proliferation rate were increased several fold in $Fgfr4^{A/A}$ cells compared to $Fgfr4^{G/G}$ cells as assessed by dual luciferase reporter and BrdU incorporation assays, respectively. Moreover, upon knock-down of endogenous FGFR4 in human cancer cell lines, tyrosine phosphorylation of STAT3 was inhibited only in cells homozygous for p.Gly388Arg variant. Tissue lysates of parenchymal and lymphoid organs extracted from $Fgfr4^{A/A}$ mice exhibited elevated levels of phosphorylated STAT3 compared to that of $Fgfr4^{G/G}$ mice. Using transgenic mouse models for breast and lung cancers, we illustrate *in vivo* that p.Gly388Arg variant-induced STAT3 signaling was enhanced not only in tumors but also in cancer-promoting $CD4^{+}FOXP3^{+}CD25^{+VE}$ regulatory T cells (T_{REG}). Moreover, proportions of T_{REGs} were significantly increased, resulting in elevated serum levels of T_{REG} cytokines such as IL-10, IL-35 and decreased expansion of CD8+ T cells *in vivo*. Accordingly, tumors grew at an accelerated rate with increased BrdU incorporation and displayed reduced infiltration of CD8+ T cells. Thus, Y^{390}-(P)XXQ mediated docking of STAT3 with consequently enhanced STAT3 signaling in cancer cells and regulatory T cells represents a mechanism whereby FGFR4 p.Gly388Arg accelerates cancer progression.

Conclusions: Our findings for the first time elucidate the mechanism behind genetic association of FGFR4 (p.Gly388Arg) to cancer aggression. Our results also illustrate an example of 'gain of function' with pleiotropic effect by a naturally occurring genomic variation in the transmembrane domain of a growth factor receptor.

No conflict of interest.

447 Classification of human cutaneous metastatic melanoma cell lines and clinical samples by receptor tyrosine kinases- and phenotype-driven gene expression signatures

M. Dugo[1], S. Canevari[1], A. Anichini[1], M.L. Sensi[1]. [1]*Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy*

Introduction: Previous gene expression profiling studies of melanoma samples have identified a Melanoma-Phenotype-Specific-Expression (MPSE) gene list, able to classify them into two major groups correlating with an invasive or proliferative phenotype. Receptor tyrosine kinases (RTKs) have important roles in cancer progression and their constitutive activation has been involved in different aspects of the biology of melanoma. Here we used public datasets containing gene profiles of metastatic melanoma (MM) cell lines and clinical samples to assess whether distinct RTKs expression could identify biologically different subsets of tumors. The same samples were also assigned to the invasive or proliferative phenotype and an integrated classification was derived.

Material and Methods: We applied unsupervised hierarchical clustering using RTK and MPSE gene signatures to a discovery dataset of 58 MM cell lines contained within the Cancer Cell line Encyclopedia database. The identified subtypes were validated in independent datasets of 187 MM cell lines and of 361 MM clinical samples, retrieved from 5 and 8 publicly available datasets, respectively. ComBat adjustment was applied for batch effect removal. Cluster stability was evaluated by consensus hierarchical clustering and most representative samples of each cluster were identified by silhouette width calculation. Prediction Analysis of Microarray (PAM) algorithm with ten-fold cross-validation was used to derive the most discriminating genes between the identified subtypes. SubMap algorithm was used to compare subtypes across the clinical and melanoma cell lines datasets.

Results: Hierarchical clustering of RTKs expression profiles revealed two stable clusters in MM cell lines datasets. When compared to invasive and proliferative subtypes, the first cluster included part of invasive tumors only, whereas the second included all proliferative and the remaining invasive ones. In order to merge the two classifications we used the PAM algorithm on the discovery dataset to derive a 298 gene signature, able to distinguish the three classes with the lowest ten-fold cross-validation error. This gene signature identified three stable clusters in the validation and in the clinical dataset. The SubMap algorithm confirmed that the three clusters identified in the clinical dataset reflected the same groups discovered on the two cell lines datasets.

Conclusion: Classification of MM according to the pattern of RTKs expression enabled a refinement of molecular subtypes identified so far pointing out that previous gene signatures did not completely depict the molecular complexity of this disease. Furthermore our classification could be used to identify which MM subtype is more likely to respond to a given therapeutics and could help in the development of new molecularly targeted drugs.

No conflict of interest.

448 Promoter hypermethylation and overexpression of putative oncomiRs may contribute to silencing of the tumor suppressor gene PCDH17 in laryngeal squamous cell carcinoma

E. Byzia[1], M. Szaumkessel[1], K. Kiwerska[1], M. Kostrzewska-Poczekaj[1], M. Jarmuz-Szymczak[1], N. Zemke[1], R. Grenman[2], K. Szyfter[1], M. Giefing[1]. [1]*Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland,* [2]*Department of Otorhinolaryngology − Head and Neck Surgery, Turku University Central Hospital and Turku University, Turku, Finland*

Introduction: The *PCDH17* (protocadherin 17) gene encodes a transmembrane protein that belongs to a large group of mammalian cadherins associated primarily with cell adhesion. Recent reports showed however recurrent downregulation of expression of cadherins in cancer what might suggest the involvement of these proteins in tumor suppression. In line with this assumption, we have recently demonstrated that the *PCDH17* gene is recurrently targeted by homozygous deletions in laryngeal squamous cell carcinoma (LSCC) cell lines. Moreover, in cell lines with no homozygous deletion of the *PCDH17* gene we observed a complete lack of *PCDH17* mRNA and protein expression as compared to non-tumor laryngeal controls. This indicates that other mechanisms to deletions must be responsible for the observed loss of expression in these cell lines.

Material and Method: To identify these putative mechanisms, we have performed a mutation screen of the *PCDH17* gene in 8 LSCC cell lines by Sanger sequencing. We have moreover searched for epigenetic alterations using DNA methylation and miRNA expression analyses. In detail, the promoter regions of *PCDH17* was bisulfite pyrosequenced in 16 LSCC cell lines and 81 primary LSCC tumors and the methylation profiles were compared to profiles obtained for 10 non-tumor controls (buccal swabs from healthy volunteers). Whereas, to identify aberrantly expressed miRNAs that could contribute to *PCDH17* silencing we used the Human Microarray 60K miRNA (Agilent) data and screened for overexpressed miRNAs targeting *PCDH17* in 16 LSCC cell lines and 5 primary tumors compared to 3 non-tumor laryngeal controls.

Results and Discussion: Using sequencing analysis, we could indeed identify one homozygous mutation (chr13: 58299018 G>T; Ala>Ser; hg19) in one of the analyzed cell lines. But a detailed *in silico* analysis of the resulting amino acid change did suggest only minor effects on the protein functionality. In contrast, by the bisulfite pyrosequencing of the *PCDH17* promoter region we identified hypermethylation (methylation level >80%) in all 16 LSCC cell lines and elevated methylation level in 77/81 (95%) primary tumors as compared to non-tumor controls. In 12/81 (15%) of the primary tumors the methylation level exceeded 50%. Moreover, we have identified two miRNAs significantly overexpressed ($p < 0.05$) in LSCC, namely hsa-miR-15b-5p and hsa-miR-16-2-3p that are predicted to target *PCDH17* (Target Scan Human, June 2012 database). These miRNAs are good candidates for novel oncomiRs in LSCC.

Conclusion: In summary, beside the previously published recurrent homozygous deletions targeting the *PCDH17* gene, the presented results suggest epigenetic alterations including aberrant methylation and overexpression of putative oncomiRs as the most frequent mechanisms underlying the observed silencing of *PCDH17* in LSCC. The data further corroborate the significance of *PCDH17* loss in LSCC.

No conflict of interest.

449 Gene-specific methylation profiles in male breast cancer

P. Rizzolo[1], V. Silvestri[1], A.S. Navazio[1], V. Valentini[1], V. Zelli[1], M. Falchetti[1], I. Zanna[2], S. Bianchi[3], D. Palli[2], L. Ottini[1]. [1]*"Sapienza" University of Rome, Department of Molecular Medicine, Rome, Italy,* [2]*Cancer Research and Prevention Institute (ISPO), Molecular and Nutritional Epidemiology Unit, Florence, Italy,* [3]*University of Florence, Division of Pathological Anatomy Department of Medical and Surgical Critical Care, Florence, Italy*

Background: There is growing evidence that methylation plays an important role in breast cancer (BC) development and that characterization of tumor-specific methylation profiles may allow the identification of specific biomarkers to discriminate BC subtypes. Significant differences in tumor-associated gene methylation patterns have been related to ER, PR and HER2 status.

The contribution of DNA methylation and the precise targets of epigenetic alterations in male breast cancer (MBC) development have not yet well investigated.

Materials and Methods: Using candidate-gene approach, we performed promoter methylation analysis of a panel of BC-related genes (*hTERT, ESR1, RASSF1, AR, BCL2, MYC, WNT1, BRCA1,* and *CHEK2*), in tumors and matched normal (blood) samples from 69 MBC patients. The analysis was performed by Pyrosequencing, a highly sensitive and reproducible method, which provides absolute quantitative information on bases at each CpG site analyzed. Methylation levels were determined by calculating the average of methylation for each gene, respectively in tumor and normal samples. ANOVA and Kruskal–Wallis tests were used to identify significant differences in methylation levels: (1) between normal and tumor samples; (2) among tumors

stratified according to relevant clinical-pathologic features, including BRCA1/2 mutation.
Results: Promoter methylation of all the 9 genes analyzed was found in the 69 MBC cases. A gene specific variability in methylation levels was observed, with *MYC* showing the lowest and *CHEK2* the highest methylation level. Overall, methylation levels of each gene considered were higher in tumors than in the corresponding normal samples, and, in particular, significant differences emerged for *hTERT* ($p < 0.0001$), *ESR1* ($p < 0.0001$), *RASSF1* ($p < 0.0001$), *MYC* ($p < 0.0001$) and *WNT1* ($p = 0.047$). Furthermore, significant association emerged between *BRCA1/2* mutations and higher *RASSF1* methylation level ($p = 0.008$), *BRCA1/2* wild-type and higher AR methylation level ($p = 0.016$), HER2+ status and higher *RASSF1* methylation level ($p = 0.01$), G3 (high tumor grade) and higher *RASSF1* methylation level ($p = 0.007$) and between PR− status and higher *WNT1* methylation level ($p = 0.014$).
Conclusion: Overall, our results indicate that alterations in gene methylation profiles are common in MBC and that tumor-associated gene methylation patterns may identify specific MBC subgroups.
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450 The consequence of SETD2 mutation in clear cell renal cell carcinoma progenitor cells

J. Li[1], J. Osinga[1], P. Ferronika[1], M.B. Van Werkhoven[2], M.M. Terpstra[2], P. Van der Vlies[1], G. Duns[3], H. Westers[1], R.H. Sijmons[1], K. Kok[1]. [1]*University Medical Centre Groningen, Genetics, Groningen, Netherlands,* [2]*University Medical Centre Groningen, Internal Medicine, Groningen, Netherlands,* [3]*BC Cancer Agency, Genome Sciences Centre, Vancouver, Canada*

Background: Clear Cell Renal Cell Cancer (ccRCC) is thought to arise from the Proximal Tubular Epithelial Cells (PTECs) in the kidney. Beside the well-established association between the tumor and von Hippel–Lindau (VHL)-hypoxia inducible factor (HIF) signaling pathway, we and others identified 3 new candidate ccRCC tumor suppressor genes that are located on 3p: SETD2, PBRM1, and BAP1. In mammalian cells SETD2 is specific responsible for the tri-methylation of histone 3 lysine 36 (H3K36me3), which is present on the body of actively transcribed genes and plays a role in transcription elongation and/or primary transcript processing. Although clinical data indicate that inactivating SETD2 mutations correlate with higher grade tumors, the biological consequence of its inactivation in ccRCC is still unknown.
Material and Method: We first performed Chip-seq to determine which genes in PTECs carry the H3K36me3 mark, and correlated these data with the level of gene expression. Stable knock down of SETD2 for three independent cultures of PTECs was achieved by transduction with lentiviral particles containing shRNA sequences against SETD2. Growth competition assays are performed by co-culturing normal PTECs and SETD2 knock down PTECs followed by measuring the GFP positive rate. β-Galactosidase staining is used to test the status of senescence. RNA-seq is carried out for expressing profiling, as well as isoform expression analysis.
Results: Genes carrying the H3K36me3 mark are on average highly expressed in PTECs. The knock down of SETD2 in PTECs led to a strong decrease of the level of H3K36me3. Preliminary data suggests that knock down of SETD2 in PTECs may enable the cells to escape from the status of senescence and go into proliferation. The expression profiling data is still under analysis. First results will be presented at the meeting.
Conclusion: Our study confirmed the role of SETD2 mutation in the early development of ccRCC from normal PTECs, it can make PTECs escape from the status of senescence to proliferate, as one of the mechanism of tumorigenesis.
No conflict of interest.

451 Epigenetic changes and dietary factors in human and experimental breast cancer

E. Escrich[1], C. Rodríguez-Miguel[1], N. Braviz[1], A. Modolell[2], T. Checa[2], R. Moral[1]. [1]*Universitat Autonoma de Barcelona, Cell Biology Physiology and Immunology, Barcelona, Spain,* [2]*IDOC Centre Mèdic, Medical Oncology, Barcelona, Spain*

Introduction: Disruption of epigenetic patterns is a major change occurring in all types of tumors. Besides genetic and epigenetic factors, environment has an important role in the etiology of breast cancer, and especially dietetic factors. In this study we aimed to investigate changes in methylation of genes with a role in the hallmarks of cancer, in blood, mammary gland and locally advanced tumors in humans. Moreover, in an experimental model, we analyzed methylation modifications in mammary glands and tumors, and the effects of high fat diets on such changes.
Material and Methods: Blood were obtained from healthy volunteers and locally advanced breast cancer patients. From such patients we also obtained biopsies of mammary gland and tumor. We also analyzed mammary gland and experimental tumor from female rats induced with DMBA. Animals were fed a control diet (LF), a high corn oil diet from weaning (HCO) or from induction (LF-HCO), or a high extra-virgin olive oil diet from weaning (HEVOO) or from induction (LF-HEVOO). We analyzed by methylation-specific PCR (MSP) the genes BRCA1, p16, RARB2, ESR1, PRB, REH3P21, NES1, CDH1, TWIST, HLA-A, CXCL12 and Maspin. Global methylation was determined by pyrosequencing of LINE1.
Results: The study of specific genes in the human samples showed a different methylation profile among blood and mammary tissues. Some differences were observed in blood from healthy volunteers and cancer patients, but results did not reach statistic significance. Gene methylation was always increased in tumor in comparison with mammary gland, such increase being significant in seven genes. We found a decrease in global methylation in mammary tissues in comparison with blood. Moreover global methylation was decreased in tumor versus mammary gland. In the experimental model, MSP analysis of REH3P21 showed a similar pattern than in human tissues, although no differences were found in tumors by effect of the high fat diets. Global methylation showed no differences in mammary gland and tumor in controls and HCO groups, and higher methylation of tumors in animals fed the HEVOO diet.
Conclusion: The study of a panel of 12 genes in human samples showed hypermethylatiòn in most of them in tumor respect mammary gland, accompanied by a decreased in global methylation. In experimental mammary cancer high fats diets may have an influence on the global methylation of tumor cells, but their possible effects on specific genes need further investigation.
No conflict of interest.

452 Modifications in gene expression profile of mammary gland and experimental tumors by effect of high fat diets

R. Moral[1], R. Escrich[1], M. Solanas[1], E. Vela[1], M.C. Ruíz de Villa[2], E. Escrich[1]. [1]*Universitat Autonoma de Barcelona, Cell Biology Physiology and Immunology, Bellaterra (Barcelona), Spain,* [2]*Universitat de Barcelona, Department of Statistics, Barcelona, Spain*

Introduction: Breast cancer is the most frequent malignant tumor among women worldwide. Environment, especially nutritional factors, has an important role in the etiology of this neoplasia. We have previously demonstrated the differential effect of high fat diets on experimental mammary carcinogenesis. Thus, whereas a high corn oil diet (HCO) has a clear stimulating effect, a diet high in extra virgin olive oil (HEVOO) has a negative modulatory influence. The aim of this work is to analyze the effects of those diets on the gene expression profile of mammary gland and experimental tumors.
Material and Methods: Female Sprague-Dawley rats were distributed into 5 groups depending on the type and timing of diet administration: LF (control low fat diet from weaning), HCO from weaning (HCO group), or from induction (LF-HCO group), HEVOO from weaning (HEVOO group) or from induction (LF-HEVOO group). Animals were induced with 7,12-dimethylbenz[a]anthracene at 53 days. For each group, gene expression profile of 6 adenocarcinomas, and 3 abdominal mammary glands at different ages (36 days, 51 days, 100 days and 246 days) was determined using GeneChip® Rat Exon 1.0 ST Array (Affymetrix).
Results: Mammary gland presented a higher number of differentially expressed genes by effect of diets than tumors. After short dietary intervention, the genes modulated in mammary gland were specific for each diet. Data mining revealed different enriched functional categories, the HCO downregulating the expression of genes related to immune system and apoptosis, whereas HEVOO modified the expression of metabolism genes. Longer dietary exposure showed higher number of common genes (modulated in more than one high fat group), mainly related to metabolism. In tumors, modulated genes were mostly specific of each group.
Conclusion: HCO and HEVOO diets differentially modified the expression profile of mammary gland and experimental tumors. Both diets modulated metabolism genes, but only HCO significantly downregulated the expression of immune system and apoptosis genes. Data also suggest the importance than dietetic habits from an early age may have on breast cancer susceptibility.
No conflict of interest.

453 Enhancer of Zeste Homolog 2 (EZH2) modulation in either embryonal or PAX3-FOXO1 alveolar rhabdomyosarcoma shows different anti-tumoral effects

R. Rota[1], E. Carcarino[2], M. De Salvo[1], L. Adesso[1], R. Ciarapica[1], V.E. Marquez[3], A. Mai[4], P.L. Puri[5], D. Palacios[2], F. Locatelli[1]. [1]*Ospedale Pediatrico Bambino Gesù, Oncohematology, Roma, Italy,* [2]*Dulbecco Telethon Institute at EBRI/Santa Lucia, Muscle Diseases, Roma, Italy,* [3]*CCR National Cancer Institute NIH, Frederick National Laboratory for Cancer Research, Frederick MA, USA,* [4]*Istituto Pasteur Fondazione Cenci Bolognetti Sapienza University, Dipartimento di Chimica e Tecnologie del Farmaco, Roma, Italy,* [5]*Sanford-Burnham Medical Research Institute, Muscle Development and Regeneration Program, La Jolla CA, USA*

Introduction: The Polycomb group (PcG) protein Enhancer of Zeste Homolog 2 (EZH2) prevents anticipated differentiation of skeletal muscle progenitors. EZH2 is the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2)