The only region of amplification that differed significantly between MEC and NMEC was 8q24. It was amplified in 2/5 (40%) of MEC versus none (0%) of 20 NMEC tumours (P = 0.03). The region started at 8q24.12, upstream of *EIF3H*, and ended at 8q24.13, downstream of *MYC*. It encompassed the *DEPDC6* (upstream) to *HAS2* (downstream) and contained *COL14A1* (collagen, type XIV), *MTBP* (Mdm2-binding protein, which stabilizes MDM2 and in this way increases p53 degradation) and *SNTB1* (syntrophin, beta 1, which is a dystrophin binding protein).
Of the putative targets of 8q24 amplification identified, the *HAS2* gene merits particular attention. It encodes hyaluronan synthase, and has been found overexpressed in many tumour types. The resulting high concentration of hyaluronan has been used as a tumour marker as its direct measurement in urine and serum samples has shown very good predictive values for cancer detection and grading. Also, in vitro silencing of the *HAS2* gene has reversed the aggressive potential of cancer cells and is hoped to entice pharmacological potential. The gene also encodes for a *cis*-antisense mRNA (HAS2AS gene), which regulates HAS2 transcription. Thus, if confirmed as a target of 8q24 amplification, the mechanism by which this increases cancer growth should also be elucidated. Further investigation of the significance of the 8q24 genes for EC aggressiveness is warranted. If confirmed, their amplification could reveal new knowledge on the mechanism behind the metastatic process.

812 Transcriptional profiling of early onset colorectal cancer identifies CLC as a potential cancer susceptibility gene

T.H. Ågesen[1,2], M. Berg[1,2], L. Cekaite[1,2], E. Thiis-Evensen[3], M. Merok[4,5], A. Nesbakken[4,5], R.A. Lothe[1,2], R.I. Skotheim[1,2]. *[1]Department of Cancer Prevention, Institute for Cancer Research, Oslo University Hospital, The Norwegian Radium Hospital, Oslo, Norway, [2]Centre for Cancer Biomedicine, University of Oslo, Oslo, Norway, [3]Medical Department, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [4]Department of Gastrointestinal Surgery, Oslo University Hospital, Aker, Oslo, Norway, [5]Faculty of Medicine, University of Oslo, Oslo, Norway*

Background: Genetic predisposition is estimated to be the cause of colorectal cancer (CRC) in up to 30% of all cases, including the known inherited CRC syndromes (*e.g.* Lynch syndrome and familial adenomatous polyposis) that accounts for only about 5%. The incidence of CRC increases with age and an early onset of disease is an indication of genetic predisposition. In an attempt to identify cancer susceptibility genes, whole genome transcriptional differences between samples from patients diagnose with CRC at an early age and samples from sporadic/late onset CRC were compared.
Material and Methods: CRC samples were obtained from hospitals in the South-East region of Norway. In total, 24 primary tumours from patients diagnosed at an early age (mean 43 years), 17 sporadic tumours from elderly patients (mean 79 years) and four normal mucosa samples were included. All tumours were microsatellite stable, and samples from both genders and from the different Dukes' stages were represented equally. Applied Biosystems AB1700 microarrays were used, which enables measurements of gene expression using 32,878 unique probes.
Results: Principle component and cluster analysis showed an equal overall expression profile when comparing the early and late onset tumours. Nevertheless, although not all reaching statistical significance, we identified 20 protein coding genes differentially expressed in the early onset tumours compared to those with a late onset. *CLC* was the overall most significant gene with an increased expression in the early onset samples. Gene Set Enrichment Analysis identified chromosome band 19q13 as the most significant region with an enrichment of genes with an increased expression in the early onset samples, a region that includes *CLC*. Supporting these findings, the expression data has in parallel been integrated with corresponding DNA copy number data and chromosome band 19q13.2 was one of the loci identified with concomitantly DNA copy number gain and increased mRNA expression (Berg *et al.*, unpublished).
Conclusions: Minor differences were found when comparing the overall transcriptome profiles of early and late onset CRC. Nevertheless, we have identified several genes which serve as potential cancer susceptibility genes warranting further investigation in the continuing search for inherited genetic alteration in CRC.

813 The scaffolding adaptor GAB2 promotes anchorage independence and drives a transcriptional program associated to metastatic progression of breast cancer

E. Medico[1], C. Isella[1]. *[1]Institute for Cancer Research and Treatment (IRCC), Laboratory of Oncogenomics, Candiolo, Italy*

Background: The ability to grow in the absence of anchorage to the extracellular matrix represents a key oncogenic property of cancer cells. To screen for genes conferring anchorage independence, we exploited a novel functional genomics approach recently developed in our laboratory.
Material and Methods: The screening was based on transduction of MCF10A human breast cells with a murine retroviral expression library. Transduced cells were then selected for anchorage independence by culturing them on polyhema-coated dishes. Exogenous cDNAs enriched after selection were identified by one-shot, species-specific quantitative tracing with murine DNA microarray analysis before and after selection.
Results: Independent infection-selection experiments highlighted significant and reproducible enrichment for murine cDNAs encoding the Gab2 protein, suggesting a role for this scaffolding adaptor in anchorage-independent growth. Gab2 was confirmed to strongly promote anchorage-independent growth when overexpressed. Such effect did not involve protection from detachment-induced apoptosis, but rather the maintenance of a proliferative status also in the absence of the consensus provided by integrin engagement. Interestingly, downregulation by RNA interference of endogenous Gab2 in neoplastic cells did not affect their adherent growth, but abrogated their growth in soft agar. Gab2-driven anchorage independence was found to specifically involve activation of the Src-Stat3 signaling axis. A transcriptional "signature" of 205 genes was obtained from GAB2-transduced, anchorage-independent MCF10A cells, and found to contain two main functional modules, respectively controlling proliferation and cell adhesion/migration/invasion. Notably, the signature was enriched in genes discriminating responsiveness of breast cancer cell lines to Dasatininb, a Src-family kinase inhibitor. Extensive validation on breast cancer datasets showed that the Gab2-signature provides a robust prognostic classifier for breast cancer metastatic relapse, largely independent from existing clinical and genomic indicators and from estrogen receptor status.
Conclusions: This work highlights a pivotal role for GAB2 and its transcriptional targets in anchorage-independent growth and breast cancer metastatic progression. Moreover, it delivers a transcriptional signature capturing metastatic propensity of breast cancer with high sensitivity and accuracy.

814 Men genotyped for BRCA1/2 mutations: how does it affect them?

S. Shiloh[1], E. Dagan[2], I. Friedman[3], N. Blank[1], E. Friedman[4]. *[1]Tel Aviv University, Psychology, Tel Aviv, Israel, [2]Rambam Medical Center, Genetics, Haifa, Israel, [3]Sheba Medical Center, Onco-genetics, Ramat Gan, Israel, [4]Tel Aviv University and Sheba Medical Center, Human Genetics, Tel Aviv and Ramat Gan, Israel*

Background: Men who undergo genetic testing for harboring germline mutations in breast/ovarian cancer associated genes (*BRCA1*/*BRCA2*) represent an interesting and under investigated population. The reasons for testing are often a family history of either neoplasm or finding a mutation in a female relative. Testing for a "feminine" disease may have significant effects on men who are mutation carriers. The purpose of the study was to examine the cognitive, emotional and behavioral impacts that BRCA1/2 testing has on those tested men, by comparing carriers to non-carriers in a follow-up study.
Material and Methods: Fifty-one male carriers of a mutation in either the BRCA1 or BRCA2 genes were compared to a similar group of ethnically matched non-carriers on a questionnaire that measured changes in health behaviors since testing, within family communication about test results, risk perceptions, cognitive representations of breast cancer, and emotional reactions to test results. Participants filled-out the questionnaire in a telephone interview a few months after receiving test results and counseling in an oncogenetic clinic.
Results and Conclusions: Comparisons between carriers and non-carriers will be presented. Undergoing genetic testing for BRCA1/2 mutations may be experienced by some men as a threat to their manhood. Men found to be carriers may need support in adjusting to their genetic status and the impact that it has on their own health and that of their female offspring.

815 Fast statistical analysis of high density CGH and SNP arrays

G. Rigaill[1], T. Dubois[2], E. Barillot[3], S. Robin[4]. *[1]Institut Curie, AgroParisTech, INRA, Département de Transfert, MIA UMR 518, Paris, France, [2]Institut Curie, Département de Transfert, Paris, France, [3]Institut Curie, INSERM U900, Mines ParisTech, Paris, France, [4]AgroParisTech, INRA, MIA UMR 518, Paris, France*

Background: Array-based Comparative Genomic Hybridization (array-CGH) and Single Nucleotide Polymorphism (SNP) arrays are used for studying the genetics of cancer. Their bioinformatical and statistical analysis is a critical step to identify gained and lost regions containing potential oncogenes or tumour suppressor genes.
Material and Methods: The CGHseg method (Picard et al 2005), which uses a dynamic programming algorithm, was shown to be one of the best methods (Lai et al 2006) to analyze CGH arrays and detect DNA copy number alterations. However, its application to very high density CGH and SNP arrays measuring the DNA copy number on more than 1 million loci per patient was limited due to algorithm complexity.
Results: We have found shortcuts in the dynamic programming algorithm and have implemented an improved version of the CGHseg method. The new algorithm recovers exactly the same result as the previous one in a drastically

reduced amount of time, enabling the analysis of million points copy number profiles in a matter of minutes. We applied our new algorithm to a series of 200 Affymetrix SNP 6.0 breast tumour samples.
Conclusions: The CGHseg algorithm is now well suited for high density CGH and SNP array analyses and efficiently detects DNA copy number alterations.

816 FGFR3 mutations in prostate cancer and other tumours

S. de Muga[1], S. Hernández[2], L. Agell[1], N. Juanpere[1], R. Esgueva[1], M. Lorenzo[1], S. Serrano[1]. [1]*Hospital del Mar (Fundación IMIM), Pathology, Barcelona, Spain,* [2]*Pompeu Fabra University, Health and Experimental Sciences, Barcelona, Spain*

Background: We previously reported *FGFR3* mutations in prostate carcinoma (PCa). *FGFR3* mutations were associated with low-grade PCa, and also with PCa found in patients with concurrent bladder cancer or skin tumours. The aim of this work has been to further investigate the relationship between *FGFR3* alterations in PCa and the presence of concurrent tumours.
Material and Methods: 41 cases with PCa and other associated tumour types were studied. The PCa series consisted of: 22 incidental (cystoprostatectomy) tumours and 19 clinically significant (biopsy or prostatectomy) cases. Twenty-three PCa were Gleason grade $\leqslant$6, 11 were Gleason grade 7 and 7 were Gleason grade $\geqslant$8. In each case, we studied the PCa and the concurrent tumour (prostate and bladder cancer, n = 32; prostate and skin tumour, n = 6; prostate and colon cancer, n = 2; prostate and lung cancer, n = 1). *FGFR3* exons 7 and 10 were analysed by PCR and direct sequencing.
Results: Eight of 41 (19.5%) PCa presented a mutation in *FGFR3*. From these, 6 were Gleason grade $\leqslant$6, and 2 were Gleason grade 7. Four of 32 (13%) patients with PCa and bladder cancer harboured a *FGFR3* mutation in the PCa, and 5 other cases (16%) in the bladder tumour. In the PCa-skin tumour group, 3 of 6 (50%) PCa presented a *FGFR3* mutation, and other 2 different cases (33%) in the skin tumour. One case harboured *FGFR3* mutations in both tumours, but in different codons. Finally, one case with PCa and colon cancer also had a *FGFR3* mutation in PCa.
Conclusions: *FGFR3* mutations in PCa are associated with an increased frequency of concurrent tumours in other organs, mainly skin and bladder. The lack of coincidence in the presence of *FGFR3* mutations in both the PCa and the associated tumours suggests that they evolve through different pathways.
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817 Integration of gene and miRNA expression profiles in clear cell renal carcinoma cell lines and relationship with VHL gene status

C. Battaglia[1], V. Tinaglia[1], I. Cifola[2], F. Frascati[2], E. Mangano[2], M. Biasolo[3], S. Bortoluzzi[3], S. Bombelli[4], C. Bianchi[4], R. Perego[4]. [1]*Università Degli Studi di Milano, Dipartamento Scienze e Tecnologie Biomediche (Disteb), Milan, Italy,* [2]*National Research Council (Cnr), Institute for Biomedical Technologies (Itb), Segratemilan, Italy,* [3]*University of Padua, Department of Biology, Padua, Italy,* [4]*University of Milano-Bicocca, Department of Experimental Medicine, Milan, Italy*

Clear cell renal cell carcinoma (ccRCC) is the predominant form of kidney cancer, representing 75−80% of primary malignancies of the kidney. The status of the von Hippel-Lindau (*VHL*) tumour suppressor gene, an important regulator of the hypoxia pathway via the hypoxia-inducible factors (HIFs), has been correlated to RCC pathogenesis. *VHL* biallelic inactivation occurs in 80% of sporadic ccRCCs and in all inherited cases, while the remaining 20% harbours wild type gene, thus this molecular heterogeneity needs further investigations. The role of microRNAs (miRNAs) in cancer development and progression is expanding, since a number of evidences suggested that miRNA expression is implicated in tumourigenesis, and miRNAs might function as tumour suppressors and oncogenes in a contest-dependent way.
We used Caki-2 and A498 cell lines as in vitro model of ccRCC pathology, and HK-2 (normal proximal tubular epithelial cell line) as reference sample. We characterized the *VHL* status by direct sequencing and the HIF status by western blot. Affymetrix microarray platforms were applied to assess miRNA profiles (onto GeneChip® miRNA Array, comprising 847 human mature miRNAs) and gene expression profiles (onto GeneChip® Human Gene 1.0 ST Array, including 19,793 annotated genes).
Analysis of differentially expressed miRNAs (DEMs) outlined specific miRNAs in both Caki-2 and A498 that have been found related to ccRCC (e.g. miR-155, miR-21 and miR-221), and in addition some DEMs found only in A498 (*VHL*−/−) that are involved in hypoxia pathway (e.g. miR-210). Functional enrichment analysis showed that some modulated gene (DEG) have a known role in hypoxia and p53 signalling pathways. Additionally, we performed an integrated analysis to combine gene and miRNA expression profiles, under the assumption that, since miRNAs tend to down-regulate their targets, expression profiles of miRNAs and real targets are expected to be anti-correlated.
This integrated analysis exploits miRNAs and target expression information in order to identify most probable functional regulatory interactions occurring in the ccRCC cells, and to reconstruct and study the corresponding post-transcriptional regulatory network. The further integration of these results with DEGs and DEMs will facilitate the elucidation of regulatory circuits important for tumourigenesis and biological processes under relevant post-transcriptional regulation in ccRCC and the interpretation of these results on the basis of *VHL* status.

818 Expression correlations of NFkB signaling and miR146 a/b miR21 and let-7 expression in primary human head and neck squamous cell carcinomas

G. Pajkos[1], I. Sejben[1], K. Gombos[2], I. Ember[2]. [1]*BKM Center Hospital, Pathology, Kecskemét, Hungary,* [2]*Pécs University, Public Health, Pécs, Hungary*

Background: The NFkB signal transduction pathway plays as an important link between inflammation and cancer and serves as a promising target for molecular cancer therapy in head and neck squamous cell carcinoma. HNSCCs are characterized by elevated constitutive activity or aberrant regulation of NFkB that acts as a survival factor for malignant cells by its predominantly anti apoptotic function. While the post translation regulation of the NFκB signaling is deep and detailed its post transcriptional regulation is still unclear and there is sparse of data about the expression of the potential miRNA regulators of the NFκB releated genes in primary human HNSCC.
Materials and Methods: Total RNA isolated from fresh frozen primary tumour tissues (n = 10) and formalin fixed paraffin embedded (FFPE) primary tumour tissues (n = 35), fresh frozen non diseased head and neck epithelial tissues (n = 6) and FFPE normal epithelial tissues (n = 8) were analyzed by quantitative real-time PCR for the expression of *Nfκb p65*, *Rel A*, *Ikk1*, *Pparγ*, *Pten*, *Gadd45α*, *Jnk1* and miR146 a/b, miR-21 and let-7.
Results: Significant expression alterations of the investigated genes were found in 92% of the tumour samples. We also found consequent converse and inverse correlation between mRNA and miRNA expressions, especially regarding the *Rel A*, *Pparγ* and the miR-21. Expressions of the fresh frozen samples did not differ significantly from those found in the FFPE samples.
Conclusions: Our data confirm parallel disregulation of miR146 a/b, miR21 and let-7 and their potential mRNA targets in primary human HNSCCs, that could be useful for molecular diagnostics and therapy.

819 Integrated analysis reveals overexpression of miRNA clusters in osteosarcomas

H.M. Namløs[1], S.H. Kresse[1], I.H.G. Østensen[2], R. Duim[3], A.M. Cleton-Jansen[3], L.A. Meza-Zepeda[1], O. Myklebost[1]. [1]*Institute for Cancer Research, Oslo University Hospital, Oslo, Norway,* [2]*Norwegian Microarray Consortium, University of Oslo, Oslo, Norway,* [3]*Department of Pathology, Leiden University Medical Centre, Leiden, The Netherlands*

Introduction: Osteosarcoma (OS) is the most common primary malignant tumour of bone, and almost all most conventional osteosarcomas are high-grade tumours with complex genomic aberrations. Many studies have shown that miRNAs are aberrantly regulated in different human cancers. Elucidating what pathways are affected by a change in miRNA pattern could reveal new avenues for diagnosis or therapy in osteosarcomas.
Material and Methods: We have performed global microarray analysis of a well-characterised panel of 19 OS cell lines, collected within the EU network EuroBoNeT (www.eurobonet.eu), and 4 normal bone samples in addition to mRNA expression data for 71 OS patient samples. Global miRNA expression patterns have been analyzed using the Agilent miRNA array v2.0, mRNA expression patterns using the Illumina HumanWG-6 Expression BeadChip, and DNA copy number changes using the Affymetrix Genome-Wide Human SNP Array 6.0. We used TargetScan 5.1 to predict the most likely targets of the miRNAs and integrated the miRNA and mRNA expression data by calculating the Pearson's Correlation for each of the predicted miRNA-mRNA pairs across all the samples.
Results: We identified 4000 mRNAs that were significantly differentially expressed in OS cell lines compared to bone, of which 40% were confirmed to have the same pattern in the OS patient data. 148 miRNAs were found to separate the OS cell lines from the normal bones. For the target prediction, only conserved miRNA families and conserved target sites with an aggregated P_{CT} value >0.5 were selected. In addition, miRNA-mRNA pairs with low or positive correlation were removed, setting a cut-off at Pearson's correlation <−0.6. This resulted in a set of 38 miRNAs and 119 putative mRNA targets, making up 323 pairs of miRNA-mRNA.
A high number of the miRNAs identified in this study co-localize in clusters in the genome and belong to common miRNA families. These miRNAs have been found to be overexpressed in several solid tumours, but their involvement has not been reported in osteosarcomas. The putative mRNA targets are involved in development and hormone signaling pathways and networks, and have important functions in both bone and cancer.
Conclusions: We have identified miRNAs and mRNAs that are differentially expressed between osteosarcomas and normal bone samples, and integration