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

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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

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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

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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*.
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1020 POSTER

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

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