Material and Methods: We detected CSCs from an esophagus ascites with the stemgent alkaline phosphatase staining kit II and characterised CD133+ cells by separating Lin- and CD133+ cells with the miltenyi diamond separating kit. These cells have been cultured with the esophagus CSC medium from cellsystem and analysed by determined surface marker (CD133, CD166) with FACS, quantitative gene expression of stem cell transcription factors (Oct3/4, Sox2, Nanog, cMyc) and their behaviour of migration under TGFβ stimulation.
Results: After separation of Lin- CD133+ from an esophagus ascites we cultured the cells under specific condition for CSCs. We could show that the number of CD133+ cells increase over a period of 21 days from 1% up to 7.8%. Furthermore we could demonstrate that 4% of CD133+ also positive for CD166. The gene expression of stem cell markers in CD133+ cells compared to CD133- cells showed an up regulation of different markers e.g. Oct3/4, Nanog. We also obtained differences in migration behaviour.
Conclusion: We not only demonstrated that cells with stemness characteristics exist in ascites, but also isolated and characterized them. These cells exhibit markers like CD133 and CD166 which describe for CSCs. Only in CD133+ cells is an up regulation of specific stem cell transcription factors. These results indicate that CD133+ cells from ascites feature a stem cell potential and maybe play an important role in metastases. This hypothesis is supported by the observance that the CD133+ cells showed different migration behaviour compared to CD133- cells. The presented data not only demonstrated the importance of understanding CSCs but also to develop a clinical treatment.

1017 POSTER

Over Expression of ALDH1 as Stem Cell Marker, Is Associated With Mutated BRCA1 in Breast Carcinomas

Z. Madjd[1], B. Ramezani[1], M. Kadiver[1], M. Asadi-Lari[2]. [1]*Tehran University of Medical University, Pathology, Tehran,* [2]*Tehran University of Medical University, Epidemiology, Tehran, Iran*

Background: Cancer stem cells (CSC) have been described in a variety of malignancies including breast carcinomas. Among several markers aldehyde dehydrogenase 1 (ALDH1) has been identified as a reliable marker for breast cancer stem cells. Knockdown of BRCA1 in primary breast epithelial cells leads to an increase in cells expressing ALDH1.
Methods: We examined 127 breast carcinomas for expression of prospective CSC markers ALDH1, using by immunohistochemistry and correlated with clinicopathological parameters, also with the status of BRAC1 in breast carcinomas.
Results: Cytoplasmic expression of ALDH1 was significantly higher in aggressive tumours (p-value = 0.023), whereas, no significant association was detected between expression of ALDH1 and other prognostic factors. Comparing the results for both ALDH1 and BRCA1 expression showed a significant inverse association between expression of ALDH1 and BRCA, indicating that reduced BRCA1 was more often seen in breast cancer cells expressing ALDH1 (p-value = 0.044).
Combining the results for these two markers, a total of 24/ 110 (22%) of tumours displayed the ALDH1 +/BRCA1 −/low phenotype, which occurred more frequently in higher grade tumours. (P-value = 0.042).
Conclusion: Taken together, our finding suggests that increased ALDH1 was significantly more frequent in aggressive tumours and significantly correlated with reduced BRCA1 in breast carcinoma. Therefore, ALDH1 positive (cancer stem) cells with mutated BRCA1 phenotype intended to be more aggressive and this may indicate a subset of patients for whom more aggressive adjuvant treatment is appropriate.

1018 POSTER

Delayed Cell Death Associated With Mitotic Catastrophe in Gamma-Irradiated Stem-like Glioma Cells

G. Niedermann[1], E. Firat[1], S. Gaedicke[1], C. Tsurumi[1], A. Weyerbrock[2]. [1]*University Clinic Freiburg, Department of Radiation Oncology, Freiburg,* [2]*University Clinic Freiburg, Department of Neurosurgery, Freiburg, Germany*

Background and Purpose: Stem-like tumour cells are regarded as highly resistant to ionizing radiation (IR). Previous studies have focused on apoptosis early after irradiation, and the apoptosis resistance observed has been attributed to reduced DNA damage or enhanced DNA repair compared to non-stem tumour cells. Here, early and late radioresponse of patient-derived stem-like glioma cells (SLGCs) and differentiated cells directly derived from them were examined for cell death mode and the influence of stem cell-specific growth factors.
Materials and Methods: Primary SLGCs were propagated in serum-free medium with the stem-cell mitogens epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2). Differentiation was induced by medium containing serum without EGF and FGF. Radiation sensitivity was evaluated by assessing proliferation, clonogenic survival, apoptosis, and mitotic catastrophe. DNA damage-associated γH2AX as well as p53 and p21 expression were determined by Western blots.
Results: SLGCs failed to apoptose in the first 4 days after irradiation even at high single doses up to 10 Gy, but we observed substantial cell death later than 4 days postirradiation in 3 of 6 SLGC lines treated with 5 or 10 Gy. This delayed cell death was observed in 3 of the 4 SLGC lines with nonfunctional p53, was associated with mitotic catastrophe and occurred via apoptosis. The early apoptosis resistance of the SLGCs was associated with lower γH2AX compared to differentiated cells, but we found that the stem-cell culture cytokines EGF plus FGF-2 strongly reduce γH2AX levels. Nonetheless, γIR-induced apoptosis even correlated with EGF/FGF-induced proliferation and mitotic catastrophe in two p53-deficient SLGC lines examined. In a line containing CD133-positive and -negative stem-like cells, the CD133-positive cells proliferated faster and underwent more γIR-induced mitotic catastrophe.
Conclusions: Our results suggest the importance of delayed apoptosis, associated mitotic catastrophe, and cellular proliferation for γIR-induced death of p53-deficient SLGCs. This may have therapeutic implications. We further show that the stem-cell culture cytokines EGF plus FGF-2 activate DNA repair and thus confound *in vitro* comparisons of DNA damage repair between stem-like and more differentiated tumour cells.

1019 POSTER

CD133-/low HT29 Cells Rapidly Re-express CD133 in Vitro and in Vivo and Show Enhanced Survival Under Physiological Oxygen Conditions

J. Waurig[1], C. Dittfeld[1], Y. Garbe[1], S. Peickert[1], A. Dietrich[1], L.A. Kunz-Schughart[1]. [1]*Oncoray − National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus − University of Technology Dresden, Dresden, Germany*

Background: In primary colorectal carcinomas (CRC) CD133 surface expression has been described to enrich for cells with a cancer stem cell like phenotype. The biomarker, however, is under fear debate and we found CD133 expression to not define tumour-propagating cell populations in established CRC cell lines. However, when we studied the *in vitro* properties of $CD133^{-/low}$ vs. $CD133^{+}$ HT29 subpopulations, $CD133^{+}$ cells showed a significantly higher clonogenic survival than their $CD133^{-/low}$ counterparts. This difference unexpectedly neither correlated with response to treatment nor did it translate to loss of tumorigenic potential *in vivo*. We therefore aimed at identifying the rationale for this *in vitro − in vivo* discrepancy.
Materials and Methods: $CD133^{+}$ and $CD133^{-/low}$ HT29 cells were separated via fluorescence-activated cell sorting and studied *in vitro* and *in vivo*. CD133 protein expression was analyzed by flow cytometry and western blotting directly after sort and in short-term 2-D and 3-D cultures. Xenograft tumour formation was monitored in a limiting dilution approach using as low as 10 cells for subcutaneous injection in NMRI (nu/nu) mice. Selected xenografts were extracted, dissociated and CD133 distribution was recorded.
Results: The $CD133^{-/low}$ HT29 subpopulation frequently showed a slight signal shift in flow cytometry and a dim CD133 protein band in western blot analyses directly after sorting. During both 2-D and 3-D culture of the $CD133^{-/low}$ HT29 population, a rapid and massive increase in CD133 expression was observed resulting in a redistribution of $CD133^{+}$/$CD133^{-/low}$ populations within 19 days of culture. A similar redistribution was seen in xenografts derived from $CD133^{-/low}$ HT29 cells. Because we found HT29 to show enhanced clonogenic survival under physiologic oxygen concentrations, we also analyzed cell survival of the subpopulations at 4% oxygen *in vitro* and interestingly found the survival advantage to be higher for the $CD133^{-/low}$ than for the $CD133^{+}$ subpopulation.
Conclusions: The survival advantage of $CD133^{-/low}$ HT29 under physiological oxygen conditions in parallel to the plasticity in CD133 expression are likely to contribute to the lack of phenotypic difference of $CD133^{+}$ vs. $CD133^{-/low}$ HT29 populations *in vivo*.
Work was supported by DFG grant KU 971/7.

1020 POSTER

Detection of Circulating Tumour Cells (CTCs) in Gastrointestinal Tumours Using Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Method

S. Kucukyildirim[1], B. Erdem[1], Z. Polat[2], H. Mergen[1]. [1]*Hacettepe University, Biology, Ankara,* [2]*Gulhane Military Medical Academy, Gastroenterology, Ankara, Turkey*

Background: Most cancer deaths are caused by haematogenous metastatic spread and subsequent growth of tumour cells at distant organs.

Recent molecular and clinical studies have shown that invasion and metastasis may occur very early in tumour development, thus emphasizing the potential importance of spesific and sensitive detection of circulating tumour cells (CTC). With the ability of refined technologies the identification of CTCs from peripheral blood is emerging as a useful tool for the detection of malignancy, monitoring disease progression and measuring response to therapy.
The goal of this study was to identify optimal marker or marker combinations for detection of CTCs in the gastrointestinal malignancies using RT-PCR.
Materials and Methods: To detect the presence of CTCs, we analyzed Cytokeratin 19 (CK19), Cytokeratin 20 (CK20) and Mucin 1 (MUC1) mRNA in the peripheral blood of 31 patients with gastrointestinal (gastric, stomach and colorectal) carcinoma and 30 healthy individuals.
Results: In RT-PCR analysis of the peripheral blood, 77.4% (24/31), 58.06% (18/31) and 3.22% (1/31) of cancer patients were positive for MUC1, CK20 and CK19 mRNA respectively. The sensivity and specificity for any one of mRNA detected in peripheral blood is 83.3% and 66.6% respectively, with an acurracy of 59%.
Conclusions: Our study suggest that MUC1 and CK20 mRNAs in the peripheral blood could be useful molecular markers for gastrointestinal tumours. Combination of these two tumour-spesific mRNA markers would increase the detection rate and may be clinically helpful in predicting the tumour presence and colorectal cancer metastasis.

1021 POSTER

Docosahexaenoic Acid Induces Cell Death Through ROS-dependent ERK and JNK Activation in Human Ovarian Cancer Cells

K. Lim[1], S. Jeong[1], K.S. Song[1], N. Kim[1], K. Jing[1], S. Shin[1], H. Oh[1], J.I. Park[1], W.H. Yoon[1], B.D. Hwang[1]. [1]*Chungnam National University Medical School, Biochemistry, Daejeon, Korea*

Background: Ovarian cancer is the main cause of cancer death from gynecologic tumours. Although there are abundant experimental evidences showing that the ω3-polyunsaturated fatty acids (ω3-PUFAs) rich in fish oil, such as DHA and EPA, inhibit cancer development and growth, the molecular mechanisms on the anti-cancer actions of ω3-PUFAs remain incompletely understood. In the present study, anticancer action of docosahexaenoic acid (DHA) has been investigated in ovarian cancer cells.
Material and Methods: The effects of DHA on cell proliferation and cell cycle were examined by MTT assay and FACS. DHA-induced apoptosis was analyzed using the TUNEL assay, caspase activity assay, and western blot. Dihydroethidium (DHE) was used in PA-1cells for reactive oxygen species (ROS) measurement.
Results: DHA induced cell cytotoxicity in ovarian cancer cell lines including PA-1, MDAH2774 and ID8. Following treatment of DHA, the cell proliferation of PA-1 cells was decreased in a dose and time-dependent manner. Further study showed that the DHA-induced cytotoxicity was mainly associated with apoptosis as caspase 3 activity, TUNEL-positive cells and the portion of sub-G1 cells were significantly increased in the cells treated with DHA. DHA dramatically increased cellular level of phospho-ERK and phospho-JNK Pretreatment of ERK inhibitor, U0126, partially protected the cell death caused by DHA, indicating protective role of ERK activation in DHA-induced cell death. In addition, an oxidative process was implicated in apoptosis induced by DHA since DHA increased ROS level in PA-1 cells. ROS scavenger, NAC, prevented DHA-increased ROS production and cytotoxicity. Finally, pretreatment of PA-1 cells with NAC attenuated ERK and JNK activation and cell death induced by DHA.
Conclusions: DHA induces cell death through ROS-dependent ERK and JNK activation in human ovarian cancer cells, thus providing important evidence and molecular insights for the use of ω3-PUFAs in chemoprevention and treatment of ovarian cancer.
This work was supported by basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (2010–0001290).

1022 POSTER

Microtubule − a Target of Withaferin-A Induced Cell Death

K. Ghosh[1], A. Das[2], A. Lahiri[1], G. Chakraborti[2], S. Sengupta[1]. [1]*University of Calcutta, Biophysics Molecular Biology and Genetics, Calcutta,* [2]*University of Calcutta, Biotechnology, Calcutta, India*

Background: The tubulin-microtubule equilibrium in a cell has been a very popular target for anticancer therapy; while paclitaxel and vinblastine are two widely used drugs employed for treating cancer, many natural products are getting importance in this field of search. Withaferin-A (WA), derived from Withania-somnifera-Dunal, induces cell cycle arrest and apoptosis in different carcinomas. WA alters cytoskeletal structure by targeting actin microfilament, as well as intermediate filament protein vimentin. Heretofore, no evidence has been reported regarding its effect on microtubule assembly. This study is to elucidate the mechanism of inhibiting microtubule assembly by WA.
Methods: The IC-50 value of WA was determined by MTT assay, apoptosis and cell cycle assay was performed by FACS analysis, wound healing assay was done to see cellular migration, immune fluorescent detection was done to check microtubular network of WA treated cells. Effect of WA on tubulin polymerization in vitro was studied by light scattering assay, binding measurements of WA to tubulin was determined by flurometric assay and probable WA-tubulin interaction site was proposed by molecular modeling method.
Results: We found, WA inhibited proliferation of MCF7, A549 and HeLa cells by inducing apoptosis in concert with cell cycle arrest in different phases, effectively inhibited cellular migration, caused significant disruption of interphase and spindle microtubular organization. WA inhibits microtubule polymerization of purified goat brain tubulin *in vitro*. Binding of WA to tubulin quenches tryptophan fluorescence of tubulin, alters fluorescence of ANS-tubulin complex and stiochiometry of WA is to tubulin binding was 1:1 (molar ratio) with a dissociation constant of $14.30 \pm 5.35\ \mu M$. Competition assay showed no binding of WA to colchicine binding site of tubulin. Molecular docking simulations indicated that WA preferentially binds to a novel site to tubulin.
Conclusion: It is evident that WA suppresses microtubule dynamics by directly binding to tubulin which sheds light on mechanisms behind its anti-proliferative activity.

1023 POSTER

Role of the E2F1 Transcription Factor in Transforming Growth Factor-β-mediated Apoptosis

J. Korah[1], N. Falah[1], J.J. Lebrun[1]. [1]*McGill University Health Centre, Department of Medicine, Montreal, Canada*

Tumour formation is linked to a series of well-defined events that occur in most human cancers, including the ability of cells to attain immortalization and to evade apoptosis. Transforming growth factor-β (TGFβ) exerts its tumour suppressive effects by inhibiting cell growth, preventing immortalization through inhibition of telomerase, and inducing apoptosis. In a previous study, we showed that TGFβ-mediated inhibition of telomerase requires an intermediate molecule that we identified as the transcription factor E2F1. The E2F family of transcription factors plays a central role in regulating cell-cycle progression, and deregulation of these factors is a common event in most human cancers. The transcriptional activity of E2F1 is regulated primarily via its association with the retinoblastoma tumour suppressor protein, pRb. Interestingly, E2F1 has been shown to induce both cell cycle progression and apoptosis, though the mechanisms of E2F-mediated apoptosis have not been fully elucidated. As TGFβ itself is a potent proapoptotic factor, we investigated the potential implication of E2F1 in TGFβ-mediated apoptosis.
Expression analysis by Western blot demonstrated that E2F1 itself is upregulated by TGFβ in a number of varying cancer types and cell lines. Transient siRNA knockdown of the Smad proteins indicated that these canonical downstream effectors of TGFβ signalling are implicated in this regulation. Analysis by quantitative real-time PCR indicated that numerous proapoptotic genes known to be induced by E2F1 are also regulated by TGFβ and, in particular, we identified Smac/DIABLO as a novel TGFβ target. In addition to regulating specific apoptotic genes, TGFβ reduced cell viability and induced apoptosis in a number of cell lines, as assessed by MTT and Calcein-AM viability assays as well as AnnexinV staining and PARP cleavage. Importantly, transient siRNA knockdown experiments indicated that loss of E2F1 antagonized these TGFβ proapoptotic effects. Overexpression experiments with dominant negative E2F1 mutants revealed that pRb binding to E2F1 is implicated in TGFβ-mediated apoptosis. Immunoprecipitation studies confirmed that TGFβ induces association of E2F1 and pRb and additionally revealed the recruitment of the histone acetyltransferase P/CAF to this complex in response to TGFβ. Moreover, chromatin immunoprecipitation (ChIP) analysis indicated that this E2F1-pRb-P/CAF complex is recruited to the promoters of a number of proapoptotic genes in response to TGFβ.
These data strongly support a proapoptotic role for the E2F1 pathway downstream of TGFβ and provide a potential mechanism for the TGFβ-mediated activation of E2F1-responsive proapoptotic genes. Together, our results highlight E2F1 as a critical regulator of TGFβ tumour suppressive effects; in addition to its role in mediating TGFβ-induced inhibition of telomerase, we now show that E2F1 is also central to TGFβ-mediated apoptosis.