C8. Gene therapy and cellular reprogramming

Lectures

L17.1

How to program myogenic differentiation of stem cells *in vivo* and *in vitro*

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Skeletal muscle regeneration relies on the pool of satellite cells that in adult muscle reside between sarcolemma and basal lamina surrounding muscle fiber. Once skeletal muscle becomes injured these cells are activated, re-enter cell cycle, fuse into multinucleate myotubes, and finally reconstruct the injured tissue. However, some pathological conditions, such as reoccurring skeletal muscle injuries/regeneration accompanying muscular dystrophies, can lead to the irreversible exhaustion of satellite cells and to the failure in muscle repair. Mobilization of endogenous or implantation of exogenous stem cells are considered as the therapeutic approaches aiming at the improvement of regeneration. In order to be used participate in tissue repair exogenous cells need to be able to undergo myogenic differentiation, to form myotubes, and then myofibres. Among the cells extensively tested as a candidates for "replacement" cells are embryonic stem and induced pluripotent stem cells, and also variety of multipotent stem cells isolated from fetal or adult tissues. The simplest and non-offensive, however, the least efficient means of the induction of stem cells differentiation relies on their exposure to the myogenic environment, i.e. *in vitro* co-culture with myoblasts, or transplantation into injured skeletal muscle. Other techniques inducing myogenic differentiation base on the treatment of these cells either with various growth factors, or with reagents impacting at the overall gene expression leading to the induction of myogenic program. The most efficient way to obtain myoblasts from stem cells relies, however, on the overexpression of crucial factors regulating myogenesis, e.g. Pax3, Pax7, MyoD. Regardless of the system used detailed molecular, cellular, and functional analyses are required to prove that the stem cells differentiation was efficient and proper, and thus, that these cells can be possibly considered as suitable for the therapy of injured or diseased muscles.

L17.2

Heme oxygenase-1 inhibits myoblast differentiation by targetting myomirs

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Heme oxygenase-1 (HO-1) is a rate limiting enzyme degrading heme to ferrous iron, carbon monoxide and biliverdin, the latter converted to bilirubin. Apart from the well-recognized anti-inflammatory, anti-oxidant and anti-apoptotic effects, the HO-1 facilitates angiogenesis, playing an important role in cancer development and cell differentiation. Here we investigated the role of HO-1 in myoblast maturation. First, we found that differentiation of satellite cells into myotubes was inhibited in cells isolated from HO-1-deficient mice, as evidenced by cell morphology and reduced expression of myogenin, MyoD, and myomirs. Accordingly, 10-fold upregulation of HO-1 activity in C2C12 myoblast line stably overexpressing HO-1, improved the cell proliferation and survival under oxidative stress, while inhibited the differentiation, as indicated by reduced formation of myotubes, diminished activity of creatine phosphokinase, and decreased expression of MyoD and myogenin. Interestingly, overexpression of HO-1 affected the genes involved in regulation of miRNA processing: among others it reduced the expression of DGCR8, a heme-dependent dimeric protein. Furthermore, total pool of pre-miRNA and miRNA was lower in C2C12-Luc-GFP-HO1 myoblasts, the effect reversed by enforced overexpression of DGCR8. Transcriptome analysis demonstrated that ~18% of miRNA were differentially expressed in C2C12-Luc-GFP-HO-1 cells. Especially, a group of muscle specific myomirs, namely miRNAs 1, 133a, 133b, and 206, was almost completely blocked in cells overexpressing HO-1. On the other hand, miR-146, regarded as an inhibitor of muscle differentiation was strongly increased. Partial reversion of these effects by HO-1 siRNA or SnPP confirmed the specificity of HO-1 action. Also, analysis of myomirs in primary satellite cells demonstrated a higher generation of miR-1, miR-133a, miR-133b and miR-206 in cells isolated from the HO-1 deficient mice than in satellite cells possessing at least one functional HO-1 allele. This observation additionally supports the inhibitory effect of HO-1 on myomir production. Finally, the enforced expression of miR-133b and miR-206 partially restored the generation of myogenin, and myosin, whereas overexpresion of MyoD did not influence significantly the generation of myomirs. In summary, HO-1 inhibits myoblast maturation acting, in part, by inhibition of miR-133b and miR-206 cluster.

L17.3
Towards generation of human iPS cells for clinical applications

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Human induced pluripotent stem cells (iPS) are generated by reprogramming of somatic cells through enforced expression of embryonic transcription factors. However, clinical applications requires that expression of introduced transgenes must be permanently switched off in the iPS cells and obtained differentiated progenies. Here, we took advantage of epigenetic switch that relies on doxycycline(dox)-controllable binding of tTRKRAB transrepressor to tetO element. In the presence of dox tTRKAB is sequestrated from tetO allowing for transgenes expression. In contrary, dox removal allows for tTRKRAB binding to tetO that results in tight transcriptional repression of proximal promoter through heterochromatin formation. In order to apply this system for reprogramming, the tetO element was inserted into pSTEMCCA lentiviral vector carrying OCT4, SOX2, KLF4 and cMYC under control of EF-1alpha promoter. Co-transduction of human skin fibroblasts or human umbilical vein endothelial cells with obtained pSTEMCCA-tet and pLV-HK carrying tTRKRAB cDNA followed by culture in presence of doxycycline allowed for expression of reprogramming factors and thus efficient generation of human iPS clones. Obtained clones were picked and further cultured in the absence of dox. Tight repression of introduced transgenes in all human iPS clones was analysed by RT-qPCR and confirmed full functionality of our system. Pluripotent phenotype of iPS cells was revealed by analysis of endogenous embryonic genes expression using RT-PCR and cell surface protein markers by immunofluorescence. tTRKRAB-mediated epigenetic repression persisted through prolonged culture of in obtained iPS cell lines in feeder-free and xeno-free conditions. Importantly, expression of introduced transgenes remained undetectable after differentiation into embryonic bodies or neuronal progenitors.
Our results confirm that our epigenetic switch effectively prohibits re-expression of embryonic transgenes in human iPS cells and their differentiated progenies paving the way for their applications in various fields of regenerative medicine, disease modelling and drug discovery.

L17.4
Development of therapeutic cancer vaccines

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Active cancer immunotherapy including therapeutic cancer vaccines is recently gathering attention of scientists and pharma sector. Therapeutic cancer vaccines in order to elicit effective long lasting anticancer immune response require appropriate adjuvants. We have developed two designer molecular adjuvants Hyper-IL-6 (H6) and Hyper-IL-11 (H11) for construction of whole tumor cell therapeutic vaccines. Both directly target gp130 on immune cells what leads to breaking tolerance mechanisms, triggering innate immunity, recruitment, activation and maturation of antigen presenting cells, what translates to induction of effective mechanisms eliminating cancer cells.
H6 and H11 are fusion proteins comprising IL-6 or IL-11 and their soluble α-subunits of the receptor, respectively. H6 and H11 were tested in an ectopic (subcutaneous) and orthotopic (organ specific) murine renal cell carcinoma (RENCA) models. H6 or H11 cDNA were transduced into RENCA cells. Mice were immunized with RENCA-H6 or -H11, or control vaccine (RENCA-IRR) in prophylactic, adjuvant and therapeutic settings. Tumor formation and survival were the clinical end points of the study. Moreover, immune mechanisms stimulated by H6 and H11 adjuvant in induction and effector phases of anti-cancer response were studied.
H6 was further used for construction of therapeutic human melanoma vaccine (AGI-101). Two allogeneic melanoma cell lines (Mich-1 and Mich-2) were retrovirally transduced with H6. AGI-101 vaccine primes at three different levels. First H6. It acts in autocrine and paracrine manner. It auto activates vaccine cells what alters their phenotype (secreation of IL-2, IL-8, IL-12, INFγ, GM-CSF, VEGF, RANTES). In the paracrine manner at the site of vaccination H6 inhibits formation of T regs, induces maturation of DCs and presentation of cryptic antigens, induces production of GM-CSF by T lymphocytes, activates NK cells, enhances formation of CD4+ and CD8+ memory cells and balances immune response towards Th1 type. HLA alloantigens provide next (second) co-stimulatory signal, further enhanced by H6. Third, vaccine cells are coated with trace amounts of FCS, coming from the culture medium, which elucidate cellular and antibody responses in immunized patients and further augment ant-melanoma immune response.
AGI-101 was tested in clinical trials (6 studies, about 500 patients) in advanced melanoma patients. In adjuvant setting (Phase II, 200 patients) it significantly increased overall survival.

Oral presentations

O17.1

Telomerase targeting with siRNA as a new approach to cancer treatment

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High telomerase activity in cancer cells is supposed to be responsible for unlimited division of cancer cells. The enzyme has been shown to be active in over 90% of cancer cells while it is generally inactive, or at least significantly much less active, in most normal cells. The telomerase activity maintains the telomeres that results in protection of the ends of chromosomes from damage and prevents the adverse recombination. On the other hand it has been suggested that telomeres act as a "mitotic clock" that limits the cell division number. Thus, telomerase activity might constitute an attractive target in anticancer therapy.
The aim of the study was to analyze the efficiency and consequence of silencing the key subunits of the telomerase complex TERT, TERC and TEPP1 in human breast cancer MCF7 (estrogen-dependent) and MDA-MB-231(estrogen-independent) cells. For the transfection a pool siRNA was chosen (Dharmacon and SantaCruz Biotechnology) and Lipofectamine2000 (Invitrogen) as a transfection reagent. The cytotoxic and/or antiproliferative effect of siRNA was measured by SRB assay, and telomerase activity was assessed by a TRAP assay (Roche Diagnostics) followed by PAGE and ELISA assays. The influence of telomerase silencing was measured using flow cytometer (FACScan, BD) and qPCR assay (LC480, Roche Diagnostics).
It was revealed that treatment of breast cancer cells with different concentrations of siRNA (10-375 nmol) resulted in a time-dependent inhibition of telomerase activity up to over 40% comparing to control cells. The highest efficiency was observed when the cells were retransfected after 48 hours and cultured for another 48 h (2×48 incubation altogether). In a proliferation study neither cytotoxic effect of specific siRNA, nor non-specific influence of control siRNA were observed. Interestingly, telomerase downregulation resulted in induction of numerous genes engaged in proapoptotic mechanism followed by apoptosis.
It was revealed that targeting TERT in MCF7 was more efficient than in MDA-MB-231 and resulted in telomerase downregulation as well as apoptosis induction. Interestingly, an inverted effect was observed in MDA-MB-231 cells where targeting TERC was more efficient in telomerase silencing showing a cell type-dependent mechanism of regulation.
We conclude that telomerase targeting siRNA might be considered as a useful adjuvant therapy agent in anticancer therapy.

Acknowledgements
The present study was supported by N N401 223 534 research grant.

O17.2

HO-1 gene transfer has pro-survival and regenerative properties in ischemic skeletal muscles

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Heme oxygenase-1 (HO-1) is an enzyme degrading heme to carbon monoxide (CO), bilirubin, and iron ions. Through these compounds, HO-1 exerts pro-survival effects that include anti-apoptotic and/or anti-inflammatory activity. Uncontrolled overexpression of HO-1 can, however, cause some detrimental effects. Here, we report the successful use of plasmid vector expressing HO-1 under the regulation of three hypoxia-responsive elements (3HRE) and a minimal cytomegalovirus promoter (CMVmp) for the treatment of limb ischemia. Hypoxia-induced expression of HO-1 significantly improved the post-ischemic foot blood flow and decreased the incidence of toe necrosis in murine hindlimbs injected with pHRE-HO-1 shortly before femoral artery ligation. This protective *in vivo* effects were associated with reduction of inflammation (IL-8 and IL-6) and decreased levels of pro-apoptotic caspase-3. Moreover, overexpression of HO-1 seems to influence the regenerative potential of myocytes as it significantly modulated the expression of transcriptional (myogenin) and post-transcriptional (miR-146a and miR-206) regulators of genes involved in satellite cell differentiation and skeletal muscle regeneration. Our results demonstrate that pHRE-HO-1 vector can be further investigated as a tool to provide cytoprotection in ischemic cardiovascular diseases.

Acknowledgements
Supported by grant No: POIG No. 01.01.02-00-109/09, 01.01.02-00-069/09, 02.01.00-12-064/08 and 02.02.00-00-014/08 (European Union structural funds) and No. N N301 314837 (Ministry of Science and Higher Education).

O17.3

Myogenic potential of pluripotent stem cells

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Regeneration of mammalian skeletal muscle relies on the presence of muscle specific stem cells, i.e. satellite cells. Under some pathological conditions, such as muscle dystrophies, the pool of satellite cells becomes prematurely exhausted, and as a result skeletal muscles fail to regenerate. Thus, there is an unquestionable need to find the "replacement" cells that can be used in therapies supporting muscle regeneration. Among promising sources of the cells that could substitute for satellite cells are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), characterized by their ability to self-renew and differentiate into any given tissue, including skeletal muscle, both *in vivo* and *in vitro*. However, it is not clear whether the mechanisms regulating their myogenic differentiation reflect these operating during the specification and differentiation of myogenic precursor cells. Finding the answer to this question seems to be crucial for the progress of the studies on pluripotent stem cells to use them for regenerative medicine.
In our work we focused on the molecular and cellular processes accompanying myogenic differentiation of murine ESCs and iPSCs. We characterized those cells, their morphology, expression of pluripotency and myogenic markers. Our results show that both types of cells co-cultured with C2C12 myoblasts are able to undergo myogenic differentiation and form hybrid myotubes. Moreover, to trace myogenic differentiation we used the method of embryoid body formation from ESCs. We analyzed the expression of genes coding markers of pluripotency or myogenesis in embryoid bodies and in their outgrowths, at different times of culture. We found that in cells differentiating in embryoid bodies, expression of markers of myogenic differentiation can be detected. Next, we will analyze iPSCs, and finally use this system to analyze pathways governing myogenic differentiation of pluripotent stem cells.

O17.4

Induction of monocyte antitumour response by human cancer cells transduced with TNF-GFP fusion gene

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These studies were undertaken to determine how human pancreatic cancer (HPC-4) cells transduced with TNF-GFP fusion gene (TLG) alter antitumour response of human monocytes *in vitro* and whether they can act as antitumour vaccine. In our model, HPC-4 cells were transduced with retroviral vector harbouring TLG gene and designated as HPC-4_{TLG}. The TLG protein expression was confirmed by Western blot and flow cytometry analysis. Monocytes were cocultured with transduced and control HPC-4 cells. The secretion of TNF, IL-10 and IL-12 was measured by ELISA. Cytotoxicity of monocytes against HPC-4 cells was determined by MTT test. The results show, that the HPC-4_{TLG} cells expressed membrane-bound, intracellular and secretory TLG protein. When cultured with HPC-4_{TLG} cells monocytes released a higher amount of TNF and IL-10 but IL-12 secretion was inhibited. The preexposure of monocytes to HPC-4_{TLG}, but not to HPC-4, cells did not decrease TNF nor increase IL-10 production thus not leading to monocyte deactivation. Also, the antitumour cytotoxicity of monocytes stimulated with HPC-4_{TLG} was not downregulated, which occurred when nontransduced HPC-4 cells were used. In conclusion, comparing to parental HPC-4 cells, TLG gene transduced HPC-4 cells induced stronger antitumour response of monocytes *in vitro* and prevented deactivation of monocytes.

Poster

P17.1

Toxin — antitoxin system pasA/pasB from plasmid pTF-FC2 maintains activity in human cancer cell lines

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Bacteria posses specialized systems that regulate programmed cell death called Addiction modules or toxin - antitoxin modules. In most cases these modules consist of two components: stable toxin and unstable antidote proteins. Until now several families of toxin-antitoxin modules have been identified on chromosomes of bacteria, as well as on extrachromosomal DNA elements like plasmids. Typically, the toxin is a small protein (95-115 aa) that can interact with important cellular functions like translation or replication. Toxin remains active until it binds to antitoxin protein (65-85 aa) – forming stable, inactive complex. When carried on plasmids, TA systems constitute a maintenance mechanism or "plasmid addiction" system. Certain modules, such as Kis/Kid, RelE/RelB are found to be active as well in eukaryotic cells. Due to the fact that toxin possesses its natural inhibitor and the small size of both compounds toxin-antitoxin modules have ideal properties for use as a model of dual – component agent in anticancer therapy.
Despite the fact that toxin – antitoxin modules are widely distributed among bacteria, our knowledge of them is still very limited. In our research we were testing addictive module pasA/pasB (74 and 90 aa respectively) from 12.2 kb plasmid pTF-FC2 derived from *Thiobacillus ferrooxidans*. Our main goal was to determine if this module remains active in human tumor cell lines. Both components of pasA/pasB system were cloned into pEGFP-n1 protein and transfected into selected human tumor cell lines. Cytotoxicity assay and transfectants pasA/pasB ratio showed significant difference suggesting that TA-system pasA/pasB from *Thiobacillus ferrooxidans* remains active in eukaryotic cells.

P17.2

Towards convenient generation of induced pluripotent stem cells

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Introduction: Introduction of pluripotency-specific genes into differentiated somatic cells causes reversal of cell differentiation and generation of induced pluripotent stem (iPS) cells which exhibit self-renewal and proliferative abilities identical to embryonic stem cells. This approach gives chance for generation of cells of any lineage directly from patient's somatic cells and may become an accessible source of tissue material for regenerative medicine.
Aims: The aim of this study was introduction of iPS generation protocol to our laboratory. The protocol covers production of vectors for gene transfer and preparation of feeder cells (mice embryonic fibroblasts-MEF), used as support of undifferentiated growth of iPS cells. An isolation of human keratinocytes from plucked hair was performed as an example of convenient and non-invasive method for obtaining somatic cells.
Methods: Genes responsible for pluripotency (KLF4, SOX2, OCT3 and NANOG) were cloned into lentiviral expression system plasmid. This generated four expression clones which were used for production of lentiviral vectors. To derive MEF cells, pregnant mice were sacrificed in 10–14 day of gestation. Embryos were eviscerated and carcasses were finely minced with scalpel blade. Homogenized tissue was briefly trypsynized and resuspended in DMEM High Glucose supplemented with 20% FBS. Passage 0 cells were grown overnight and then passaged. After 1–2 days 80–90% confluent cells of passage 1 were inactivated with mitomycin C to form feeder layer. To derive keratinocytes, few dozen hairs were plucked from head of a volunteer male donor. Plucked hairs were placed in 35 mm Petri dish containing 100 µl hESC medium. The next day 1 ml of hESC medium was added. After 2–4 days keratinocyte outgrowth was observed and the cells were subsequently propagated in keratinocyte medium for two weeks.
Results: We have succeeded in introduction of four pluripotency genes into lentiviral expression system plasmids and completed production of four lentiviral vector populations harboring aforementioned genes. To add to it, we have introduced and optimized protocol for efficient isolation and maintenance of mouse embryonic fibroblasts, as a feeder layer for iPS cells. Finally, we have introduced and optimized a method of keratinocyte derivation from plucked hair. It has shown that merely 30 hairs after two weeks of incubation can give sufficient amount of cells for reprogramming

P17.3

Heme oxygenase-1 regulates functioning of myogenic progenitor

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Muscle satellite cells (mSC) are key players in postnatal muscle growth. Under normal conditions mSC remain quiescent, but after muscle damage they become activated, proliferate and differentiate into mature myocytes. Our aim was to explore the role of heme oxygenase-1 (HO-1), a rate-limiting enzyme in a heme degradation pathway, in physiology of these cells. Well known antiapoptotic, antioxidant, antiinflammatory and proangiogenic properties of HO-1 exerted in different cell types make it a well suited candidate for an improvement of the major problem of mSC-therapy - massive apoptosis after transplantation.

Experiments performed on immortalized myoblast cell line (C2C12 cells) overexpressing control genes or additionally HO-1, as well as with primary satellite cells isolated from HO-$1^{+/+}$, HO-$1^{+/-}$ and HO-$1^{-/-}$ mice demonstrated, that HO-1 improves viability under oxidative stress and induces proliferation of myogenic precursors, as well as elevates SDF-1a. However, it also decreases expression of myogenic markers (muscle regulatory factors) and muscle specific miRNAs (myomirs) leading to inhibition of myogenic differentiation. These effects were confirmed in an *in vivo* experiment, where the number of C2C12-Luc-GFP cells injected into hind limbs of NOD-SCID remained stable up to 3 weeks and they contributed to formation of muscle fibers. In contrast, C2C12-Luc-GFP-HO-1 were proliferating continuously and formed big, hyperplastic tumors, infiltrating the surrounding tissues.

To investigate possible mechanism of HO-1-induced abrogation of differentiation it was revealed, that both C2C12-Luc-GFP-HO-1 cells and control cells stimulated with one of the HO-1 metabolites (carbon monoxide) have elevated expression of p38 kinase, but decreased level of C/EBP transcription factor and DGCR8 enzyme. Transfection of C2C12-Luc-GFP-HO-1 cells with DGCR8 and C/EBP restored the miRNA pool and myogenic markers expression, respectively. Although HO-1 inhibits reactive oxygen spices formation, this effect has no influence on differentiation of these cells.

We therefore indicate, that although HO-1 induction may promote cell survival and proliferation after transplantation, it also decreases differentiation potential of myogenic cells *via* downregulation of C/EBP and DGCR8.

Acknowledgements

Supported by No. 01.01.02-00-109/09, 01.01.02-00-069/09 (European Union structural funds) and No. N N301 035240 (Ministry of Science and Higher Education).

P17.4

Cationic polyprenyl derivatives proposed as effective DNA carriers

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The challenge of finding efficient and safe carriers for the introduction of therapeutic genes into cells invites scientists to design of new synthetic vectors. Among non-viral vectors, carriers based on cationic amphiphiles have been widely employed. In the present investigation, we tested a potential new class of polyprenyl-based cationic lipids for gene transfer.

Polyprenols were extracted from the plant tissues. Crude lipid extracts were subjected to the alkaline hydrolysis and single prenologues were isolated chromatographically. Semisynthetic polyprenyltrimethylammonium iodides (PTAI) were synthesized from corresponding alcohols via phthalimide and amine. In vitro transfection activities were tested alone or with the co-lipid DOPE (dioleylphosphatidylethanolamine) by means of GFP-expression assay.

The chemical structure of synthesized PTAI was confirmed by NMR and MS methods. They were chemically stable (according to TLC) within 3 years of storage. PTAI:DOPE (molar ratio of 1.5:1 or 1:1) lipoplexes induced efficient transfection of DU145 cells (APren-7–60%, APren-8–62%, APren-11–64%) without any toxic effect. Interestingly, these results are comparable to the highest transfection efficiency of commercially available lipofecting agents tested in our model (66%).

In conclusion, cationic isoprenoids constitute a new class of cationic lipids for DNA transfer.

Acknowledgements

Project is part-financed by the European Union within the European Regional Development Fund under the Operational Programme Innovative Economy.

P17.5

Inhibition of STAT-3 signaling and P-glycoprotein (MDR-1) expression by RNA interference decreases bladder cancer cells survival

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STAT3 protein is constitutively activated in many human cancer cells, and aberrant STAT3 signaling is implicated as an important process in malignant progression. Antitumor activity or safety of specific agents may depend on drug metabolizing enzymes and transporters. The product of the *MDR1* gene, P-glycoprotein (PGP), represents membrane protein of the ATP binding cassette transporter family. It is an active efflux pump for a variety of drugs The short interfering RNA molecules are one of most specific and efficient tools for silencing the gene expression within cells. The main goal of presented study was the modulation of the multidrug resistance phenotype of bladder cancer cells by silencing of *STAT-3* and *MDR1* gene expression using RNAi methods. Short hairpin (shRNA) RNA_s were prepared, cloned under H1 promoter into pSUPER expression system, and their activity in posttranscriptional gene silencing was evaluated. Using Real Time™ PCR method, (QRTPCR), the relative mRNA level of was estimated in cellular material, it is: stable human bladder cancer cell line T24. The gene expression on mRNA level was determined. The influence of gene silencing on bladder cancer chemoinvasiveness, as well as a lymphocyte T adhesion on modulated cancer cells was described.
Obtained results for RNA interference in bladder cancer cells against *STAT-3* and *MDR1* gene have shown strong activity of shRNA in reversing of multidrug resistance phenotype, and this activity was much stronger than detected one for standard modulator cyclosporine A.

Acknowledgements
This study was financially supported by research grants: KNW-1-151/10 and KNW-1-036/P/1/0, subsidized by the Medical University of Silesia.

P17.6

The use of aminoprenols as gene carriers in angiogenic gene therapy

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Gene therapy is based on introducing proper gene as a therapeutic factor to the cell, where this gene is damaged or missing. Efficiency of gene transfer methods is dependent to a high degree on progress of research work devoted to gene carriers – vectors. The most commonly used in clinical trials are viral vectors, but many studies are dedicated to non-viral methods of gene delivery, mostly because of the higher biosafety level.
Experiments concern determining cells transfection efficiency, where plasmid specimens obtained by direct complexation of amino-prenols -7, -8, -11,-15 with plasmid DNA coding reporter proteins were utilized, mostly in vitro conditions. Three cell lines were chosen (B16-F10, HEK293 and LNCaP), on which amino-prenol:pDNA formulas were directly applied. Laboratory animals were also used in preliminary trials during which amino-prenol11:pVEGF complexes were injected to them. These tests allowed to determine the proangiogenic activity of such complexes.
Optimal complexation ratio of pDNA with amino-prenols was obtained using gel retardation assay. Moreover MTT test demonstrated that amino-prenyl carriers toxicity increases with lessening carbon chain length.
According to results obtained from transfections of B16-F10 cells, the use of amino-pren-15:pDNA and amino-pren-8:pDNA complexes resulted in the most significant transfection efficiency. Interestilngly, it was comparable to that obtained with use of commercial lipid agents. Preliminary studies conducted on mice showed, that amino-pren-11:pVEGF complexes have a positive impact on new vessel formation on skin.
Investigated amino-prenyl carriers have transfection properties, which can be concluded from research carried out in the project.
Based on previous notifications that condensation of plasmid DNA into cationic liposomes enhances gene delivery and also that some of the amino-prenols have lipofecting activity, there is work in progress concerning preparations of cationic liposomes. Amino-pren-15, comprising the cationic compound, is combined with neutral lipid, then mixed with plasmid DNA and finally this lipofection mixture is used to transfect the cells, in order to test whether such formula can bring better transfection efficiency.

Acknowledgements
Project is partially-financed by the European Union within the European Regional Development Fund under the Operational Program Innovative Economy UDA-POIG 01.03.01-14-036/09.