# The role of TFF3 in the development of thyroid cancer

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**Aim:** Trefoil factor 3 (TFF3) is overexpressed in a variety of solid epithelial cancers including breast cancer where it has been shown to promote migration, invasion, proliferation, survival and angiogenesis, whereas it is downregulated in the majority of thyroid tumors derived from follicular cells. However, its role in the development of thyroid cancer remains unknown. The purpose of the present study was to elucidate the regulation of TFF3 expression by estrogen signaling and its functional role in development of thyroid cancer. **Methods:** TFF3 expression was analyzed by immunohistochemistry using a tissue array comprising 72 specimens representing various benign, inflammatory and malignant thyroid diseases and normal tissues. The effects of estradiol stimulation on TFF3 expression were studied in thyroid and breast cancer cells and by meta-analysis of gene expression datasets. The functional effects of forced TFF3 expression in anaplastic thyroid and breast cancer cell lines were analyzed by assays for cell proliferation, invasion and gene expression profiling. **Results:** TFF3 exhibited strong cytoplasmic staining of thyroid follicular cells and colloid that was increased in hyperfunctioning thyroid nodules while its expression was decreased in all thyroid cancers of follicular cell origin. In thyroid cancer cells, on the contrary to the breast cancer, the expression of *TFF3* was downregulated by estrogen signaling and forced expression of TFF3 resulted in decreased cell proliferation and entry into the S phase. Furthermore, it induced changes in the cell morphology and expression of the functional differentiation markers of thyroid follicular cells, such as *TPO, TSHR, THRB, SLC26A4* and *SLC5A5*, epithelial marker *CDH1*, and transcription factors implicated in the thyroid morphogenesis and function *FOXE1* and *WWTR1*. **Conclusions:** In thyroid cancer, TFF3 expression is decreased by estrogen signaling and its restoration results in the decreased cell proliferation, and induces the differentiation towards the follicular cell phenotype thus suggesting that TFF3 may act as a tumor suppressor or an oncogene depending on the cellular context.

## CHI3L1 AND TSC22 proteins involvement in cellular response to extracellular stress

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**Purpose**. High intratumoral heterogeneity and significant chemotherapy resistance to different agents are distinctive features of glioblastoma, the most aggressive tumor among all central nervous system neoplasia in adults. Low efficiency of anticancer therapies using antioxidants to prevent the progress of tumor could be associated with a higher resistance of malignantly transformed cells to reactive oxygen species. Chitinase-3-like 1 (*CHI3L1*) gene is extremely overexpressed in glioblastoma cells, while *TSC22* gene encoding the TSC22 protein (transforming growth factor-beta 1 [TGF-β1]-stimulated clone 22 domain family, member 1) is significantly down-regulated in glioblastoma as compared to normal human brain. The main purpose of this work was to determine the contribution of *CHI3L1* oncogene and *TSC22* tumor suppressor gene to the processes of survival and apoptosis in human glioblastoma U87 cells under oxidative stress. **Methods and results.** We investigated the sensitivity of CHI3L1-producing cells U87 to reactive oxygen species and identified capabilities of tumor suppressor protein TSC22 to reduce the viability and to recover the sensitivity of these cells to the action of oxidants. Thus, U87 cells transfected with a construct containing full-length *TSC22* cDNA and treated for 24 h with hydrogen peroxide in a concentration range of 0.05-5 mM showed a significant reduction of cell viability by 16 % with 0.5 mM hydrogen peroxide according to the MTT test results, whereas cells without transfection of *TSC22* did not undergo cytotoxic effect within the same concentration of $H_2O_2$. The number of metabolically active U87 cells increased exponentially for 7 days, but the stable production of TSC22 suppressor protein led to 2.6-fold reduction in number of metabolically active cells during the same period. In the presence of TSC22 protein, it was observed the increased phosphorylation of p53 protein and reduction of CHI3L1 protein production as it was shown by Western blot analysis. To elucidate if TSC22 as transcription repressor could regulate the transcription of CHI3L1 we performed reverse transcription-PCR analysis with primers to *CHI3L1* coding sequence, however, overexpression of TSC22 did not inhibit *CHI3L1* transcription in U87 cells. **Conclusions.** Overproduction of tumor suppressor TSC22 protein inhibits viability and restores sensitivity of U87 cells to the action of reactive oxygen species, supposing a new oncogene-dependent mechanism of chemotherapy resistance in glioblastoma. The presence of TSC22 led to increased p53 phosphorylation and caused down-regulation of CHI3L1 content in U87 cells.

# Acceleration of the peroxynitrite decomposition rate, DNA binding and oxidative stress reduction by 1,4-dihidropiridine derivatives

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**Aim.** Peroxinitrite is a powerful oxidant exhibiting a wide array of cell damaging effects. It can cause strand breaks in DNA, introducing base pairs and sugar-phosphate backbone oxidative modifications. In order to evaluate ability of 1,4-DHP to protect cells and DNA against peroxynitrite-produced damage the peroxynitrite decomposition rate was determined in presence of different 1,4-DHP derivatives. The same group of compounds was tested for ability to protect the cell DNA and to interact with the DNA molecule. **Methods.** The peroxynitrite decomposition rate in the presence of different 1,4-DHP had been studied by means of UV-VIS spectroscopy using the kinetics analysis. The peroxynitrite decomposition was detected measuring the absorption changes at 302 nm. Several 1,4-DHP derivatives: AV-153-Na and its analogues, J-8-120 and its analogues, PP-544- $NH_4$ ,cerebrocrast, etaftorone un fenotorone were tested for ability to accelerate the peroxynitrite decomposition. Ability of the above compounds to interact with DNA was determined by measuring fluorescence intensity of the compounds in presence of increasing concentrations of DNA. Ability of the compounds to protect the HeLa cell DNA against peroxynitrite-induced DNA single-strand breaks was analysed by means of single-cell gel electrophoresis in alkaline conditions. **Results.** Results of the kinetics analysis did not reveal any influence on peroxynitrite decomposition rate by AV-153-Na, J-8-120 and PP-544-$NH_4$. Fenoftorone and etaftorone accelerated the decomposition reaction three times, cerebrocrast – 6 times.AV-153, etaftorone and cerebrocrast can interact with DNA molecule. Intensity of AV-153-Na fluorescence (λex = 350, λem = 480) increased in presence of increasing DNA concentrations. The binding constant (K) of AV-153-Na is equal to 6,4 x $10^3$ $M^{-1}$. The inverse effect was noticed in case of cerebrocrast (λex = 370, λem = 440), the fluorescence was quenched with increasing the DNA concentrations. The Stern-Volmer quenching constant of cerebrokrast (Ksv) is equal to 2,2 x $10^3$ $M^{-1}$.The single-cell gel electrophoresis results showed, that cerebrocrast and etaftorone could significantly protect HeLa cells preincubated with these compounds against peroxynitrite-induced DNA damage. AV-153 produced a similar effect. **Conclusion.** Cerebrocrast ,etaftorone, fenoftorone react with peroxynitrite *in vitro*. 2. AV-153-Na, cerebrocrasts and etaftorone protect the DNA against peroxynitrite-induced damage in living cells. 3. Cerebrocrast and AV-153-Na can interact with DNA.

## Ubiquitin, SUMO, the nuclear pore complex and the DNA damage response

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**Aim**. The Nuclear Pore Complex, a gigantic machine of the nuclear envelope recently emerged as a "hub" coordinating nuclear transport, gene expression, chromatin organization and genome integrity. In this context, persistent double-strand breaks, arrested replication forks and eroded telomeres have been shown to relocate to the yeast Nuclear Pore Complex (NPC) and some NPCs proteins even influence DNA repair both in yeast and mammalian cells. However the limited number of NPC per cell imposes a certain hierarchy of these functions in time and space. Our hypothesis is that dynamic post-translational modifications control the plasticity of the NPC structural organization and functions.

**Results**. Our recent systematic analysis of the ubiquitylation of yeast nuclear pore complex proteins (Nups) revealed that more than 50 % of them are ubiquitylated, mostly by mono-ubiquitylation suggesting a non-degradative role of this post-translational modification. In addition we found that Nups ubiquitylation involves different components of the ubiquitin conjugation machinery. A detailed analysis of Nup159 mono-ubiquitylation, a Nup exclusively localized on the cytoplasmic face of the NPC, revealed a unexpected function in nuclear segregation at the onset of mitosis.

More recently, we focused our attention on modifications of the components of nuclear basket substructure of the NPC and found that these Nups are extensively modified by ubiquitylation, sumoylation and phosphorylation. We precisely analyzed the dynamics and interplay of these modifications during cell cycle progression, their functions in controlling the plasticity of the nuclear basket as well as the efficiency of the DNA damage response.

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# Parameters of nitric oxide metabolism and DNA integrity in patients with type 1 diabetes mellitus

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**Objectives:** Hyperglycemia in diabetes mellitus type 1 (DMT1) increases nitric oxide (NO) and superoxide anion ($O_2^-$ ) production resulting in peroxynitrite formation, which leads to increased DNA damage linked to formation of late -onset complications of DMT1. Our goal was to determine the parameters of NO metabolism and DNA integrity in patients with DMT1.

**Methods**: 88 patients with DMT1 (67 % of them normoalbuminuric, 16 % microalbuminuric, 17 % macroalbuminuric and with end stage renal disease) and 44 healthy controls matched for age, sex and BMI were involved. Anamnesis and other data was collected through a questionnaire, blood biochemical analysis was performed in a clinical laboratory. NO production in blood was detected by the electron paramagnetic resonance spectroscopy, nitrite and nitrate ($NO_2^-/NO_3^-$) concentrations in serum and urine with the Griess reaction and DNA damage in nucleated blood cells by the alkaline single cell gel electrophoresis.

**Results**: NO and DNA damage were significantly higher in DMT1 patients ($p < 0.0001$), while $NO_2^-/NO_3^-$ in serum ($p < 0.007$) and urine ($p < 0.04$) were lower compared to the controls. In patients, NO was inversely proportional to $NO_2^-/NO_3^-$ in serum ($p < 0.04$) and urine ($p = 0.05$), thus, suggesting NO synthase-independent NO production.

In patients, $NO_2^-/NO_3^-$ in serum were directly proportional ($p < 0.0001$) to the stages of diabetic nephropathy, whilst inversely proportional to glomerular filtration rate (GFR) ($p < 0.001$), suggesting decreased output in case of a low GFR. $NO_2^-/NO_3^-$ in urine were inversely proportional ($p < 0.02$) to the stages of diabetic nephropathy, but directly proportional to glomerular filtration rate (GFR) ($p < 0.0001$), suggesting decreased output in case of a low GFR.

We have also revealed a direct correlation between NO and DNA break levels (if compared to the average of controls) with HbA1c ($p < 0,04$) and the duration of the illness ($p < 0,05$). In comparison to the average level of controls NO and DNA break levels were high in 74.14 % of patients and only in 16.28 % of controls. But in 26 % of patients NO was inversely correlated to the DNA break levels ($p < 0,0001$), thereby NO and DNA breaks are probably adjacent factors, which are characteristic of DMT1.

**Conclusions**: Higher levels of NO production and DNA breaks in blood cells are characteristic of DMT1. These parameters might be used as therapeutic targets for pathogenetic therapy of DMT1. In future these parameters might be used as therapeutic targets for pathogenetic therapy of DMT1.

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## The cooperativity and fractality in the functional genome organization

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One of the most intriguing and still unresolved questions of gene regulation is how the genome coordinates all genes for development and tissue differentiation. While the data from studies of the genome organisation provide some hints on the hierarchial cooperativity, starting with Rabl orientation and ending with loop-domains, the gene-based data were hitherto exlusively elementaristic. However, in recent time, there appeared the publications (Masa Tsuchyia et al., 2010 and other) based on the whole transcriptome analysis which revealed on the neutrophil differentiation models the fractal-like gene ensembles acting as vehicles for differentiation attractor deciding cell fate. It will be discussed in presentation how it may fit with the known data on the structural and dynamic genome organisation.

## Evaluation of the *PSMB5* (rs11543947) and *PSMA3* (rs2348071) gene polymorphisms on the association with multiple sclerosis in Latvians

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**Aim**: Functional realization of many signalling proteins and transcription factors implicated in development and progression of multiple sclerosis are mediated by proteasomes. This case-control study aimed to evaluate genetic variations in the *PSMB5* and *PSMA3* genes encoding proteasomal subunits on susceptibility to multiple sclerosis in Latvians.

**Methods**: The rs11543947 (*PSMB5)* and rs2348071 (*PSMA3*) polymorphisms were genotyped in 291 multiple sclerosis patients and 305 healthy individuals and analysed on general, subtype and sex-specific associations with the disease.

**Results**: Loci rs11543947 and rs2348071 were identified as disease neutral and susceptible respectively. The rs2348071 heterozygous genotype GA showed strong main effect ($P < 0.001$; OR=1.891, 95% CI [1.360 − 2.628]), and moderate ($P < 0.01$; OR=1.663, 95% CI [1.152 − 2.402]) and strong ($P < 0.001$; OR=2.459, 95% CI [1.534 – 3.943]) association with relapsing-remitting and secondary progressive phases of disease respectively. No genotype-sex interaction associated with multiple sclerosis has been detected.

**Conclusions**: Our results suggest susceptibility of the rs2348071 heterozygous genotype to multiple sclerosis in Latvians.

# Interaction of BER factors with pleiotropic protein YB-1 in oxidative clustered DNA lesions repair

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**Aim.** Single oxidative DNA lesions (such as abasic sites, single-stranded DNA breaks, oxidized bases) and their combinations that are referred to as *oxidative clustered DNA lesions – OCDLs* are generally corrected by base excision repair pathway (BER). During BER, the damaged DNA strand is cleaved by AP endonuclease APE1 or bifunctional DNA glycosylases like NEIL1 that results in nicked intermediate. Due to this fact, repair of abasic sites located within bistranded OCDLs or single-stranded DNA regions is closely connected with the risk of high cytotoxic DNA double strand breaks formation. Pleiotropic protein YB-1 is extensively studied in the context of cellular response to genotoxic stress, but its unmediated role in DNA repair is still open to question. *The aim* of present research was to investigate multifunctional protein YB-1 influence on repair of abasic sites located within oxidative clustered DNA lesions (OCDLs) or single-stranded DNA fragments. **Methods.** In present work bistranded OCDLs in DNA were imitated by combination of abasic site and 5-formyluracil (potential product of thymine oxidation) in opposite DNA strand. YB-1 interaction with oxidative DNA lesions was determined by $NaBH_4$-mediated crosslinking. YB-1 influence on major components of BER machinery was performed by functional studies and gel retardation assays. Posttranslational modification of YB-1 was analyzed by Western-blot analysis and Coomassie staining. **Results.** The data obtained demonstrated that YB-1 directly interacts with abasic site and 5-formyluracil in DNA via intermediate Schiff base formation. YB-1 was shown to promote APE1- and NEIL1-depending cleavage of either single abasic sites or AP sites within OCDLs. YB-1 influence on APE1 occurs during substrate-binding step. Presence of high YB-1 concentrations resulted in blocking APE1-depending pathway via inhibition of APE1 activity as well as polβ dRP lyase activity. With AP sites being in the single-stranded DNA regions, YB-1 inhibited both APE1 and NEIL1 activities. *In vitro* studies revealed a possibility of a novel posttranslational modification of YB-1 that could influence on its interaction with DNA. **Conclusions.** Pleiotropic protein YB-1 directly interacts with AP sites and 5-formyluracil within DNA and possesses the capability of modulating of APE1- and NEIL1-depending AP site cleavage. YB-1 is able to go through posttranslational modification that can affect the role of YB-1 in DNA repair.

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## New active compounds derived from molecular diversity.

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Molecular diversity corresponds to the set of molecules that can be found in various biological objects or artificially generated. From this diversity can be extracted compounds able at regulating biological functions. In applied research, drug discovery more and more involves the use of molecular diversity for screening against targets. Selection of regulators able to bind targets of all types generally follows iterative processes leading to the identification of molecules (peptides, nucleic acids…) from a large randomized pool (library). Among them: *Phage display using filamentous bacteriophages represents one of the most successful in vitro ligand selection technology. It is an *in vitro* process starting from a library of peptides or antibodies. A pool of degenerated oligonucleotides comprising the required diversity is cloned in fusion with a coat protein of the phage. In the resulting library, each phage displays a unique random peptide or antibody. This maintains a physical link between genotype and phenotype: analysis of selected sequences can thus be performed by DNA sequencing. Each round of selection is ended by phagepeptide amplification using bacterial infection. In literature, phage display technology has been wealthily described with versatile applications [1]. *SELEX (Systematic Evolution of Ligands by Exponential enrichment) starts from a library of synthetic nucleic acids (DNA or RNA) [2] . In this case, the diversity of the library is not only due to the sequence variability of single-stranded oligonucleotides but also to the three-dimensional structures they may adopt (loops, hairpins, stems...).SELEX method relies both on chemistry for library synthesis and nucleic acids modifications and on molecular biology techniques for the roundamplification of selected entities. It has been widely used to identify high affinity aptamers against various targets (proteins, dyes…) [3].

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## A RecG helicase involved in the maintenance of the plant organellar genomes.

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**Aim**. The dynamic state of the plant mtDNA is due to recombination processes involving repeated sequences that modulate its structure. Large repeats are interconvertible by homologous recombination (HR) and contribute to the multipartite structure of mtDNA, while rare ectopic recombination occurs among small repeats and generate alternative mtDNA configurations at substoechiometric levels, which contribute to the rapid evolution of the mtDNA structure in plants. In addition, both the mtDNA and the cpDNA are believed to replicate by recombination-dependent pathways reminiscent of the mechanisms of replication of certain bacteriophages. HR dependent processes have also a predominant role in the repair of the plant organellar genomes. Our aim is to identify the factors implicated in the control of organellar HR or in mechanisms of HR-dependent repair. Among those, DNA helicases should have a predominant role in recombination surveillance, and are postulated to be required for rejection of ectopic HR intermediates.
**Results.** We identified an Arabidopsis DNA helicase homologous to the bacterial DNA helicase RecG, that we called RECG1. Bacterial RecG acts as a translocase in DNA recombination, repair and replication. It promotes holliday junctions translocation, the regression of replication forks and avoiding replication re-initiation when replication forks collide. RECG1 is found in all plant species, including algae and mosses, but not in animals or fungi. Transgenic Arabidopsis plants expressing RecG1:GFP fusion showed that RECG1 is dually targeted to mitochondria and plastids, suggesting that assumes similar functions in both organelles. Promoter-GUS fusion showed that the gene is expressed in most plant tissues, in particular in leafs and root vascular systems. The Arabidopsis RECG1 is able to complement its bacterial orthologue for the repair of UV-induced DNA repair, but not the bacterial DNA translocase RuvAB. Using T-DNA insertion mutants we tested the roles of RECG1 in the surveillance of ectopic HR and the repair of DNA damage induced by genotoxic agents. We found that RECG1 affects the efficiency of mtDNA repair by recombination. In addition, loss of RECG1 results in increased ectopic HR of the mtDNA having as consequence specific changes in the segregation of mtDNA sequences. Surprisingly no effects were observed in cpDNA replication and segregation, suggesting that compensatory mechanisms exist in chloroplasts. Plants deficient in RECG1 expression have no remarkable growth phenotypes, but double mutants of RECG1 and other factors involved in mtDNA repair by recombination and/or recombination surveillance display exacerbated phenotypes of leaf distortion and variegation. Analysis of the relative copy numbers of the organellar genomes sequences by qPCR show that these phenotypes apparently result from instability of the mtDNA by recombination, resulting in abnormal segregation of mtDNA subgenomes. **Conclusions.** Our results show that RECG1 is required for recombination surveillance, suppressing ectopic HR involving repeated sequences that compromise the stability and stoichiometric segregation of the plant mtDNA.
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## Long chemically modified siRNA provide an efficient and specific gene silencing

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RNA interference (RNAi) is an evolutionarily conserved cellular mechanism of sequence-specific gene silencing mediated by diverse classes of double-stranded RNAs. Because of the outstanding potency and specificity in comparison with other loss-of-function technologies, RNAi has quickly developed into a potent biological tool for the specific inhibition of gene expression. Synthetic 21 bp RNA duplexes can be introduced into cells and will function of mimics of the natural siRNAs that result from Dicer processing. Recent reports have proven that longer duplexes (25-30 bp in length) are processed by Dicer into 21 bp siRNAs and loaded into RISC more efficiently than canonical siRNAs and demonstrate better gene silencing. However, it has been shown, that longer synthetic RNA duplexes could trigger an innate immune response. The chemical modifications known to prevent the recognition of longer RNA duplexes by the innate immune system without influencing silencing activity have been assumed. These chemical modifications can block the immune response and we can suppose that theoretically nothing prevents the use of partly modified dsRNAs of any length. In our study, we used selectively modified RNA duplexes 21 bp, 42 bp and 63 bp in length targeted MDR1 mRNA and examined their ability to silence the expression of the target gene and the specificity of their action. These siRNAs were found to silence the expression of P-glycoprotein and restore the sensitivity of drug-resistant cancer cells to vinblastine more efficiently that canonical siRNA with the same sequence. Our data revealed that 2′-OMe modification of CpA, UpA and UpG motives in 42 bp and 63 bp siRNAs effectively prevents activation of the innate immunity response and did not change the expression levels of two key interferon response genes, PKR and β-actin, in KB-8-5 cells. To our surprise, no substantial processing of dsRNAs by Dicer was detected inside the cells. Our results remove the length limits for the design of RNAi effectors, and add another example in the list of novel RNAi-inducing molecules differing from the classical siRNA. For these reasons, we believe that these structures have great potential for the development of efficient therapeutics.

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## Etoposide induced dual expression of opposite regulators of self- renewal and senecence OCT4/P21CIP1 in embryonal carcinoma cells PA1 is modulated by the serum concentration changes

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Tumour cellular senescence induced by genotoxic treatments was typically considered a terminal cell fate, however recently it was shown to be to some extent reversible, while the mechanisms of reversion, in particular in cancer stem cells are unclear [1]. Previously we found on embryonal teratocarcinoma PA1 serving as a model of cancer stem cells that the induced by etoposide (ETO) treatment senescence was intimately associated with self-renewal. We revealed that during the 2-3 days long G2-arrest most cells simultaneously express in their nuclei the opposite regulators – self-renewal master transcription factor OCT4A and inhibitor of cell cycle and mediator of senescence P21CIP1. Both depend on activation of TP53, while its silencing caused divergence for irreversible senescence and premature aberrant mitoses [2]. Here we report on further exploration of the character of this duality by studies on the influence of serum concentration in the time course. The expression of opposite regulators was detected in individual cells by the two-channel immunofluorescence measurements using flow and image cytometry. Distributions were characterized by geometrical parameters of cell coordinates on dot plot, showing p21CIP1/OCT4A expression. In non-treated cells, the two-dimensional distribution of the intensity of OCT4A and background p21CIP1 staining in individual cells forms a compact cluster, while in the ETO-treated cells the contents of both factors is enhanced several-fold (confirmed by Western blotting) and the variability amplitude increases even more. Nevertheless on day 2-3 the both p53-dependent regulators are correlating positively indicating to fluctuations of p53 itself. However, a ”swing” of the regulators increases in the time course, with the opposing outliers (P21CIP1>>OCT4A vs. OCT4A>>P21CIP1) appearing upon the time of release from the G2-arrest (days 4-5). This is followed by death of the majority and further dissociation of the remaining minority for proliferating and terminally senescing cells. P21CIP1>>OCT4A fraction increased by cell starvation, while the size of regrowing clones was reduced. On the contrary, increased serum concentration demonstrated OCT4 prevailing over p21CIP1 and larger recovery clones. Interestingly, however, serum concentrations did not affect the timing of regrowth (from day 7). Using the hypothesis of ergodicity, we suggest that high amplitude of variation of both regulators in individual cells reflects the temporal fluctuations of the steady state in individual cells characterising it as bi-potential and metastable. This state holds a possibility to keep the both opposite networks potentially functional, however preventing for the time being each other from accomplishing their destination. Besides, cell vitality supported by serum concentration is an important factor supporting this bi-potential state and tumour cell recovery after treatment with Etoposide.

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## Gene expression signature of glial tumors and participation of oncogene *CHI3L1* in malignant transformation

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Glioblastoma is the most aggressive intracranial malignancy characterized by high invasiveness, recurrence and poor responce to chemo- and radiotherapy. Heterogenous character of glioblastoma eliminates the value of single molecular markers. High-throughput gene expression analysis provides huge amounts of data that could be used to identify characteristic gene expression profiles (signatures), associated with specific tumor properties. Previously, glioblastomas were divided into three subtypes that correlated with patient survival or into four subtypes using unsupervised cluster analysis. However, gene expression signatures alone provide limited possibility to understand the biology of tumor.. Analysis and integration of various signatures, created using different approaches, can clarify common pathways involved in tumor development, which is not apparent at the level of individual genes. We used cluster analysis with k-means approach to search for the differences in expression of 12480 genes in 224 glioblastomas samples obtained earlier, and found two subtypes. 15 genes were upregulated and 401 genes were downregulated two-fold in the first subtype of glioblastoma (80 samples) as compared to the second subtype (144 samples). 10 from 15 genes with increased expression in the first subtype encoded proteins involved in the regulation of cell cycle and proliferation. Significant part of 401 genes were found to be involved in synaptic transduction, neurogenesis, formation of myelin sheath, and axon development. Kohonen map, built on the basis of expression of 15 genes with increased expression in the first subtype, and 60 of 401 genes with increased expression in the second subtype also showed good separation of two determined subtypes in same glioblastoma samples. «Mesenchymal» subtype of gliobastoma, described earlier, is characterized by overexpression of the set of genes, and among them the gene, encoding chitinase-3-like protein 1 (*CHI3L1*), which involved in abnormal cell proliferation and tumor angiogenesis. However, it is not amplified in glioblastomas and mechanisms of its overexpression are unknown. In our previous work we demonstrated that MYC/MAX complex was important but not crucial for overexpression of *CHI3L1* in glioblastoma as single nucleotide mutation in its promoter region, which disrupted MYC/MAX binding site, led to decreased *CHI3L1* expression. As we revealed, ectopic overexpression of *TP53* in glioblastoma U87 cells led to the decreased CHI3L1-level. This effect was achieved also either by activation of p53 by antitumor agent resveratrol or by inhibiting p53 degradation mediated by SIRT1, using its specific inhibitor sirtinol. We showed that CHI3L1 level in glioblastoma cells is p53-dependent and could be decreased by p53 activation. To clarify structural peculiarities of CHI3L1, we compared 3D structures of CHI3L1 and its closest human homologue CHI3L2 and revealed the cluster of positively charged amino acids 144-RRDKQH-149, located on the protein surface. Site-directed mutagenesis of this potential heparin-binding region in CHI3L1 revealed that residues Arg144, Arg145, and Lys147 are crucial for interaction with heparin. Soft agar assay demonstrated that mutation in heparin-binding site significantly decreased colony formation efficiency. Thus, we demonstrated that single mutation in the MYC/MAX binding site in promoter region of *CHI3L1* led to decreased *CHI3L1* expression in glioblastoma, in the absence of it expression of *CHI3L1* is more variable. Second, the p53 protein is a negative regulator of *CHI3L1* expression in glioblastoma U87 cells. Inactivation of p53 in tumors can lead to the overexpression of *CHI3L1*, and consequently, increase CHI3L1-mediated pathological effects, such as abnormal cell proliferation and angiogenesis. CHI3L1 enhanced malignant properties of 293 cells after its ectopic expression, disruption of its heparin-binding site decreased the cell ability for substrate-independent growth in soft agar.

## Antitumor and antimetastatic effect of short immunostimulating dsRNA against hepatocellular carcinoma G29 and melanoma B16

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Recently, we designed a series of short double-stranded RNAs that possess pronounced antiproliferative activity in cancer cells and did not have substantial homology to any human or mouse mRNAs. These compounds were shown to induce immunostimulatory effects in culture of human adherent peripheral blood mononuclear cells (PBMCs) and in mice. The aim was to evaluate the antitumor and antimethatatic potential of selected 19-bp RNA duplexes with 3-nucleotid overhangs in the 3'-ends (here and after immunostimulating RNA, or isRNA). **Methods.** Antiproliferative activity of isRNA *in vitro* was analyzed in KB-3-1 (throat), A-549 (lung) and SK-N-MC (neuroblastoma) cancer cell lines by MTT test. The percentage of apoptotic and dead cells in the population was assayed by flow cytometry. Selective knock down of different cellular sensors of dsRNAs was achieved using lentiviral vectors expressing defined shRNAs. The transplantable hepatocellular carcinoma (HCC) G-29 tumor in CBA/LacSto (CBA) mice and melanoma B16 in C57BL/6 mice were used to study the effects of isRNA on tumor growth and metastases spreading. The levels of IFN-α and cytokines in human PBMC cultural medium and in mouse serum were measured using ELISA. **Results.** The results of *in vitro* experiments demonstrate that isRNAs under study efficiently inhibit growth of human cancer cells (KB-3-1, A-549, SK-N-MC), and induce synthesis of IFN-α and pro-inflammatory cytokines IL-6 and TNF-α in the human PBMCs. The examination of isRNAs sequence/activity relationships revealed that the introduction of substitutions in the middle part of the isRNA sequence does not alter the antiproliferative and immunostimulating activities, while substitutions in the 3'-end region of isRNA substantially reduce the activities. Our research demonstrated that antiproliferative effects of isRNAs are related to cell growth arrest, rather than the induction of apoptosis. To define dsRNA-binding receptors involved in stimulation of innate immunity and inhibition of cancer cell proliferation by isRNAs, we used we used a set of cell lines with selectively silenced dsRNA sensors. The data showed that the dsRNA-binding protein kinase (PKR) is essential for the biological activity of isRNA**.** The results of *in vivo* experiments show that single intravenous injection of isRNA complexed with transfection reagent efficiently increases the level of IFN-α, and to a lesser degree the level of pro-inflammatory cytokine IL-6 in mice blood serum. We found that isRNA reduces the metastases area in the liver, kidneys and heart of CBA/LacSto mice with HCC G-29 and cause a slight, but reliable inhibition of the primary tumor growth. The results of experiments with C57BL mice with implanted melanoma B16 show that isRNA efficiently inhibits tumor growth and metastasis spreading in the lungs. **Conclusion.** The obtained results revealed the pronounced immunostimulatory, anti-tumor and antimethastatic properties of the studied isRNAs. So, this short double stranded RNA can be considered as potential adjuvant for the therapy of oncological diseases.

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## Mechanisms of heat stress-induced cell senescence.

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**Aim***:* The aim of this study was to investigate delayed effects of heat stress on mammalian cells.
**Methods***:* Comet assay and modified PFGE were used to measure DNA damage. Indirect immunofluorescence was applied to track DNA damage response events. FISH was used to quantify the levels of hyper-replication of certain genomic loci. Gene expression analysis was performed using qRT-PCR and Western blot hybridization.
**Results***:* In the present study we demonstrated that acute heat stress (HS) induces cell senescence in human cells – HS results in a robust G2/M cell cycle arrest within approximately one cell cycle. Heat-treated human cells acquire most of the cell senescence marks (proliferation arrest, senescence-associated (SA) beta-gal activity, changes in cell size/morphology, etc.) in few days after the treatment. Interestingly, HS does not lead to formation of SA heterochromatin foci (SAHF) – only ring-like heterochromatin domains are formed. We demonstrated that HS-induced cell senescence state is maintained by p21. We found that persistent DNA damage response, which depends on DNA hyper-replication, is the trigger of HS-induced cell senescence.
**Conclusion***:* Heat stress induces p21-dependent cell senescence.
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## Complex treatment of chemoresistant glial brain tumors and delivery of drugs to the tumor cells

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Most tumorigenic processes are multi-factorial in nature. They are affected or controlled by multiple effectors in sequential or parallel form. A dominant effector usually requires the synergy of one or more secondary effectors. Its loss of function is often compensated for by other secondary effectors. In order to effectively control a pathological process, it is necessary to modulate multiple related effectors concomitantly. Glioblastoma, the most common and aggressive malignant tumor of central nervous system in adults is known to be highly resistant to chemotherapy. The first-line cytotoxic chemotherapy (temozolomide) is unable to induce long-term remission in the majority of patients. Despite the certain clinical success, only a portion of patients responds and durability of response is limited, majority of patients exhibit an aggressive clinical course with a median survival time of 1 to 2 years. Glioblastoma is driven by multiple molecular aberrations that cannot be controlled by a single targeted agent, chromosomal instability and tumor heterogeneity makes every tumor rather "mobile" than "frozen" target. So, the development of new treatment modalities is desperately needed for this malignant disease. Although targeting oncogenes becomes a clinical reality, the therapies aimed at a single target may not be effective: massive biological tumor heterogeneity dictates the necessity for a therapy directed not at individual genes but rather at the pathological effects that they cause. One of such pathological effects is angiogenesis, which plays a key role in tumor vascularization. In anticancer therapy, considerable attention was paid to anti-angiogenic drugs. Previously, it was shown that CHI3L1 had strong angiogenic properties. We found that knockdown of *CHI3L1* in HeLa_*CHI3L1* and glial U87 cells by specific siRNAs led to 90 % inhibition of CHI3L1 production in cell culture and in the same time suppressed the transformed cells proliferation and their ability to grow in soft agar, the main feature of malignantly transformed cells. However, the success of anti-angiogenic drugs in clinic is temporary. Drug resistance, tumor re-emergence, and rapid appearance of new vessels are manifested at the end of therapy. Angiogenic (oncogenic) redundancy is a significant obstacle to the success of targeted therapy. It is supposed that simultaneous treatment with anti-CHI3L1 together with other anti-angiogenic drugs as for example bradykinin antagonists should give better positive result. Bradykinin antagonists were shown to be quite promising as new potential anti-cancer compounds for complex therapy. Several mechanisms of resistance have already been proposed, however changes in chromosome stability of glioblastoma cells after acquisition of resistance to temozolomide have not been elucidated. The project was focused on the characterization of karyotype and phenotype changes in 293 immortalized cells and U373 glial cells after acquisition of resistance to this drug. This project provides a simultaneous application of new bioactive chemical reagents of different types, specific siRNAs or new antisense strategies, namely stable in bloodstream morpholino oligonucleotides together with conventional chemotherapeutics used in clinics. Anti-tumor properties of different combinatorial drug schemes were investigated *in vitro* in several malignant cell lines of different origin and *in vivo* in mouse and rat tumor models. It is the goal to develop the highly effective multitargeted pharmaceuticals, which could modulate multiple related effectors (drug targets) concurrently. It is possible to expect that the combinatorial anti-cancer therapy aimed simultaneously at different elements of tumor formation mechanisms will be more effective and will promote the extension of patients' life. For multiple-target drugs delivery to gliomas we are going to use a specific nanovector Polycefin (Ljubimova et al. 2012), to one molecule of which inhibitors of multiple molecular targets may be attached easily.

## Timing characteristics of degeneration of nigrostriatal dopaminergic neurons in mice at the model of the early clinical stage of Parkinson's disease

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Degeneration of nigrostriatal dopaminergic (DA-ergic) neurons leads to a dopamine (DA) deficit in the striatum and development of Parkinson's disease (PD). Long-term asymptomatic development of PD is explained by activation of compensatory processes in the brain. Motor dysfunctions of PD, resting tremor, rigidity and bradykinesia, appear after a threshold degradation of the DA-ergic neurons (a loss of 50-60 % of nigral cell bodies and 70 % of striatal axons) and depletion of the brain compensatory reserves. The **aim** of this study was to examine the timing characteristics of degeneration of nigrostriatal DA-ergic neurons in mice with modelled early clinical stage of PD for a subsequent use of this model for searching potential neuroprotectors. **Materials and Methods.** The model of the early clinical stage of PD was obtained by 4 injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a precursor of DA-ergic neurons neurotoxin, at the dose of 12 mg/kg with a 2 h interval between injections (Ugrumov et al., 2011). The timing characteristics of nigrostriatal DA-ergic neurons degeneration in MPTP-treated mice were evaluated by quantification of surviving neurons, nigral cell bodies and striatal axons. Surviving DA-ergic neurons were identified by immunostaining of tyrosine hydroxylase (TH), the rate-limiting enzyme of DA synthesis. Moreover, contents of DA and TH in cell bodies and axonal terminals of DA-ergic neurons were estimated as an index of the functional state of surviving DA-ergic neurons. **Results.** An initial loss of DA-ergic cell bodies in the SN was observed 3 h after the last MPTP injection. At that time, the number of DA-ergic axonal terminals in the striatum was reduced by 65 % compared to the control. A loss of DA-ergic cell bodies and axons continued for the next 3 h, whereas their number was not changed for the next 18 h. The TH concentration in nigral cell bodies remained at the control level during the whole studied period, 24 h after the last injection of MPTP. The DA content in the SN decreased by 70 % 3 h after the last injection of MPTP, followed by its continuous increase in subsequent 21 h up to 70 % of the control value. Obtained data suggest that TH activity decreased in first few hours after the last injection of MPTP. The concentration of DA in the striatum was reduced by 90 % compared to the control over the period from 3 to 24 h after the last injection of MPTP. The TH concentration in the axonal terminals decreased by approximately 25 % and 40 % after 3 and 6 h after the last injection of MPTP, respectively, without any change over next 6 h, and increased up to 82 % of the control value 24 h. A partial loss of DA in the striatum may be either result of decrease of its synthesis caused by reduced TH activity or a compensatory increase of DA release in the striatum. The availability of our parkinsonian model for searching and testing of potential neuroprotectors was evaluated by using nomifensine, the inhibitor of the membrane transporter of DA and MPTP (MPP+). It was shown that the number of axonal terminals was reduced only by 10 % without any loss of DA in the striatum 12 h after their simultaneous treatment with MPTP and nomifensine. By contrast, the mice treatment with only MPTP resulted in a loss of 50 % of striatal DA-ergic axons. **Conclusions.** Timing characteristics of degeneration of the nigrostriatal dopaminergic neurons in MPTP-treated mice were evaluated in this study showing availability of this model for searching potential neuroprotectors.

## Alternative mechanisms account for plant mitochondrial competence

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**Aim.** Up to now, such question as how DNA molecules of different size traverse outer and inner mitochondrial membranes is still unclear. However, the resolution of this problem is the critical prerequisite for the development of efficient gene transfer system in mitochondria of plants and mammals. Earlier it was shown that mitochondria, isolated from various plant species (potato, maize, cauliflower, tobacco cell suspension culture) and from mammals (rat liver, human cell cultures) are capable to import double-stranded linear DNA molecules of a reasonable size ( < 10 kb) via an active mechanism, nonspecific to DNA sequence and involving the permeability transition pore complex in plant mitochondria (Koulintchenko et al., 2003; 2006). The main substrate for mitochondrial DNA import in such a studies was the PCR-product corresponding to the sequence of mitochondrial plasmid of 2.3 kb from maize. It was shown that (i) DNA import into plant mitochondria does not depend on nucleotide sequence of the imported molecule; (ii) the import efficiency decreases with increasing of imported DNA molecule size; (iii) import of circular DNA molecules is not as effective as that of linear molecules; (iv) single-stranded DNA is not imported into mitochondria. But in further study (Ibrahim et al., 2011) it was established that the DNA import into plant mitochondria is specific with respect to large DNA substrates. For DNA import into plant mitochondria and into mitochondria of human cell culture the linear plasmid from rapeseed mitochondria (*Brassica napus* L.) with the size 11.6 kb was used. This plasmid, like the 2.3 kb plasmid from maize is characterized by the terminal inverted repeats (TIRs) present at each end of the molecules. It's known that inside the mitochondria TIRs are covalently bound to proteins involved in replication and maintaining of the plasmids. It was shown that (i) the efficiency of large DNA molecules import into plant mitochondria depends on molecule sequence; (ii) the specificity of DNA import is mediated by the presence of TIRs. The aim of the present study was to clarify the question, there is one or multiple paths for different size DNA molecules specific transport into plant mitochondria. **Methods.** To study different size DNA molecules import efficiency the freshly isolated intact mitochondria from potato tubers (*Solanum tuberosum*) were used. The respiration control value of mitochondria used in experiments was ca 4.0 with succinate as respiratory substrate. To estimate the efficiency of exogenous DNA uptake by mitochondria two methods were used (evaluation of isotopic labeling DNA transport into mitochondria and quantitative PCR). **Results.** We had showed that the efficiency of 9 kb DNA molecules import into isolated potato mitochondria is increased substantially in the case of incorporation in their structure of terminal inverted repeats (327 bp) of the linear 11.6 kb plasmid from *Brassica napus* mitochondria. Another important finding is the different sensitivity of import activity in case of using different size DNA substrates (109 bp, 269 bp, 717 bp, 1540 bp, 3700 bp, 6000 bp, 9000 bp) to the action of such an inhibitor of porin (VDAC) in the outer mitochondrial membrane – Ruthenium Red and inhibitors of ADP/ATP carrier in the inner mitochondrial membrane – atractyloside and carboxyatractyloside. We revealed that the treatment of potato mitochondria by Ruthenium Red even increased substantially the import efficacy for definite size of DNA molecules. **Conclusions.** The DNA import into plant mitochondria is presumably specific with respect to substrate sequence context and size. Therefore, we propose the alternative biochemical pathways for transfer of different size DNA molecules into plant mitochondria.

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# Haplotype HLA-DQ frequency distribution in Latvian patients with Lyme disease

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**Introduction.** Lyme disease (Lyme borreliosis) is caused by infection with the tick- borne bacterium *Borrelia burgdorferi*. Human pathogen, *B. burgdorferi* causes a multisystem disease that may affect the skin, nervous system, heart, or joints. The disease incidence in Latvia is one of the highest in Europe. The genes encoding the HLA-DQ heterodimer molecules, DQA1 and DQB1, have been found to have the association with Lyme borreliosis risk, although there is cumulative evidence for the effect of other gene loci within the major histocompatibility complex gene region. **Objective.** The purpose of this study was to determine of HLA -DQ haplotypes in patients with clinical, epidemiological and laboratory approved Lyme borreliosis diagnosis. **Materials and methods.** The study included 91 patients with clinical stage – erythema migrans and 100 control (healthy) persons. The diagnosis was confirmed and imposed Latvian Infectology Center. Immunogenetic examinations were performed RSU Clinical Immunology and Immunogenetic Laboratory with PCR-SSP method. **Results.** The frequency of haplotypes: DQA1*02:01:01/DQB1*03:02:01 (OR 3.27; p < 0,040);
HLA- DQA1*05:01:01/DQB1*02:01:01 (OR 2.54; p < 0,048)
and -DQA1*01:01:01/DQB1*02:01:01 (OR 2.11; p < 0,031) were significantly increased in the Lyme *disease* patients compared with the control groups.But, the haplotypes of DQA1*01:02:01/DQB1*06:02:01(OR 0.24; p < 0.036); DQA1*01:03:01/DQB1*06:02:01 (OR 0.27; p < 0.046) and DQA1*01:02:01/DQB1*06:02:01 (OR 0.31; p < 0.029) were smaller in *Borreliosis* patients and significantly higher in controls. **Conclusions.** This data suggest that HLA-DQ haplotypes may have a considerable effect on susceptibility/or protection to Lyme borreliosis. In particular, HLA -DQA1*02:01:01/DQB1*03:02:01; -DQA1*05:01:01/DQB1*02:01:01; and -DQA1*01:01:01/DQB1*02:01:01 haplotypes definitely contributes to a genetic predisposition to Borrelia burgdorferi infection in Latvian population, which may have implications in our understanding of pathogenesis of this disease.

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## Selenoproteins in health and disease. Towards understanding their peculiar mechanism of biosynthesis

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**Aim**. Selenoproteins harbor the selenium-containing amino acid selenocysteine (Sec) in the active site. These proteins fulfill as varied functions as defence against reactive oxygen species, sperm and thyroid hormone maturation, muscle biogenesis. Selenoprotein biosynthesis is not a standard mechanism because UGA, otherwise a stop codon for translation, encodes selenocysteine. Reading selenocysteine instead of stop at the UGA Sec codon is a complex event that requires a dedicated machinery with specialized RNAs and proteins, including the SECIS stem-loop in the 3'UTR of selenoprotein mRNAs and the SECIS binding protein SBP2. Mutations in these or other components lead to embryonic lethality or various pathologies, attesting the importance of selenoproteins in health and disease. At the mechanistic level, the central question is to understand how assembly of a selenoprotein mRNA-multicomponent complex occurs in an orderly and temporal fashion so that approaching ribosomes are cleared at the UGA Sec codon. As little was known regarding the issue, our goal was to understand where and when selenoprotein mRNAs and components of the machinery interact with the ribosome in the course of translation. **Methods**. We used synthetic mini-selenoprotein mRNAs carrying photoactivatable groups in the SECIS motif for UV cross-linking to ribosomes; hydroxyl radical footprinting of SBP2-human ribosomes complexes; bi-functional reagents to cross-link SBP2 to purified human ribosomes. RNA-protein cross-links were revealed by gel electrophoresis. **Results**. We showed that SBP2 is bound to the SECIS at the pre-initiation step of translation; it then binds the ribosome at the pre-translocation step to bring the Sec-$tRNA^{Sec}$ to the UGA Sec codon; subsequently, SBP2 returns to the SECIS after the transpeptidation step. We also identified the binding site of SBP2 on the human ribosome. **Conclusion**. Altogther, our findings led for the first time to broaden our understanding about the unique mechanism of selenoprotein incorporation in mammals. Also, our study established that SBP2 makes direct contacts with a discrete region of the human 28S ribosomal RNA.

## PARP1 as a mediator of the catalytic activity of the enzymes processing apurinic/apyrimidinic site

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The apurinic/apyrimidinic (AP) site is considered to be a common lesion in genomic DNA. If unrepaired, AP sites present mutagenic and cytotoxic consequences to the cell [1]. The capacity of human poly( ADP-ribose) polymerase 1 (PARP1) to interact with the intact AP sites in DNA has been demonstrated by us earlier [2].The specificity of PARP1 interaction with AP sites was confirmed by the mutual tests. The identity of PARP1 cross-linked to AP sites in DNA in crude cell extracts was confirmed by mass spectrometry. PARP1 was weakly activated to conduct poly(ADP-ribose) synthesis upon binding to the AP site containing DNA. PARP1 interacting with AP sites catalyzes DNA strand incision by its internal AP lyase activity. PARP1 demonstrates also 5'dRP lyase activity. The interaction of PARP1 with AP sites modulates activity of the enzymes which catalyze the AP-site cleavage during the base excision repair (BER). The major enzyme which is responsible for the AP site cleavage activity is apurinic/apyrimidinic endonuclease 1 (APE1). The activity of this enzyme was influenced by PARP1 in the process of cleavage of DNA containing single or clustered AP sites. The capacity of the other enzyme, human tyrosyl-DNA phosphodieasterase (Tdp1), to the AP site cleavage was detected by us [3]. Tdp1 catalyzes the cleavage of an AP site and its synthetic analog, 3-hydroxy-2(hydroxymethyl)-tetrahydrofuran (THF), in DNA by hydrolysis of the phosphodiester bond between the substituent and 5'-adjacent phosphate. The product of the Tdp1 cleavage in the case of the AP site is unstable and is hydrolyzed with the formation of 3'- and 5'-margin phosphates. The following repair demands the ordered action of polynucleotide kinase phosphatase, with XRCC1, DNA polymerase β and DNA ligase. In the case of THF, Tdp1 generates break with the 5'-THF and the 3'-phosphate termini. Tdp1 is also able to effectively cleave the non-nucleotide insertions in DNA, decanediol and diethyleneglycol moieties, by the same mechanism as in the case of the THF cleavage. The efficiency of the Tdp1 catalyzed hydrolysis of the AP-site analog correlates with the DNA helix distortion induced by the substituent. The following repair of 5'-THF and the other AP site analogs can be processed by the long-patch base excision repair pathway. PARP1 stimulates activity of Tdp1 in the AP site cleavage. The protein–protein interactions of PARP1 with APE1 and Tdp1 were detected by various techniques. These interactions were suggested as a basis of the modulation of APE1 and Tdp1 activities by PARP1 in the AP site processing.

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## Tyrosyl-DNA phosphodiesterase 1 initiates repair of apurinic/apyrimidinic sites in single stranded DNA

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Tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the hydrolysis of the phosphodiester linkage between the DNA 3′ phosphate and a tyrosine residue as well as a variety of other DNA 3′ damaged termini. Recently we determined that human Tdp1 interacts with AP sites and catalyzes the AP-site-cleavage reaction to generate breaks with the 3′- and 5′-phosphate termini. Tdp1, unlike APE1, much more efficiently cleaves the AP-site, located in the single-stranded DNA regions or opposite to bulky DNA damages in the regular DNA duplexes. This property of Tdp1 suggesting that this enzyme has the potential to be involved in AP site repair in the single-stranded regions of the genomic DNA, resulting in its metabolic processes, including during replication, DNA repair and transcription. It should be noted that the destabilization of the double helix DNA and, consequently, the occurrence of single-stranded regions in it significantly increases the likelihood of AP sites, as a result of spontaneous apurination / apyrimidination of DNA and under the action of DNA glycosylases, removing the modified (oxidized or alkylated) base. The introduction of bulky DNA damage does not affect the binding of Tdp1 with DNA containing a "bubble", but reduces the efficiency of the AP-site cleavage relative to DNA structure without a bulky lesion, possibly due to steric hindrance created by the bulky lesions. The observed effect may have biological significance, because such DNA structure represents the intermediate of nucleotide excision repair (NER) process, formed as a result of the partial unwinding of the DNA duplex around the bulky lesion by the NER factors.

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## Effects of Wnt/β-catenin pathway and RET/PTC oncogene on tumour progression and treatment of papillary thyroid carcinoma

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Junction oncogenes are the consequence of genomic rearrangements, leading to intragenic gene fusion; they represent around 20 % of total cancers. In fact, deregulation of gene expression is a common feature in cancers. The possibility to inhibit the expression of an oncogene at the mRNA level, instead of blocking the function of the gene product, has elicited for a long time a great interest as the potential therapeutic applications are obvious. Therefore, RNA interference offers promising new opportunities to target very specifically genes deregulated in cancers carrying junction oncogenes. We aimed in one hand to inhibit dedifferentiation due to RET/PTC junction oncogene by siRNA and in the other hand to investigate the effect of the inhibition on the thyroid transcription factor 1 (TTF-1), a tissue-specific transcription factor essential for the differentiation of the thyroid. **Methods.** First, we designed siRNA against RET/PTCs (siRNARET/PTC1 and RET/PTC3) and tested their efficiencies and specificities by RT-qPCR and by western blot in cell lines expressing RET/PTCs junction oncogenes. The effects on cell cycle growth (MTT tests), cell cycle (flow cytometry), and apoptosis (caspase-3 clivage and TUNEL method) were studied. Then the siRNA RET/PTCs were conjugated to squalene (SQ) and the corresponding nanoparticles (NPs) were prepared by nanoprecipitation and characterised for their size and zeta potential. *In vivo* gene silencing efficiency of NPs was assessed by administration in nude mice either *via i.v.* or intra tumoral (*i.t.*) routes. Tumour growth was followed during the course of experiment while gene and protein inhibitions were assessed by RT-qPCR and Western blotting. To investigated if the Wnt/β-catenin pathway might regulate TTF-1 expression in a human PTC model we knock-down the Wnt/β-catenin components by siRNAs and mimicking the activation of Wnt signaling by lithium chloride. Functional promoter studies and ChIP analysis were also performed to localize the TCF/LEF binding site into TTF-1 promoter. **Results.** Concerning RET/PTCs junction oncogenes, the designed siRNA against RET/PTC1 and RET/PTC3 were found to decrease oncogene expression > 80 %. This inhibition of mRNA levels is paralleled with a reduction of protein content that persists for at least 72h. Furthermore, the siRNAs are able to decrease cell proliferation of about 50 % and to increase apoptotic index and to cleave caspase-3. Interestingly, *in vivo,* on a mice xenografted RET/PTC experimental model, RET/PTCs-SQ NPs were found to inhibit tumor growth and RET/PTCs oncogene and oncoprotein expression. Then, we investigated whether the Wnt/β-catenin pathway regulate TTF-1 expression. Knocking-down the Wnt/β-catenin components by siRNAs inhibited both TTF-1 transcript and protein expression. Activation of Wnt signalling by lithium chloride induced TTF-1 gene and protein expression. Functional promoter studies and ChIP analysis showed that the Wnt/β-catenin pathway exerts its effect by means of the binding of β-catenin to TCF/LEF transcription factors on the level of an active TCF/LEF response element at [-798, -792 bp] in TTF-1 promoter. Moreover, immunohistological studies performed on human normal thyroid and PTC tissues revealed that TTF-1 and transcription factor 4 (TCF4) proteins were expressed in the same areas. **Conclusions.** These results showed that we success to establish siRNA efficient to RET/PTC able to inhibit tumour growth *in vivo.* Moreover, we demonstrated that the Wnt/β-catenin pathway is a direct and forward driver of the TTF-1 expression.

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## Differential Regulation by ppGpp vs pppGpp

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Background: Multidrug resistance caused by bacterial persisters poses a serious problem in the treatment of reoccurring or chronically bacterial infections. Bacterial persisters are transiently drug tolerant bacteria that enter a physiological state allowing the escape from lethal action of drugs in a mechanism different from antibiotic resistance. The bacterial stress alarmone (p)ppGpp plays an important role in pathogenesis and in particular in persistence. Binding of (p)ppGpp to RNA polymerase results in reprogramming of bacteria by affecting the expression of hundreds of genes. Both ppGpp and pppGpp are thought to function collectively as second messenger. There are few indications that their regulatory effects might be different, however this question has been largely unexplored for lack of an ability to experimentally manipulate the relative abundance of ppGpp and pppGpp.

**Aim**: In order to understand the molecular mechanism underlying (p)ppGpp controlled mechanisms better we wanted compare the effects of ppGpp and pppGpp on stringently controlled processes.

**Methods**: Preferential accumulation of either ppGpp or pppGpp was achieved in *E. coli* strains through induction of different *Streptococcal* (p)ppGpp synthetase fragments. In addition, expression of *E. coli* GppA, a pppGpp 5'-gamma phosphate hydrolase that converts pppGpp to ppGpp, was manipulated to fine tune differential accumulation of ppGpp and pppGpp.

**Results**: *In vivo* and *in vitro* experiments show that pppGpp is less potent than ppGpp with respect to regulation of growth rate, RNA/DNA ratios, ribosomal RNA P1 promoter transcription inhibition, threonine operon promoter activation and RpoS induction. To provide further insights into regulation by (p)ppGpp, we have also determined crystal structures of *E. coli* RNA polymerase-$\sigma^{70}$ holoenzyme with ppGpp and pppGpp. We find that both nucleotides bind to a site at the interface between β' and ω subunits.

# Endocytic scaffold ITSN1: novel connections and way of regulation in neurons

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ITSN1 is an endocytic scaffold that has a considerable impact in functioning of a nervous system. Changes in its expression level or disruption of its function were reported to affect synaptic vesicle endocytosis and dendritic spine morphology. Overexpression of *Itsn1* gene is associated with development of Down syndrome and Alzheimer's disease. Thus, a role of ITSN1 in neuronal development and functioning is an important theme for research.

It is widely accepted that many cellular events in neurons are regulated by change in concentration of $Ca^{2+}$ ions. ITSN1 molecule possesses two EF-hand motifs that can potentially bind $Ca^{2+}$ ions within its EH domains. However, we have shown that presence of $Ca^{2+}$ does not affect binding of ITSN1 EH domains with their interactor epsin 1. Additionally, bioinformatic predictions indicate that ITSN1 can potentially be phosphorylated by $Ca^{2+}$/calmodulin-dependent kinases. In order to test this hypothesis we purified fraction of Ca/calmodulin-binding proteins from mouse brain lysate by affinity chromatography and used them for *in vitro* kinase reaction with recombinant fragments of ITSN1. We have shown that at least two fragments of ITSN1 containing coiled-coil and SH3 domain-containing regions are phosphorylated in presence of $Ca^{2+}$ ions and calmodulin. Using tandem mass spectrometry we have identified 9 sites of $Ca^{2+}$/calmodulin-dependent phosphorylation of ITSN1, suggesting existence of $Ca^{2+}$-dependent regulation of ITSN1 functioning.

Using in vitro-binding assay and MALDI-TOF mass spectrometry we have identified novel neuronal ITSN1-binding protein STOP (stable tubule-only polypeptide) — a microtubule-associated protein that is required for formation of cold-stable subpopulations of microtubules in neurons and some other cell types. Complexes of ITSN1 and STOP were precipitated from mouse brain lysate by both antibodies against STOP and ITSN1. Moreover, we found that ITSN1 and STOP are partially co-localized in primary rat hippocampal neurons. Functional role of interaction between ITSN1 and STOP remains unknown. Considering the role of microtubules in formation of dendritic outgrowth, we used shRNA-mediated knock-down assay in order to reveal the effect of ITSN1 level decrease on morphology of cultivated hippocampal neurons. We found that total dendritic length is decreased in shRNA-transfected cells compared to cells transfected with empty vector. Further studies will be aimed to uncover the mechanism of ITSN1 influence on dendritic growth and its possible role in formation of alterations in Down syndrome brains that were reported previously.

## Charge-dependent mechanism for retention of proteins in the nucleoli via dynamic interaction with nucleolar RNAs

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**Aim.** The majority of known nucleolar proteins examined thus far are highly mobile: these proteins diffuse rapidly in the nucleoplasm and typically exchange quickly with their binding sites. The key questions are how macromolecules find their target sites inside the nucleus, and how they accumulate in these target sites. One way of protein retention in the nucleolus is associated with the presence of specific short amino acid sequences – nucleolar localization signals (NoLSs). The aim of the present study was to investigate mechanism by which NoLSs may lead to the nucleolar accumulation of the proteins. **Methods.** There are several ways for NoLS identification and investigation. We have used the approach based of analysis of tested sequence as factor for nucleolar accumulation of marker fluorescent protein (EGFP). To quantitatively estimate the effect of tested sequence on the nucleolar accumulation of GFP, the NoLS activity (concentration of EGFP in the nucleoli/concentration of EGFP in the nucleoplasm) was measured for all fusions obtained. **Results.** Three groups of observations are in agreement with the hypothesis of that charge-dependent (electrostatic) interactions of NoLSs with nucleolar components lead to nucleolar accumulation. (i) Known NoLSs are enriched in positively charged amino acids, but the structure of NoLSs is highly heterogeneous, and it is not possible to identify a consensus sequence for this type of signal. (ii) In two analyzed proteins (NF-κB inducing kinase and HIV Tat), the NoLS coincide with the boundaries of the region enriched with positively charged amino acids. Using the method of quantitative estimation of nucleolar accumulation, after substitution of charged amino acids to non-charged we were able to demonstrate that there is a strong correlation between the charge of protein fragment tested as a NoLS and its ability to accumulate marker protein (EGFP) in the nucleoli. (iii) The sequences containing only lysine or arginine (which were referred to as imitative NoLSs, iNoLSs) were accumulated in the nucleoli in a charge-dependent manner. We investigated localization of the strongest iNoLS, containing 19 agrinines (EGFP-R19), and found that the localization and some of properties were closely similar to that of B23, the protein of late rRNA processing. Using this iNoLS, we demonstrated that charge-dependent accumulation inside nucleoli was dependent on interaction with nucleolar RNAs. **Conclusions.** These results are in agreement with the hypothesis that the accumulation of proteins in the nucleolus by NoLSs can be determined by the electrostatic interaction of positively charged signals with nucleolar RNAs.

## PUB-NChIP – "*in vivo* biotinylation" approach to study chromatin in proximity to a protein of interest

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**Background** : Chromatin Immunoprecipitation (ChIP) has been widely used for over a decade to study the in vivo association of a particular DNA sequence with regulatory proteins. However, due to the need to use chemical crosslinking, the protein part of chromatin is typically discarded in this approach. On the other hand, the analysis of histone modifications and replacement histone variants could provide valuable information about the state of chromatin around the particular protein of interest.
**Aims** : Our aim was to develop an alternative approach to ChIP that would allow us to preserve the protein part of chromatin associated with a protein of interest, in order to study it with proteomics methodology.
**Methods** : Our method is based on coexpression of a) a protein of interest, fused with the bacterial biotin ligase BirA together with b) a histone fused to BAP biotin acceptor peptide, which is specifically biotinylated by BirA fusion in the proximity of the protein of interest.
**Results** : Using RAD18 protein as a model, we demonstrate that the RAD18-proximal chromatin is enriched in some H4 acetylated species. Moreover, the RAD18-proximal chromatin containing a replacement histone H2AZ has a different pattern of H4 acetylation. Finally, biotin pulse-chase experiments show that H4 acetylation pattern starts to resemble acetylation pattern of total H4 after the proximity of chromatin to RAD18 has been lost.
**Conclusions** : We hope that the new method will find a wide use in the fields of chromatin studies and epigenetics.

# Changes in bone marrow mesenchymal stem cell count during osteoreflextherapy

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**Aim.** The human organism ages over its lifetime, and that may lead to different diseases including Alzheimer's disease, atherosclerosis, osteoporosis etc. Many age-related illnesses can be treated with osteoreflextherapy (ORT), which is based on stimulation of the bone sensory system. Osteoreceptors are in close contact with the central nervous system and thus different organs and organ systems can be affected through reflex arcs. ORT entails the injection of physiological solution into the marrow of porous bone, thereby stimulating osteoreceptors. Among the cell types in bone marrow are multipotent mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, chondrocytes, muscle cells and other cell types. MSCs migrate to sites of damage in the body and can regenerate multiple tissues. Since MSCs do not have one specific marker, different markers can be used to identify MSCs, including CD105 (endoglin), CD29 (integrin- ) and CD54 (intracellular adhesion molecule-1). The aim of the present study was to determine how osteoreflextherapy affects mesenchymal stem cell count in bone marrow. **Methods.** Patients ($n = 21$) undergoing ORT once a week had bone marrow harvested from different bones, depending on their illness. Females ($n = 12$) ranged from 50 to 87 years of age while males ($n = 9$) were 17 to 59 years of age. Prior approval was obtained from the Scientific Research Ethics Committee of the University of Latvia Institute of Experimental and Clinical Medicine. Marrow samples from each patient were obtained twice: prior to ORT and four weeks following the initiation of ORT. The counts of CD105, CD29 and CD54 antigen-expressing cells were determined by immunocytochemistry and light microscopy. **Results.** Prior to ORT, $CD105^+$, $CD29^+$ and $CD54^+$ cell counts were $0.08 \pm 0.02$ %, $0.08 \pm 0.02$ % and $0.08 \pm 0.03$ %, respectively, of mononuclear bone marrow cell count. Four weeks after the initiation of ORT, $CD105^+$, $CD29^+$ and $CD54^+$ cell counts were $0.12 \pm 0.03$ %, $0.10 \pm 0.03$ % and $0.11 \pm 0.03$ %, respectively, of mononuclear bone marrow cell count. This increase in antigen-expressing cell counts after initiation of ORT was statistically significant ($p < 0.001$) in all three groups, while the difference among the three groups of antigen-expressing cells was not significant ($p > 0.05$). Statistically significant ($p < 0.05$) Pearson's correlations were between $CD105^+$ and $CD54^+$ cell counts before ORT and after initiation of ORT ($r = -0.47$ and $r = -0.49$, respectively) and also between increase in $CD105^+$ cell count after ORT initiation and patient age ($r = -0.47$). **Conclusions.** The variable increase in CD105, CD29 and CD54 antigen-expressing cell count following initiation of ORT could be attributable to the limited number of mesenchymal stem cells in human bone marrow. Minimal increases observed in MSC count following ORT initiation could be due to an initial MSC count close to the upper limit in bone marrow, while greater increases in MSC count could be due to low pre-ORT MSC levels. This difference seemed to be age-dependent, as increases in MSC count in older patients were not as pronounced as those observed in younger patients. Increased MSC counts in bone marrow through osteoreflextherapy could be one of successful therapy factors.

## Association of the *PSMB5* (rs11543947), *PSMC6* (rs2295826, rs2295827) genes polymorphismswith obesity in the Latvian population

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**Aim.** The ubiquitin-proteasome system (UPS) is the major nonlysosomal proteolytic pathway that affects crucial intracellular processes including the cell cycle, the "quality control" of newly synthesized proteins, transcription factors machinery, gene expression, cell differentiation, homeostasis and apoptosis. Multiple data have proved that UPS has the potential to be a therapeutic target for treatment of some diseases (vascular disorders, diabetes mellitus, autoimmune diseases, cancer and its outcome). Importance of proteasomes in both normal and pathological processes triggers interest for search of sequence variations in the proteasome genes to be associated with human pathologies. The genetic variations of the *PSMA6, PSMC6* and *PSMA3* proteasome genes were previously shown to be associated with JIA, T2DM, BA and cardiovascular disorders. Aim of the present study is to investigate polymorphisms in the *PSMB5* and *PSMC6* genes for association with children obesity in Latvian population.

**Methods.** The rs11543947 (*PSMB5)*, rs2295826 and rs2295827 (*PSMC6)* loci were genotyped in 94 overweight children versus 191 controls. Stratification was performed by sex and family history of obesity. Both the two-tailed Fisher's exact test and the $\chi^2$ test were applied. Only 2< OR < 0.5 was considered to be clinically significant.

**Results.** In both case and control cohorts the genotyping call rate was 100% and markers were found to be in Hardy-Weinberg equilibrium. The rs2295826 and rs2295827 were found to be in linkage in Latvians (D' =1; $r^2$=1). In obesity and family obesity groups nominal risk effect for rare allele's variations G/T ($P$<0.05) was detected. Risk 2-loci genotype AG/CT showed nominal association ($P$<0.01) with obesity and obesity with family history.

For rs11543947 rare allele T detected nominal risk effect ($P$<0.01) for obesity and obesity with family history. The heterozygous genotype CT showed strong association ($P$<0.001) with obesity and family obesity (OR = 2.641 [95% CI 1.484 − 4.699] and (OR = 2.966 [95% CI 1.542 −5.706] respectively. In cases for obesity without family history all markers were considered to be the diseases neutral ($P$ > 0.05).

**Conclutions:** Genetic variation of *PSMB5* (rs11543947) and *PSMC6* (rs2295826 and rs2295827) 14q proteasomal genes may influence children obesity in Latvian population.

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## Prolonged culture of skin-derived mesenchymal stem cells changes their cell cycle and proliferation marker expression

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**Aim.** Mesenchymal stem cells (MSCs) are present in many tissues, including bone marrow, adipose tissues, skin and dental pulp. Skin-derived MSCs can be induced to differentiate into mesodermal lineages as well as neuronal and glial cells which make them suitable candidates for regenerative therapies, however, there is a substantial need for evidence that cultured MSCs would be safe for patients if used as therapeutic agents. The aim of this study was to analyse changes of different cell cycle and proliferation marker (TERT, Ki-67, polyploidy) expression during prolonged culture of human skin derived mesenchymal stem cells. **Methods.** Skin derived MSC cultures were propagated in DMEM/F12 medium supplemented with fetal calf serum. Cultured MSC were characterized for expression of MSC markers (CD90, CD73, CD105) by flow cytometry. For TERT expression analysis, cells were seeded on coverslips and propagated for 48 hours followed by immunofluorescence analysis. Results were confirmed by Western blot analysis. Cell cycle marker Ki-67 expression and polyploidy was analysed by flow cytometry. **Results.** Prolonged culture of skin-derived mesencymal stem cells changes their cell cycle and proliferation marker expression: later passages of MSC tend to show higher TERT expression, but Ki-67 expression decreases with a later passage number. Flow cytometry data also indicate that later passage number is associated with an increase of polyploidy in cultured MSC. These common trends remained to show up regardless of donor-to-donor differences in cell cycle marker expression between different MSC lineages. **Conclusions.** There is a strong correlation between MSC culture passage number and increased expression of telomerase activity marker TERT and decreased expression of Ki-67. Between TERT and Ki-67, there is a strong inverse relation, i.e., higher TERT expression is associated with lower Ki-67 expression, and *vice versa*. Prolonged propagation of MSC causes increase of polyploidy. However, the possible effect of these findings on potential therapeutic applications of skin-derived MSC still remains to be elucidated.

**Interactions of the NER factors with DNA are modulated by PARP1 activity**

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Nucleotide excision repair (NER) is one of the major mechanisms to prevent genomic DNA instability. This process removes a wide range of lesions distorting the double helix and bulky chemical adducts resulting from environmental factors or chemotherapeutic agents. The coordination of the assembly of the NER complexes and the sequential individual reactions is achieved through multiple DNA-protein and protein-protein interactions. The interactions of key protein factors of the NER process, XPC-RAD23B, XPA, and RPA with DNA structures mimicking NER intermediates and their modulation by PARP1 activity have been analyzed. **Methods.** The topography of the NER protein complexes with damaged DNA was determined by photoaffinity labeling technique using DNA structures containing photoreactive 5I-dUMP residues in the certain positions either in damaged or in undamaged strands and fluorescein group linked to uridine residue as a lesion. Electrophoretic mobility shift assay (EMSA) and fluorescent depolarization measurements were used for the analysis of DNA-protein binding. Western blotting analysis was used to determine ADP-ribosylated proteins. **Results.** Positioning of human and yeast DNA damage recognition complexes, XPC-RAD23B and Rad4-Rad23, respectively, on damaged DNA was determined. Under conditions of equimolar binding to DNA both proteins exhibited the highest level of crosslinks to 5I-dUMP located exactly opposite the damaged nucleotide. Both proteins bind to the damaged 15 nt bubble-DNA structure mimicking in size the “transcription bubble” DNA intermediate with the highest affinity that is reduced in the following order: damaged bubble>undamaged bubble>damaged duplex>undamaged duplex. The affinity of XPC/Rad4 for various DNA structures correlates with DNA bending angle. RPA and XPA are very abundant proteins that are absolutely required for NER functioning. Both RPA and XPA proteins stimulate XPC binding to the damaged DNA and enhance the level of XPC-DNA crosslinks. Poly(ADP-ribose)polymerase-1 (PARP-1) is one of the candidate to participate in NER regulation. PARP-1 is rapidly activated in response to DNA damage using $NAD^+$ as a substrate to form poly(ADP-ribose) (PAR) subunits. Using EMSA, the influence of PARP1 on binding of XPC-RAD23B and XPA to model DNA-structures modulated by PAR synthesis was revealed. Both XPC and RAD23B subunits of DNA damage recognition complex were found to be ADP-ribosylated by PARP1. **Conclusions.** Obtained data fill the gap between biochemical results for XPC-RAD23B and X-ray structure for yeast ortholog Rad4-Rad23 and exhibit a significance of protein-protein interactions for the correct assembling of the NER machinery. PARP1 can be regarded as the universal regulator in DNA repair processes.

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# Mobility of broken proto-oncogenes within nuclear space of cells treated with DNA topoisomerase poisons.

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*Aim:* Today it is known that treatment of cells with topoisomerase poisons leads to the formation of double stranded DNA breaks in breakpoint cluster regions (BCRs) of known proto-oncogenes such as *MLL* or *AML1*. And that incorrect repair of these breaks may cause chromosomal rearrangements which in turn may induce the so-called treatment-related leukaemias. But the exact mechanisms of these rearrangements remain unclear.
*Methods:* We have treated cultured human lymphoid cells (Jurkat) with etoposide or camptothecin and then visualized the chromosomal territories and proto-oncogenes with using 3D-FISH technique. 3D images have been processed by using software which detects differently colored parts of proto-oncogenes (upstream and downstream of BCR) and labeled chromosomal territories.
*Results:* We have found that exposure of Jurkat cells to etoposide resulted in frequent cleavage of *MLL* and *AML1* genes, with the flanks of the break located distant from each other and are often found outside of chromosomal territories. Therewith it was no differences between 5'- and 3'- flank of the breaks. It was also observed that spatially separated flanks of proto-oncogenes are more frequently located outside of chromosomal territory than non-separated ones. Treatment of cells with camptothecin does not affect the integrity of *MLL* and *AML1* genes.
*Conclusion*: Our findings demonstrate that flanks of the breaks introduced in BCR of *MLL* or *AML1* gene under inhibition of DNA topoisomerase II acquire additional mobility inside the nucleus and tends to be on the edge or even outside of corresponding chromosomal territory. And this may in turn contribute to the more probable meeting and incorrect joining of translocation partners.
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## Poly(ADP-ribose) glycohydrolase in DNA repair and replication: towards potential applications in anticancer strategies ?

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**AIM:** Poly(ADP-ribosyl)ation is a post-translational modification of proteins involved in a wide number of biological processes including DNA repair, transcription, cell differentiation or cell death. The regulation of poly(ADP-ribose) produced in response to DNA damage by the poly(ADP-ribose) polymerases (PARPs), and degraded by poly(ADP-ribose) polymerase (PARG) is critical for the damaged cell fate. The role of the founding member of the PARP family PARP-1 is highly documented in the DNA damage response. Moreover, PARP inhibitors are involved in clinical trials to potentiate the action of anticancer clastogenic drugs as well as for their cytotoxic effect on tumours harbouring mutations in genes involved in double strand breaks repair by homologous recombination, such as BRCA1/2. Far less is known about PARG and the role of its different isoforms in the cell response to DNA damage. A major goal of our laboratory is to determine whether PARG could also be considered as a promising target for anticancer strategies. We have shown previously that the absence of PARG increases radiosensitivity and affects the repair of radioinduced single (SSB) and double (DSB) strand breaks. We have also demonstrated a functional link between PARG and the repair/replication factor PCNA: binding to PCNA contributes to PARG recruitment to laser induced DNA damage sites and to replication foci. This latter observation prompted us to investigate the contribution of PARG in DNA replication and replication stress. Replication stress is a source of DNA damage, leading to transient stalling of replication forks or to their collapse followed by the generation of double strand breaks (DSB). The involvement of PARP-1 in replicative stress response has been described, whereas the consequences of a deregulated PAR catabolism are not yet well established. **RESULTS:** Here, we show that wheFreas PARG appeared dispensable for normal replication, PARG-deficient cells showed increased sensitivity to the replication inhibitor hydroxyurea (HU). PARG is dispensable to recover from transient replicative stress but is necessary to avoid massive PAR production upon prolonged replicative stress, conditions leading to fork collapse and DSB. Extensive PAR accumulation impairs replication protein A (RPA) association with collapsed forks resulting in compromised DSB repair via homologous recombination. Our results highlight the critical role of PARG in tightly controlling PAR levels produced upon genotoxic stress to prevent the detrimental effects of PAR over-accumulation
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## Immunoregulatory role of Hsp60 at thyrod cancer progression

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The molecular chaperones (HSPs) are involved in numerous diseases, including cancer, revealing changes of their expression and cellular localization. The expression of Hsp60 in neoplasia has been implicated in the regulation of apoptosis, as a modulator of p53 function, in the immune response against tumors, and in multidrug resistance. A wide range of tumor cells or tissues have been shown to express atypical level or localization of HSPs. For example, Hsp60 expression in breast or gastric cancer is associated with poor prognosis and resistance to chemotherapy or radiation therapy. Such observations have led to suggestions that HSPs could be used as biomarkers. Molecular chaperon (chaperonin) Hsp60 is a special interest due to recently observed pro-inflammation potential of Hsp60 and anti-Hsp60 autoantibodies which have been detected in circulation of diseased and healthy persons as well. Hsp60 expression in thyroid cancer has not been studied extensively. The aim of our study was to evaluate the possible changes in Hsp60 cellular content and localization in thyroid gland' cells in normal state and upon pathology by quantitative immunohistochemical analysis and to exam the correlation between anti-Hsp60 autoantibodies level and the degree of thyroid gland lesions in this group of patients. ***Methods***. Sera from 49 patients (46 females and 3 males, 20-57 years old, 12 – nodular hyperplasia of thyroid gland, 12 – Hashimoto's thyroiditis, 18 – follicular adenoma, 6 –papillary and 1 follicular carcinoma) and 12 healthy donors (as a control) were used for determination of anti-Hsp60 antibodies level by ELISA. To evaluate the content and localization of Hsp60 in thyroid tissue of such patients and upon pathology we used immunohystochemical analysis. As a control, autopsy material of 12 thyroid tissue without morphological signs of thyroid pathology was used. Hsp60 expression was evaluated according to the percentage of positively stained cells. ***Results***. The increased anti-Hsp60 autoantibodies level has been detected in sera more than 50 % of patients with non-malignant thyroid gland pathology (Hashimoto's thyroiditis, nodular hyperplasia, follicular adenoma) and in 86 % of TC patients sera by ELISA method. This finding correlates with the degree of thyroid gland lesions in this group of patients using immunohistochemical studies. ***Conclusion.*** Significant increase of anti-Hsp60 antibodies level was determined in sera of patients with thyroid pathology. The highest titers of anti-Hsp60 antibodies in sera of patients with thyroid cancer has been revealed. Changes of Hsp60 expression and cellular localization in thyroid cancer tissue have been detected in comparison with normal ones. The increase of Hsp60 expression and elevated level of anti-Hsp60 autoantibodies was associated with morphological signs of pathology - lymphoid infiltration and sclerotic changes of tissue. The working model of Hsp60 involvement in thyroid cancer progression is proposed.

## Ki-67 and Oct3/4 expression in two types of breast cancer histological samples

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Breast cancer is the most common oncological disease in women. Improvement in treatment efficiency is a major priority. There is known more types of breast cancer, and each of them are characterised by biological markers who describe cancer malignancy.

**Aim.** To determine cancer cell resistance, cell population heterogeneity in luminal and triple negative breast cancer cell population using paraffin sections.

**Methods.** Women breast cancer primary operation material in paraffin sections. Two groups of breast cancer samples, luminal (n = 32) and triple negative breast cancer (n = 11). For each case were five cuts (1.5µm) from paraffin section. Biological markers (CD44, CD24, ALDH, Oct3/4) expression were detected by semi-quantitative method, where 0- expression was not detected, 1- low expression, 2- expression was detected in average low, 3- high expression, but not in whole sample, 4- strong expression in whole sample. In this study patient clinical data was summarized: age, tumour size and histological grade, and metastases in axillar lymph nodes, Ki67, ER, PgR and HER2.

**Results.** In both cancer types we observed very strong CD44 expression in different cell types but not in all histological structures. In triple negative breast cancer, CD44 is negatively correlated with lymph node metastases and positively correlated with proliferation marker Ki-67. In luminal breast cancer, type CD44 is positively correlated with histological grade.

In triple negative breast cancer, proliferation marker Ki-67 correlates with embryonic stem cell marker Oct3/4 and lymph node metastases. In luminal breast cancer, however, Ki-67 is negatively correlated with hormone receptors (oestrogen receptors and progesterone receptor).

**Conclusions.** Marker CD44 overexpression was observed in different cell types, among them, microcells, which possibly can be cancer stem cells. In triple negative breast cancer, proliferation marker Ki-67 correlates with embryonic stem cell marker Oct3/4 and lymph node metastases. The embryonic cell marker Oct3/4 was observed to be dominant in two cell types: polyploid cells and microcells.

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## Detection at the single molecule level of interaction of poly(ADP-ribose) polymerases 1 and 2 with base excision repair DNA intermediates by Atomic Force Microscopy

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**Aim.** Atomic force microscopy (AFM) is considered as one of the unique instrument for studying direct DNA-protein interactions. Here we designed synthesis of long 1400 bp DNA fragments containing single DNA damaged site, uracil or synthetic analog of apurinic/apirimidinic (AP) site –tetrahydrofuran (THF). Both AP site and uracil are common DNA lesions, which are repaired via base excision repair (BER) process. The repair process can be initiated by DNA glycosylase (UDG) action to produce an AP site, and then apurinic/apyrimidinic endonuclease (APE1) cleaves the phosphodiester bond 5′ to the AP site, generating a single strand break. The present biochemical studies indicate that the initial stage of BER may be modulated by other proteins involved in the process, among them PARP1 and PARP2. Although PARP1 (PARP2) do not have BER-related enzymatic activities, but the proteins interact with DNA intermediates containing AP site or single-strand breaks. To estimate the efficiency of interaction of these proteins with BER intermediates at single molecule level we tested the interaction of PARP1 and PARP2 with 1400 bp DNA fragment containing AP site or with one nucleotide gap using imaging of PARP1(PARP2)-DNA complexes with AFM. **Methods.** We prepared the 1400 bp DNA fragment by ligation of 30-mer DNA duplex with THF or uracil at a defined position into the pEGFP plasmid with followed by digestion of the plasmid with restriction enzymes (AseI and Not I) to generate 1400 bp fragment. Obtained DNA fragments have single THF or uracil in precise localization on DNA. We shown that the 1400 bp DNAs are ***processed*** by BER enzymes in system reconstituted from purified proteins, like UDG, APE1, DNA polymerase β and DNA ligase III. We developed experimental conditions for adsorption of PARP1 (PARP2) complexes with the 1400 bp DNA on Mica surface using putrescine ($Pu^{2+}$) in AFM deposition buffer. Such conditions enable to visualize the PARP1(PARP2)-DNA complexes and compare binding specificity of PARP-1 and PARP-2 with AP site or single nucleotide gap generated after AP site cleavage by APE1. **Results.** AFM-based quantitative data analysis shows that PARP1 interacts with similar efficiency both with TFH-site and with one nucleotide gap on 1400 bp DNA . The amount of PARP1-DNA complexes is slightly increased at damaged site after incision of AP site with APE1. In the contrast to PARP1, PARP2 shows low efficiency in interaction both with TFH-site and with single nucleotide gap. **Conclusion**. Thus, AFM data presented here support the notion that PARP1 has important role in modulation of BER process after forming of AP site and single nucleotide gap, while PARP2 do not play a critical role at these early stages of BER.

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## Potential implication of aminoacyl-tRNA synthetases in tumourigenesis


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Aminoacyl-tRNA synthetases (aaRSs) are ancient ubiquitous “house-keeping” enzymes that are responsible for cellular protein synthesis and cell viability. Also nontranslational functions of vertebrate aaRSs have recently been discovered. Taken together, new finding suggest that aaRSs have critical mechanistic roles in a variety of cellular processes which are relevant for disease development and pathology, and these roles may be used as one possible avenue for improvement of diagnostics and open a new dimension for cancer therapy.

To uncover the biomarkers related with tumorigenesis and behavior of cancer we have studied of differently expressed genes of some aaRSs in tissues of kidney and colon cancers by the quantitative polymerase chain reaction (qPCR) method.

Firstly, we have investigated the level of gene expression of cytoplasmic leucyl-tRNA synthetase (*LARS)* in A549 cell line and normal pulmonary epithelial cell line, and in tissues of kidney cancer and tissues of normal kidney. The level of mRNA expression of *LARS* in cancer cells A549 was more than 2 times higher than in cells of normal epithelium. In the case of kidney cancer from 16 samples of tumor tissue, increased expression of *LARS* was observed in 13, and only two were noted its slight decrease. However, only three samples showed a more than twofold increased expression, and one – more than three times. Thus, from a data on the samples of tissues of kidney tumors we used one can see a tendency for increasing of *LARS* expression.

In the case of human colon cancer we have observed the expression profile for *LARS*, histidyl-tRNA synthetase (*HARS*), seryl-tRNA synthetase (*SARS*) and lysyl-tRNA synthetase (*KARS*) in 18 primary cancer samples. We have found that genes of *HARS* and *KARS* underwent most changes. These genes might be used for diagnosis of colon tumors.

## Optimization of cell motility evaluation in scratch assay

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Migration is one of the most important cell functions that includes any directed movement and results in change of cell position within the body. It plays a key role in embryonic development, wound healing, immune response and a number of others fundamental biological processes. A scratch test is one of the most popular methods of classical cell migration assay in monolayer culture. At the same time, the scratch assay has some disadvantages that can be easily corrected.

**Aim:** The aim of the study is to justify and demonstrate that the detection of migration distance calculated from experimental wound surface area is more objective and minimizes the main drawbacks of scratch assay in contrast to the classical width measuring.

**Methods:** The cell migration activity was estimated using a scratch assay.

**Results:** The scratch assay results expressed as routine detected width and mean distance between opposite cell layers calculated from the area of experimental wound does not significantly differ.

**Conclusions:** The procedure of wound width measure calculated from the area between the edges of an experimental wound in scratch assay is more effective. Theoretically this approach gives more accurate results, practically such detection is much more simple and removes the main disadvantage of the method, namely human factor.

## A role of SDF1/CXCR4 signaling and mesenchymal stem cells in pathophysiology of facioscapulohumeral dystrophy

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**Background:** Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant hereditary neuromuscular disorder linked to the deletion of an integral number of 3.3 Kb-long macrosatellite repeats (D4Z4) within the subtelomeric region of chromosome 4q. Several genes identified in this region are overexpressed in FSHD myoblasts including the double homeobox genes DUX4 and DUX4c.
**Methods:** We used immunostaining and cell mobility assays to assess the role of SDF1/CXCR4 signaling in FSHD.
**Results:** We have shown that DUX4 induces an overexpression of the SDF1 ligand and its receptor CXCR4 in FSHD muscle. This results in adipocytes and mesenchymal stem cells being attracted to damaged muscles, and subsequent muscle invasion contributing to the disease phenotype.
**Conclusion:** SDF1/CXCR4 signaling contributes to FSHD phenotype.

## The role of resveratrol and miRNAs in regulation of proto-oncogenic eukaryotic translation elongation factor 1A2 expression.


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*Aim:* The eukaryotic translation elongation factor 1A2 (eEF1A2), besides functioning in translation, is a known proto-oncogene which is detected in human tumors of different localization. The overexpression of the gene is not related to the genetic or epigenetic modifications in the *eef1a2* locus suggesting a possibility of posttranscriptional regulation. We proposed that the eEF1A2 expression in cancer tissues is controlled by miRNAs.
We aimed to discover miRNAs that can regulate eEF1A2 expression and to investigate the effect of the miRNA-mediated eEF1A2 down-regulation on the MCF7 breast cancer cell line proliferation.
*Methods:* Changes in the miRNAs and EEF1A2 mRNA levels were quantified by qPCR. Dual-luciferase assay was applied to detect the influence of miRNAs on the EEF1A2 expression. For proliferation assay, the stained cells were counted using the Operetta® High Content Imaging System (Perkin Elmer).
*Results:* We found that eEF1A2 is a direct target of two oncosuppressor miRNAs miR-663 and miR-744. Both miRNAs were able to downregulate the expression of luciferase gene attached to the 3'UTR of EEF1A2. Moreover, overexpression of miR-663 and miR-744 in MCF7 cells reduced the endogenous EEF1A2 mRNA level by 45 % and 70 % and decreased the eEF1A2 protein level by 20 and 40 % respectively. In line with that, overexpression of both miR-663 and miR-744 negatively influenced the proliferation of MCF7 cancer cells.
miR-663 is well-known oncosuppressor which is up-regulated during treatment of THP-1 and SW-480 cells with resveratrol. Resveratrol also inhibits the eEF1A2 expression in serum- or insulin-stimulated PA-1 cells. We observed direct correlation between the up-regulation of miR-663 and miR-744 and decrease in the eEF1A2 amount in resveratrol-treated MCF7 cells. Transfection of MCF7 cells with miR-663 and miR-744 inhibitors eliminated inhibitory effect of the drug, suggesting that resveratrol may influence the EEF1A2 expression through miRNA-dependent pathway.
*Conclusion:* Our findings demonstrate that expression of proto-oncogenic eEF1A2 is controlled by miR-663 and miR-744. Thus, essential increase in the eEF1A2 amount in tumor tissues may be caused by the loss of microRNA-mediated post-transcriptional control.

## Rearrangement of intracellular pathways in tumor cells caused by RNase A treatment: full transcriptome analysis

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Recently, we have shown that the pancreatic RNase A is capable of inhibiting tumor and metastasis growth and one of the mechanisms is associated with the change of miRNA profiles in the blood and tumor cells (Mironova et al, 2013). Here, in order to find intracellular targets of RNase A, we performed an analysis of whole transcriptome of Lewis lung carcinoma tissue after treatment of tumor-bearing mice with RNase A by high-throughput sequencing using SOLiD 5.5 platform.
Analysis of sequencing data revealed that inhibition of tumor and metastasis growth by RNase A is accompanied by up-regulation of 320 genes and down-regulation of 645 genes in tumor cells. Top 20 of the mostly down-regulated genes includes snoRNA class (both C/D and H/ACA boxes) elevated in tumor cells; genes encoding proteins with cell-growth promoting and transforming activity (*PARK7*), genes encoding proteins functioned as negatively regulators of MAP kinase superfamily and tumor suppressor (*DUSP6*), which is associated with cellular proliferation and differentiation, anti-apoptotic genes (*LCN2*). Among top 20 of the mostly up-regulated genes are negative regulators (*FAM89B*) of TGF-beta signaling known to exert metastasis-promoting activity associated with epithelial-to-mesenchymal transition, modulation of cancer microenvironment and extracellular matrix components, inflammation and immune suppression at the later stage of tumor progression. Also we observed the increase in expression of genes encoding phosphatidylserine receptors (*JMJD6*) involved in phagocytosis of apoptotic cells, p53-inducible proteins (*STEAP3*), regulators of p53/TP53 stability (*USP10*).
Obtained data give the evidence that RNase A caused in tumor cells rearrangement of intracellular pathways associated with malignant transformation and tumor escape from immunological surveillance towards normalization.
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