evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **Apoferritin and liposome: structure and possible use in gene therapy**

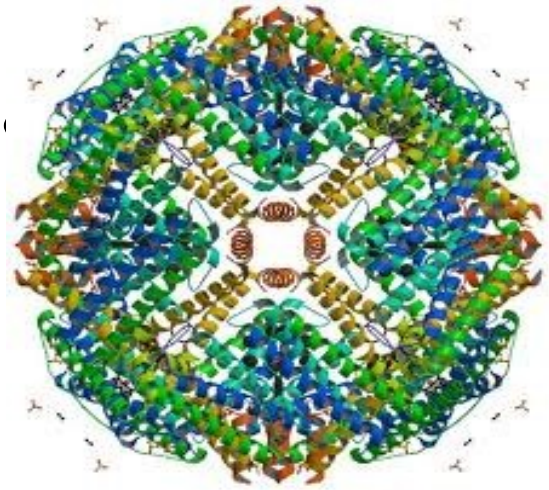
Školitel: **MVDr. Ludmila Krejčová**

Datum: 14.6.2013

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

Apoferritin



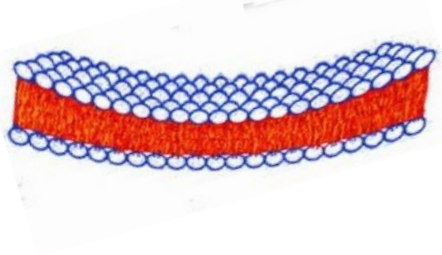
- ferritins are composed of 24 subunits
- sub-units of two types the heavy chain (H-ferritin) and light chain (L-ferritin)
- iron storage and detoxification proteins (4500 iron ions)
- water-soluble spherical macromolecule

- hollow nanocage 12 nm in diameter (8 nm cavity)
- within the ferritin nanocage, ferrous iron ion is oxidized by the ferroxidase center of H-ferritin

- ferritin consists of an outer protein shell surrounding the core, consisting of Fe^{3+} and phosphates

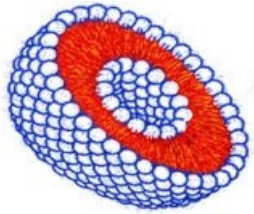
- apoferritin is obtained by removing atoms of iron from ferritin

Liposome

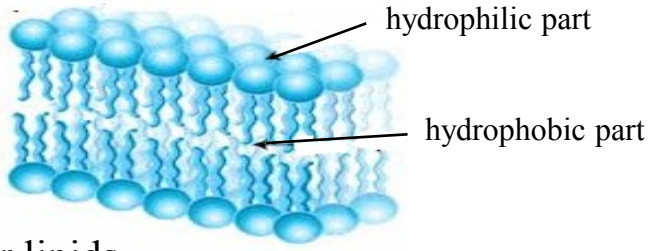


lipid bilayer

micelle



liposome



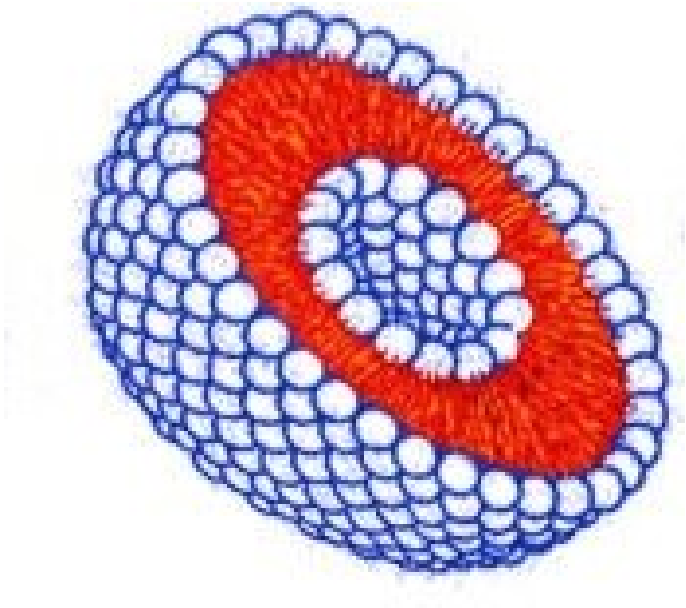
- artificially prepared closed vesicles
- composed of a lipid bilayer
- formed by sonication an aqueous suspension of suitable polar lipids
- most used: lecithin (egg yolk)
- liposomes diametr are usually between 1-2 μm

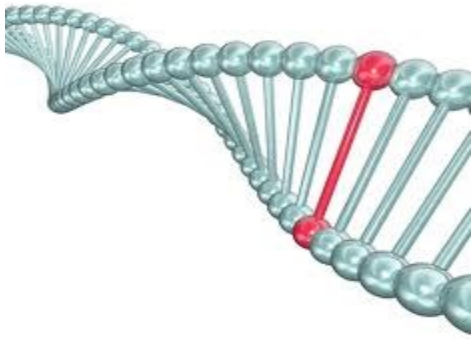
- if the liposome is formed in medium, containing soluble components (salts, proteins… drugs), these ingredients are enclosed in the internal vesicle

- penetrate into the cell via endocytosis

- can serve as a transport medium (vector) for a number of drugs (doxorubicine)

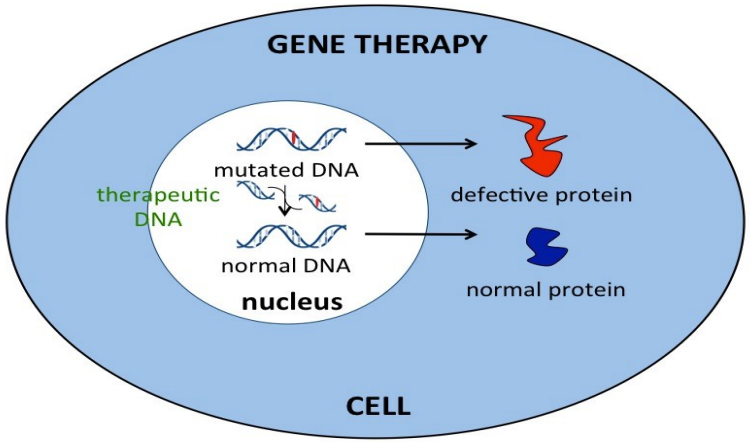
Apoferritin **x** **Liposome**





Gene theraphy – introduction

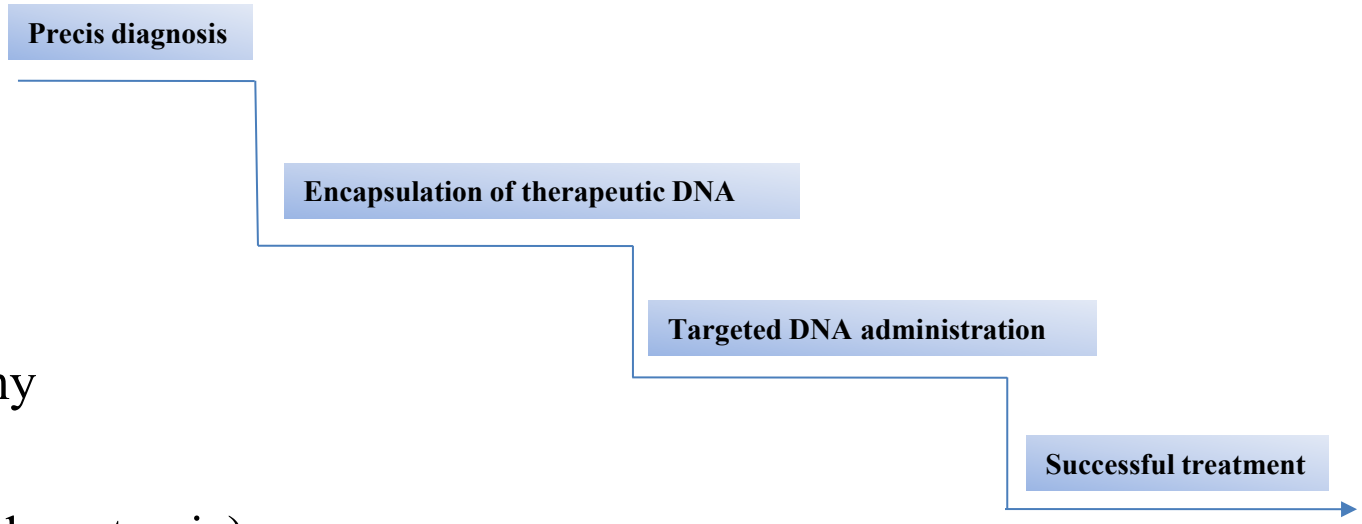
- therapeutic DNA sequence is inserted into the patient genome, wherein the target sequence encodes a missing or malfunctioning protein
- can be a candidate to cure diseases (inherited disorders, some types of cancer, certain viral infections)
- has not yet been introduced into medical practice (only experimental medicine)



<u>Essential issue to bring this technology into practice</u>

- knowledge of pathological process of the disease (low quantity of gene, mutated gene product , etc.)
- knowledge of the sequence of the examined gene
- cell-specific targeting and selection of the appropriate vector
- the patient's consent

Main objectives of treatment

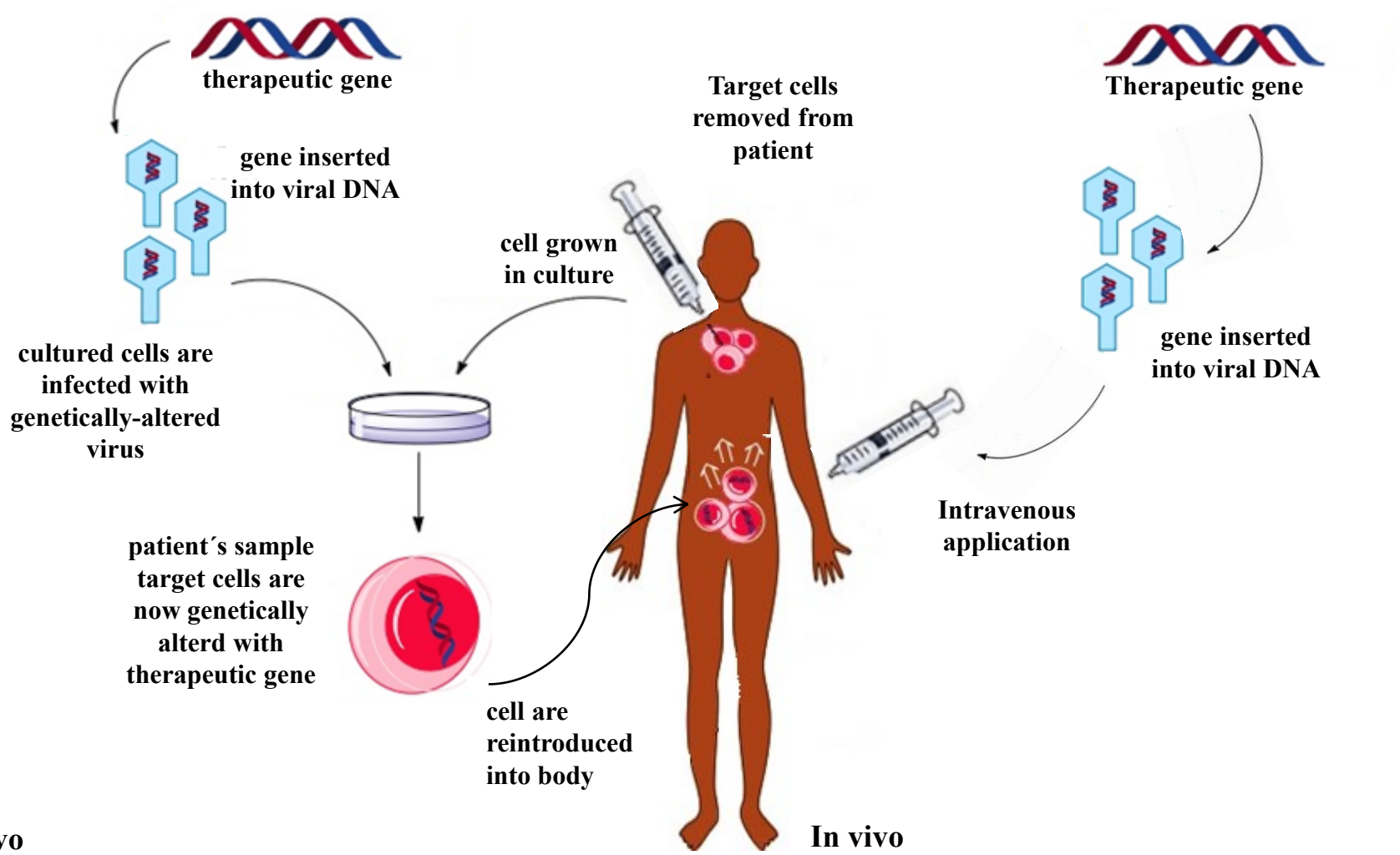


Cystic fibrosis
Muscular dystrophy
Haemophilia
Thalassemia (trombocytemia)
Sickle cell anemia
Retinal disease
X-linked SCID, ADA-SCID
Chronic lymphocytic leukemia, acute lymphocytic leukemia,
Multiple myeloma
Parkinson's disease

Strategy of gene theraphy

EX VIVO TECHNIQUE

IN VIVO TECHNIQUE



Ex vivo

- „sick“ cells are surgically removed
- application of the healthy gene to the „sick“ cells
- cultivation (requires many hours)
- cultivated cell will reinjected back into the tissue

In vivo

- does not require anesthesia or surgical treatment
- application of modified gene into the body cells
- use of carriers (vector) of modified gene (viral or nonviral)

Types of gene therapy

Somatic gene therapy
the therapeutic genes are transferred into the somatic cells
not inherited by the patient's offspring or later generations
therapeutic DNA transgene is used to treat a disease in an individual

Germ line gene therapy
sperm or eggs modified by the introduction of functional genes
be heritable and passed on to later generations
jurisdictions prohibit this for application in human beings

Vectors in gene therapy
delivery of DNA into cells,
by recombinant viruses (viral vectors)
naked DNA or DNA complexes (non-viral methods)

Viral vector: using viruses capable of transferring its DNA into the genome of host cell
strategy for gene therapy: removing the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA.
(retrovirus, adenovirus, lentivirus, herpes simplex virus, vaccinia, pox virus, and adeno-associated virus)

Non-viral methods: large scale production and low host immunogenicity
Previously, low levels of transfection and expression, recent advances upgrade efficiencies
including the injection of naked DNA, electroporation, the gene gun, sonoporation, magnetofection, and the use of oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles.

Antisense therapy
not strictly a form of gene therapy, is a related, genetically mediated therapy

Advantages and disadvantage of gene theraphy
(and target delivery by liposomes or apoferritins)

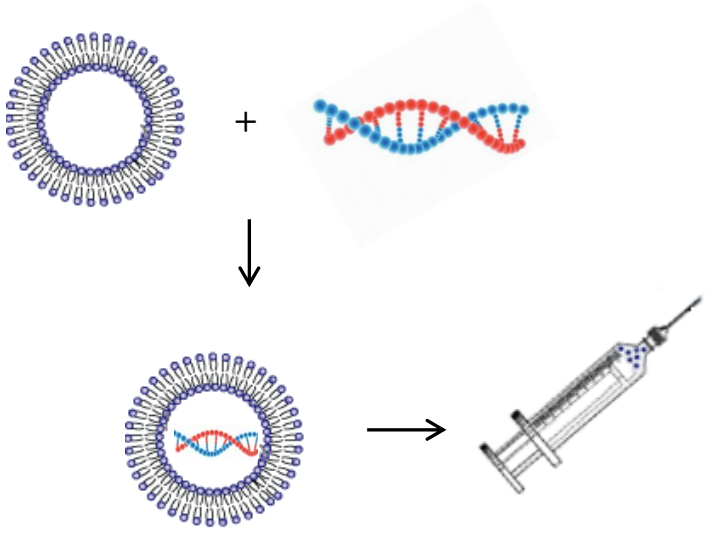
Effectiveness of treatment

Drug quantity
Side effects
Treatment period
Recovery period

Although it was successfully treated several patients by gene theraphy, some of whom died. Therapeutic gene got into another place and caused leukemia.

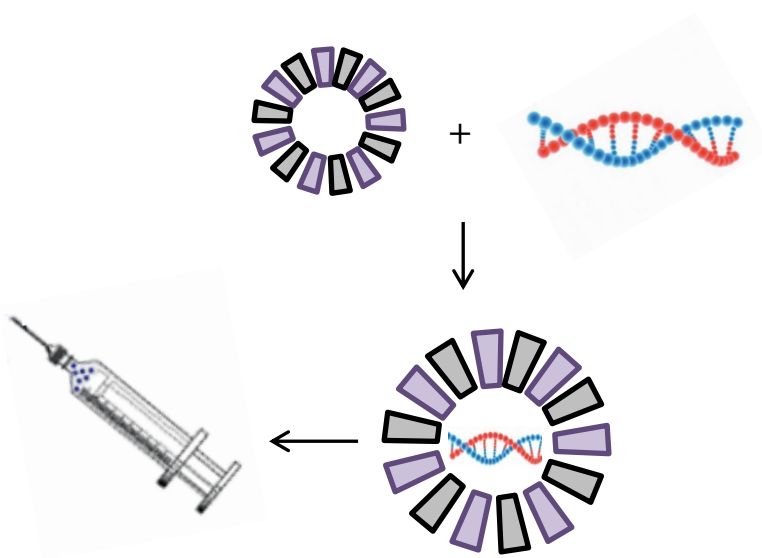
Liposome and Apoferritin -potential use in gene therapy

Liposome



- application without immune response
- already used (encapsulated form doxo)
- target transporting apoferritin would be feasible due to surface modification of apoferritin
- drug delivery by fusion of membranes
- target transporting due to surface modification
- larger cage (liposome size is 200-700 µm)

Apoferritin



- application without immune response
- handling of apoferritin is dependent on the pH of the solution (pH 3 = open, pH = 7closed)
- DNA amount enclosed into apoferritin is dependent on the concentration of DNA in solution
- target transporting apoferritin would be feasible due to surface modification of apoferritin
- limited by large of apoferritin nanocage (8 nm)

Conclusion

Gene therapy:

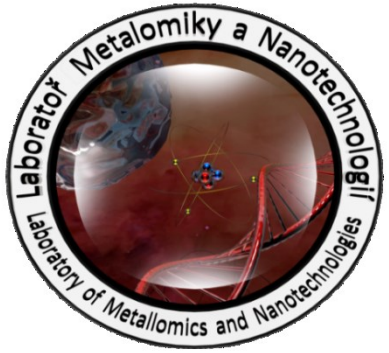
- therapy: inherited, cancer, viral diseases
- target (sick) DNA sequence is replaced by therapeutic sequence
- missing, low quantity or malfunctioning protein is replaced by proper protein

Using of liposomes or apoferritins command a good view of therapeutic sequences transportation.

Due to:

- structure (cavity: apoferritin 8 nm x liposome 200-700nm)
- without immune response (body´s own)
- target delivery by membrane fusion or surface modification
- reduction of amount of drug, side effects and convalescence period

Thank you for your attention!



esf evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ
Mendel University in Brno