(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