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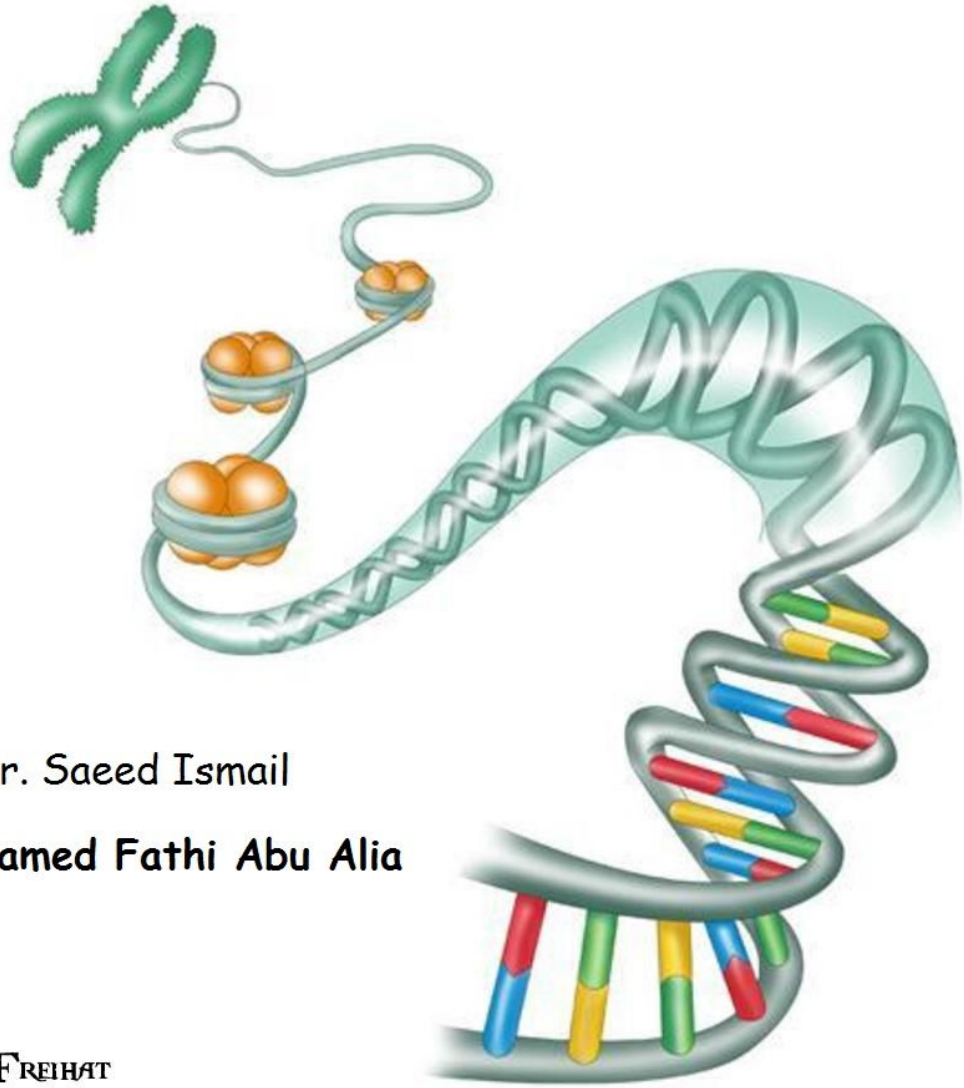
Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Sheet#: 22

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Gene Regulation and the Molecular bases of Cancer

This will cover the rest of chapter 16, and an introduction to chapter 18.

The Regulation of gene expression:

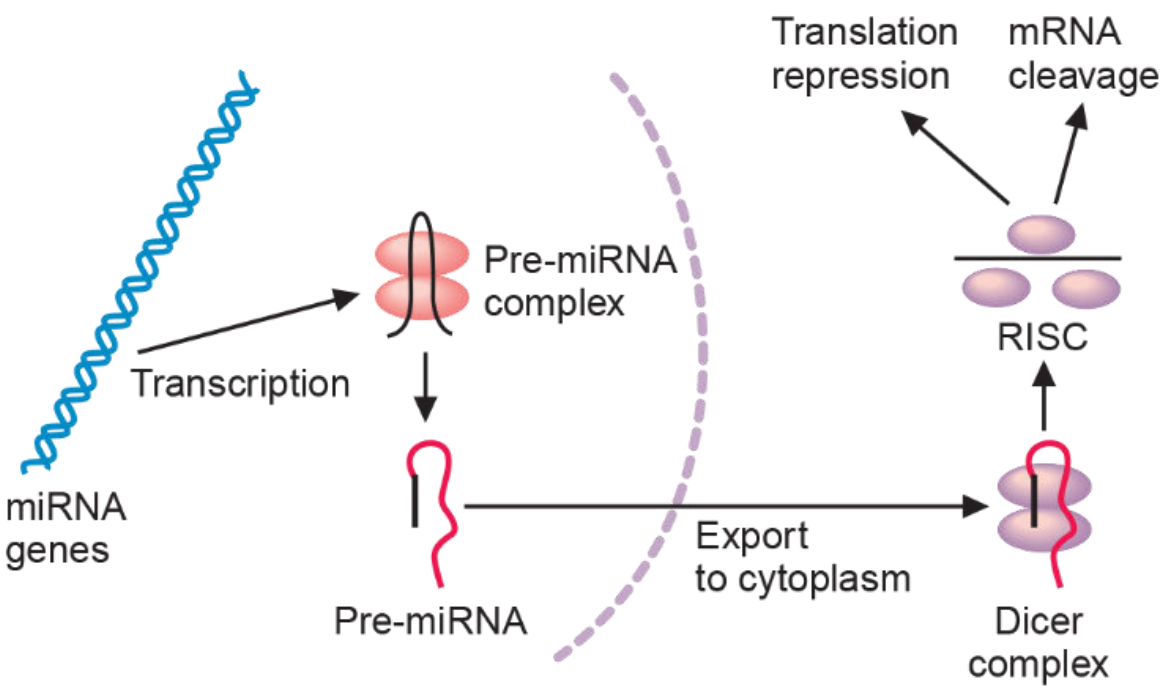
The cell can control the activity of its genes, whether by witching them on or off or controlling the level of expression.

In eukaryotes, this regulation is divided into five levels: DNA level (chromatin remodeling, DNA methylation), most importantly: transcriptional level (*cis*-acting elements – promoters and enhancers, *trans*-acting elements – specific and general transcription factors), post-transcriptional level (quantitatively and qualitatively), translational level (how much protein is produced) and post-translational level.

More on level 4: Translational level:

One more example on this level is micro RNA (miRNA), although some would argue that it is an example on post-transcriptional level.

A way to control already processed mRNA, miRNA is one of the most important discoveries in molecular biology in the past ten years. They have suddenly found a double-stranded RNA for the first time, it was called micro RNA. So a long piece of double-stranded RNA (pre-miRNA) composed of repetitive sequences gets cut by a protein complex called DICER into small (21 to 25-nucleotide long) double-stranded RNAs (later on, they are changed to a single stranded-RNAs), these small pieces of RNA are complementary to a sequence of an mRNA that the cell wants to down regulate. Here, miRNA is a marker to cleave the mRNA of a gene to be switched off.



As you see, the miRNA (now has become single-stranded to bind an mRNA molecule) can be complementary to a sequence on the 3' UTR end of the gene's mRNA. Once it is bound, a protein complex (RISC) starts cleaving the mRNA. So we have accelerated the degradation of unwanted mRNA – remember that the mRNA already has a short half-life, so we use the miRNA to make it much shorter and to prevent translation.

We have discovered hundreds of genes that their expression can be inhibited by their own miRNAs. This shows us the importance of the discovery of miRNA, the applications that can use them are enormous. For example, in cancer therapy we can activate the production of the miRNA of the mRNA of an oncogene to accelerate its breakdown, or inhibit the production of a miRNA of a tumor suppressor gene. Also, in genetic engineering in the past, in order to discover the function of a certain gene, they used to *knock out* the gene, this means that they delete this gene at the embryonic stage of a mouse, then they observe the mouse as it grows up and notice the change. Today, we can *knock down* the gene (switch it off) and

then switch it back on to see if we can reverse the condition. This is done by synthesizing an RNA molecule that will act as a miRNA and add it to the cell to temporarily switch a gene off to see the change, when we stop adding this RNA the function will be restored and we then can be 100% sure about the function of that gene. For distinguishing, the natural inhibitory RNAs are called miRNA, while the ones we make to change the condition of a specific gene are called "small interfering RNA" or siRNA.

Level 5: Post-translational level:

Now, the protein is synthesized, and the cell is trying to control the protein itself. This can be done in several ways:

The cell can accelerate the breakdown of the protein by adding a **ubiquitin** molecule, which acts as a tag for the protein to be degraded. In a similar manner, miRNA was acting with its target mRNA.

Another way is **conjugation**, as in phosphoproteins, the presence of the phosphate group(s) can either activate or inactivate the protein.

In control by **localization**, we can for example move a nuclear protein which functions in the nucleus to the cytoplasm, this way, we have prevented the protein from doing its function by changing its place. The glucocorticoid receptor is an example on a protein that, in the absence of a glucocorticoid, is held in the cytoplasm by the HSP, but when it is bound to its ligand it gets tranlsocated to the nucleus where it acts as a transcription factor.

Chapter 18: The molecular biology of cancer

The study of cancer and its molecular biology is very fundamental, in addition to understanding the disease, it allowed us to understand many molecular processes within cells. For example, knowing that cancer is defined as uncontrolled cell proliferation, scientists initially have tried to understand how *normal* cells divide. And it is true to say that some of those who were behind the discovery of the **cell cycle** were actually studying cancer. Also, knowing that cancer cells are said to be *immortal*, studies involved the understanding of the mortality of cells, including ideas such as **apoptosis** and cell **senescence**. **Telomeres and telomerase** were discovered when scientists have found that the ends of the chromosomes of the cancer cells are a bit shorter than those of other cells. But cancer telomeres are stable in length and never get shorter unlike normal cells.

Cancer itself is a very important disease, it is called "the plague of the age." Cancer is a group of *different* diseases, all characterized by an unregulated and unlimited cell division.

In the past twenty years, we started to understand cancer at the molecular level, and we have realized that it is a group of different genetic disorders, surpassing the superficial view of pathology to the disease as a mass of cells. In many cases, pathologist fail to differentiate actually between normal and transformed cells from a microscopic investigation. This difficulty came from the fact that cancer cells are normal human cells that had some genetic changes that allowed it to divide continuously. So the cells don't have to look different – even though sometimes they do acquire some apparent changes. Taking hematological malignancies for example, looking in a blood sample for transformed cells is a near impossible job when the number of cells is very small. The ability to detect an abnormal cell among normal ones is called the "sensitivity" of a test, an expert pathologist can pick a cancerous cell in a sample with a sensitivity of 1 to 100 or 1000, which is not great when we talk about cancer. One transformed cell in a million normal ones –which is impossible to detect- is more than enough to

start a cancer, since cancer only needs one cell to develop, so we should diagnose cancer on the genetic level not only on the morphological level.

The characteristics of cancer cells in vitro:

Here is the description of the behavior of cancer cells when put in a culture dish (in vitro):

- They lose contact inhibition: normal cells continue to divide in a dish until they touch each other, then they stop dividing as they become over crowding the dish, forming a monolayer of cells. Cancer cells, however, don't stop growing into multilayers.
- They don't need stimulation by growth signals: normal cells need growth factors to grow in culture, while some –not all- cancer cells don't need lots of growth factors, it is sometimes said: "throw them in the sink, they can grow".
- They acquire resistance to growth inhibitory signals.
- They are resistant to apoptosis, *immortal*. And they don't become senescent, they are always *youthful*. Remember that senescence is a short period of non-dividing state preceding apoptosis.
- Loss of anchorage dependence, they don't need structural support.
- They require less serum in the medium of culture: serum normally brings a lot of growth factors that are difficult to be produced commercially – it is derived from cows. Cancer cells mostly need less growth factors and serum.
- They form tumors in nude mice: nude mice are immunocompromised mice, they can receive anything. The immunity genes that are knocked out in nude mice involved genes related to hair, and thus the name: nude (hairless). They can readily receive human cells, including cancer cells that can form human tumors in these mice – called Xenograft tumors.

Some related terminology:

- **Benign vs. malignant**: among many distinctions, invasiveness is the most important.
- **Metastasis:** the spread of tumor cells form the location of the primary tumor to another location to make a secondary tumor. Metastasis has been lately thoroughly discussed, it was found that it is not a random process, it is well-planned, and cells apparently *know* where they are going. It is thought that the primary tumor sends *scouts* that look for the best place to invade next. When they select a location, they start nesting *a soil* (making a suitable environment for cancer growth) in preparation for the *seed* (cancer stem cells) of the tumor to arrive and grow, it is then called *Seed and Soil theory.*

  Cancer stem cell theory is another attractive theory on cancer that has changed our understanding of cancer. It can be related in part to the concept of selective metastasis, which comes together with studies that show, for example, that 70% of the patients of a certain breast cancer have lung metastases. So it seems that cancer cells do know where they are going. Many scientists believe that this theory will be the main concept of explaining cancer in all textbooks.

  Cancer stem cells are the real "bad guys" of a cancer at its core. Those cells, that probably represent 1 or 2% of cancer cells, are the only cells capable to renew the tumor back, and also one of them can form a new tumor in a secondary location.

  The theory also explains drug resistance of tumors and remission, which is the huge reduction of tumor size upon chemotherapy, which makes the tumor invisible to radiology and gives the impression that the tumor is gone. However, in a certain patient, the tumor relapses after a couple of years. Why? Because, according to this theory, the huge bulk of the tumor is made of *normal* cancer cells, they can divide but they cannot reproduce the tumor as the cancer stem cells. Cancer

stem cells seem to be very resistant to many chemotherapeutic drugs so they renew the tumor again.

As a light of hope, researchers are trying to target these cells. They have identified specific receptors on cancer stem cells, such as CD133 or CD44, and they have designed small ligands (called aptamers) that can identify these receptors with high specificity, the aptamers are attached from the other side to liposomes (a sphere of lipid substance) filled with chemotherapeutic drugs. So when these small molecules are given in blood, they selectively bind cancer stem cells (from their CD44) and when their liposomes fuse with the cells' membranes they release their content of drugs and destroy the *roots* of the tumor. This is known as smart or targeted drugs or "golden bullets" ; they know where they are going.

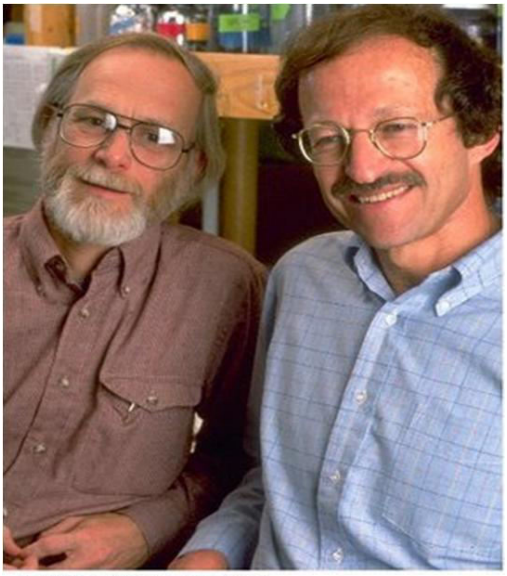
The *blind*, non-selective chemo drugs that destroy any normally dividing cell creating many side effects will become something of the past.

Most likely, the future therapies will include both the drugs that kill the bulk of the tumor and the targeted drugs for the stem cells.

- **Angiogenesis:** formation of blood vessels to provide more nutrients to the rapidly dividing cells of the tumor. Many modern drugs actually target the growth factors that promote angiogenesis, such as VEGFR; targeting it will deprive the tumor from its ability to synthesize more blood capillaries, so it will get shrunk and die.
- **Carcinogens:** any mutagen is a potential carcinogen, a mutagen is a factor that can make a mutation in the DNA, if this mutation happens to affect an oncogene or a tumor suppressor gene then it is a carcinogen. It can be a chemical, radiation, a virus or genetic predisposition to cancer. Genetic predisposition means that one would be born with a mutation that makes them more susceptible to a certain cancer.
- **Accumulation of mutation:** cancer does not result from a single mutation, it requires 4 to 7 (in some books 5 to 10) mutations, a minimum of four mutations involving upregulated oncogenes and downregulated tumor suppressors.

- **Proto-oncogene:** genes that need to be upregulated in cancers. They are similar to speed pedals in cars, meaning that oncogenes normally are found to give a normal function to cells, which is making cells grow and divide under a very controlled way. The speed pedals of a car are there to make it move but not too quickly that can cause an accident. In the case of oncogenes, cancer cells need to **overactivate only one** copy of the gene to transform. As only one stuck speed pedal, if there is two, can cause a deadly accident.
- **Tumor suppressor genes:** they need to be downregulated in cancers. They similar to the brakes, they prevent the effect of an overly active proto-oncogene. Even if you have one speed pedal stuck, you can still have some hold control by using the brakes. In case we lose these *brakes* (tumor suppressors) at the same time the *speed pedal* is stuck (oncogenes) we just lose control, and we have the cancer cell loose of any control. In the case of tumor suppressors, cancer cells have to **knock down both copies** of the gene to transform. Just as two brakes, if two are there, should be broken to be unable to prevent the accident.

J. Michael Bishop and Harold E. Varmus have won the Noble prize in proving that **viruses,** specifically retroviruses, can cause cancer. A previous female scientist has suggested this relationship between some viruses and cancer, and that these viruses have genes that are similar to some of our genes, and that some of them can cause cancers in chicken so some other varieties can cause cancers in humans. However, no one took her seriously at that time, until the two scientists in the picture proved it practically.



J. Michael Bishop (1936 -)
Harold E. Varmus (1939 -)

The End