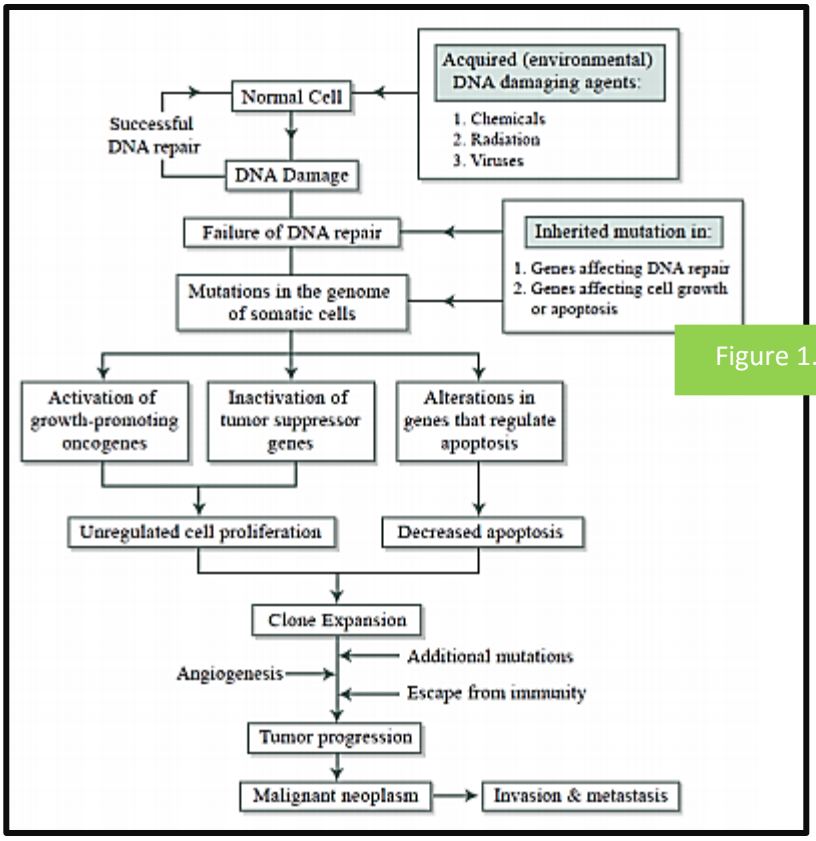


Figure 1.

Natural selection

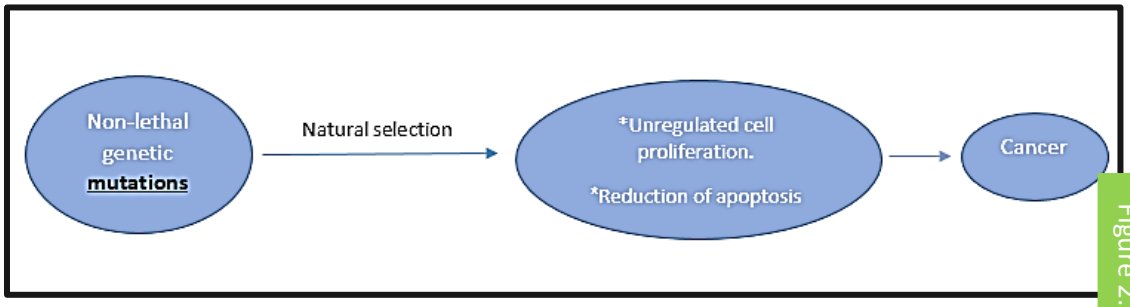


Figure 2.

Something that selects a cell to continue when it gains something that gives it an advantage over the surrounding cells.

cells which undergo non-lethal genetic mutations that activate an oncogene, inhibit a tumor suppressor gene or affect genes that regulate apoptosis, can be naturally selected because they give the cell an advantage over all the surrounding cells, so the cell can now survive apoptotic signals or proliferate uncontrollably either by activation of oncogenes or inhibition of tumor suppressor genes.

When this occurs, you get unregulated cell proliferation or reduction of apoptosis.

That one cell that has gained one of these major changes, that is the initial clone, all cancers are considered to be clonal, i.e. they arose from one cell, although by time the cancer is detectable if you survey the different cells in the cancer you will find that they are heterogeneous because some cells have gained different characteristics than other cells, some cells are capable of invading whereas other cells stay in the core of the tumor, some cells are capable of metastasizing whereas other cells aren't. When that clone has expanded it can gain additional mutations like the ability to escape from the immune system, the ability to invade, the ability to stimulate angiogenesis, all of these extra abilities beyond oncogenes, tumor suppressor genes & apoptotic genes, are the abilities that allow a tumor to progress, this is the phase of tumor progression.

**non-lethal genetic damage →natural selection→cancer.

→Sometimes, if the mutation is either activating a tumor suppressor gene or inhibiting an oncogene or is lethal, the cell dies off and you don't see that cell (no cancerous proliferation).

**lethal genetic damage→no natural selection→no cancer.

Four principal targets of genetic damage in cancerous proliferation:

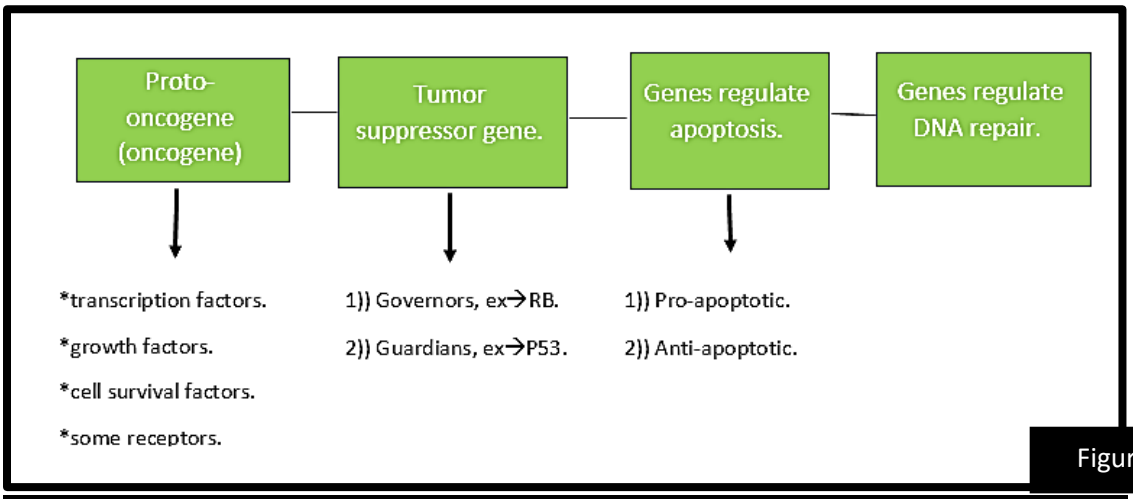


Figure 3.

Oncogenes:

An oncogene is a gene that has the potential to cause cancer, and it's a mutation/over-expression of a proto-oncogene, these are typically:

a. Transcription factors.

b. Growth factors.

c. Cell survival factors.

d. Receptors that allow cells to interact with the matrix

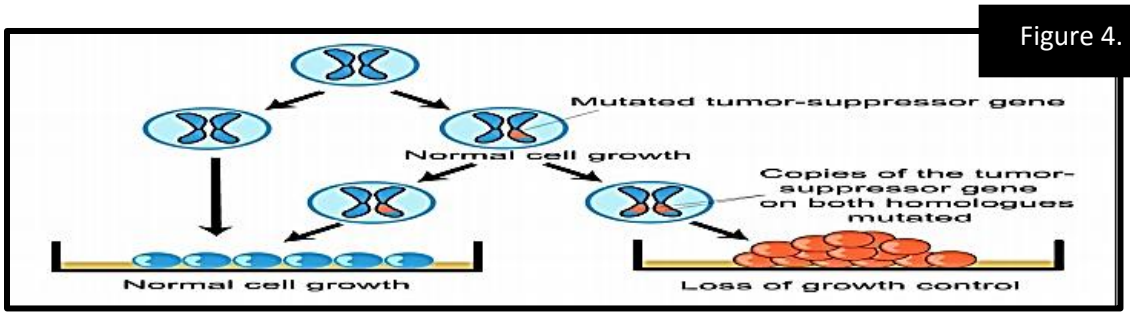
This leads to cellular transformation and this is typically autosomal dominant, i.e. one allele is enough for the characteristic. Upon receiving a cancer promoting agent, these proto-oncogenes will get mutated or over-expressed and then transform to an oncogene (cancerous gene). These oncogenes allow the cell that is supposed to undergo apoptosis to survive and proliferate instead. Proto-oncogenes are normal genes that code for proteins, which help regulate cell growth, differentiation, survival, cell-matrix interactions and cell-cell interactions.

Tumor suppressor genes:

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

A mutation results again in uncontrolled growth/transformation, these typically work as autosomal recessive (you need to use both alleles), however you could lose one allele (now you're heterozygous for that location) and then only after you lose the second allele through a different mechanism do you get the disease, this is called loss of heterozygousity, typically the first allele loss is through a mutation, the second allele loss is through deletion.

Figure 4.



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 another type of haploinsufficiency where even a small reduction of function can promote transformation. It is not always genetic damage that leads to cancer; there are non-genetic changes that can lead to cancer.

Tumor suppressor genes are divided into:

[1]- Governors: like the retinoblastoma gene. (Retinoblastoma is recessive on the molecular level, but one allele defines the disease as an increased risk of getting cancer).

[2]-Guardians: sense DNA damage, like P53. This doesn't necessarily lead to a change that causes uncontrolled proliferation, but loss of P53 means that if there is a mutation it's going to go undetected and the cell cycle is no longer going to be stopped, DNA repair machineries aren't going to be activated, apoptosis isn't going to be activated allowing another mutation from passing by that check point and causing damage. This is called a mutator phenotype, where patients who have P53 mutations accumulate further mutations far more easily than patients who have a normal P53 system.

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

Genes regulate apoptosis:

There are two types of genes that regulate apoptosis:

1- Pro-apoptotic gene. 2- Anti-apoptotic genes.

If you inhibit a pro-apoptotic gene or stimulate an antiapoptotic gene (both are beneficial for cancer cells), the cell will be naturally selected because this will give it an advantage over the surrounding cells.

New topic ☺

Genetic lesions in cancer

Karyotypic changes in Tumors:

1)) Balanced Translocations.

2)) Deletions.

3)) Gene amplification.

4)) Aneuploidy.

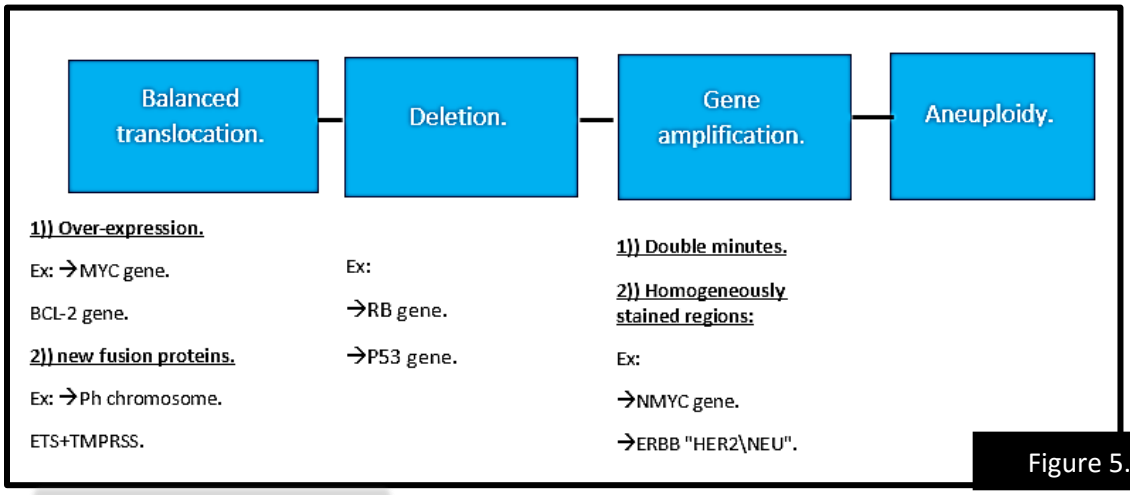


Figure 5.

Balanced translocations:

A translocation is an exchange of genetic material between two nonhomologous chromosomes, a 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. A 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 (a transcription factor which is normally regulated by its own promoter) 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 **Burkitt lymphoma**.

Another example about this point is a translocation between chromosome 14 and chromosome 18 which leads to overexpression 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), these tumors don't proliferate very quickly (normal proliferation rate). When BCL-2 is over-expressed it becomes an oncogene. This translocation is associated with **follicular B cell Lymphoma.**

[2]. The creation of **new fusion proteins** (a break in the middle of a gene, and you stick it onto another gene which creates a whole new protein) that didn't exist in the past and have gained a function that wasn't present in the past, which is exemplified by the **Philadelphia (Ph)** chromosome translocation in 90% of the cases of **chronic myelogenous leukemia**. More than 90% of the translocations 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 the

ABL gene on chromosome 9 that encodes for Tyrosine Kinase activity becomes fused with the BCR gene on chromosome 22. So we gain tyrosine kinase activity, while no tyrosine kinase activity was present in the past. Now ABL is taking control of BCR products applying tyrosine kinase activity. Normally ABL has regulatory elements that control 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.

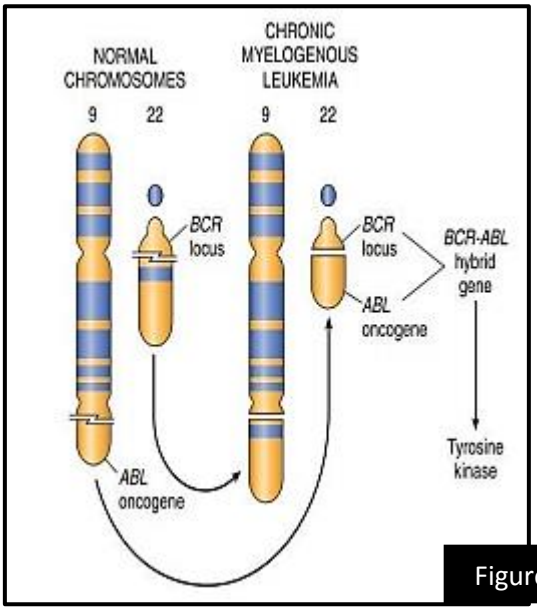


Figure 6.

As we noticed, lymphoid cells are most commonly the target of gene translocations because these cells make double stranded DNA breaks all the time and rearrange the genes responsible for the synthesis of the antibody receptors. This happens for the recombination process when they want to memorize new antigens that enter the body which makes them highly susceptible for these genetic mutations. And that's why these translocations are common in lymphoid cells. 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 androgenically responsive whereas ETS is not androgenically responsive for testosterone, what happens in this translocation is that a fusion gene will be created between TMPRSS gene and ETS gene making ETS gene androgenically responsive (which is normally not responsive) and over-expressed in response to testosterone and as we know, ETS growth of the cell, when it's over-expressed gene is a transcription factor for the cancer will occur .

It means that a particular gene on the chromosome is being copied and copied and copied all over again inappropriately; protooncogenes can be converted to oncogenes by this amplification followed by overexpression.

Deletion:

Deletions in the retinoblastoma gene on chromosome 13q & the P53 gene on chromosome 17p.

* p is the short arm on a chromosome and q is the long arm.

You get multiple copies of the gene, and this could show up as:

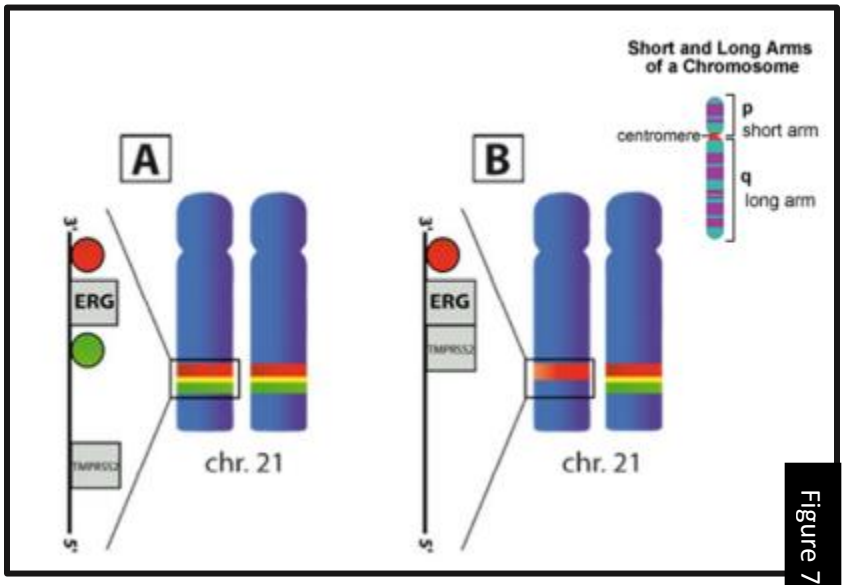


Figure 7.

Gene amplification:

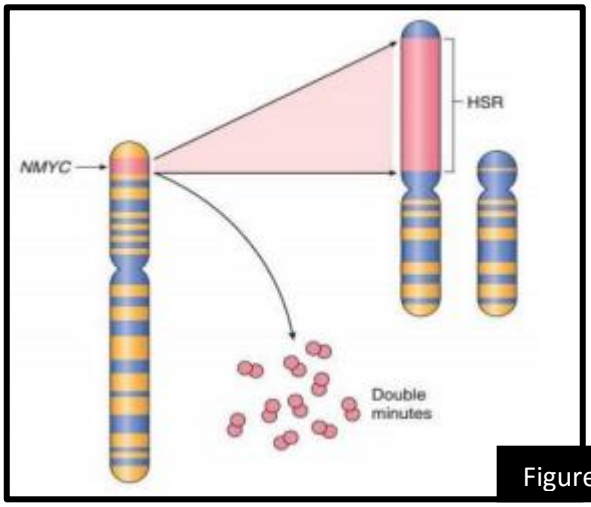


Figure 8.

1. Double minutes: extra chromosomal fragments of the DNA swimming in the nucleus, increasing their amount leads to over expression of that particular gene they carry (could be a protooncogene).

2. Amplification of the gene in the same chromosome, and that will give rise to homogenously staining regions (regions that contain the same gene) Examples about Gene amplification:-

a. Amplification of NMYC gene (present in neurons specifically) which is a transcription factor, is associated with 25-30 % of the cases of neuroblastomas and it's associated with poor prognosis (because we don't have the proper molecular techniques to diagnose it and turn it off, but in the next 5-10 years this can happen)…

b. ERBB2 gene also known as 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 prognosis but now it's better 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 chromosomes in a somatic cell nucleus is a multiple = 46. This does not occur in Aneuploidy. The cause of Aneuploidy is abnormalities in the mitotic check points, at normal conditions we need to grab each sister chromatid of each chromosome and separate them into 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, 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 commonly in solid tumors, particularly carcinomas. Whether this is a cause or an effect of the original insult, we don't know. There are two possibilities:

a. Mitotic checkpoint failure leads to Aneuploidy.

b. 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 mutate) this will lead to more Aneuploidy, or we have mutations that lead to transformation of these cells and genetic instability and those abnormalities led to Aneuploidy.

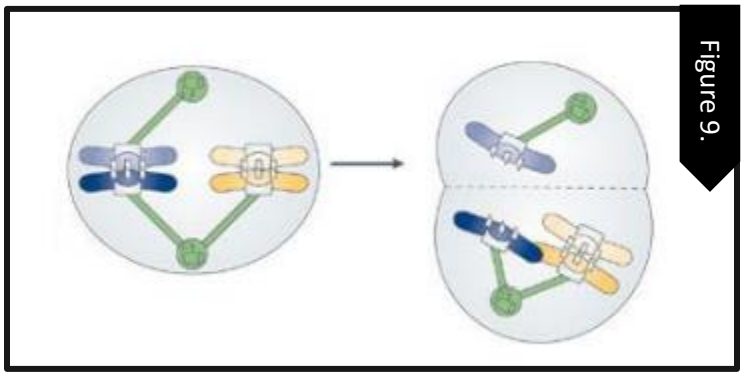


Figure 9.

The mechanism in which Aneuploidy causes cancer is not well established but the doctor said that it's probably caused by over-expression of proto-oncogenes because when a cell has 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, their whole function is to fine tune how much you transcribe a gene, mRNA could be transcribed, degraded or left not doing anything, miRNA is what fine tunes this process it functions as a negative regulator for gene expression and it inhibits translation of mRNA (They inhibit gene expression posttranscriptionally by repressing translation or by mRNA cleavage).

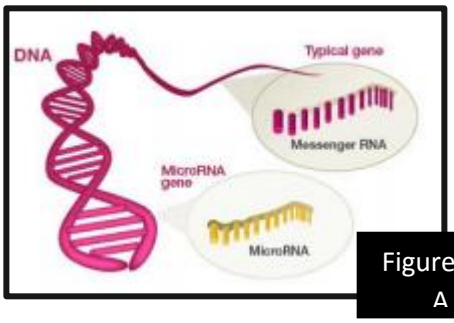
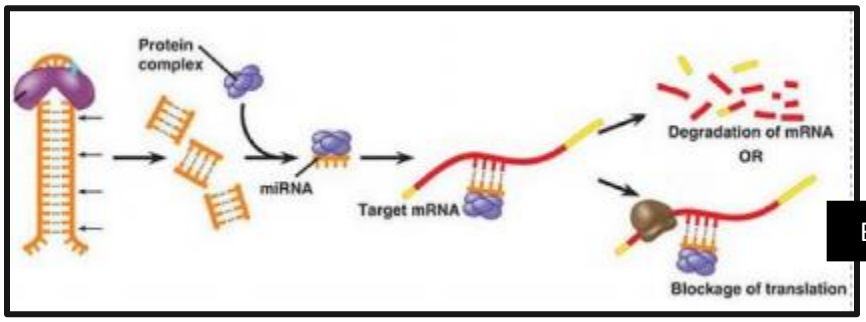


Figure 10. A



B

Pieces of mRNA could have a complementary sequence 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 an miRNA that inhibits production of an oncogene was reduced this will lead to overexpression of the oncogene; because there is no miRNA to stop the translation process and cancer will occur.

On the other hand if there is an miRNA that specifically binds to the mRNA of a tumor suppressor gene and you over-express that miRNA, this will lead to decreasing the amount of tumor suppressor genes, which will cause cancer.

Examples on the role of miRNA in carcinogenesis:

1. Down-regulation 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. Up-regulation of the amount of miRNA of **RAS** (called RAS because it was found in a Rat sarcoma), an oncogene present in lung cancer, and **MYC** oncogenes (miRNA for MYC is the exact opposite for BCL-2 ,MYC is a transcription factor that

Very important note : the doctor said that tumors that arise from overexpression of the 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

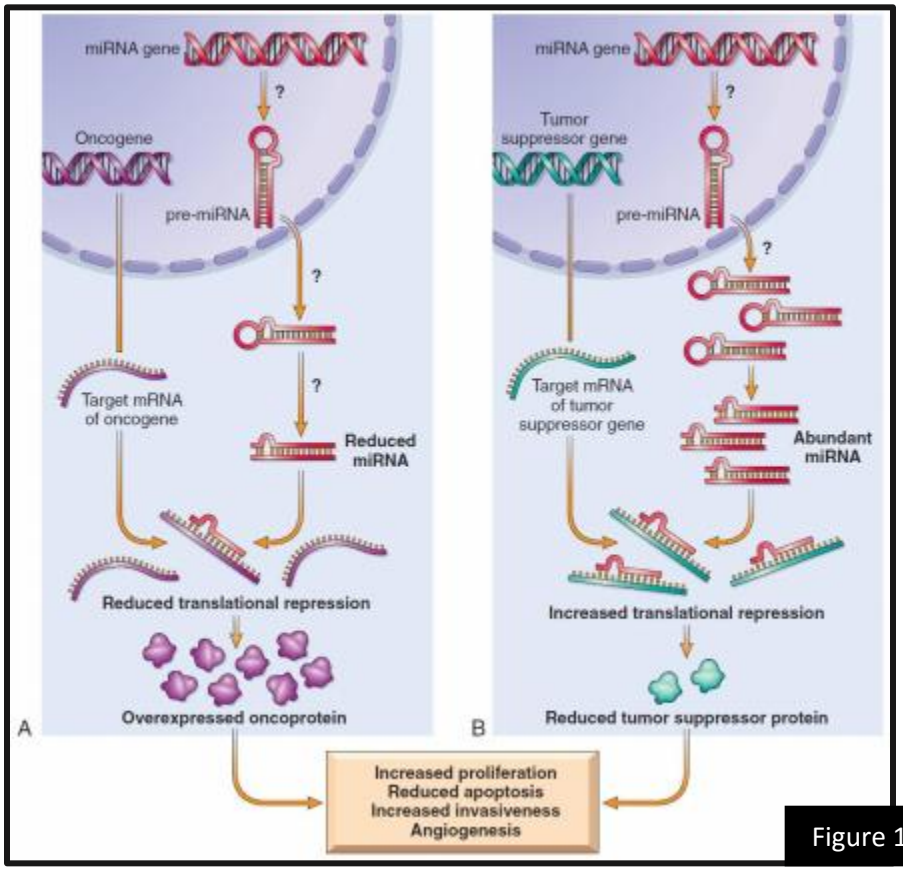
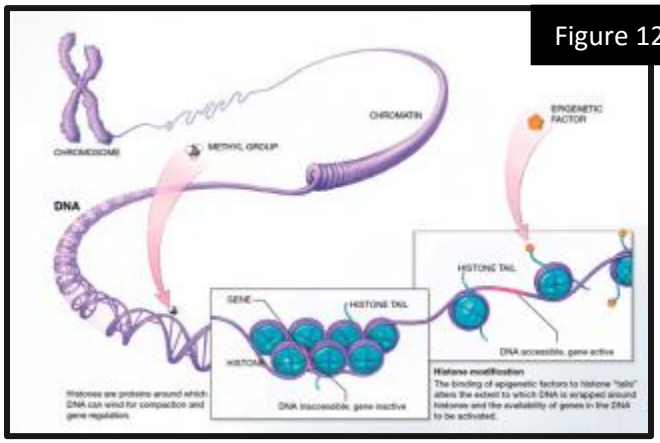


Figure 11.

Epigenetic Modifications and cancer.

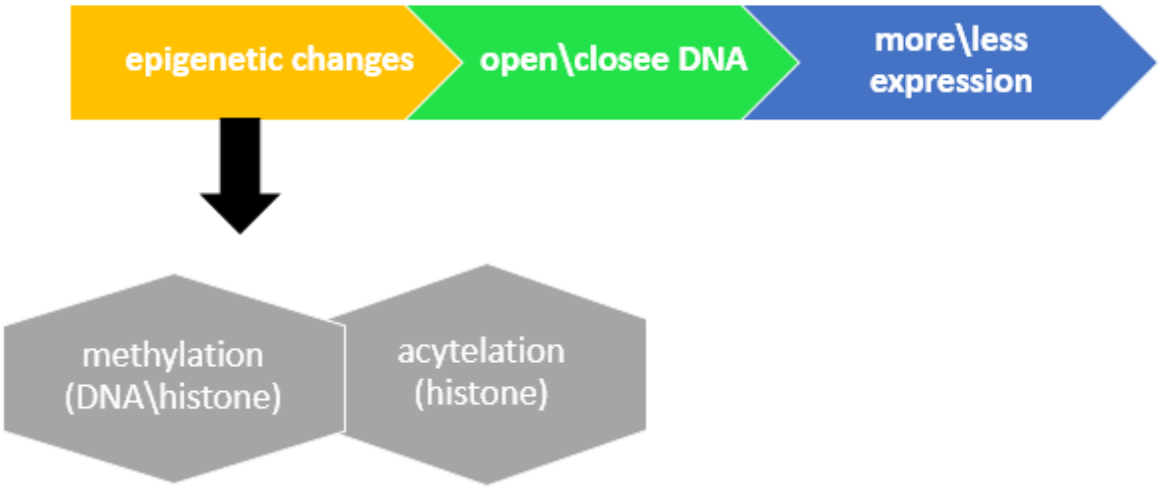
Epigenetics are reversible, heritable, non-mutated genes. Every cell has all the genetic material required for its life but if you look at each cell alone you will notice that each cell has different areas which are dense or loose. How do you open or close your DNA? You increase the twist of histones and you close it up or you untwist them and you open it up, how? Helicase, how does it know to twist or untwist a region? There are markers that are put on the DNA itself or on the histones. These are called epigenetic changes, they are heritable but reversible changes that don't result in mutations, you're not changing your genetic code, you're changing markers for different enzymes to open or close a region of DNA.

Figure 12.



Epigenetic changes could be:

1. Methylate DNA: when you methylate a certain gene on the DNA you turn it off (inactivate it).
2. Methylate histones
3. Acytelate histones. And there are multiple other changes you could do to histones as well. This will tell helicase, among other enzymes, to open and close DNA.



For example: if you had an oncogene that is normally in a region of DNA that is closed and you change the methylation or acetylation status of that region, this leads helicase and other enzymes to open that area, you've allowed expression of an oncogene that normally isn't expressed in that cell. This is an epigenetic change, there's no mutation, there's no change in your genetic code, but there's a change in what area is open or closed. So if there are certain mutations that affect DNA methylation or histone modification, you will be able to turn certain genes on or off

Note: DNA methylation Cancer cells in relation to epigenetics can rise by either whole DNA hypomethylation or selective and localized DNA Hypermethylation. 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 leads to cancer and make the cancer cells gain "stemcell-ness" property

Example about cancer epigenetics: CDKN2A (Cycline Dependent Kinase Inhibitor 2A), a complex locus, 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 of inactivating p14/ARF gene by hypermethylation, it's associated with colon and gastric cancers. This is an example of turning off this particular gene. Since this locus produces two different genes (these genes are

responsible for retinoblastoma and p53 activity) hypermethylating its promoter takes out two important steps: Our ability to sense DNA damage, and our ability to stop G1-S transition.

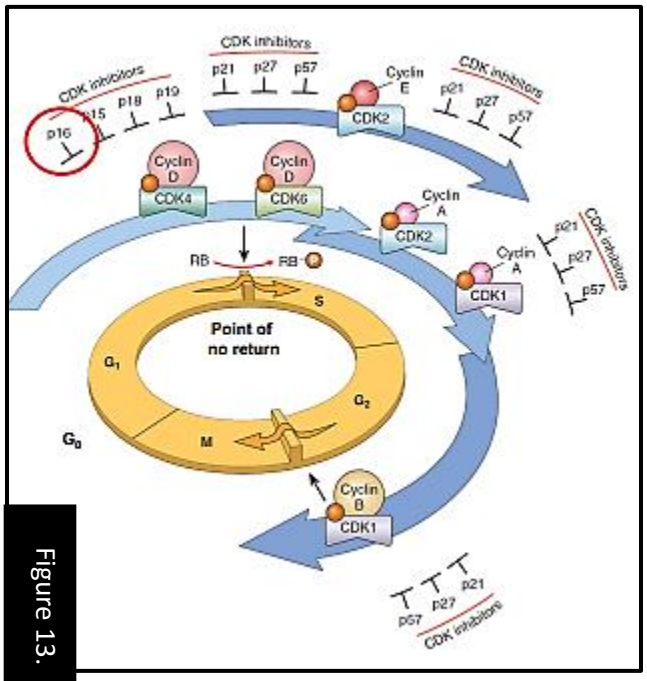


Figure 13.

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) (inhibits mdm2, thus promoting p53) affects p53 and 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 that it gets degraded.

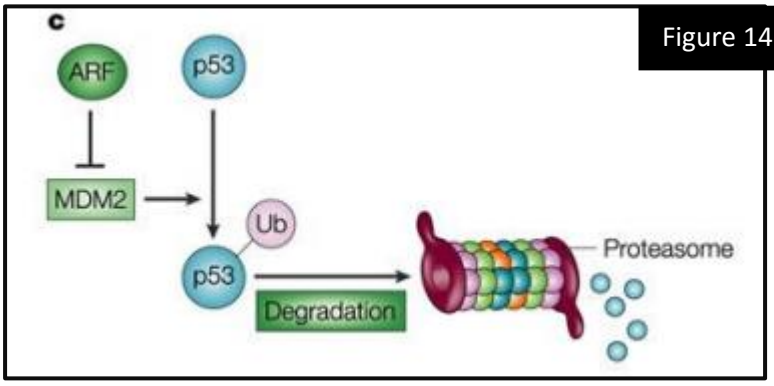


Figure 14.

So if you inhibit theproduction 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 in cancer:

Epigenetic context means the epigenetic state of a 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, It depends on which genes are open and which genes are closed.

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.

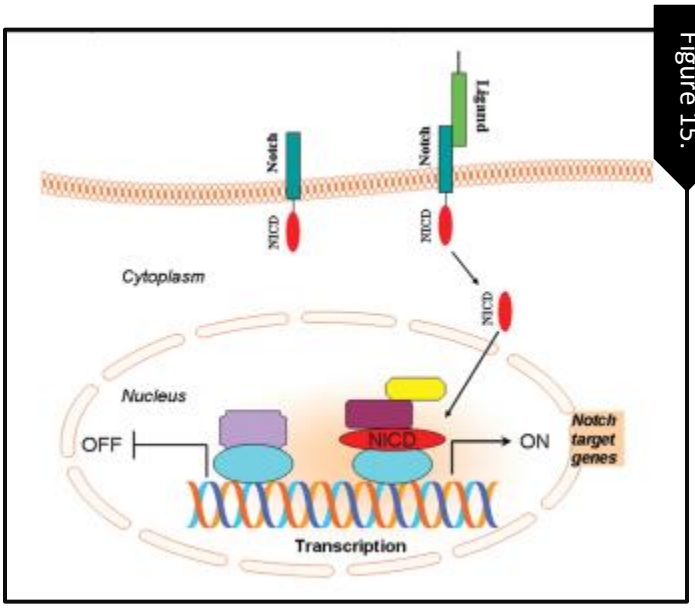


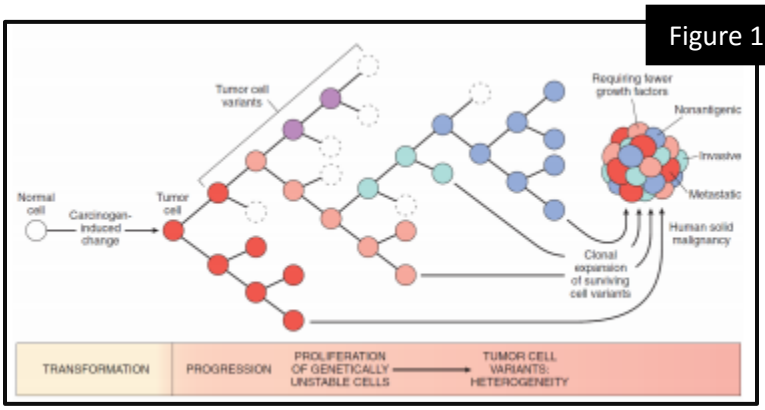
Figure 15.

NOTCH1 gene has an oncogenic role in T cell leukemia (one of the target genes is MYC, which is open in this case) and has a protective role against cancer in Keratinocytes (one of the target genes is P21, inhibitor of the cyclin dependent kinase which is responsible for moving along the cell cycle) (different response to the same signal which explains epigenetic context) when NOTCH binds to the receptor of T-cells it activates the expression of MYC gene and causes leukemia, but when it binds to the surface of keratinocytes it activates a tumor suppressor genes like p21 protecting the cell from becoming cancerous.

All of that together are the steps that a cancer cell has to go through, either there's a genetic change, epigenetic change, karyotypic change, point-mutation change, non-genetic changes at the protein levels that can affect how a cell

responds to external stimuli or changes internally. All of this is what feeds into natural selection, none of these are targeted or pointed, it's all random, so if something random happens that kills the cell → cell gone, if something random happens that enhances the proliferative ability of the cell, it's selected above other cells. That's all it is! It's a random process "natural selection". Think about darwin's finches, in one season there's a particular seed that died off, and only finches with curved beaks could get that particular food source, the rest died off because they weren't able to get food, next season, all of the finches had curved beaks. This is natural selection, the ones that couldn't adapt died. Cancer cells are essentially the same thing, they gain a function, cells that don't gain that function die and those that gain the function survive. So it's a multistep process, and this is why after the first mutation, different cells will gain different characteristics, which is why in the end, even though we say cancer is clonal the cancer is very heterogeneous.

Figure 16.



Cancers are clonal, they originate and replicate from a single mutated cell, and 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 immune 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