These results were confirmed by western blots also showing an increase of γH2AX in Cks2 KO cells. The level of γH2AX in Cks2 KO cells was rescued by the expression of Cks2mCherry.
Conclusions: We have found that the Cks2 protein maintains cell cycle length and that in the absence of Cks2, cells have a faster division time. Cells without Cks2 also have increased levels of γH2AX compared to WT cells or Cks2 KO cells rescued by the expression of Cks2. In Cks2 KO cells, defects in DNA replication may be exasperated by cell cycle deregulation.

768 Reproducibility of gene expression measurements in microarray studies relies on filtering of expressed genes: implications for the understanding of childhood papillary thyroid cancer transcriptome

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Aim of the study: The aim of the study was to assess the use of filtering based on repeatability-obtained parameters as a strategy for gene selection in microarray studies, performed by validation of genes responsible for tumour-normal difference in papillary thyroid cancer (PTC). Comprehensive analysis of technical accuracy of thyroid cancer gene expression measurement by oligonucleotide microarrays, done in the same samples in two independent laboratories was performed.
Material and Methods: We analysed 19 samples of RNA obtained from childhood PTC (12 samples) and normal thyroid from the same patient (7 samples). Two participating laboratories, IOG and ULB carried cRNA synthesis and microarray hybridization to HG-U133 Plus 2.0 according to the local routine of each laboratory. In total, 38 CEL files were obtained, 19 from each laboratory. Each set was normalised separately by GC-RMA method, and the normalization of all samples from both laboratories in one batch was performed. All samples were also normalized by MAS5 algorithm, with scaling to TGT = 100.
Results: Although the overall correlation between the gene expression profiles was excellent (R = 0.99 when GC-RMA preprocessing was done and R = 0.8–0.92 in MAS5 normalized data), only a subpopulation of probesets showed such correlation in transcript by transcript analysis, over R = 0.8 (about 16500 probesets for GCRMA and 10000 probesets for MAS5).
As it was clearly observed that gene expression level influenced the correlation, where the variance had lower impact on it, we analyzed both factors starting with gene expression level and subdividing the genes in each 100-gene set into 3 subgroups (low variance: below 25 centile in each set; average variance: between 25−75 centile and high variance, over 75 centile in each set). Analysing the relationships, we subdivided the sets according to the gene expression level and variance, to discriminate between sets with good, moderate and poor correlation. Assessment of technical repetitions let us to discriminate a group of genes with poor reproducibility where genes responsible for tumour-normal difference were almost absent (below 0.5%).
Conclusions: We present a method for selection of reproducible genes which allows to increase the sensitivity of detection of significant differences in gene expression profile by reduction of the number of comparisons and which is especially necessary when subtle differences are looked for. A small sized experiment of some 20 microarray analyses repeated in two laboratories is necessary for each type of tumours analyzed.
Supported by Genrisk-T project no 036495.

769 c-Myb promotes invasivity of breast cancer cells

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Background: The c-*myb* gene codes for transcription factor that is essential for regulation of hematopoiesis in vertebrates. Deregulated expression and/or mutation of c-*myb* can result in leukemias. In addition to hematopoietic malignancies, the role of c-Myb in development of solid tumours has been documented as well. c-Myb was shown to promote proliferation and inhibit differentiation/apoptosis of various cancer cells. While the role of c-Myb in control of these processes has been extensively studied, there are only a few indications that c-Myb can be involved in cancer cell invasion and metastatic spread. The aim of this study was to assess the role of the c-Myb protein in control of invasivity of breast cancer cells.
Material and Methods: MDA-MB-231 breast cancer cells were transfected with the c-Myb coding cDNA to prepare MDA-MB-231MYBup derivatives. The effects of c-Myb overexpression on migration and invasion capacity of these cells were assessed using Cultrex Cell invasion assay (RnD Systems). In order to reveal dynamics of these processes, we performed real-time analysis of cell migration and invasion using the xCELLigence RT-CA system (Roche). This system is based on non-invasive impedance-based monitoring of the transition of cells through the microporous membrane in real time.
Results: MDA-MB-231MYBup cells were significantly more active in both motility and invasion than controls as determined by Cultrex cell invasion assay. This was clearly confirmed by real-time analysis of cell migration/invasion. To address the mechanism of c-Myb-enhanced breast cancer cell invasion, we examined the role of c-Myb in control of expression and activity of some of the proteases involved in degradation of extracellular matrix. We found that c-Myb enhanced production of cathepsin D and matrix metaloproteinases in MDA-MB-231MYBup cells.
Conclusions: c-Myb promotes motility and invasivity of breast cancer MDA-MB-231 cells and this effect at least partially results from deregulation of expression/activity of cathepsin D and some matrix metaloproteinases. These results suggest a novel role of c-Myb protein in control of tumour invasion and metastatic progression.
This work was supported by grants 301/09/1115 of GACR, IAA501630801 of GAAV CR, MUNI/C/0099/2009 of Masaryk University and MSM0021622415 of Ministry of Education CR.

770 Transactivation by temperature-dependent p53 mutants in yeast and human cells

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Background: The p53 protein plays an important role in cancer prevention. In response to stress signals, p53 controls essential cell functions by regulating expression of its target genes. Full or partial loss of the p53 function in cancer cells usually results from mutations of the p53 gene. Some of them are temperature-dependent, allowing reactivation of the p53 function in certain temperature. These mutations can alter general transactivation ability of the p53 protein or they modify its transactivation only towards specific genes.
Material and Methods: We analyzed transactivation of p21-, bax- and mdm2 genes by 23 temperature-dependent p53 mutants in transiently transfected human lung H1299 cells (p53-null) by luciferase reporter assay. Then, we prepared isogenic H1299/p53 cells and studied expression of the endogenous p53-target genes at mRNA and protein levels. The results obtained were compared to the results of functional analyses performed in yeast cells by FASAY we published earlier.
Results and Conclusions: We confirmed temperature-dependency and discriminative character of the most p53 mutants and stratified them into four functional groups. Despite the differences of yeast and human cells, they allowed similar transactivation rates to the p53 mutants, thus providing evidence that functional analysis of separated alleles in yeast is valuable tool for assessment of the human p53 status.
This work was supported by grant NS/10448−3/2009 of the Internal Grant Agency of the Ministry of Health of the Czech Republic, MSM0021622415 of the Ministry of Education, Youth and Sports of the Czech Republic.

771 A 9 series microRNA signature differentiates between germinal centre and activated B-cell-like diffuse large B-cell lymphoma cell lines

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Background: The microRNAs are endogenous, non-coding RNAs that play key roles in a range of pathophysiological processes by negatively regulating gene expression. Recent studies have shown that some microRNAs have oncogenic or tumour suppressor activity.
Diffuse Large B-cell Lymphoma (DLBCL) is an aggressive non-Hodgkin's lymphoma with a heterogeneous biology which has impeded the clinical assessment of patients. The currently-used clinically-based IPI provides useful information for treatment decision making, but has limited predictive power. Recent immunohistochemical approaches have identified two different prognostic groups: the more indolent germinal centre (GC) – and the higher risk activated B-cell (ABC)-like phenotypes. Although useful, prediction based on immunophenotype has limitations.
The present study uses microRNA profiling and a number of well-characterised B-cell lymphoma cell lines to identify microRNA signatures that are correctly assigned to the DLBCL prognostic sub-groups and distinguish DLBCL from other more indolent lymphoma, including follicular lymphoma (FL).
Materials and Methods: MicroRNA microarray analysis was carried out by Miltenyi Biotec using miRXplore™ technology, based on miRBase version