samples. The specific expression profile reflects activated processes related to re-organisation of the microenvironment.
Conclusions: The DCIS lesions within the subgroup are diverse in their classic histopathological subtypes and intrinsic molecular subtypes, suggesting that the signature inherent in these lesions is common across breast cancer subtypes. This raises interesting possibilities for identification of DCIS lesions both with and without invasive characteristics, which potentially could be used in clinical assessment of a woman's risk of progression, and lead to improved management that could avoid the current over- and under-treatment of patients.

797 Modeling BRCA2 associated breast cancer progression through genomic profiling

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Background: During the development and progression of cancers, their genomes undergo different types of modifications, including DNA copy number changes by which gene expression can be affected. In our previous report, we showed that genomic alterations occur in non-random patterns throughout breast cancer genomes which, furthermore, relate to *BRCA* abnormalities and molecular phenotypes (Stefansson et al 2009). The study presented here was carried out to follow-up on results described in our previous report which suggested progression for *BRCA2* tumours involving degree of genomic complexities and histological grade.
Materials and Methods: Copy number changes in 34 breast tumours derived from *999del5 BRCA2* germline mutation carriers were analyzed by high-resolution (~7kbp) array comparative genomic hybridization (385K aCGH; NimbleGen Systems). Tumour phenotypes were established by analysis of expression using immunohistochemistry (IHC) on tissue arrays for selected biomarkers (ER, PR, HER2, EGFR, CK5/6, Ki-67, RB and p16) and histologic grade was determined by the modified Bloom-Richardson system.
Results: Molecular characteristics and patterns of copy number changes differed substantially between *BRCA2* tumours displaying luminal- and triple-negative phenotypes. The observed differences include deletions at the *BRCA2* gene locus which were strongly associated with increased growth advantages in *BRCA2* tumours displaying luminal characteristics reflected in expression of Ki-67 proteins. The same was not found for triple-negative *BRCA2* wherein the event of *BRCA2* deletion appears to be stochastic. Network analysis for copy number changes identified several candidate genes that may cooperate with loss at the *BRCA2* gene locus.
Conclusions: The differences identified between *BRCA2* tumours displaying luminal- and triple-negative phenotype suggests that they have developed in different ways and we show here that this involves the *BRCA2* gene locus. These results have potential implications regarding therapeutic choice for future patients with *BRCA2* germline mutations.

798 Transcriptional modules predicting response of colorectal cancer to EGFR-targeted therapy

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Background: Only a fraction of colorectal cancer (CRC) patients respond to antibodies targeting Epidermal Growth Factor Receptor (EGFR), such as cetuximab. It is known that oncogenic mutations in KRAS or BRAF, downstream effectors of EGFR, impair such response. Such cases however account for only about 70% of the non-responder cases. We considered that gene expression profiling could provide new response predictors for CRC cases with wild-type KRAS or BRAF, and developed two complementary molecular signatures respectively linked to "untractable" and "tractable" EGFR pathway activation, and therefore associated with resistance and sensitivity to EGFR-blocking therapy.
Material and Methods: We collected tissue samples from 93 liver metastases of CRC and carried out global gene expression profiling and mutational profiling for KRAS and BRAF. Using mutational information, we derived a transcriptional signature of genes associated to KRAS mutation, whose summarized expression was defined as the "KRAS signature". We also carried out transcriptional profiling of the response to targeted therapy of various cancer cell lines addicted to EGFR or BRAF oncogenic signaling, and defined a common *in vitro* "Addiction signature", whose genes were mapped and further analyzed in CRC expression datasets.
Results: In our CRC dataset, the KRAS signature sharply distinguished a high-score group, formed not only by samples with mutated KRAS or BRAF but also by some non-mutated samples, and a low-score group, formed by the remaining non-mutated samples. Interestingly, also the Addiction signature partitioned the samples in well-distinguished subgroups, but the partition was only partially overlapping with that of the KRAS signature. We then analyzed the behavior of the two signatures in an independent dataset of CRC-liver metastases, annotated with the mutational status and with the response to cetuximab, administered after the biopsy. In samples with wild-type KRAS or BRAF, both the KRAS and the Addiction signatures were correlated to responsiveness in an opposite manner: drug resistance was associated to either very high KRAS signature or very low Addiction signature. In one case, therefore, the RAS pathway was "untractable", similar that of mutated KRAS-driven cases, in the other case the pathway was not active at all, and therefore not responsive to inhibition. According to this hypothesis, the combination of the two signatures (Addiction signature minus KRAS signature) yielded a much more robust response predictor, confirming that responders must have an active EGFR pathway (high Addiction signature), but still a "tractable" one (low KRAS signature).
Conclusions: These data show that gene expression profiling can be successfully used to dissect the molecular alterations that take place in colorectal cancer and to define how they determine response to targeted therapy also in cases without KRAS or BRAF oncogenic activation.

799 Molecular subgroups of breast cancer show distinct genomic profiles and different clinical courses: a novel definition of disease subclasses

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Background: Breast cancer is notoriously heterogeneous and molecular studies have fostered great expectations in the prospect of defining a renewed classification of breast cancer. However the definition of breast cancer molecular subgroups has been debated, pointing at their instability and elevated dependence on the original set of samples or genes. Three broad classes of breast tumours, commonly used in the clinic, were drawn along their ER, PR and HER2 status but this simple classification lacks precision.
Materials and Methods: We believe that breast cancer can be broken down in smaller and more homogeneous subsets based on their genetic characteristics. To reach such a goal we worked on a large dataset (comprising 712 breast tumours 537 analyzed for expression Affy U133A chips and 655 by BAC-array CGH) in order to enhance statistical power and build a robust molecular classification.
Results: Using a combination of supervised and unsupervised analysis of expression profiling data we defined 6 well-delineated molecular subgroups. Array-CGH analysis revealed that each of the 6 molecular subgroups showed distinct profiles of copy number and associated gene expression changes that will be presented. Of particular notice were the findings of chromosomal regions showing inverse patterns (gain in one subgroup/loss in another). We could associate to each molecular subgroup a specific set of signaling pathways and interaction networks. These differences at the molecular level were consonant with significant differences in tumour grade, metastatic sites, relapse free survival among molecular subgroups. Furthermore, we determined the existence of important differences in response to chemotherapy among subgroups and showed that our classification bore independent prognostic power.
Conclusion: Owing the strong prognostic significance of this classification we propose that it could be the keystone of future investigations aiming at identifying novel prognostic factors or therapeutical targets in breast cancer.

800 Genome copy number variation and gene mutation profiling show different somatic development of colorectal cancers in young and elderly patients

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Background: Colorectal cancer (CRC) is one of the most common cancers in the Western world, and has an average age of diagnosis around 70 years. The majority of patients have tumours with sporadic origin, and less than five percent of all CRC cases have a known hereditary defect causing the disease. However, young age at diagnosis, and/or familial clustering of cancers without known hereditary cancer syndromes, indicate a genetic increased risk and the

tumourigenesis in this subgroup remain poorly described. We have analysed copy number aberrations and mutation profile of known target genes in tumours from early and late onset CRC patients.
Material and Methods: High resolution array CGH analysis (385 000 features), was performed in 23 patients with CRC diagnosis at young age (range: 28−53 years, median: 44 years), and 17 patients with CRC diagnosis at old age (range: 69−78 years, median: 79 years). Furthermore, mutation profiles were analysed in a larger series of carcinomas stratified according to microsatellite instability analysis (MSI) status. These patients included 45 young-at-onset (range; 27−50 years, median; 43 years), and 69 old-at-onset (n = 69, range; 71−93 years, median; 81 years). A panel of five genes, TP53, KRAS, BRAF, PTEN and PIK3CA, were investigated for mutations by sequencing.
Results: The overall genome copy number profiles were similar between carcinomas from patients in the two age groups. However, some chromosomal stretches were found to have statistically significant ($p < 0.05$) more aberrations in the young patients compared to the old-at-onset group (not visa versa); DNA sequences within 2q, 10q, 19q, were more often gained, and sequences within 1p, 1q, 2q, 4p, 4q 10p and 19p, were more frequently lost. KRAS and PTEN mutations were distributed equally between the patient groups, whereas the mutation frequencies of TP53 and PIK3CA differed between the groups. BRAF mutations were not significantly correlated with MSI in the young-at-onset group.
Conclusions: We have identified genomic and gene specific differences in colorectal carcinomas related to time of disease onset. The somatic genomic changes that occur preferentially in tumours from young patients pinpoint potential genetic risk loci that will be further examined.

801 Downstream targets of the TMPRSS2-ERG rearrangement in prostate cancer: cysteine-rich secretory protein-3 (CRISP3) is strongly up-regulated in fusion-positive carcinomas

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Background: A large percentage of prostate cancers harbor *TMPRSS2-ERG* fusions, leading to the aberrant overexpression of the transcription factor *ERG*. The target genes deregulated by this rearrangement, however, remain mostly unknown, precluding additional therapeutic strategies directed at the downstream effectors.
Material and Methods: Genome-wide RNA expression analysis on a subset of 24 prostate carcinomas with (n = 16) and without (n = 8) *ERG* rearrangements provided a list of candidate targets significantly associated with the fusion event. RNA expression data for the top-most deregulated genes was validated on an independent series of 200 tumours using Real-time PCR, whereas protein levels were assessed in an extended series of clinical samples comprising morphologically normal prostate, benign hyperplasic tissue, and prostate carcinomas (n = 77).
Results: Within the group of genes significantly over-expressed in fusion-positive lesions, Cysteine-rich secretory protein-3 (*CRISP3*) showed a striking 38-fold increase in expression when compared to fusion-negative carcinomas, being almost absent in normal and benign prostate tissue. In the independent validation series, *ERG* and *CRISP3* expression levels were strongly correlated ($r_s = 0.84$, $p < 0.001$), and a median 43-fold increase was observed for *CRISP3* in *ERG*-positive tumours. Imunohistochemistry results showed a marked overexpression of *CRISP3* in 66% of the carcinomas, but no clear distinction could be made between fusion-positive and fusion-negative lesions at the protein level.
Conclusions: *CRISP3* mRNA is strongly up-regulated in *ERG*-rearranged tumours, providing a good surrogate marker for the TMPRSS2-ERG fusion.

802 The epidermal growth factor receptor (EGFR) is frequently overexpressed in uveal melanoma as a consequence of chromosome 7 polysomy and miRNA128b downregulation

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Background: Intraocular (uveal) melanomas represent 5% of all melanomas and about 40% of patients develop metastases, usually in the liver, during the first 5 years after diagnosis. Despite advances in the prognostic assessment through cytogenetics and molecular genetic techniques, current therapies are inefficacious for metastatic disease. In order to identify drug targets, we are analyzing gene expression profiles of primary uveal melanomas.
Methods: Expression profiling was performed on 40 samples using Affymetrix HGU133Plus2 arrays. Microarrays for miRNA screening were produced using the Exiqon library version 10.0. Differentially expressed miRNA and mRNAs were validated by qRT-PCR. Copy number of chromosomes 3, 8 and 7 was assessed by fluorescent in situ hybridization (FISH) on histological sections. Array based comparative genome hybridization (CGH) was performed on selected samples using Affymetrix 250K SNP arrays.
Results: mRNA expression analysis identified highly differential expression of several drug targets among which EGFR, a target of specific kinase inhibitors and therapeutic antibodies. We found a significant inverse correlation between the expression miR-128b and its target, EGFR. EGFR overexpression alone does not significantly correlate with recurrence yet the combination with miR-128b does. Polysomy of chromosome 7 also contributes to the overexpression of the receptor. Array CGH and FISH analyses reveal the presence of uveal melanoma subtypes with polysomic chromosomes 7.
Conclusions: EGFR expression, chromosome 7 polysomy and miR-128b expression may contribute to prognosis in uveal melanoma. EGFR is an interesting target for personalized therapy since over-expression is observed in a defined subset of patients who might benefit from therapy with inactivating antibodies.

803 PI3K signalling pathway is activated by PIK3CA gain and overexpression in prostate tumours, but PI3KCA, BRAF, KRAS AND AKT1 mutations are an infrequent event

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Background: The PI3K-AKT and RAS-MAPK pathways are deregulated in a wide range of human cancers by gain- or loss-of-function in several of their components, including *PIK3CA, KRAS, BRAF* and *AKT1*. Our purpose has been to identify genetic alterations in members of these pathways in prostate cancer.
Material and Methods: Eighty-one prostate tumours, 58 from prostate cancer alone (group G1) and 23 from bladder and prostate cancer patients (G2) are the subject of this study. In 20 of these 23, the bladder tumours were also analysed. *PIK3CA, KRAS, BRAF* and *AKT1* hot spot codons and the surrounding exon regions were studied by PCR and direct sequencing, and *BRAF* also by pyrosequecing. *PIK3CA* mRNA expression was tested by qRT-PCR in 19 prostate tumours, and 32 samples were analyzed by FISH to test copy number gain of *PI3KCA* gene. Immunohistochemistry for AKT and pAKT was performed in 46 and 20 prostate tumours respectively.
Results: Five of 19 (26.3%) prostate tumours with Gleason score $\geqslant 7$ showed *PI3KCA* mRNA overexpression, and *PI3KCA* copy gain was detected in 9 of 32 (28%) prostate tumours. Three of 20 (15%) matched bladder tumours, displayed mutations in *PI3KCA*, *KRAS* and *AKT1*, the corresponding prostate tumours being *wt.* We also detected a not previously described *PIK3CA* polymorphism (IVS9+91) in two prostate tumours. Thirty-four percent of samples overexpressed AKT protein, and there is a statistical association ($p = 0.013$) between strong immunostaining and mRNA overexpression and/or copy number gain of *PI3KCA* gene.
Conclusions: *PI3KCA* gene is deregulated by mRNA overexpression and DNA gain in about 26−28% of prostate tumours and the presence of these gene alterations is statistically related to AKT protein overexpression ($p = 0.013$). There is an association between mRNA overexpression and high-grade tumours ($p = 0.040$), but not with FISH status or AKT protein. *PI3KCA*, *BRAF*, *KRAS* and *AKT1* mutations are a very infrequent event in prostate tumours. Gene is deregulated by mRNA overexpression and DNA gain in about 26−28% of prostate tumours and the presence of these gene alterations is statistically related to AKT protein overexpression ($p = 0.013$). There is an association between mRNA overexpression and high-grade tumours ($p = 0.040$), but not with FISH status or AKT protein and mutations are a very infrequent event in prostate tumours.
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