NANOBIOTECHNOLOGY AND HUMAN THERAPY: PRESENT AND PERSPECTIVES. NANODEVICES – A NEW TECHNOLOGY IN HUMAN THERAPY

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Abstract. *Human therapy benefit by biological sciences progress in generally and by genetics progress in particularly. Spring up at the second half of the XX century and quoted as a certain permission, the recombinant DNA technology recorded notable successful in the artificial synthesis of numerous bioactive substances, as well as removing of the affected or mutant genes with normal genes. Together with the deciphering of the human genome (at the limit between II and III M millenniums), the use of the stem cell conduct to obtaining of some remarkable successes in the human therapy and medicine field. The development of technology, conduct to spring of the nanotechnologies, with applications in different sciences domains, inclusive in medicine and human therapy. Numerous investigations performed in the last two decades, underlined the importance of the titanium dioxide used, initially single, and then complexes with other elements, and in the last time under nanodevices compounds, a molecular hybrid between titanium dioxide (a metal) and a bioactive substance (inclusive DNA or RNA oligonucleotides). Researches performed in different scientific centers from the all world, inclusive in Romania, reveal the importance of these new investigations and treatment methods in the human therapy.*

Keywords: *nanobiotechnology, nanodevice, human therapy, titanium dioxide, cyclodextrins.*

Rezumat. Nanotehnologiile şi terapia umană: prezent şi perspective. Nananodevice – o nouă tehnologie în terapia umană. *Terapia umana beneficiază de progresele din ştiinţele biologice în general şi genetică în special. Aparută în cea de a doua parte a secolului XX şi cotată ca o certă promisiune, tehnologia DNA recombinant, a repurtat progrese notabile în sinteza artificială a numeroase substanţe bioactive, precum şi în înlocuirea genelor afectate sau mutante cu gene normală. Odată cu descifrarea genomului uman (la trecerea dintre mileniile II si III), a fost rândul celulelor stem să conducă la progrese remarcabile în domeniul terapiei umane şi al medicinii. Dezvoltarea tehnologiei a condus la apariţia nanotehnologiilor, care au aplicare în diferite domenii ale stiinţei, inclusiv în medicina şi terapia umană. Numeroase investigaţii efectuate în ultimele două decenii au subliniat importanţa utilizării dioxidului de titan, întâi singur, apoi complexat cu alte metale, iar în ultimul timp, sub formă de nanoidevice, un hibrid molecular între un metal şi o substanţă biologic activă (inclusiv oligonucleotide RNA, sau DNA). Cercetarile efectuate în diferite centre stiinţifice ale lumii, inclusiv în România au relevat importanţa acestei noi metode de investigaţie şi tratament în terapuia umană.*

Cuvinte cheie: *nanobiotehnologie, nanodevice, terapia umană, dioxid de titan, ciclodextrine.*

GENETIC ENGINEERING AND GENE THERAPY

Developed in the second half of the XX century, the **recombinant DNA technology (genetic engineering)**, constitute o great promise in the human therapy. This technology consists in jointing of DNA molecules *in vitro* (originating from different genotypes) and introducing them into living cells where they replicate (KING & STANSFIELD, 2002). Summary, this technique consists into the inclusion of an interest gene (a DNA-insert molecule) in a cloning vector (preferably in a shuttle vector) and obtaining of a DNA-recombinant molecule (Fig. 1). After his cloning, his is introduced in a host cell who replaces the mutant or defect gene. With help of this technique was obtaining some prestigious success in the synthesis of bioactive substances (JACKSON et al., 1972, SMITHIES et al., 1985, MITREA VASILESCU et al., 2001; CORNEANU & CORNEANU, 2007, a/o), as well as in gene therapy. This technology is laborious, heavy and expensive.

Gene therapy, consist in alter or replace defective genes. A defective gene, present an adulterated sequence of nucleotides, as result of a mutation at the DNA level. As a consequence, and the sequence of amino acids from protein (enzyme) is adulterated, as well as his properties. Technique currently consist in transfer of a normal genes into the genetic material of the cell to replace the defective gene and the use of microRNA's to "knock out" defective genes in certain tissues. For this purpose are use retroviruses as vectors for transferring genes into cells, liposomes, a/o (Oxford Dictionary of Biology, 2008).

Researches performed by SHARP (2008), represent a new promising in the control of the gene activity through the help of the small RNAs. These small RNAs are components in networks of regulatory factors where their synthesis and activities are controlled by proteins that in turn can be regulated either directly or indirectly by small RNAs. Thus, evidence suggest that over two thirds of all genes in vertebrates are regulated by a class of small RNAs, microRNAs, that are encoded as hairpin-type sequences in their genome (SHARP, 2008). Each of these microRNAs is thought to regulate about 1-200 genes by recognition of target sequences in their 3' UTRs. These microRNAs suppress the stability and/or translation of these target mRNAs. "The degree of regulation by microRNAs depends upon the proliferative state of cells since was found that about half of all mammalian genes are expressed with short 3' UTRs in dividing cells as compared to quiescent cells. Many different types of small RNAs have been characterized in various organisms. Other than microRNAs, the only other class of small RNAs whose synthesis is clear documented in mammalian systems is the germline specific piRNAs" (SHARP, 2008).

STEM CELLS

After deciphering of the human genome code, a major attention was accorded at the use of the **stem cells** for the therapeutic purposes. The **stem cells** are undifferentiated cells, apt for quick multiplication. They can undergo unlimited divisions to form other cells, which can remain as stem cells, or can differentiate to form specialized cells. After their origin, they are of two types: embryonic and adult.

Embryonic stem cells are pluripotent or totipotent, being derivate from early developmental stages of metazoan organisms. Theoretical they are able to differentiate into any type of somatic or germ cell. In vitro, embryonic stem cells differentiate into all kids of tissues, and when injected under the skin of immunological mice, they grow into teratomas (totipotent valence).

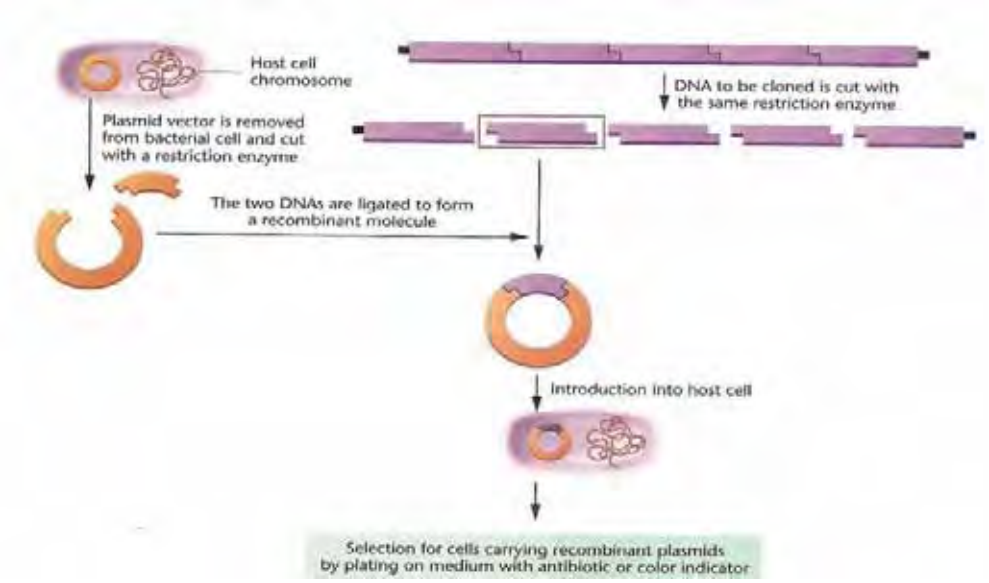

Fig. 1. Recombinant DNA technology (KLUG et al., 2006).
Fig. 1. Tehnologia ADN-recombinant (KLUG et al., 2006).

Adult stem cells, serve to replenish certain somatic cells that die during the life of the metazoan organism. An example of somatic stem cells is the bone marrow cells of mammalian that divide to produce a continuing supply of blood cells (Fig. 2). Also, in the gonads, germinal stem cells generate eggs or sperm (KING & STANSFIELD, 2002).

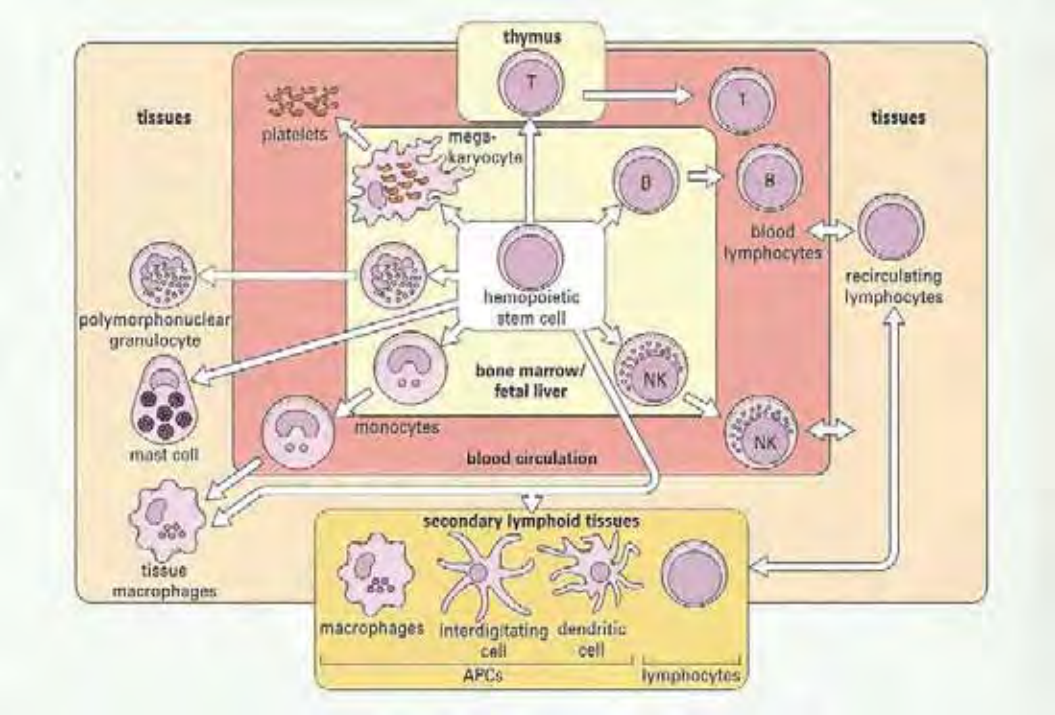

Fig. 2. Origin of cells of the immune system (MALE et al., 2006).
Fig. 2. Originea celulelor sistemului imun (MALE et al., 2006).

In therapeutic purpose, are used the stem cells, originated from a normal organism, with present normal genes. They are introduced in the affected organ and after quick multiplication they will replaced the affected or mutant cells. The use of the stem cells is a great promise in human therapy.

The use of the therapy with stem cells, permit the gene expression in specific cells (erythrocytes, neurons, a/o), and regulation of the gene expression at physiological signals. They are applied in hematological affections (leukemia, multiple melanomas, anemia with sticky cells), autoimmune affections (systemic lupus erythromatous, Crohn disease), affections with immune deficiency (Wiscott-Aldrich disease), cardiovascular affections (myocardic ischemia), metabolic affections (diabetic, osteoporosis, Gaucher disease), osseous-muscular affections (Duchene muscular dystrophy), neurologic affections (Parkinson disease), a/o.

NANOBIOTECHNOLOGY

Another promising method in human therapy is from the nanobiotechnology domain.

Nanobiotechnology is a new research domain, resulted from the joint of life science with nanotechnology. Nanotechnology is the science branch which researches the nanoparticles with dimensions between 1 nm – 100 nm. The nanobiotechnology purpose is the use of the biomolecules and the processes supported by these, for the development of a new functional materials and devices, and at end, of nanomachines and nanorobotics.

One of promising and perspective nanoparticles is represented by titanium and his compounds.

Titanium is a white-gray metal, a common element on the Earth planet, very uncreative, having five isotopes (Ti-46 – Ti-50). Titanium dioxide is a white powder, used in pharmaceutical industry, in medicine, industry and in nanobiotechnology. Introduced in the human body, titanium dioxide can traverse three possibilities: (a) is deposited in organs with a macrophage activity; (b) is transported by blood from the mononuclear cells; (c) induce the forming of the species of oxygen reactive (SOR) in alveolar macrophages.

Numerous researchers were analyzed the TiO_2 nanoparticles effect on the carcinogen cells. SHENG HU et al. (2005) studied the TiO_2 nanoparticles effect on the hepatom cells, through their incubation time of one or eight hours. The ultrastructural features analysis, performed that these nanoparticles can present a lethal effect on the carcinogen cells level. They reported the presence of adulteration of mitochondria with disorganized cristae.

The nanotechnology integration with biology and medicine was limited until the conjugated nanoparticles were used. Conjugated with other metals (gold, silver, platinum iron, a/o), titanium dioxide nanoparticles manifest an enhanced of their photo catalytic activity. Nanoparticles conjugated with gold, present large applications in the nanomaterials and nanotechnology field. The size and chemical properties of the gold nanoparticles surface (G-NP) can be controlled with the help of some thyol groups (KISAILUS et al., 2005). CSÁKI et al. (2002) considered that the nanoparticles doped or not with gold, can be used as DNA diagnostic method, because of their very small size and a radionuclide potential. Were reported numerous researches which confirmed the obtaining different bimetallic particles, with gold and other metal: Ag, Pd, Pt, TiO_2, Fe, Zn, Cu, ZrO_2, CdS, Fe_2O_3 or Eu (DANIEL & ASTRUC, 2004). The characterization of the bimetallic nanoparticles was performed for the first by SCHMID et al. (1991). The gold nanoparticles (AuNP) conjugation with oligonucleotides, permit their use in experiments of molecular biology, and nanobiotechnology. Also, were elaborated techniques for their use for diagnostic of some diseases and for establishes the genes expression. (MIRKIN & LETSINGER, Northwestern University, Chicago and ALIVISATOS & SCHULTZ, Berkeley University; ALIVISATOS et al., 1996).

Similar experiments were performed with **magnetic fluids**. They are constituted by magnetite (Fe_3O_4), dispersed in a carrier liquid (water, petroleum, transformer oil, a/o). Magnetite is a mixed compound, constituted by FeO and Fe_2O_3, in different percentage. Experiments performed by different authors, point out the use in biology and in biotechnology. His effect is depending on the species, ratio between bivalent and trivalent iron, carrier liquid, the nanoparticles size and their amount in the cell, biological considered process, a/o. In experiments performed at plants, at *in vitro* culture, CORNEANU et al. (1995, 1998, 2000) underlined the magnetic fluids effect on different developmental, biochemical and genetics processes at a many species of plant (*Cactaceae*, *Chrysanthemum* sp., *Aloe* sp., *Prunus* sp., *Dendrobium* sp., a/o), or at Cyanobacteria (*Spirulina platensis*).

NANODEVICES AND GENE THERAPY

Nanodevice, is a finely entity, capable to accomplish or to determine an activity (a small circulated particle in blood, capable to carry a drug or a bioactive substance).

In the experimental biology are used nanodevices performed from a carried substance (titanium dioxide, magnetite, a/o), and an active biological substance, represented through proteins, oligonucleotides (DNA or RNA), sugar, or other different bioactive substances.

Thus, in nanobiotechnology, ***nanodevice***, is a chemical-biological hybrid, constituted from an organic molecule (a bioactive substance) covalent attached at a nanoparticle (titanium dioxide) with size until 10 nm. PAUNESCU And WOLOSCHAK (2004) obtained ultrastructures of nanodevices type, capable to use a DNA target (WOLOSCHAK et al., 2006). Through this genetic manipulation, is possible the nanodevices use in gene therapy. Similar researches were reported by RAJIH et al. (2006). They obtaining titanium dioxide hybrid nanoparticles, linkage with PNA

oligonucleotids which binding at a bicentenary DNA target (Fig. 3). The authors underlined that through technique is possible obtaining of restrictive enzyme very efficient, controlling through the light use. The titanium dioxide nanoparticles linkage at DNA or PNA, present a specific locus of attaching situated on the bicentenary DNA vector.

Also, were obtained other types of AuNP biconjugate with peptides, lipids, enzymes, drugs or viruses, with application in nanobiotechnology, nanomedicine and/or in gene therapy. In the present study were used bimetallic particles of AuNP and FeNP types, conjugate with α- or β-cyclodextrin, the ratio between TiO_2 and cyclodextrine being 1:1 or 1:2.

Because some biomolecules (oligonucleotides, peptides, saccharoses, a/o) can be attached at titanium dioxide nanoparticles, is possible their use in therapy. WOLOSCHAK (2006) affirm" if a DNA molecule or a peptide molecule can be attached and transferred in a particular locus in cell, result that other peptide can be transferred by this method".

Nanodevices can be used as a simple alternative for the delivered of the drugs in organism or in cell. Also, the antisense gene attached by titanium dioxide nanoparticles can be delivered in a particular locus in cell, when this is lighting. Because the ionizing radiation can also induced the nanodevices photo reactivation, the delivered of the therapeutic genes or of the drugs, can be applied in combination with radiotherapy, at the patients with cancerous tumor.

The study performed *in vitro*, pointed out that the TiO_2 - DNA nanodevices, can induced scissions in the DNA molecule, in a specific way. Because the TiO_2 – DNA (nuclear or mitochondrial) is attached at a specific regions, subsequent take place gradual removing from the all compartment cell (WOLLOSCHAK et al., 2006).

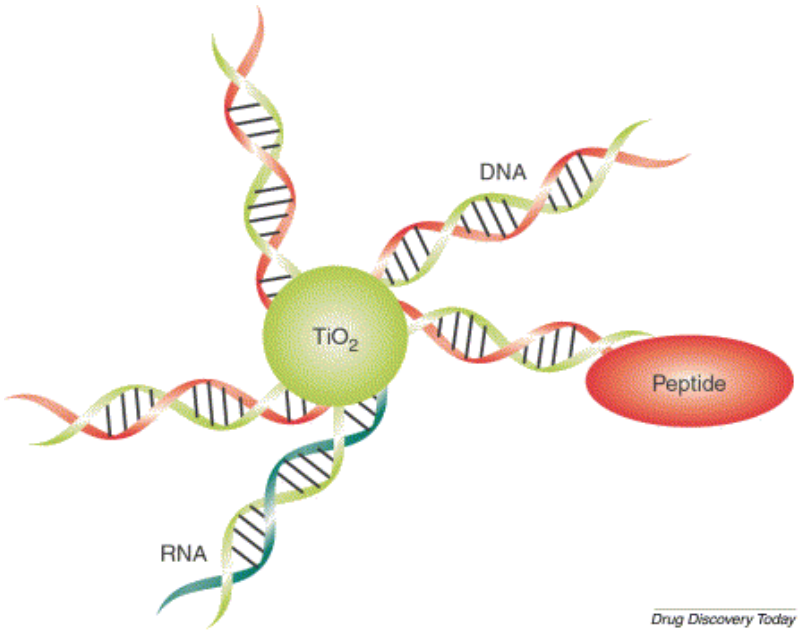

Fig. 3. A nanodevice, constitute from TiO_2, RNA and DNA oligonucleotide, and a peptide molecule (WOLOSCHAK, 2006).
Fig. 3. Un nanodevice de tipul TiO_2-oligonucleotide RNA şi DNA şi o moleculă de peptid (WOLOSCHAK, 2006).

The use of nanodevices of TiO_2-oligonucleotids type in the gene therapy constitutes a concept which can be applied in practice. In present is difficult to establish a particular cell target, as well as the subcellular location. But the nanodevices of TiO_2-DNA type can be transfectate in the cell nucleus. Also, can be attached DNA oligonucleotides with a sequence of 50-60 p.b. Can be establishing the cellular location. In vitro, nanodevices are excited by exposure at white light or ionizing radiations, which determine the DNA scission. The cleavage reaction of the DNA is influenced by: the time of illumination incubation temperature post-illumination, the presence of the radionuclide in oligonucleotids, the nanocomposite concentration, presence or not of a lesion/deletion in the DNA molecule attached at TiO_2 molecule, a/o.

In an experiment performed with nanodevice (similar ***nanocomposites***) constitute from an organic substance (α- or β-cyclodextrin) and titanium dioxide nanoparticles, was analyzed the radioprotective effect against the X-rays, at a tester genetic specie (*Nigella damascena*, Fam. *Ranunculaceae*). Analysis the percentage of the normal phase's mitotic cycle, as well as the effect at the chromosome level, point out a different effect, depending on the cyclodextrin type, the ratio between titanium dioxide nanoparticles and cyclodextrin, the radiobiological point analyzed (CORNEANU et al., 2008). The α-cyclodextrin, induced a high acentric fragments percentage in anaphase and telophase, and not facilitates the reunion processes of the chromosomes broken end (the bridges absence from cells). For this reason the variant TiO_2~α-cyclodextrin (ratio 1:2), can be used for the progressive elimination, from organism, of the cells with a high mitotic multiplication rate, respective the carcinogen cells (CORNEANU et al., 2008). The TiO_2 conjugate or not with a metal (gold or iron), complexes or not with a cyclodextrin, induced in a low percentage,

metabolic modification of the chromosomes, being affected their compaction degree in prophase and metaphase (**PCC** and **DCC**), as well as inactivation of the kinetochore or mitotic spindle.

PHOTODYMANIC THERAPY

Photodynamic therapy, imply the destroy of the cancerous cells (XU et al., 2007). TiO_2 nanoparticles constitute an important photocatalist, because posses photo catalytic properties very good (CAI et al., 1991; SAKAI et al., 1995; ZHANG & SUN, 2004, a/o). When the TiO_2 nanoparticles are exposed at UV light with a wavelength under 385 nm, are produced electrons and gap-s. They can react with water and OH· ions, forming strong oxidative radicals OH*, H_2O_2* capable to destroy the compounds of the tumor cells (CAI et al., 1991). Thus, the TiO_2 nanoparticles, manifest a very good antitumorous activity, under UV irradiation. But, the photo generated gap-s can easily react with photo induced electrons, process which reduced the photo catalytic inactivation of TiO_2.

Nanocomposites of the Au-TiO_2 type (doped with gold), were tested for their efficiency at the bacteria destroy and decomposition of the organic compounds (LI et al., 2007; FU et al., 2005). Experimental results performed with the Au/TiO_2 nanocomposites, point out their role in inactivation of the human colon cancer (XU et al., 2007). Thus this method represents a promising fir the cancer therapy in future.

CONCLUSIONS

The developed of the science in generally and of the genetics in special, permit discovery of new investigation method. For the correction and for treatment of different diseases, in addition to recombinant DNA technology, are used with successfully the stem cells and nanodevices, complexes with oligonucleotids or other bioactive substances.

The experimental investigations performed with a nanodevice constitute by titanium dioxide nanoparticles (single or conjugate with gold or iron), encapsulated in α- or β-cyclodextrin, was point out a different effect towards the X-rays action.

The α-cyclodextrin, which induced a high acentric fragments percentage in anaphase and telophase, not facilitate the reunion processes of the chromosomes broken end. For this reason these nanodevices can be used for the progressive elimination, from organism, of the cells with a high mitotic multiplication rate, respective the carcinogen cells.

Experiments performed with magnetic fluids at *in vitro* plant culture, point out their use in the vegetal biotechnology as a substitute of usual phytohormones (auxin and kinetin) from the medium culture composition. Their amount and features are dependent on the vegetal species, the biological processes purposed (rhysogenesis, caulogenesis or calusogenesis), and others.

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