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