University of Jordan
Faculty of Medicine
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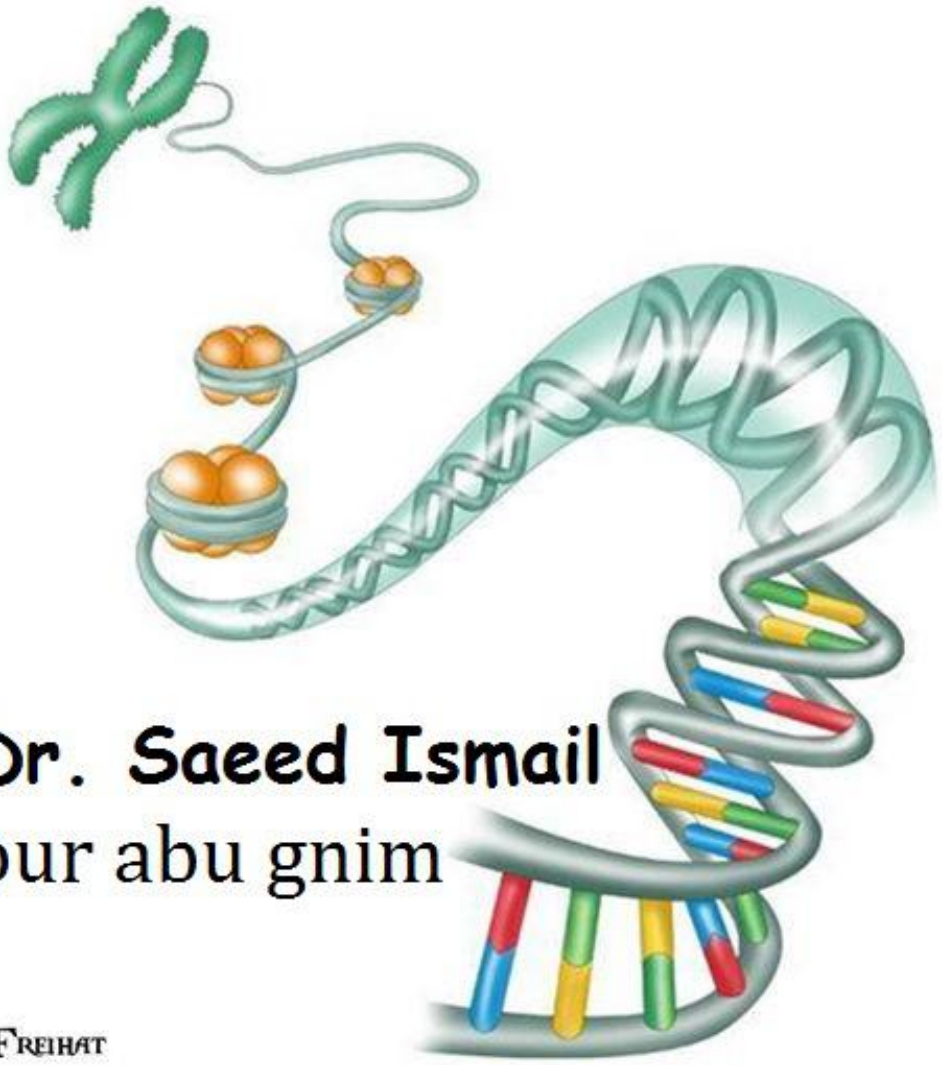
Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



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Molecular biology of cancer

We start with chapter 18 that talk about molecular biology of cancer, and we said that you can look to the cancer as a genetic disorder not as heredity disorder, since it explained at the gene level, how?

We know that cancer results from uncontrolled cell division, and these cell divisions happen because there are mutations in specific genes (oncogenes and tumor suppressors), so we understood what cancer is all about at the gene level. It is basically a group of mutations in specific area or specific gene sequence within our genome.

Now like we said before there are two group of genes involved in cancer transformation , oncogenes and tumor suppressors and we said that oncogenes are like the gasoline pedal in the car and tumor suppressor are like the brakes, we agreed that **four to seven mutation** or as other books said five to ten mutation they should involve some tumor suppressor and some oncogenes, up- regulate the oncogene or down- regulate tumor suppressors , actually the more accurate term is gain of function of oncogenes and loss of function of tumor suppressor.

What is the different between gain of function and over expression of gene? Why we said that the gain of function is more general, more appropriate and more true than over expression?
for molecular biology it doesn't make a difference
The answer is that over expression is the increase of the production of the RNA and protein , but it's not always the case, it could happen that we have gain of function without increase in the expression, which means that oncogenes are there for a short period of time to drive limited cell proliferation, they appear shortly and stay until the end of the cell division and then they go back, now out of control growth or proliferation could happen in two ways:

1. it could be too much of that onco-protein (over-expression).
2. it could be continues activation of that protein , which called **constitutive activation**, so the protein is there in it's normal level, it's not increased but it's there for longer than it should be or it's there for ever.

This protein (oncogene protein) should appear for short time until the end of one, two, three… divisions then it goes down but it may stay their, here there is no over expression, there is prolong or continuous or the right world is the constitutive

activation. So the term "gain of function" more accurate when we describe what really happen than over expression (the increase of the production).

Now from that we should say that in there normal functions, in there normal state they are called proto-oncogenes and when they gain function they will become oncogene. So proto oncogenes are normal, and oncogenes are abnormal.

How the gain of function mutation in proto-oncogene happen? How the normal proto-oncogene converted to the abnormal oncogene?

There are an examples on that, it could be:

1. **Radiation or chemicals:** where there are thousands of million of chemicals that can mutate our genome which hit the promoter region or at the coding region. If they hit the promoter how it could lead to over expression in this case or gain of function? Basically the promoters of many oncogenes are intentionally made suboptimal, they don't have the appropriate or the best sequence, they have suboptimal sequences so they are weak promoter, for example the sequence in specific place in the promoter is G (which make the promoter very strong), we remove the G and put A in the promoter to make it weak promoter intentionally (because it's the optimal state for the normal cell) so because of that the expression of the gene is weak, then by accident a mutation happens which will substitute the A for a G so suddenly the mutation make the promoter that should be weak a "strong promoter" so the gene will work better, that's the mutation hitting the promoter region.

Another mutation in the coding region, so many of these proto-oncogene favours free fold conformation of the protein, it could be a tyrosin kinase involved in signal transduction (that is the oncogene) and there is an optimal shape for it but its naturally made inactive by intentional rotation that twist the active site making it not in the optimal shape, so that it's not very active in signal transduction so the proliferation is suboptimal which is better to control for the proliferation like in the car when it's better to make gasoline pedal hard in order not to press on it very hard and cause accidents.. So a mutation could happen by an accident and suddenly it hits that place and make the shape of the protein in the optimal shape (stronger) it gives the conformation it's better 3D conformation, that's lead to an increase in the activity of the enzyme but this very unlikely events .

remember these words till the end of this chapter we will refer to this point and some people are not very convinced that four to seven mutations or five to ten mutations

could happen in a single cell in a very specific places and they will go ahead and tell you it's like a winning a lottery seven time in your life time which is almost impossible.

Last week a guy in England won the lottery twice and they ware talking about the chance is one in two hundred of billion of time but he did it, but when the chance of winning seven times is like one in a zillion of chances which almost impossible, so not any mutation could happen in the promoter and not any mutation could happen in the coding region and not in any where in our huge genome, it should be in a specific region and not in any cell it can happen.

A student ask a question about the relation between the promoter and the mutation and the dr. answer was: the mutations and proto oncogenes could happen in one of the following they could hit the promoter region making it a better promoter ,stronger promoter, what that's mean? A strong promoter is a promoter having the ideal consensus sequences, it should be in one sequence when we remove one it will be weak promoter remove another the promoter will die, the cell in purpose has a weak promoter, but by accident a mutation retain the promoter in the stronger formation, it could be in the coding region, in the coding region doing what? It convert the protein from the suboptimal shape to the more optimal or it could happen in another way, imagine there is an oncoprotein and it has two buttons, one that switch it on and one to switch it off ,so what happens is that the mutation could hit the switch off button so you switch it on and then it will start working as an onco protein and then all of the sudden you want to switch it off because you have enough of divisions and then you discover that the switch off button is not working, there is a mutation on the control area that control the protein so here there will be constitutively active protein, so it may make the promoter more active or the protein itself or by the shape it make it more active or less controllable by hit the negative controlling region.

There is another way for switching off which is by the protein inhibitor that when it attach to the protein, it inhibit its activity but when there is a mutation in the inhibitor binding protein site so this inhibitor protein can no longer bind which lead also to constitutively active protein.

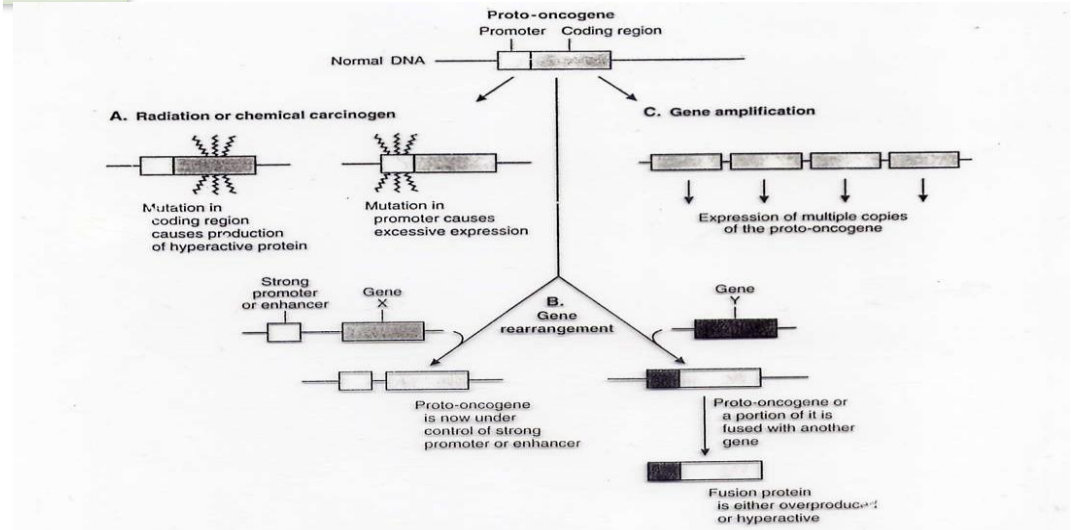


Fig. 18.3. Transforming mutations in proto-oncogenes. (A) Effect of radiation or chemical carcinogens on proto-oncogenes or their promoters. The mutations may be point mutations, deletions, or insertions. (B) Gene rearrangements as caused by transposition or translocation of a proto-oncogene or proto-oncogene fragment. (C) Amplification of a proto-oncogene allows more protein to be produced. The proto-oncogene and the resulting oncogene are shown in blue.

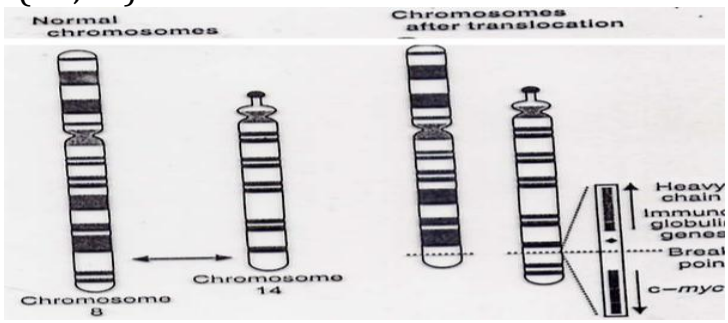
2. **Gene amplification:** another scenario where you have the gain of function in proto oncogene, this is actually over expression, because we have two copies of proto oncogene and suddenly you can end up with a hundred of these and this happen only in cancer cells like in the HER2/NEU which is a member in the EGFR family, it is amplificated in breast cancer, even if each copy is giving me a little of the protein but because we are having high number of those protein copies the effect on the cell will be amplified .

3. **Translocations:** the perfect example for this category is the translocation of (8;14) in Burrkitt's lymphoma, it happen for some reason radiation for example, they break chromosome and when another chromosome broken they could exchange this broken bits , now what happen is that most of these bits are harmless ,they do nothing but sometimes some of these translocation could do the following for example there is a very powerful oncogene at chromosome 8, which transcriptionally inactive region so it could be for example heterochromatin, so intentionally the cell would have condense chromatin at this broken bit of chromosome eight because it know that here there is a potent-oncogene called MYC, suddenly a translocation happen and these chromosomes are broken down here and chromosome 14 also broken in another place and they will exchange the two broken bits, the part from chromosome 8 will go to chromosome 14 and the piece from 14 goes to 8, but in chromosome 14 in the ***B cell*** (we should remember that this translocation happen in the lymphoma) because in other cells it will be harmless, why? Because that region of chromosome 14 is only active in B cells. what happen is that MYC found itself suddenly in chromosome 14 in B

cell, where the region that we can found the immunoglobulin gene which make the antibody, now originally what is the function of B cell? To Make antibody, so where is the very active region in the whole genome of the B cell?
In the pat bit of the long arm in chromosome 14, so its:

- Euchromatin (transcriptionally active).
- There is no methylation on the promoters.
- Plenty of transcription factors.

So as we said MYC found it self suddenly in the 14 chromosome next to very active genes there is a fact in molecular biology "promoter proximity effect", which when you have a strong promoter next to a weak promoter ,the weak promoter will take advantage of the strong promoter and get activated how? because its not it's job (the weak promoter) any more to bring in transcription factors, whose attracting the transcription factor? The strong promoter, you have strong and weak promoters so when the weak come near to the strong one, now its not the responsibility of the weak any more to bring the transcriptional factor, when the stronger attract the transcription factor it will be near to the weak so the weak will also use it, so by that the weak get benefit. So MYC gene found it self next to very strong gene (the immunoglobulin region) so it will get over expressed. Now this translocation when its found in any other cell it will be useless because only the B cells have the immunoglobulin region, all the B lymphoma cells have translocation involving the long arm of chromosome 14, (1;14), (8;14)… all certain translocations bringing different oncogenes next to that active area, Burrkitt's lymphoma (8;14), follicular lymphoma t(14;18) …



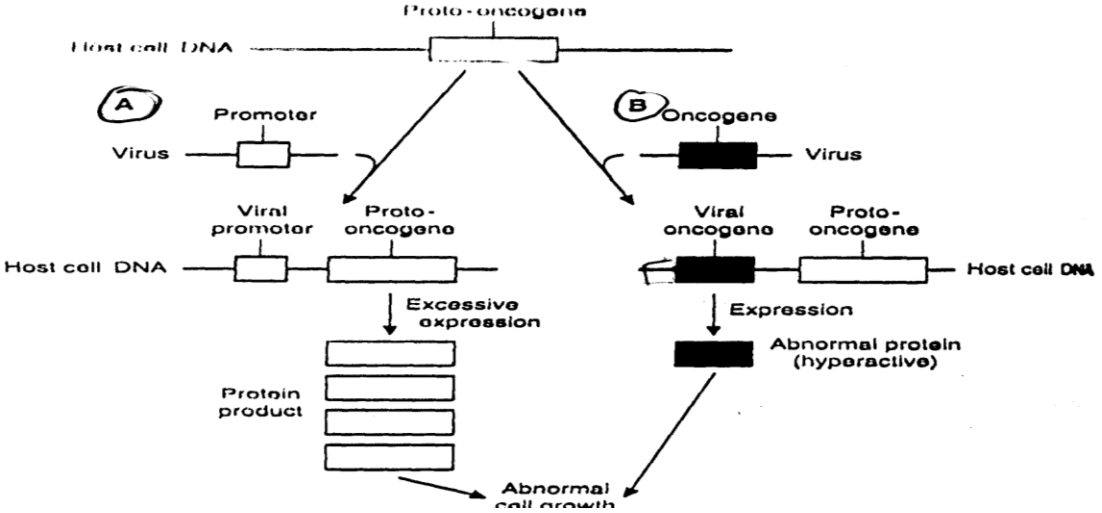
4. **Onco-viruses:** converting proto oncogenes to oncogenes, how do viruses cause cancer? In one of two way:

- They come in with strong promoters: if the virus come with strong promoter, we said before that many oncogenes have weak promoters intentionally so the virus

come and put the strong promoter on the weak promoter and activate the gene that have week promoter.

- The virus brings its own oncogenes: the virus will not do integration, it enters in with it's own strong promoter and oncogene and activates cellular proliferation and cause cancer , many of retro-viruses can do that, some of adenoviruses also. They work on the gene level not the protein level .

Now the important thing to now about this onco viruses if they are to bring there own oncogenes and switch on the proliferation of that cell, it means that there oncogenes should be like our oncogenes other ways they will not be compatible with that cell, so almost all of our oncogenes or the main one are found on this virus, how this viruses get our oncogenes and they can give us cancer in some stages? We take it from them, they take it from our cell, no body knows its like the egg and chicken story. But the virus that was in our cell and get out with a piece of our genome and this piece has an oncogenes , immediately this virus has survival advantage, because this virus when it goes to the next cell it will spread, how? By the faster and numerous divisions of the cell, how? By the virus oncogene.



Loss of function mutations in anti-tumor genes

The other half of the story is the loss of function in tumor suppressor or anti- tumor genes (here take care about the terminology), here we talk about the anti-tumor genes and one of them is the tumor suppressors genes, but again at the end of this chapter we will talk about all of them as tumor suppressor so they are used interchangeably, you can say anti-tumor gene or tumor suppressors gene, why its like that? Because

historically the term tumor suppressors gene it was just for a group of gene that just stop the cell cycle or proteins that arrest the cell cycle and then it become more general to include other things that have nothing to do with cell cycle directly ,for example protein responsible for cell adhesion are being considered now as anti-tumor gene or tumor suppressor genes, why? They have nothing to do with the proliferation of the cell but they have something to do with the transformation in the last stages of cancer "metastasis" because if they are still intact they can prevent metastasis.

1. **DNA repair enzyme:** they are considered as tumor suppressor genes because if we can retain mutations affecting oncogenes then we are preventing the transformation to cancer cells.

Here we have to remember BRCA 1 & BRCA2 and mismatch repair genes, 5-10% of all cancers cases are familial the rest is sporadic and most of them are BRCA 1 & BRCA2 or mismatch repair genes in breast cancer or colon cancer. 5-10% of breast cancer are familial and 90% of them are carrying BRCA 1 & BRCA2 mutations, 5-10% of colon are also familial and the vast of it 70-80% are mismatch repair genes. Both of them are DNA repair enzymes and they are considered as tumor suppressor because they can repair damage that could produce oncogenesis.

BRCA 1 & BRCA2 are two huge genes each consisting of around 21-22 different exons and the difficult thing about detecting this mutation in the affected families is that each family has a different mutation at a different exon some time in some oncogene or gene in general ,they can have a Hot Spot (a disease) its mean that 90% of the patient around the world the mutation that they have its found in exon number 13 so its easy for diagnosis to go for exon 13 mutation but in this case it's a huge gene, so many exons and every family has a different exon mutation so you have to sequence the whole thing not just BRCA 1 but also BRCA2 so you have 40 different sequence, no a day by the NGS (next generation sequencing) its more easy to detect, the breast cancer now a day in Jordan is a big problem which is the number one even if you put the two sixes together, so its more than the lung and colorectal cancer, and we can do something about the 10% familial because you can discover the mutation early in life and you can tell which time to test by looking to the very short family history, there are certain criteria that tell if any female know that her mother or her aunt… under the age of 40 or 50 has a breast cancer or ovarian cancer (they are related), very likely to be familial so they have just to look for BRCA 1 & BRCA2 genes mostly BRCA1, So if there is three sisters, one of them has the mutation she has to be very carful and the others don't have the mutation they are absolutely normal and who has the mutation 80-90% likely to get the breast cancer or ovarian cancer, here what she can do ether

more regular checks and discover it earlier which is really curable or she can do surgery (mastectomy). But the question is: Why when there is a BRCA 1 & BRCA2 mutation we look for breast and ovarian cancers? Because these enzymes are more active in this tissue,so this tissues have more reliance on the function of BRACA1&2, so if they are gone this tissue will be the most effective, the same things happen with mismatch repair which more active in epithelial lining in colon and if there is mutation on them, most likely there will be colon cancer.

2. **Activation of apoptosis:**Apoptosis related proteins as we will see at the end of the chapter can be divided half and half, half called tumor suppressor gene and half called oncogenes for example activators of apoptosis are tumor suppressors and they will lead to the death of that cell, while inhibitors of apoptosis are considered oncogenes.

3. **Tumor suppressor gene:** p53 loss its function in more thane 90% of cancers.

Oncogenes:

Proto-oncogenes : they don't just give us cancer, they are found for a reason and that reason basically is making cell divide and grow in a controlled manor, now how do cells grow?

That's the typical scenario, typically a cell does not divide unless it receives an order from outside in a form of growth factor, who receives this growth factor? A growth factor receptor, then what happen is that the binding of the growth factor on the receptor induces conformational changes at the inner side (the intercellular side) so it recruits G- proteins and start signalling to the nucleus because the starting point of cell division start at the nucleus, so the massage should come from the surface of the cell (receptor) to the nucleus, who does transmit the massage from the cell surface to the nucleus?

Signal transduction protein, so different signal transduction pathways that take the massage all the way to the nucleus, what happen in the nucleus is the activation of gene responsible for the cell cycle, so transcriptional factor will be activated in the nucleus and these transcriptional factors will activate genes for the cell cycle and then start cycling. Now any member of this chain of order is a potential proto-oncogene (G.F, G.F receptor, signal transduction protein, transcription factors…), so any of them can get loss of control upstream of it or some time parallel will be converted into oncogene.(the Dr. tell a story to clarify things, imagine you are doing an exam in the

exam room and the Dr. sit in his office and he told to the guy who sit on his door to till the other guy to tell the other that we can start the exam until the news reach the guy in our door we get the massage and we start writing, an hour later the same thing happen but with the massage exam is over so we stop this is the pathway, and we are all proto-oncogene and any of us who get out of order we will be in trouble) Each member of the cascade of order has a guy next to him that stop him, so it could be oncogene over doing of him or simply the inhibitor (tumor suppressor) that lost its function, so there is always a tumor suppressor working at the same level of these oncogene and some time it's the gain of function of oncogene or the loss of function of the tumor suppressor gene that cause continues signal.

Finally, remember the PDGF (platelet derived growth factor), which produced by the platelet and make it to divide to close the injury and it found in all tissue but firstly discover in the platelet, the receptor that receive it is called PDGFR. Examples on the signal transduction protein, one of the first protein that receive the signal is RAS protein, its so important and when we describe an oncogene as important its mean that it implicated in several cancer, RAS for example found in many solid tumor, so it's a main one. MYC is a transcription factor one, RAS will activate Raf and Raf will activate MEK and it will activate MAP kinase and then the massage will go to the nucleus, they are activate each other by phosphorylation, they are all kinases. Remember MYC as a transcription factor oncogene, PDGF as an example of growth factor some time they called theme CIS and PDGFR as an example of receptors, remember also the erb B1 and erb B2(HER1&2) as an example of singal transduction protein.

The end

"climb mountains not so the world can see you, but so you can see the world"

Done by: Nour abu gnim