Genetics

Subject : Gene therapy

Lec no : 32 (the last lecture)

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وَقُل رَّبِّ زِدْنِي عِلْمًا

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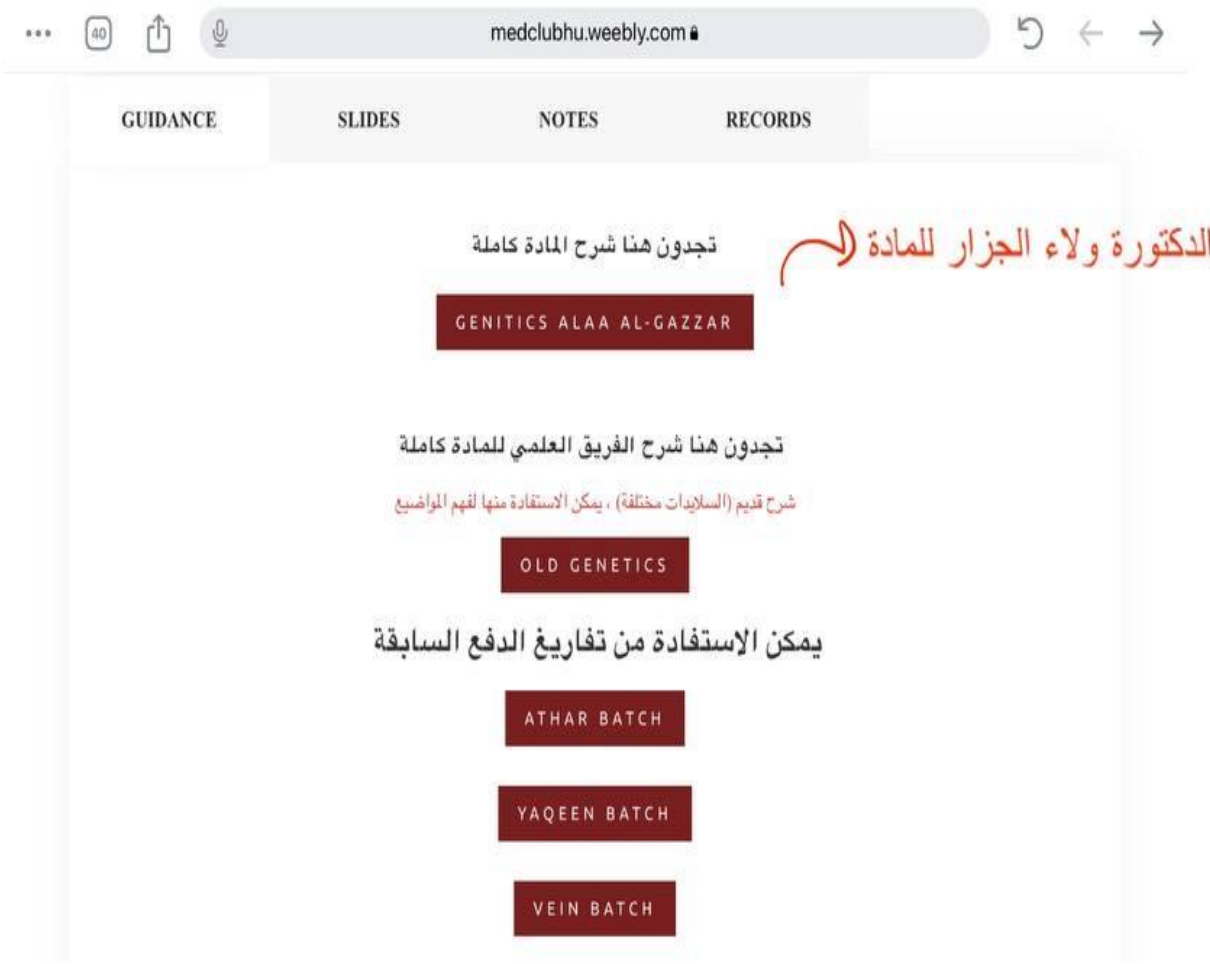

شرح الدكتورة ولاء الجزار للمادة

كل اعمال الفريق العلمي تنشر على قناة التيليغرام

GENE THERAPY

By
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* بس احنا رح نحكي عن ال diseases الي بتنتج من single gene defects / اما مثلاً ال diabetes الي هو من ضمن ال (multigenic diseases) هاد صعب نعالجه بهاي الطريقة

* فكرة ال gene therapy انه مثلاً في disease معين نتيجة defect في جين معين ، لو صلحنا هاد الجين و استبدلناه بجين طبيعي هيك بنكون قضينا على المشكلة من الأساس

- Gene therapy was once considered a fantasy. However, thousands of individuals have already undergone human clinical trials.
- A great leap in medical science has taken place on the 14th September 1990, when a girl suffering from Adenosine deaminase deficiency (severe Immunodeficiency) was treated by transferring the normal gene for adenosine deaminase.

نجحو في علاج بنت كان عندها sever immunodeficiency بسبب نقص إنزيم اسمه Adenosine deaminase و هاد الإنزيم تقريباً موجود بكل الخلايا تبعتنا بس mainly الي بتأثر هو ال lymphocytes و بالتالي بتخليها غير قادرة على أنها تهاجم ال infections المختلفة الي بتدخل على جسمنا

1. What is Gene Therapy?

- It is intracellular delivery of genes to generate a therapeutic effect by correcting an existing abnormality. * كل التجارب تجرى فقط على ال somatic cells لأنه هيك مش رح تنتقل للأجيال الاخرى
- Only *somatic gene therapy*, by inserting the new gene into somatic cell of the patient is under trial (This type of gene therapy cannot be passed to a person's children.) Germ cell gene therapy is considered as unethical (it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known.)

* التجارب على ال Germ cells تعتبر unethical و غير مسموحة

2. Summary of the Procedure

- **1.** Isolate the healthy gene **along with the sequence controlling its expression.**

نستخرج ال healthy gene مع ال sequence ، حكينا زمان انه الجين بيجي قبله
promoters هو الي بتحكم بال expression تاع هاد الجين

- **2.** Incorporate this gene into a carrier or vector as ***an expression cassette***.

بنجيب الجين و بنعمله incorporation يعني بنحطه على ال vector أو ال carrier

- **3.** Finally deliver the vector to the target cells.

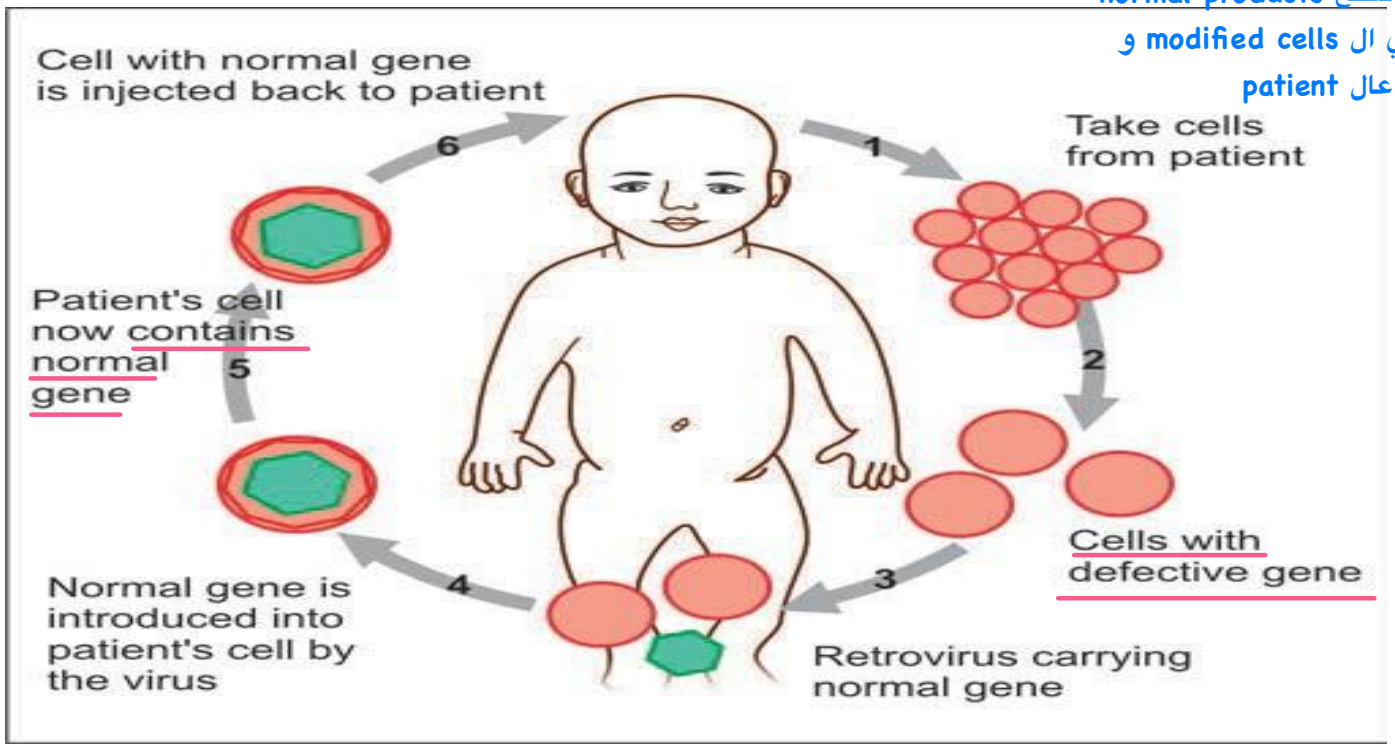
بعدها بنسلم هاد ال vector الي حامل ال normal gene لل target cell الي فيها مشكلة و هناك بصيرله expression
و بطلع normal products بعوض عن ال abnormal products الي كان بطلعه ال defective gene

3. How the Genes are Introduced?

برا جسم الانسان

- ***Ex vivo* strategy** where the patients' cells are cultured in the laboratory, the new genes are infused into the cells; and modified cells are administered back to the patient.

هون بناخد ال patient cells و بنزرعها باللاب و بندخللها ال new genes الي رح تعمل expression و تطلع normal products و بعدها بناخد هاي ال modified cells و بنرجعها عال patient



- ***In vivo* strategy**, where the vector is administered directly to the cell, e.g. CF (cystic fibrosis) gene to the respiratory tract cells.

اما بهاي الطريقة ما بناخد ال cells برا الجسم ، إنما بناخد ال vector مباشرة على ال cells زي في حالة ال cystic fibrosis بأثر على ال respiratory tract cells فبنروح بندخل لل cystic fibrosis gene عن طريق ال Respiratory tract directly و بتدخّل هاد ال vector لل cells inhalation or other specific methods

4. The Vectors

في أنواع كتير من ال vectors عشان تعمل gene delivery ، منها ال viruses و ال non viruse systems

- Different vector (carrier) systems used for gene delivery are: Retroviruses, adenoviruses. Non-virus systems include liposomes, plasmids and physical methods.
- The viruses are modified so they can't cause disease when used in people. هاي ال viruses الي رح نستخدمها بشتغلو عليها بحيث انه ما تقدر تعمل diseases جوا الناس
- Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell. بنحط ال normal gene على ال genetic material لل retrovirus و بندخل ال virus جوا ال target cell
- Other viruses, such as adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene.

* ال retrovirus يعتبر RNA virus و ال RNA viruses كان بصيرلها replication جوا ال cells عن طريق تحولها إلى DNA و ال DNA بعمل integration لل material تاعته جوا ال genome في ال human cell

* ال adenoviruses ما بتعمل integration لل nucleic acids material جوا ال genome ، انما ال DNA بكون free في ال nucleus تبعت ال host cell و ال instructions الجديدة (new genes) الموجودة في ال DNA material تبعت هاد ال virus بصيرلها transcription زيها زي ال normal genes

- **One of the problems of gene therapy using retroviruses** is insertional mutagenesis; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur.

* ال insertional mutagenesis المشكلة فيها انه ال retrovirus ممكن يعمل insertion بطريقة عشوائية فممكن يعمل insertion في نص جين معين يعني بدل ما يصلح جين راح خرب جين تاني

* ممكن يصير إشي اسوأ انه هاد ال insertion أو ال integration تاع ال viral material يخرب جين اله علاقة بال regulation of normal cell division فبصير uncontrolled cell division يعني بدل ما يعمل gene therapy راح عمل cancer

- **Disadvantages of adenovirus:** is that the expression is usually transient, the useful effect varying from a few weeks to months only, also they have the ability to trigger the immune system on subsequent exposure.

ال Adenovirus مشكلته انه ال expression تبعه بكون transient يعني لفترة قصيرة عشان هيك بنعطي هدول الناس dose تانية بس المشكلة انه بعض الناس لما نعطيهم جرعة تانية بصيرلهم immune reaction و ممكن يوصل لل death

- **Plasmid Liposome Complex:**
- It is a non-viral vector system.
- **Liposomes** are artificial lipid bilayers, which could be incorporated with plasmids carrying the normal human DNA. The complexes can enter into the target cells by fusing with the plasma membrane. Cationic liposomes (positively charged) can form complexes spontaneously with DNA (negatively charged).

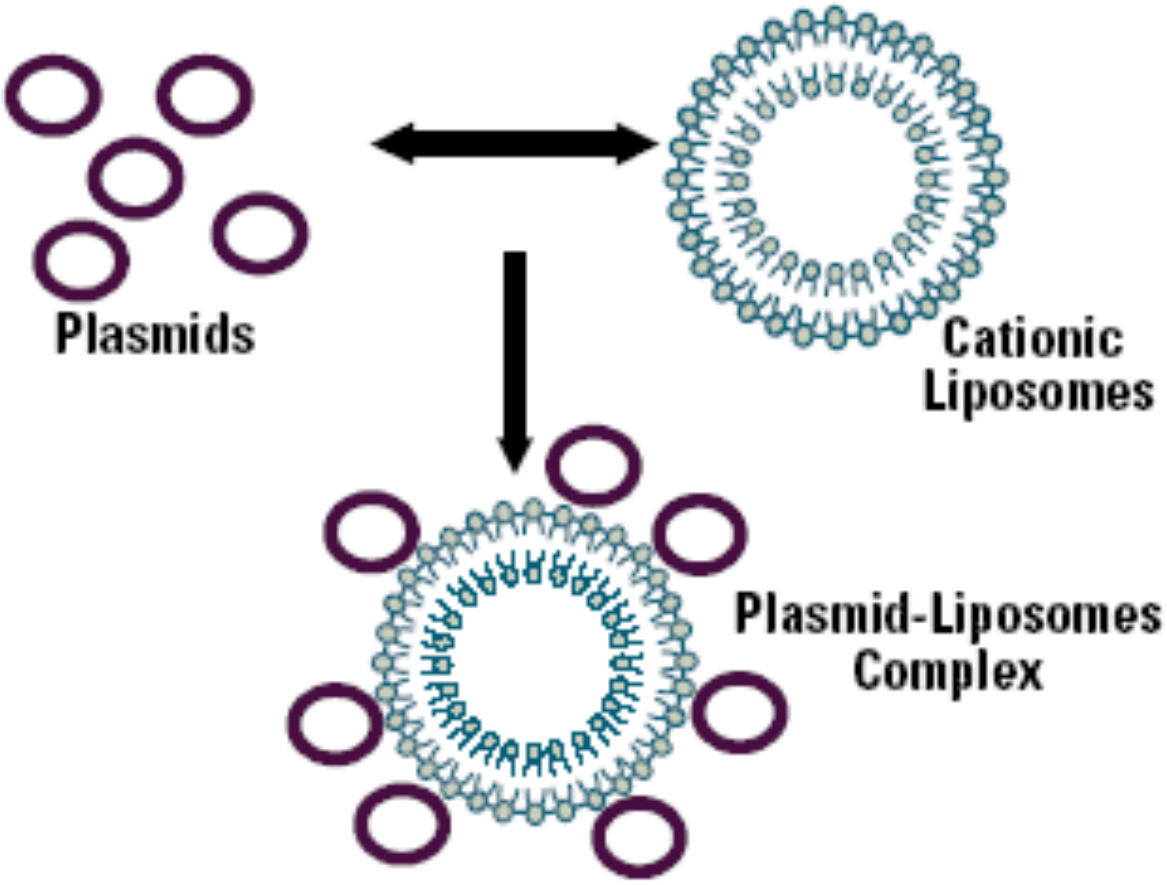
بكون جواته ال plasmid الي شايل ال normal gene

- **The advantages** with this strategy are that the vector can carry human gene of big size, do not replicate and evoke only very weak immune responses.

بقدر يدخل gene كبير / و بعمل weak immune response
عكس ال adenovirus الي كان بعمل sever immune response

- **The disadvantage** is that most of the complexes are destroyed inside the host cell, and so the efficiency of gene transfer is less.

غالباً بتكسر جوا ال host cell و بالتالي ال efficiency تاعته قليلة


Plasmids
Cationic
Liposomes
Plasmid-Liposomes
Complex

* بستخدمو آلة بحيث انه يخلو particles معينة تخترق ال target tissues الي بدنا ندخللها ال normal genetic material

- **Gene Gun Method:**

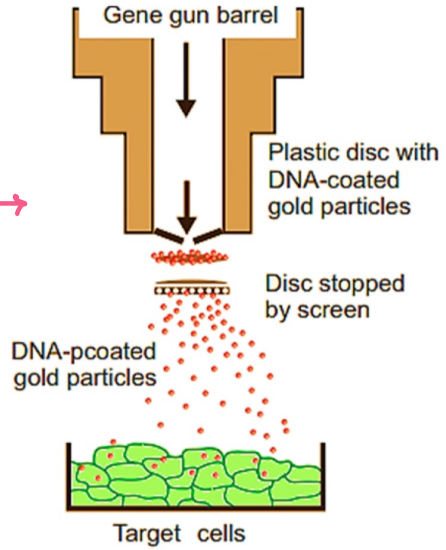
Gold particles

- Tungsten particles are coated with plasmid DNA, and accelerated by helium pressure discharge. This enables particles to penetrate the target tissues. It is quick, and could be used in almost all tissues.

بسبب ال pressure

- Cellular **damage** and **transient gene expression** are the draw backs.

هيك شكل الآلة ←



5. Accomplishments

- Gene therapy is effective in inherited disorders caused by **single genes**. Several clinical trials have been conducted. Success stories are few. The most dazzling ones are shown in the followingTable:

* الدكتورة حكت ما نحفظ اشي بس نعرف اسمائهم و اول واحد مهم لأنه حكينا عنه بالمحاضرة

Table 43.2. Success stories of gene therapy

Disease	Gene transferred by
1. Severe combined immunodeficiency (SCID)	Adenosine deaminase enzyme in chromosome 13 and 20 into lymphocytes; by retrovirus
2. Duchenne muscular dystrophy (DMD)	Dystrophin gene on short arm of X chromosome; by retrovirus
3. Cystic fibrosis (CF)	CFTR gene on chromosome 7 to bronchial epithelium;adenovirus
4. Familial hyper-cholesterolemia	LDL receptor gene on chrom 19 to hepatocytes; retrovirus
5. Hemophilia	A and B genes for factor VIII and IX into fibroblasts; retrovirus
6. Cancer	Activation of p53 (tumor suppressor gene) by liposome
7. Leber's Hereditary Optic Neuropathy	Introducing the gene for the enzyme (isomero hydrolase) using an adeno viral vector directly to the retina

* كلها diseases نتيجة defect ب single gene