(PTEN positive) and LnCaP (PTEN negative) cells in absence of androgen or in presence of the antiandrogen compound, bicalutamide to derive bicalutamide-resistant (BCLR) clones. Similarly, 22rv1 cells were grown subcutaneously in castrated and intact male nude mice receiving or not 50 mg/Kg/day bicalutamide.
We demonstrated that Akt is activated after treatment with androgen deprivation therapies or bicalutamide. In addition the Akt inhibition as well as Akt gene knock down slowed down the development of androgen independent or BCLTR cell strains. We observed also an increment in DNMT3a and DNMT3b expression as well as in HDAC-2, HDAC-4 and HDAC-6. In vitro treatment with DNMT inhibitor, 5-azacytidine, or a pan histone deacethylase inhibitor, PXD101, upmodulated PTEN levels in PTEN positive and bicalutamide resistant 22rv1 cells and reduced Akt activity down-modulating Akt protein expression both in PTEN positive or negative BCLT treated cells. Azacitidine treatment was able to slow-down the development of a BCLTR phenotype and to restore the effectiveness to bicalutamide. Our study suggests that, after exposure to androgen deprivation therapies, prostate cancer cells undergo a series of coordinated changes which eventually result in the development of androgen independence. A major factor in this process is the induction of DNMT activity by increased expression of DNMT3a and DNMT3b, responsible to de novo/gene specific DNA methylation, reducing the expression of tumour suppressor genes. Similarly the induction of HDAC activities are responsible to stabilization of several oncogenetic molecules (growth factor or intracellular key regulator such as Akt) which may contribute to the development of androgen independence through: (i) maintaining cell proliferation; (ii) inhibiting apoptosis; and/or (iii) inducing AR activation in a ligand-independent fashion. These effects may be mediated, at least in part, through activation of the PI3K/Akt pathway.

136 Validation study of the prognostic significance of β-microseminoprotein and cysteine-rich secretory protein-3 after radical prostatectomy using automated image analysis

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Background: Despite prostate cancer being the most frequent cancer in males in the Western world, there are still no clinically reliable tissue biomarkers for predicting disease recurrence after surgery. We have previously identified β-microseminoprotein (MSMB) and cysteine-rich secretory protein-3 (CRISP3) as independent outcome predictors of biochemical recurrence after radical prostatectomy. In the healthy male, MSMB is second only to prostate specific antigen (PSA) as the most predominant protein expressed, but levels are known to decrease, or even disappear in prostate cancer. In seminal plasma, MSMB can be found in a complex with CRISP3. In this study, we wanted to validate our previous findings in a larger cohort, and to use automated image analysis enabling quantitative determination of MSMB and CRISP3 expression.
Material and Methods: Tissue cores from 3 261 patients undergoing radical prostatectomy at the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005 were organised in tissue microarray blocks, and immunohistochemically stained for MSMB and CRISP3. Whole-slide digital images were captured using a 20x objective and the Aperio ScanScope CS Slide Scanner (Aperio Technologies). A positive pixel count algorithm (Aperio Technologies) was used to develop a qualitative scoring model for cytoplasmic staining.
Results: Low expression of MSMB (<20% of tumour cells staining positive) correlated with biochemical recurrence after radical prostatectomy ($P = 0.001$), and with overall survival ($P = 0.001$). High expression of CRISP3 (>80% of tumour cells staining positive) was not associated with biochemical recurrence ($P = 0.085$), but with overall survival ($P = 0.03$). Multivariate analysis revealed that MSMB expression was an independent predictor of decreased risk of recurrence (hazard ratio, 0.68; 95% confidence interval, 0.57–0.81; $P < 0.001$).
Conclusion: In the current study, we were able to validate the prognostic significance of the suggested biomarkers MSMB and CRISP3, using a large independent cohort, and novel image analysis technology. Prostate cancer tumours expressing low MSMB and high CRISP3 levels are associated with higher risk of recurrence and adverse outcome after radical prostatectomy. MSMB in particular, is a strong independent biomarker for prostate cancer recurrence.

137 A chemical genetics screen identifies novel steroid inhibitor drugs that inhibit the growth of glioma stem cells

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Background: Glioma stem cells represent a fraction of cells within a tumour mass which are postulated to be responsible for tumour re-growth. Moreover, recent studies have associated glioma stem cells with impeccable chemo-resistance mechanisms, leading to an overall poor survival and failure among patients treated by conventional adjuvant chemotherapy. Since a wide range of steroid receptors are expressed in gliomas, our objective was to investigate whether novel classes of steroid inhibitor drugs can be used efficiently to inhibit glioma growth. To achieve this, we studied the effect of these drugs on the growth of glioma stem cells.
Methods and Results: We screened using a candidate chemical structure approach, a library of 400 steroid inhibitor drugs on 5 human glioma stem cells established from surgeries ($n = 2$) and cell lines ($n = 3$), and a normal human neuroprogenitor cell line. We discovered 5 potent new steroid inhibitor drugs belonging to the methyl-piperazine family, can induce significant death of glioma stem cells ($n = 5/5$) within a 24 hour period, and with some death of normal human neuro-progenitor cells. These drugs induced significant apoptosis resulting in an overall decreased viability and proliferation of the cells in a dose dependent manner (5 μM and 10 μM). Furthermore, significant inhibition of transformation was noted.
Conclusions: We have discovered a novel chemically distinct class of drugs that can significantly inhibit the growth of glioma stem cells. Current efforts are undertaken to study more of the mechanistic function of these drugs.

138 Metastatic breast cancer survival according to triple receptor status

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Background: Although the prognosis of metastatic breast cancer (MBC) patients is poor, better knowing of useful prognostic markers could make a difference. The value of known prognostic factors is not well established, mainly because there is a lack of studiesin MBC. The aim of this study was to identify the influence of combined so called “triple receptor status” i.e. estrogen and progesteron receptor (ER, PR) and human epidermal growth factor receptor-2 (HER2) status on prognosis in MBC patients, beside other known clinicopathoogical parmeters.
Materials and Methods: The study included 109 MBC patients with known clinicopathological characteristics. ER/PR status was determined by ligand-binding assay i.e. in cytosol fraction of primary breast cancer tissue using dextran-coated method. HER2 amplification was determined by chromogenic in situ hybridization (CISH) on the same paraffin embedded primary tumour samples.
Results: According to survival analysis, among available clinicopathological parameters as relevant for follow up of MBC patients are years, DFI (disease free interval) and ER/PR status. Combined ER/PR status showed that patients with ER−PR− phenotype have poorer prognosis and that this negative effect is more pronounced with addition of the effect of HER2 amplification. (ER−PR−HER2+ phenotype). Furthermore, survival analysis of extreme receptor combinations (ER−PR−HER2+ and ER+PR+HER2−) in different age subgroups (⩽50 and >50) showed that negative impact of ER-PR-HER2+ phenotype is age related. Patients older than 50 years, with ER−PR−HER2+ phenotype, had the mortality rate 100% and median survival time of 14 months.
Conclusion: These findings confirm that biology of breast cancer could be significantly affected by patient’s age. There is a strong indication for use of combined triple receptor status for follow-up of MBC patients. Finding that ER-PR-HER2+ phenotype in a restricted subgroup of patients (>50 years) means extremely poor prognosis and a highest mortality rate, indicates further consideration regarding therapy efficiency.

139 Annexin A10 (ANXA10) is a marker for metastasis and disease progression in bladder cancer

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Background: Bladder cancer is among the most common type of cancers worldwide. Bladder cancer is clinically divided into two distinct groups; non-muscle-invasive (stages Ta and T1) treated with a local, organ-sparing approach, and muscle-invasive cancer (stages T2-T4) where radical cystectomy with lymphadenectomy is applied. Presently, no molecular biomarkers are

accepted in clinical routine. In spite of the increasing body of high-throughput generated data, molecular tools that can help better and earlier diagnosis and set the basis for a future individualized treatment are still under development.
Recently, we described Annexin A10 (ANXA10) as one of the markers included in a gene expression signature in non-muscle-invasive bladder tumours. This signature predicted both presence of concomitant CIS and progression to muscle-invasive cancer [1].
Annexins carry out biological and physiological processes including anti-coagulation, endocytosis, exocytosis, immune suppression, differentiation, tissue growth and are consistently differentially expressed in neoplasia. ANXA10 down-regulation has been correlated with poor prognosis in both hepatocellular carcinoma and gastric carcinoma [2,3].
Material and Methods: In this study, we aimed to investigate the prognostic value of ANXA10 in both non-muscle-invasive and muscle-invasive bladder cancer by immunostaining; and the function of ANXA10 following ANXA10-siRNA knock-down in bladder cancer cell lines using proliferation and wound healing techniques.
Results: Low ANXA10 nuclear staining was an independent marker for progression to muscle-invasive cancer in multivariate analysis (hazard ratio = 0.38, $P = 0.001$). In addition, low ANXA10 immunostaining in localized muscle-invasive bladder cancer ($n = 97$) was associated with development of metastatic disease ($P < 0.0000$) and short-term survival ($P < 0.0000$). The combination of ANXA10 and p53 immunostaining significantly improved the prognostic value in both non-muscle-invasive and muscle-invasive cancers.
Furthermore, ANXA10 down-regulation resulted in increased cell proliferation and migration.
Conclusions: ANXA10 can be considered an independent prognostic factor for progression to muscle-invasive disease, and for development of metastatic disease in patients with muscle-invasive bladder cancer. The combination of ANXA10 expression with other potential molecular markers as e.g. p53 and RB resulted in strong predictive models of outcome. ANXA10 may identify patients with high risk of metastatic disease that may be candidates for neo-adjuvant chemotherapy.

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140 p8 (Candidate Of Metastasis 1) drives ER-stress/autophagy/apoptosis axis induced by the synthetic cannabinoid WIN in HCC cells

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Background: Today, evidence is emerging for the role of autophagy in the regulation of life and death of tumour cells and its relationship with ER-stress signaling. Our previous results demonstrated that hepatoma HepG2 cells are sensitive to apoptotic effects induced by WIN, a synthetic cannabinoid, which acts through a mechanism involving the reduction in the levels of some survival factors and the activation of pro-apoptotic ones. Since WIN effects were observed after 36−48 hours of treatment, we investigated the possible activation of ER-stress and autophagic process in the first hours of WIN-treatment focusing our attention on p8, a factor whose expression is up-regulated in response to cannabinoid-mediated stress.
Material and Methods: ER-stress- and autophagy-related proteins were studied by RT-PCR and western blotting analysis. The autophagic morphology was estimated by MDC staining and immunofluorescence. Gene silencing was performed using small interfering RNA against p8.
Results: WIN induced ER-stress activating a pathway involving p8-CHOP-TRB3 proteins and increased the expression of the ER chaperone GRP78 which could mediate the transfer of the proapoptotic protein PAR-4 on plasma membrane. Our results indicate that WIN induced the increase in phospho-PAR-4(Thr163) level and the decrease of the pro-survival protein phospho-AKT which is responsible for an inactivating phosphorylation of PAR-4 in Ser249. Moreover, after 16 h of treatment, WIN induced the appearance of autophagic vacuoles and the increase in the lipidated form of LC3 (LC3-II) which is associated with the autophagosomal membrane. The study of beclin-1 revealed a non-canonical beclin-1 independent autophagy. To evaluate the role of p8 as an activator of death pathway we carried out experiments using specific siRNA (sip8). After p8 silencing, either the markers of ER-stress (CHOP, TRB3 and GRP78) as well as those of autophagic process (LC3-II and vacuoles formation) were significantly reduced with respect to the levels observed in WIN-treated non transfected cells.
Conclusions: These findings demonstrate that ER-stress and autophagic activation are early events in WIN-induced apoptosis of HCC cells. In particular, ER-stress-related protein p8 seems to have a key role in triggering the WIN-dependent ER-stress/autophagy/apoptosis cascade in HCC cells. Moreover, the modulation of pAKT/pPAR4 balance contributes to these events.

141 Methylation profiling in non-small cell lung cancer: clinical implications

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Background: Lung cancer is one of the most common cancer malignancies worldwide and, according to the WHO, is the leading cause of cancer death in men and second leading cause in women. Lung cancer is unique among human solid cancers in that a single environmental factor, tobacco smoke, is believed to promote sequential changes in target cells that lead to carcinogenesis. As yet, no routine screening method that enables early detection exists, and this is a key factor in the high mortality rate of this disease. Imaging and cytology-based screening strategies have been employed for early detection, and while some are sensitive, none have been demonstrated to reduce lung cancer mortality. DNA methylation has emerged as a highly promising biomarker and is being actively studied in multiple cancers. In this work, methylation of 1505 CpG loci associated with 803 cancer-related genes were studied in forty six primary non-small lung carcinomas.
Material and Methods: Forty six primary non-small cell lung carcinomas (NSCLCs) and their corresponding control tissue samples were obtained from patients who underwent potentially curative surgery between 2000 and 2005, at San Carlos Hospital in Madrid, Spain. Illumina GoldenGate Methylation® bead array was processed according to manufacturer's protocol. Illumina BeadStudio Methylation Software was use for data analysis.
Results: Sample classification based on CpG methylation profile showed a trend towards clustering tumour versus non-tumour samples. Global hypermethylation (more than 20% of the CpG islands methylated) was associated to a worse prognosis in stage IIIA NSCLCs. In a gen-by-gene comparison of CpG methylation, twelve genes showed correlations with histological type and five with differentiation grade. More interestingly, hypermethylation of genes *CALCA* and *MMP-2* were statistically associated to a worse clinical evolution of patients), whereas hypermethylation of *RASSF1* resulted a protective variable in relation to patient prognosis. These results were independent to TNM tumour stage, as demonstrated by a Cox multivariate analysis ($P = 0.06$, $RR = 2.64$; $P = 0.03$, $RR = 2.96$; $P = 0.023$; $RR = 0.53$, respectively).
Conclusions: Global hypermethylation of a wide panel of genes may be useful as a biomarker to predict prognosis in IIIA TNM stage NSCLC. Moreover, hypermethylation in *CALCA*, *MMP-2* and *RASSF1* emerged as prognostic indicators in I-IIIA TNM stage NSCLCs, independently of tumour stage.

142 Differential expression profiles for senescence and cell death pathways in non small cell lung and colorectal tumours showing telomere shortening

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Background: Differences in how pathways of senescence and cell death operate between Non Small Cell Lung Cancer (NSCLC) and Colorectal Cancer (CRC) could explain the different clinical outcome that shortening telomere reflects, as previous results from our group showed. Our aim in this work consists of investigating whether a differential expression of factors related to these pathways could determinate differential patient outcome conferred by telomere status in NSCLC and CRC.
Material and Methods: We analyzed 36 NSCLCs, 44 CRCs, and their corresponding control tissues, obtained from patients who had undergone potentially curative surgery. Telomere function was evaluated by determining telomerase activity and telomere length. Differential expression of factors related to senescence and cell death pathways was evaluated using microarrays containing a total of 113 oligonucleotide sequences corresponding to genes from these pathways. Also, using microarrays, we investigated expression profiles of 113 genes representative of 6 biological pathways involved in transformation and tumourigenesis. We tested our results by Real Time Quantitative PCR (RT-Q-PCR).
Results: Our results indicated that 75% and 72.7% of NSCLCs and CRCs showed telomerase activity. The median telomere length was 4.15 Kb in NSCLCs and 3.8 Kb in CRCs. Microarray data indicated that NSCLCs significantly overexpressed a group of genes related to senescence and cell death pathways: *BNIP3*, *NDRG1*, *DAPK1*, *AATF*, *GADD45A* and *SHC1*, after comparing NSCLCs and CRCs with telomere attrition. *EGFR* was high and significantly overexpressed in lung tumours as compared with CRCs. Expression data from arrays were confirmed investigating gene expression by RT-Q-PCR. For NSCLCs, RT-Q-PCR analysis showed that expression levels