study population 60.9% had a FIGO stage I, 9.4% FIGO stage II, 18.8% FIGO Stage III and 6.3% FIGO stage IV. 73.3% of all cases showed an Adenocarcinoma. 31.3% had a grade I tumour, 40.6% a grade II and 23.4% a grade III. A high expression of edi-1, edi-2 and edi-3 was in correlation to a significant shorter overall survival for edi-1 (p-value 0.011) and edi-3 (p-value 0.017). edi-2 was without a significant expression concerning to overall survival (p-value 0.0622). The expression was significant in correlation to recurrence free interval for all three samples. The p-value was 0.004 for edi-1, 0.014 for edi-2 and <0.001 for edi-3. In addition, NCBI blast-to-sequence analysis showed, that the initial sequence of edi-3, identified with differential display technique, is part of a hypothetical protein, named KIAA1434.
Conclusion: We found edi-1 and edi-3 in a significant correlation to Overall survival and recurrence free interval using Taqman-Assay. This analyses of differential displayed gene sequences using a second technique was done in a comparable study population. Edi-1 and edi-3 are valuable candidates for further investigations on tumour aggressiveness in endometrial cancer.

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Characterization of carbonic anhydrase 9 (CA9) overexpression: endogenous hypoxia marker and potential tumor-specific target

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Background: The transmembrane glycoprotein carbonic anhydrase 9 (CA9) has been discussed as an endogenous marker of tumor hypoxia and, due to the rarity of hypoxia in normal tissue, a target for tumor-specific treatment. Isolation of live CA9-positive cells from experimental tumors (Olive, Cancer Res 2001) may permit the separate analysis of treatment effects in normoxic and hypoxic cells. We characterized the conditions of CA9 overexpression *in vitro* and developed an improved FACS protocol for CA9 sorting.
Methods: HT 1080 human fibrosarcoma and FaDu human pharyngeal carcinoma cells were subjected to 20%, 5%, 1% or 0.1% O_2 for 10 min, 1 h, 6 h or 24 h (± reoxygenation up to 20 h; n=3–4). Treatment with 100 μM desferrioxamine (DFO) served as positive control. CA9 protein was measured by Western blotting of whole-cell lysates using the M75 antibody (Pastorekova, Virology 1992; CA9/β-actin ratio of DFO control = 100%). To evaluate the association of CA9 with radiobiologic hypoxia, cells were irradiated at the above O_2 concentrations with 2, 5 or 10 Gy (n=3–5). Modified oxygen enhancement ratios (OER') were calculated. A FACS protocol was developed using the anti-CA9 primary and FITC secondary antibodies and applied to known mixtures of normoxic and hypoxic (0.1% O_2) cells irradiated with 10 Gy before mixing. Mixtures were also plated for clonogenic survival.
Results: CA9 remained at aerobic baseline (20% in HT 1080, below 5% in FaDu) in the first 6 h of hypoxia, irrespective of O_2 concentration. At 24 h, equal CA9 overexpression of 100% was seen in HT 1080 treated with 5%, 1% or 0.1% O_2. In FaDu, 1% and 0.1% O_2 caused identical CA9 levels of 65% at 24 h (5% O_2: 37%). CA9 protein was stable over 20 h of reoxygenation. CA9 overexpression was modified by medium glucose concentration and cell density. OER' values were correlated with CA9 level in FaDu but not in HT 1080. Hypoxia (24 h, 0.1%) led to a 200-fold and 30-fold increase of anti-CA9-FITC fluorescence in HT 1080 and FaDu cell suspensions, respectively. The percentage of CA9-positive cells, as determined by FACS, in known mixtures of hypoxic/aerobic HT 1080 cells (1% to 99%) was well correlated with the known percentage of hypoxic cells and the clonogenic survival of mixtures after 10 Gy.
Conclusion: CA9 is a stable indicator of chronic hypoxia, being overexpressed already under mild hypoxia which may limit its use as a therapeutic target. The FACS protocol permits good separation of aerobic and hypoxic HT 1080 cells *in vitro*. The percentage of CA9-positive cells is correlated with hypoxic radiation resistance in mixtures of aerobic and hypoxic HT 1080 cells. The method appears suitable to study the treatment sensitivity of chronically hypoxic cells in tumors, e. g. to hypoxia-specific drugs or radiation.

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Eradication by induced apoptosis of chemoresistant infiltrating ductal carcinoma (IDC) characterised by HDAC2 overexpression and 5′CpG island hypermethylation of the FHIT, RARb2, BRCA1, APC, p16(CDKN2A), RASSF1A, CDH1(Ecadherin), stratifin, MDG1 and HIC1 oncosuppressor genes after combined treatment consisting of immunochemoconjugate of anti-DNMT1/HDAC2 bispecific F(ab)2-bsAb linked with cleavable disulfide onto vinorelbine (I-VRL)

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IDC accounts for approximately eighty percent of all cancers. Despite complete surgical resection, up to one half of patients die from recurrent disease within five years. IDC is characterised by intrinsic resistance to chemotherapy and mechanisms of this clinical resistance are poorly known and understood. One of the reasons is deacetylation and DNA methylation which causes silencing of tumour suppressor genes. To improve the prognosis of patients with IDC, a better understanding of the molecular changes involved in its pathogenesis is essential. DNMT1 interacts with HDAC2 to repress transcription of tumour suppressor genes. IDC cells obtained by surgical excision from a patient were analysed by IHC, PCR-based LOH, ChIP assay, methylation-specific PCR, RT-PCR and Northern blot. There was loss of FHIT expression, LOH at FHIT and 5′CpG island methylation of the FHIT gene. Furthermore, there was transcriptional silencing of the following tumour suppressor genes: BRCA1, RARb-2, APC, p16(CDKN2A, RASSF1A, CDH1(E-cadherin),14–3-3-s (stratifin), MDG1 and HIC1. There was overexpression of DNMT1 and HDAC2 suggesting a link between histone deacetylation, cytosine methylation, local chromatin condensation and subsequent transcriptional repression. This chemoresistant IDC was defined as (CIMP+) CpG island-methylator-phenotype positive. We treated IDC cells with immunochemoconjugate of anti-DNMT1/HDAC2 bispecific F)ab)2-bsAb linked with cleavable disulfide to vinorelbine termed as immunovinorelbine (I-VRL). Post-treatment, there was inhibition of HDAC2 and DNMT1 blocking the 5′CpG island methylation of the tumour suppressor genes resulting to transcriptional activation by upregulation of their mRNA. Furthermore, there was histone hyperacetylation which opens chromatin structure in which the DNA is more loosely wrapped around the histones making it more receptive to interaction with transcription factors. Overexpression of the tumour suppressor genes combined with the microtubule depolymerizing action of vinorelbine inhibited metabolic activity and DNA synthesis of tumour cells according to MTT and BrdU assays, respectively. Immunological analysis exhibited antibody-directed cytotoxicity (ADCC). There was induction of apoptosis in IDC cells according to TdT-mediated-DUTP-biotin nick end labeling (TUNEL) method and transmission electron microscopy(TEM). A large number of tumour cells exhibited condensed chromatin and membrane-bound small bodies (apoptotic bodies) which were phagocytosed by adjacent tumour cells leading to a bystander killing effect. Concluding, this therapeutic approach with immunochemoconjugate anti-DNMT1/HDAC2 bispecific F(ab)2-bsAb linked onto vinorelbine (I-VRL) may revolutionize IDC treatment adding significantly to the current clinical armamentarium due to potential advantages offered by I-VRL over conventional therapy such as well defined mode of action, selectivity and mainly circumvention of chemoresistance by causing DNA demethylation and histone hyperacetylation reactivating transcriptionally silenced oncosuppressor genes.

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The role of NAD(P)H: quinone oxidoreductase 1 (NQO1) in geldanamycin, 17AAG and 17AG metabolism

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Geldanamycin and related quinones 17AAG and 17AG exhibit antitumor activity primarily through the inhibition of HSP90. Previous work by others has demonstrated that NQO1 metabolizes geldanamycin and related quinones and expression of NQO1 in cancer cell lines generally increased sensitivity to these agents. We have extended these studies to examine in greater detail the role of NQO1 in geldanamycin, 17AAG and 17AG metabolism. In studies using purified recombinant human NQO1 (rhNQO1) an approx. 1:1 stoichiometric relationship was observed between NAD(P)H oxidation and quinone reduction. Oxygen uptake studies revealed only trace levels of O2 consumption during the metabolism of these compounds by rhNQO1 indicating the formation of hydroquinones resistant to autooxidation. In addition, very low levels of oxygen consumption were also detected when geldanamycin, 17AAG or 17AG was incubated with NAD(P)H in the presence of either mouse or human liver microsomes suggesting that these quinones do not rapidly undergo redox cycling reactions. The reduction of geldanamycin, 17AAG and 17AG by rhNQO1/NAD(P)H to the corresponding hydroquinones was confirmed by tandom LC-MS. To examine the role of NQO1 in 17AAG metabolism in cells we utilized the NQO1-null human breast cancer cell line MDA468 and MDA468/NQ16, a stably transfected clone that expresses high levels of NQO1 protein. Following treatment with 17AAG the MDA468\NQ16 cell line was 20-fold more sensitive to growth inhibition compared to the MDA468 cell line. The increased sensitivity of the MDA468\NQ16 cell line to 17AAG could be abolished if the cells were pretreated with a mechanism-based inhibitor of NQO1. HLPC analysis of intact cells in culture treated with 17AAG demonstrated higher concentrations of 17AAG hydroquinone in MDA468\NQ16 cells compared to MDA-468 cells and interestingly MDA468\NQ16 cells also contained greater concentrations of 17AAG. These results demonstrate that geldanamycin, 17AAG and 17AG do not undergo redox cycling reactions that generate large quantities of reactive oxygen species. Additionally, the hydroquinone formed following