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.
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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.
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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.
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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

12.0. Analysis was performed based on an unsupervised hierarchical clustering model and discriminatory microRNAs were validated by qRT-PCR.
Results: We identified a 9 microRNA signature that discriminated between ABC- and GC-like DLBCL. This included 3 newly identified microRNAs, not previously associated with DLBCL and predicted to target genes that are de-regulated in lymphoma. DLBCL was distinguished from FL by 4 microRNAs and a total of 18 microRNAs were identified that differentiated between all lymphoma and control populations. Most of the discriminatory microRNAs have been reported before to belong to known oncomiRs or act as tumour suppressors.
Conclusions: In conclusion, the present study identified a microRNA signature that correctly classified GC and ABC phenotypes in DLBCL cell lines. The numbers of microRNAs identified within each signature are manageable for potential use in a clinical setting. This signature has yet to be assessed for prediction in clinical samples. Such studies would be of great value in assessing the potential of microRNAs as biomarkers or therapeutic targets.

772 Nuclear factor kappa B expression and flowcytometric DNA analysis in Egyptian endometrial carcinoma and hyperplasia

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Background: Endometrial carcinoma is an estrogen-related common tumour, developing most probably on top of atypical endometrial hyperplasia. Inhibition of apoptosis may be a cause of tumour development prolonging cellular life-span, thus allowing accumulation of other genetic alterations. NF-kappa B is a transcription factor that may inhibit apoptosis, thus may share in tumourigenesis. Quantitative evaluation of cellular DNA (ploidy) and cell cycle kinetics (S phase fraction) are also helpful prognostic factors in human neoplasms. DNA aneuploidy in endometrial cancer identifies high risk cases, and correlates with poor survival rate, which may have an impact on clinical management of these cases. This study aimed to evaluate the role of NF-kappa B expression in the development and progression of endometrial carcinoma. Also, it determined DNA ploidy and cell cycle kinetics by measuring SPF using flowcytometry (FCM).
Material and Methods: This study investigated 72 cases of endometrial biopsies, including normal cycling endometrium in the proliferative phase, hyperplastic and adenocarcinomatous endometrium. Immunostaining for NF-Kappa B expression was done using the streptavidin-biotin-peroxidase technique. Flowcytometric studies were done by a modified Hedley method, results presented as DNA distribution histograms.
Results: NF-kappa B nuclear expression was significantly different in malignant and non malignant tissues ($P = 0.048$), also cytoplasmic expression was significantly descending from malignant to proliferative endometrial tissues passing through hyperplasia ($P = 0.000$). There was a negative correlation between both nuclear ($p < 0.01$) and cytoplasmic staining of NF-kappa B and apoptotic index ($p < 0.001$). A significant positive relation was found between both nuclear ($p < 0.05$) and cytoplasmic stains ($p < 0.05$) and mitotic index. There was a statistically significant difference regarding ploidy satus between the 3 studied groups (neoplastic vs normal $p = 0.001$, neoplastic vs hyperplastic $p = 0.014$, hyperplastic vs normal endometrium $p = 0.043$). There was a significant correlation between increased expression of NF-kappa B and both aneuploidy and high SPF.
Conclusions: NF-kappaB plays an important antiapoptotic role in the endometrium and could play a role in tumour progression. Flowcytometric evaluation of DNA ploidy and SPF allow a more precise definition of high risk groups in endometrial cancer cases.

773 MicroRNA expression analysis in human lymphoma/leukemia cells

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Background: MiRNAs are small non-coding RNAs that regulate post-transcriptional gene expression, probably by inhibiting protein translation. In recent years more and more studies have described changes of miRNA expression levels in different types of human neoplasia and their role in cancer development, and progression. The aim of our study was to determine the expression of miRNAs in human lymphomas and leukemias.
Materials and Methods: Human lymphoma/leukemia cell lines (BHD1, Nalm6, Mn60, KMH2, Jurkat, HL60, Raji, Ramos, BJAB, Daudi) were culture according to standard methods. Acute lymphoblastic leukemia (ALL) cells were isolated from the bone marrow of paediatric ALL patients by Ficoll gradient centrifugation. MiRNAs were isolated by miR Vana TM miRNA Isolation Kit, and cDNA was reverse transcribed with the TaqMan MicroRNA Reverse Transcription Kit. MiRNA expression was determined with real-time PCR using TaqMAn micro-RNA Assays (miR21, miR24, miR155, miR16, miR128b, miR142−3p, miR29b, miR223). Values were normalized to normal B- and T-cells.
Results: In the present study, the expression level of different miRNAs was analyzed in human lymphoma/leukemia cell lines, T- and B-cells, and in childhood ALL bone marrow cells. MiRNA 21 – known to be oncogenic (onco-miR) – was expressed in nearly all examined cell lines. The onco-miR 155 was overexpressed in 20% of lymphoma/leukemia cell lines. MiRNA 128b was overexpressed in all cell lines, but extremely high values were measured in Jurkat (T-ALL) and Nalm6 (B-ALL) cell lines.
Conclusions: In this study, the presence of several miRNAs was confirmed in human lymphoma/leukemia cell lines and in ALL cells. Our results suggest that different hematological malignancies have distinct miRNA expression profiles. Increasing knowledge of miRNA expression signatures may help characterize tumour subtypes, predict prognosis, and identify their regulatory role in cellular processes.
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774 WNT5A acts as an oncogene in EBV-associated nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is an Epstein–Barr Virus (EBV)-associated cancer which is particularly prevalent in Southern China and Southeast Asia. In Malaysia, NPC is the fifth most common cancer overall and third common in men. Over 70% of cases present with late stage disease and the 5-year survival rates are less than 50%. Novel therapeutic approaches to manage this disease are urgently required. Using expression microarrays, we identified the Wnt5a gene as being overexpressed in primary NPC tissue samples relative to cancer-free controls. Further, comparison with a published microarray study using 36 normal human organs revealed that the level of Wnt5a mRNA in NPC is significantly higher than in a wide range of normal organs. Wnt5a is one of the most highly studied Wnts which acts primarily through the non-canonical pathway. With respect to cancer biology, there is conflicting evidence whether Wnt5a has a tumour-promoting or -suppressing role, and its role in NPC has never been investigated. The upregulation of Wnt5a was validated in 12 NPC tissue samples by quantitative PCR, and its low expression level was confirmed in 16 normal human organs by RT-PCR. In NPC cell lines, however, the expression of Wnt5a was heterogenous. Nonetheless, a dramatic increase in the Wnt5a expression was shown in the only EBV-positive line, C666.1, suggesting a potential role of EBV in regulating the expression of Wnt5a. This data is also in accordance to our preliminary microarray data that in vitro infection of an EBV-negative NPC cell line with a recombinant EBV or individual EBV genes resulted in the upregulation of Wnt5a. In addition, we assessed the functional role of elevated Wnt5A on tumour cell behaviour in vitro. Ectopic expression of Wnt5a in NPC cell lines significantly promotes cell proliferation, migration and invasion. Taken together, Wnt5a appears to function as an oncogene in NPC, and its overexpression might be regulated by EBV. These data suggest that Wnt5a could be a useful therapeutic molecular target for NPC.

775 Plant phenols modulate JNK activity in mouse epidermis: the effect on transcription factors AP-1 and STAT

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The modulation of signaling pathways induced by tumour promoters is involved in early stage of cancer development. This includes the stimulation of protein kinases such as c-Jun N-terminal kinase (JNK). JNK is involved in activation of MAPK/AP-1 signaling pathway, responsible for regulation of inflammatory response, cell proliferation and death. Some data indicate that JNK may be also involved in activation of other transcription factors, such as STAT family (Signal Transducers and Activators of Transcription). AP-1, STAT, and JNK are considered as potential targets for chemoprevention and/or chemotherapy. Our previous study showed that both transcription factors are activated in mouse epidermis 2−4 hours after treatment with tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In this study we assessed the effects of naturally occurring plant phenolic acids, protocatechuic (PCA), tannic (TAA), and chlorogenic acid (CHA) on TPA stimulated JNK activity in mouse epidermis, and the activation of AP-1 and STAT.
Animals were treated with a single dose (10 nmol/mice) of TPA or acetone (control group). Phenolic compounds (16 μmol/mice) were applied 15 min before TPA treatment. Mice were killed at selected time points and