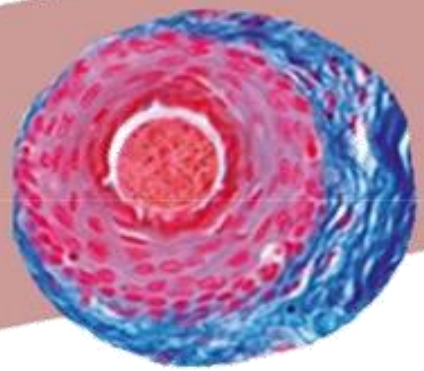
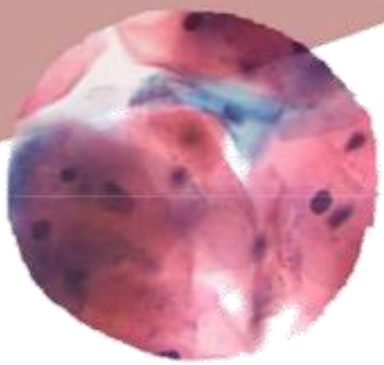
INTRODUCTION TO PATHOLOGY



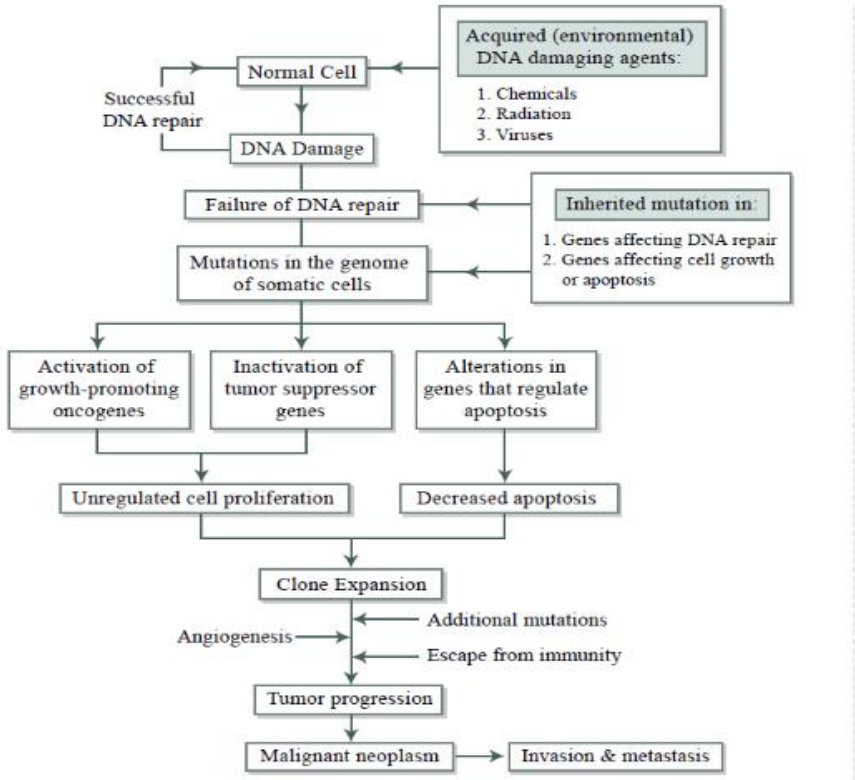


Done by Sondos Al-Najjar; Abdullah Shneikat

Corrected by Abdullah AlZibdeh

Sheet# 17

In this sheet, molecular biology of cancer is discussed. Do not forget to refer to the slides. Good luck.



By looking at the diagram above you can see that a mutation could be acquired from the *environment* by agents that damage the DNA such as chemicals, radiations and viruses, or it could be *inherited*. The inherited mutation can affect DNA repair genes, or affect the cells in a way that they are proliferating too much. Always remember that the older you are, the more likely you are prone to get cancer; because you are exposed to more environmental toxins and more accumulation of mutations.

- What happens if you stimulate the cell to constantly proliferate? You are artificially aging it. (So more likely for it to receive a mutation)

Now the mutations are in the somatic cells. Should that mutations stick in these cells? No, It can activate growth promoting genes, inactivate tumor suppressor gene, screw with the apoptotic genes (turn off the pro-apoptotic gene and turn on the anti-apoptotic gene), or damage genes involved in DNA repair.

Is there something magical in that? Are mutations magical? No, **natural selection**! Mutations are random. These mutations end up stimulating proliferation and inhibiting apoptosis. This results with **clone expansion**.

Tumors initially are clones; one cell has a mutation then proliferation occurs. But for a tumor to continue growing, invade tissues, and metastasize it needs to (1) avoid the immune system, (2) produce its own growth factors, (3) it needs to stimulate angiogenesis and it will accumulate more

mutations along the way. That means that carcinogenesis is a multi-steps process, *one mutation is not a cancer maker!* On the other hand the tumor that will be eventually found is not going to be clone anymore; not all the cells are going to receive further mutations in the same way, some cells are going to gain the ability to get up and walk somewhere else, some cells will gain the ability to induce angiogenesis, some cells are going to gain the ability to avoid the immune system, and the strongest will last more by natural selection. That's why when you find a tumor, malignant or benign, it will be a **heterogeneous group of cells**, they are not homogenous, they are not clones; they are different cells do different things.

Now by looking on this pathway, mutations or alterations in four gene groups are involved in carcinogenesis:

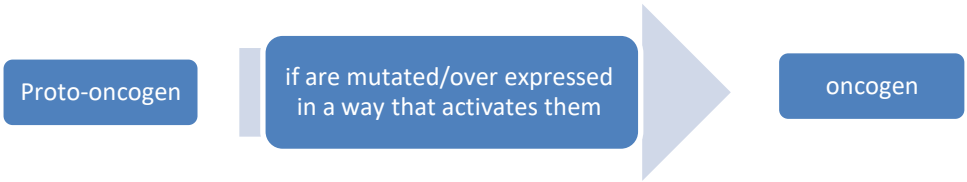
1- Oncogenes
2- tumor suppressor genes
3- genes that regulate apoptosis. (These are mentioned in apoptosis)
4- DNA repair genes.(You've already taken the DNA repair genes in chemistry)

So now we will talk only about oncogenes and tumor suppressor genes.

Oncogenes

- What are **oncogenes**?

Oncogenes are overactive, over expressed or mutated versions of normal cellular genes which are proto-oncogenes; oncogenes are those genes that induce transformation or cell survival when expressed in cells.



These are autosomal **dominant**; one damaged allele is enough to produce a tumor.

Tumor Suppressor Genes

- What are the tumor suppressor genes?

They suppress tumor growth. The presence of these genes in the active form is crucial for our life. But if these genes are mutated or inactivated, or if one allele is deleted, then a problem will result. However, these genes are typically autosomal recessive; that means that both alleles have to be lost or inactivated to get a carcinogenic result. When you lose both alleles, you are no longer having that break in the proliferation that controls the cell cycle, and also you do not have the ability to detect mutations. **NOTICE** that retinoblastoma and p53 were in the autosomal dominant table in the last lecture, but here we are saying they are autosomal recessive! What is the truth?

The autosomal dominant syndromes are defined as getting a syndrome that increases your risk of getting a tumor. Think about it this way, if you need to lose two alleles and you inherited already a damaged allele. Are you more or less likely to get a tumor by losing the second allele? Statically it's less probably for you to lose two alleles in a sequence acquired during life time. This is a syndrome if you already have an abnormal allele, the second allele can be lost a lot easier than losing two alleles in sequence, so the disease is defined as autosomal dominant because you are at an increased risk of losing the second allele, and you only gain a tumor if you lose the second allele. So, for retinoblastoma and p53, you have to lose two copies and this is called Loss of heterozygosity (LOH). If you inherited an abnormal allele, you will be heterozygous for that allele (two different alleles), one normal and one abnormal. Only if you lose your heterozygosity you will get a tumor. So, from a molecular level they are autosomal recessive, but from a syndrome/disease level they are autosomal dominant "in the syndrome, all cells are heterozygous in this allele".

Loss of one allele usually does not result with cancer "normal phenotype"; because the normal allele will compensate for the partly or completely dysfunctional allele. However, for some tumor suppressor genes, losing part of their activity or losing one allele can result in an abnormal phenotype. This is called **Haploinsuffeciency** which is a tumor requires both alleles, all the mRNA produced from those both alleles and all the proteins produced from those mRNA to be present to be phenotypically normal. If you lose some of their activity, you will end up having a problem.

For some tumor suppressor genes, as little as 20% loss of activity is enough to produce a cancer like **PTEN breast cancer**.

Not all tumor suppressor genes are recessive (but RB and P53 are, even though their syndromes are dominant).

Genetic Lesions in Cancer

- What are the genetic lesions in cancer?

Karyotype. What are we looking for in karyotypes? The number and the structure of the chromosomes, and the abnormalities in them.

The common types of nonrandom structural abnormalities in tumor cells are (1) balanced translocations, (2) deletions, and (3) cytogenetic manifestations of gene amplification.

Translocation: exchange the genetic material between non-homologous chromosomes. *Remember that the exchange of genetic material between homologous chromosomes is called cross over, which is good and producing genetic diversity.*

Translocation could be:

(1) balanced: where there is no net gain or loss of genetic material

(2) unbalanced: where there is a loss or gain of genetic material

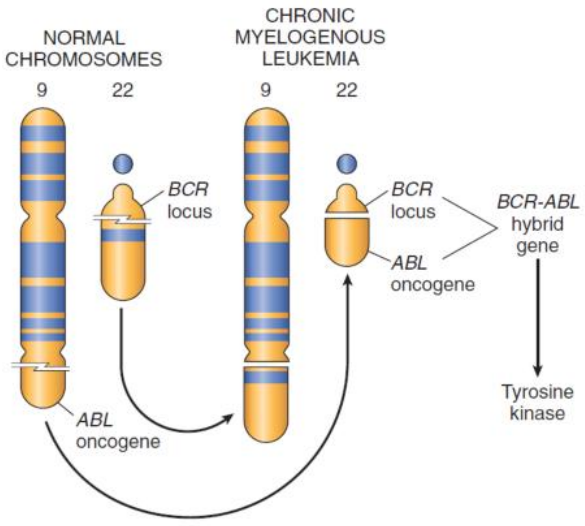
Recpricotranslocation: where there is a two-way exchange of the genetic material between the chromosomes, and it also could be balanced and unbalanced.

Translocations can activate proto-oncogenes in two ways:

1- You could **translocate a proto-oncogene** and put it downstream to a very active promoter, so you are creating more Oncogenes.

   Example: you can over express a proto-oncogene like MYC by moving it from chromosome 8 to chromosome 14 (which carries the immunoglobulin genes, a very active gene) and making it an oncogene with all the downstream proliferative pathways. By this this scheme, Burkitt lymphoma occurs. Moving **Bcl2** (an inhibitor for apoptosis) in front of a very active promoter (like the example above), so Bcl2 is over-expressed, and that inhibits apoptosis which must not happen in a cancer cell. By this scheme, follicular B cells lymphoma occurs. (Proliferation is not stimulated here so this type of cancer is very slow growing.) **notice the two last examples are lymphoid cells, why? What's so special about them that the translocation happens frequently? What do they produce? They produce T-cells and B-cells which in turn produce antibodies. We have a lot of antibodies "near $10^{\wedge 14}$ different antibodies". But we have around 20,000 genes only! And not all of them are responsible for making antibodies. So, there must be a random combination between the genes responsible for creating antibodies. And this random combination by cutting and adding which means a higher risk for translocation cancers.

2- Other oncogenic translocations create fusion genes encoding novel chimeric proteins "**fusion protein**".

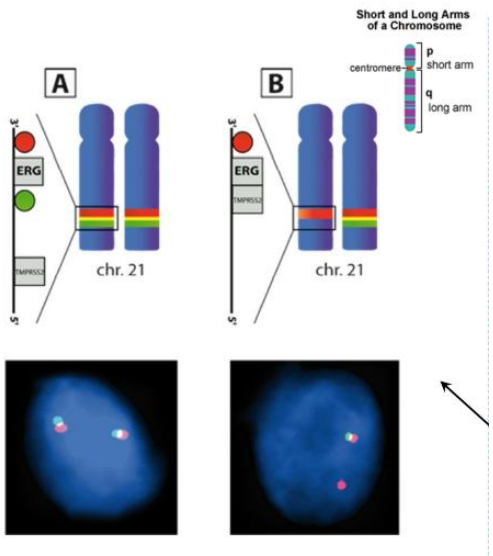


Breaking the gene that codes for a protein and sticking it to another gene creates a whole new different protein. For example, In **Philadelphia chromosome** or translocation between chromosome **9 and 22**, what happens here is that ABL kinase is removed –without its regulatory part- from chromosome 9 and moved to a different part where it is stuck to BCR gene "chromosome 22" resulting in a BCR-ABL hybrid protein. This leads to a constant active kinase and a constant proliferation. This is seen in more than 90% of all chronic mylogenous leukemia. And because there is a new produced protein we can target it only and affect the target cell without harming the body. As a result from that, the vast majority of patients respond really well to this therapy.

This is the importance of this course; you are no longer treating the phenotype, but instead you are treating the molecular basis of the disease

Other fusion products can occur even in solid tumor such as **sarcoma**. What if you take a transcription factor and put it in front of an androgen sensitive promoter, which tissues are in trouble? Prostate, because it's an organ that is androgen dependent, and the result is Prostate carcinoma. This happens also by deletion; when a gene that codes for a transcription factor "For example, place ETS family transcription factor genes" that is responsible for turning on the genes responsible for proliferation happens to be on the same chromosome as that of androgen sensitive element TMPRSS. So if you were to delete the region between them, they will come together in a way it becomes androgen sensitive transcription factor "various *TMPRSS-ETS* fusion genes found in prostate carcinomas". This will result in ETS family transcription factor genes being under the control of the TMPRSS promoter, which is activated by androgens. The net effect of these rearrangements is the inappropriate, androgen-dependent expression of ETS family transcription factors.

* in the last case you don't need to do a karyotype, because you know it's common in prostate cancer; so you could take some of these cells and put them in a dish. And by producing a colored complementary DNA probe for the region between the two genes and a region outside these genes, for each one of these probes you have a different color; 1 green, 1 red. In a normal prostate cell you should find 2 green dots and 2 red dots, because you have 2 chromosomes. If you were to have a deletion you will lose one of these dots. This is called **fluorescent in situ hybridization**.*



Keep in mind that TRANSCRIPTION FACTORS bind to the promoter sequence to initiate transcription.

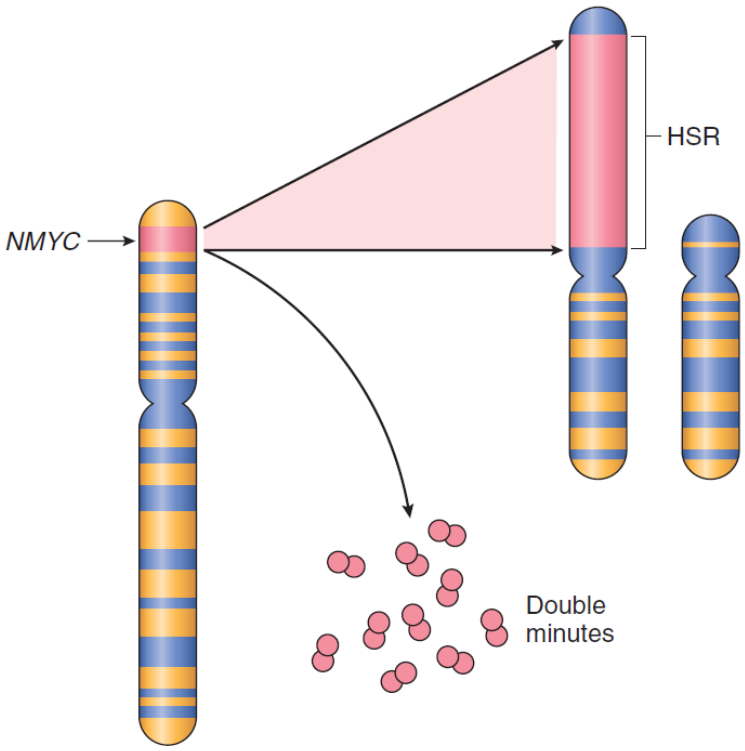
Another chromosomal abnormality is **DELETION**. Deletion can be:
1- 21q deletions (the example mentioned above)
2- 13q14 deletions RB
** don't memorize the number but know what they mean, 13 means the chromosome 13, p or q means short or tall, respectively, and the last number, 14, is the region of the short chromosome**
3- 17p deletions P53
and when you delete RB and P53, which are the guardians of the cell cycle, you allow more mutations and cancer.

Another Chromosomal abnormality is **AMPLIFICATION**.

Gene Amplification

We can amplify the gene through a problem in synthesizing DNA, when the cell is proliferating. What might happen is that you end up copying a gene on the DNA over and over and over, through a mistake in the replication machinery. This could end up as:

(1) Double minutes independent replication areas which are sub chromosomal particles that contain genes. They can be transcribed; this means that they can potentially over express a particular gene just because you have too many copies of it. You normally have two (from mother and father), but if you have more you will express more.
(2) They could stick on the chromosome as a homogeneously stained region. You stain to identify the bounds, however if you stain a region with the same sequence you can find a one big homogeneous bound.



The net result is over expression, but what are we worried about?

The answer is simple, proto-oncogene. MYC particularly NMYC is present in your nerve cells. If this gene is ove-expressed, the result will be neuroblastoma. ERBB2 (HER2/NEU) receptor kinase is one of 4 receptor tyrosine kinases that include human epidermal growth factor receptor 2. This particular receptor kinase results with breast cancer when over-expressed.

NMYC over-expression results in a very aggressive cancer (neuroblastoma) that we were not able to find a molecular treatment to treat it, and we therefore these patients are poor prognosis. However in ERBB2 case we managed to create a drug "in this case it is an antibody" that can bind to and inhibit the receptor. These patients are good prognosis patients because we figured out a molecularly targeted therapy that specifically targets cells that are over expressing ERBB2, and so treating the cancer.

Aneuploidy

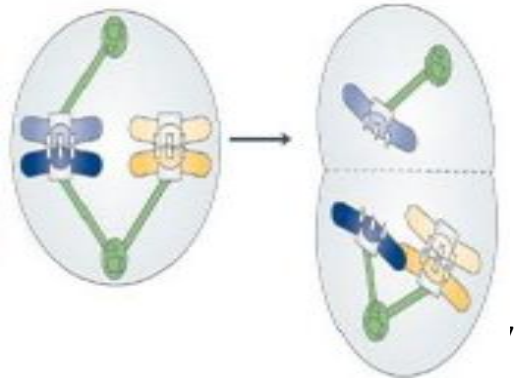
- What is our haploid number as humans? What is our diploid number?

23, 46 respectively, so anything that is not a duplicate of 23 is aneuploidy. Aneuploidy frequently occurs in solid tumors. Note, we don't know if it is the cause or the effect of the tumor, but we do know that there has been a mitotic checkpoint abnormality that caused the missegregation. Because of this missergragation, aneuploidy results. Think of this, can your cells handle more chromosones DNA or less chromosomes DNA?

Well, in the case of "less" there are missing genes that are essentially effective and only 50% of their activity is available, and that may not be enough, and the cell ends up dying. Now, think of the genetic diseases you know; Down syndrome (extra chromosome), Trisomy Edward trisomy, Klienfilter(xxy). Only Turner's syndrome (XO) patients can survive this loss of chromosome, it is not lethal, the question is why?

When you look at the female cell nucleus you find a barr body which is an inactivated X chromosome; normal female cells turn off one of their X chromosomes because cells do not need two copies of <u>most</u> of the genes on the chromosome, so they can tolerate losing one of the chromosomes. In spite of this, Turner syndrome patients are not fully normal because the chromosome is not fully inactivated; there is a small region on the chromosome called the pseudoautosomal region were two copies are needed, and because it is a small amount of DNA material that is lost, the patient survives.

Cancer cells cannot tolerate the loss of genetic material. Frequently in solid tumors, you find massive overabundance of genetic material, whether this overabundance caused the formation of the tumor, or the genetic mutations caused it "cause vs. consequence". however the cell can only permit certain increase in the genetic material before it becomes a burden rather than a boon for the cancer cells; when it becomes a burden this causes a catastrophic type of cell death, and somehow

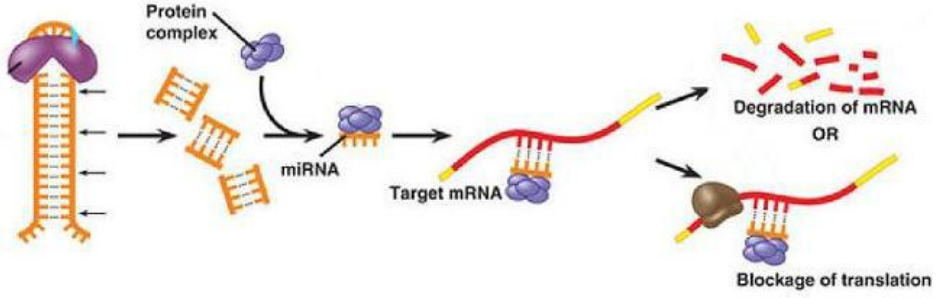


cancer cells prevent this from happening, again natural selection, some cancer cells go crazy aneuploid and then die and you never detect them, and some other cancer cells know when to stop and that's it, and when they have stopped their genome size becomes completely stable, but they still could accumulate mutations not checkpoint errors. There are current treatments to poke cancer cells or probe them to start this process up again to induce them to become aneuploid and kill themselves.

Point mutations can activate oncogenes and suppress TSG. They are random; nothing magical, natural selection also plays a role.

miRNA: from the big chromosomes to the small nucleotide sequence

There are several types of RNA; messenger RNA, ribosomal RNA, transfer RNA and *miRNA*; which is responsible for the control after transcription of the amount of protein produced from mRNA. Like radio's knob which moves between stations and its fine tuning knob which is used to make sure to get the clearest signal possible, microRNAs works as your fine tuning knob. they are approximately 22 nucleotide long. They are pieces of RNA that are complementary to certain pieces of mRNA. When mRNA becomes double stranded, it does not enter the ribosome, and so additional translation is prevented. Although double stranded RNA molecules are functional for some viruses, they are not functional in our cells, so one of the ways to combat theses viruses is to chop of the double stranded RNAs. Binding of microRNA with mRNA will result with them being chopped off.



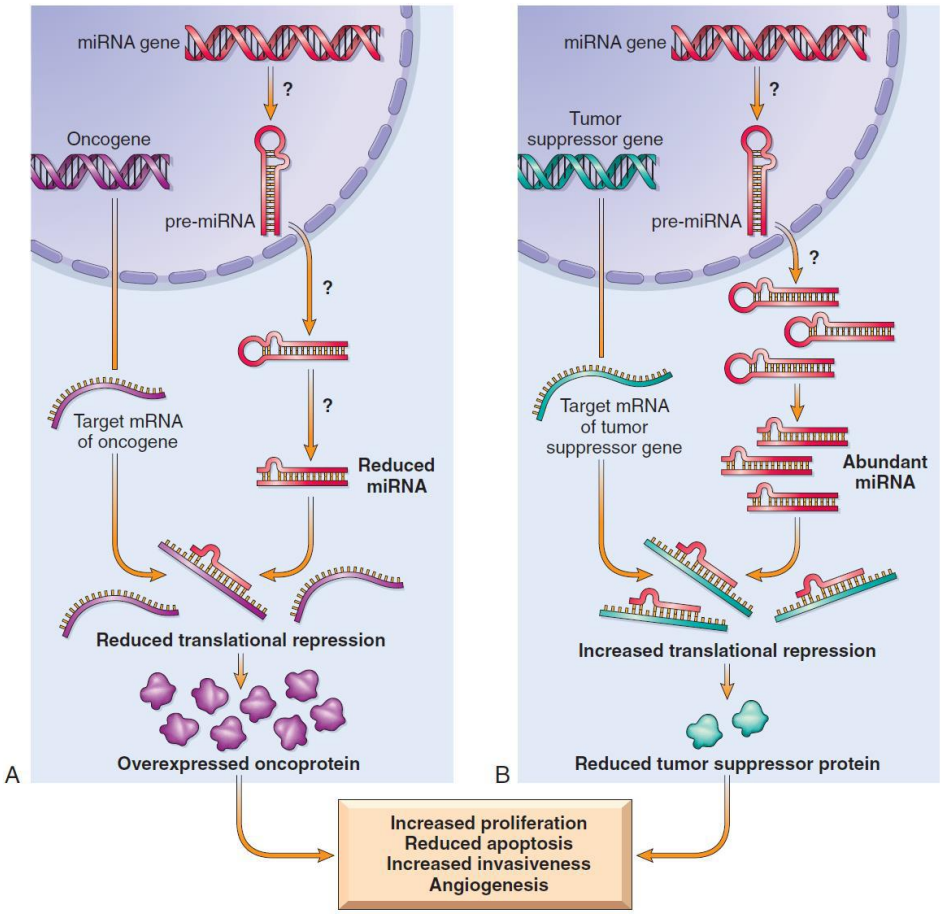
- What can you do with these miRNAs to induce cancer? (Refer to the image below)

1- <u>For miRNA molecules that bind proto-oncogenes' mRNA molecules</u>: Mutation of the genes that code for these miRNAs results with the miRNAs being unable to bind to mRNAs, and the cell ends up producing more proteins than they should, which results with the proto-oncogenes becoming oncogenes because of their over-expression.

2- <u>For miRNA molecules that bind mRNA of tumor suppressing proteins</u>: Another mechanism is the over-expression of these miRNAs, which result with the formation of a carcinogenic condition, so you don't screw with gene itself but with the regulatory element of the gene.

3- Another example is mutating the non-coding regions in the gene and prevents miRNA from binding, and the result is oncogenes "overexpressed".

For example, downregulation or deletion of certain miRNAs in some leukemias and lymphomas results in increased expression of *BCL2,* the antiapoptotic gene. Thus, by negatively regulating *BCL2,* such miRNAs behave as tumor suppressor genes. Similar miRNA-mediated upregulation of the *RAS* and *MYC* oncogenes also has been detected in lung tumors and in certain B cell leukemias, respectively.



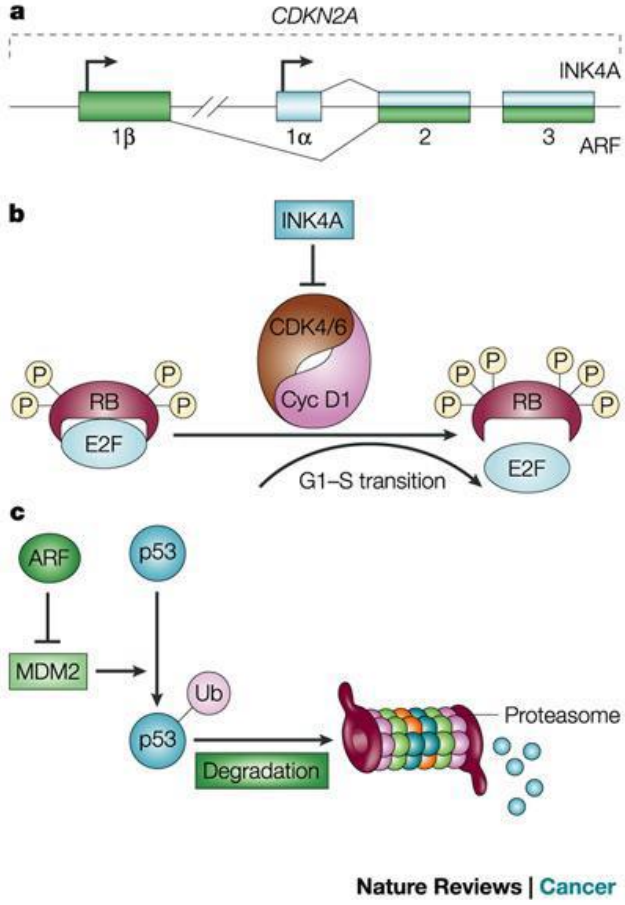
Epigenetic changes in cancer:

- All of your cells have the same DNA but what makes your skin cells as they are and cardiac cells as they are?

Gene expression; genes can be turned off and on. Euchromatin is where the genome is loose and heterochromatin is where it is tightly packed “closed.” So from this *open-transcribed*: *closed-not-transcribed* scheme, genes can be on or off; in the skin cells, the gene that produces keratin is open, whereas in muscle cells that gene is closed but the ones that are important for the action of myosin are open. We control the opening and closing through epigenetic changes which are non-mutational universal inherited changes that change the transcription of the different genes. These could be Adding a methyl group to your DNA or your histones or adding an acyl group to your histones. These modifications imply whether the gene is turned off or on, and also signal for the packing of the gene as we don’t need it anymore. Hypomethylation or reduction in methylation or acetylation of histones turns on the transcription of genes. For example; Telomerase (how cells live longer) is not expressed in somatic cells, so the gene is hypermethylated "turned off". If you remove he methyl groups, the cell will start using telomerase and this is what cancer cells do; they reactivate the telomerase, they screw with methylation markers and they are inherited "when you take molecular biology of genetics you will have a greater perspective". The way cancer cells can

screw with the methylation is by Global DNA hypomethylation; a lot of genes that are normally not expressed now become expressed, and specific promoter hypermethylation of TSG is a change in methylation markers that has a bad consequence "decreases tumor suppressor proteins". Natural selection plays a role here. Certain genetic changes in cancers may be *selected for* because they lead to alterations of the "epigenome" that favor cancer growth and survival; such as DNA methyl transferase. It is heritable because you receive your methylation markers from your mother and father. These disturbances are called *genomic imprinting,* and there diseases that can occur because of screwing up with the methylation and the genome sequence itself, so not every carcinogenic change is a mutation; there are non-mutational changes that can also affect transformation and cancer.

CDKN2A is a complex locus that encodes two tumor suppressor, p14/ARF and p16/INK4a, produced from two different reading frames; p14/ARF is epigenetically silenced "hypermethylated" in colon and gastric cancers, while p16/INK4a is silenced in a wide variety of cancers. P14 is responsible for the inhibition of the protein that sends p53 to be degraded. So if you take p14 away, p53 is constantly going to be degraded so now the function of p53 is lost "nothing happens if you get a mutation; no repair of the mutation", and proliferation will continue and the mutations will stay "good for the cancer - bad for your body". P16 is responsible for the inhibition of a protein that is responsible for your G1 to S transition. This protein affects retinoblastoma protein "pRb; governor for the cell cycle" by releasing a transcription factor that allows your cells to continue through the cycle. P16 prevents this from happening; so if you take it away, the cell cycle continues and the proliferation will not stop. Since this locus produces two tumor suppressors that affect the p53 and Rb pathways, silencing this locus has the pleasing effect (from the cancer's standpoint) of removing two checkpoints with a single alteration.



A treatment of that may be the hypomethylation "activation" of CDK2A. There are treatments that affect the methylation status of the cell. Another note on methylation; all your cells have the same genes, but some genes are closed and others are open. So, activating a pathway in skin cells is not going to be the same as in T cell and a pathway that stimulates transcription of other genes. The epigenetic state of a cell dramatically affects its response to otherwise identical signals; for example, the gene *NOTCH1* has an oncogenic role in T cell leukemia, yet acts as a tumor suppressor in squamous cell carcinomas. As it turns out, activated *NOTCH1* turns on progrowth genes in the epigenetic context of T cell progenitors (e.g., *MYC*) and tumor suppressor genes (e.g., *p21*) in the epigenetic context of keratinocytes. That's why you need to understand the molecular basis of cancer; we have left the era of chemotherapy surgery; you also need to understand the molecular basis of the patient "fully individualized"; because not all patients have the same basis.

not until we are lost do we begin to understand ourselves