492 Poster

Biodistribution and kinetics of the novel selective oncolytic adenovirus M1 after systemic administration

X. Huang[1], L. Zhuang[2], Y. Cao[1], Q. Gao[1], Z. Han[1], D. Tang[1], H. Xing[1], J. Zhou[1], D. Ma[1]
[1]Cancer Biology Research Center, TongJi Hospital, Wuhan, China; [2] Cancer Center, TongJi Hospital, Wuhan, China

Oncolytic adenoviruses represent a promising novel therapeutic option for the treatment of cancer. Despite their demonstrated safety after human clinical trials, the fundamental properties of oncolytic adenovirus biodistribution, spread, viral persistence, and replication in vivo have not been completely elucidated. The aim of this study was to evaluate the kinetics of viral distribution, spread, replication, and anti-tumoral efficacy after intravenous administration of a novel oncolytic mutant M1. This mutant consists of the E1A CR2-deleted Adv5 with a fragment of antisense plk1 cDNA inserted into the deleted 6.7K/gp19K region, which combines oncolytic properties with efficient plk1 silencing, as described in our previous reports. In the present study, we established a new human orthotopic gastric carcinoma with a high frequency metastasis mouse model, and demonstrated that M1 spread not only in local primary tumors, but also in disseminated metastases. M1 could effectively replicate in tumor cells leading to “oncolysis” and was able to eliminate expression of the targeted gene plk1 in human orthotopic gastric carcinoma model mice. Therefore, intravenous administration of M1 could prolong the survival time of tumor-bearing mice.

493 Poster

Regulation of matrix metalloproteinases and tissue inhibitor of metalloproteinases by cobalt chloride-stimulated hypoxia in primary and metastatic breast cancer cells

W.Y. Lee[1], F. Ou-Yang[2], M.F. Hou[2], S.F. Yang[3], S.C. Huang[4]
[1]Chi Mei Medical Center, Department of Pathology, Tainan, Taiwan; [2] Kaohsiung Medical University Hospital, Department of Surgery, Kaohsiung, Taiwan; [3] Kaohsiung Medical University Hospital, Department of Pathology, Kaohsiung, Taiwan; [4] Chi Mei Medical Center, Department of Obstetrics and Gynecology, Tainan, Taiwan

Tumor hypoxia promotes cancer progression. Degradation of the extracellular matrix by matrix metalloproteinases (MMPs) is required for breast cancer cell invasion and metastasis. The activity of MMPs is tightly regulated by the coordinated expression of the tissue inhibitors of metalloproteinases (TIMPs). We investigated the effect of cobalt chloride (CoCl2)-stimulated hypoxia on the transcription of MMPs and TIMPs of four breast cancer cell lines, the primary cancer cells (HCC1395 and HCC1937) and the metastatic cancer cells (MCF-7 and MDA-MB-231). The phenotypes of MMPs expression were different between primary and metastatic breast cancer cells. MCF-7 express notably less MMPs as compared to other cell lines. MMP-2 and MMP-9 (type IV collagense) measured by RT-PCR and zymography were notably expressed in primary cancer cells but not in metastatic ones. MMP-7 was also highly expressed in primary cancer cell lines. Hypoxic stimulation increased the mRNA level of MMP-1, MMP-10, MMP-13 in metastatic breast cancer cell lines, whereas only MMP-13 was up-regulated in primary HCC1937 cells by hypoxic stimulation. The expression of TIMPs was not altered induced by hypoxia; except TIMP-4 was down-regulated in MDA-MB-231 cells. Our data suggest that MMPs play no crucial roles in hypoxia-induced tumor progression in primary breast cancer cells. Furthermore, metastatic breast cancer cells express less MMPs particular MMP-2 and MMP-9. These results suggest that MMP inhibitors as anticancer agents have no beneficial effect in the patients with metastatic breast cancer.

494 Poster

Apoptosis resistance in breast cancer stem cells through the regulation of Bcl2

Z. Madjd[1], A. Zare Mehrjerdi[1], A. Sharifi[2], S. Molanaei[3], S. Zohoorian[4], M. Asadi Lari[5]
[1]Iran University of Medical Scieces, Department of Pathology, Tehran, Iran; [2] Iran University of Medical Scieces, Department of Pharmacology, Tehran, Iran; [3] Milad Hospital, Department of Pathology, Tehran, Iran; [4] Iran University of Medical Sciences, Department of Pathology, Tehran, Iran; [5] Iran University of Medical Scieces, Department of Epidemiology, Tehran, Iran

Background: Cancer stem cells are a small population of tumor cells that possess the stem cell property of self-renewal, pluripotency and immortality. They arise from their normal stem cell counterparts which undergo accumulative genetic changes until the cells acquire a malignant phenotype. Breast cancer stem cells identified as CD44+/CD24 -/low breast tumour cells which retain the ability to form new tumours in mouse models. Stem cells can resist apoptosis through some mechanism such as the regulation of Bcl2. Identification of breast cancer stem cell is important because of its implication on development of new therapeutic strategies.

Methods: We have investigated 160 primary operable breast cancer patients with a median age of 46 years for the prevalence of CD44+/CD24-/low tumour cells and their prognostic values. Immunohistochemistry was applied to identify the population of cancer stem cells in the paraffin embedded tissues of breast cancer patients. The prevalence of cancer stem cells are then correlated with level of expression of anti-apoptotic protein Bcl2, also with estrogen receptor (ER), progesterone receptor (PR) and prognostic factors including tumour grade, tumour size, lymph node stage, and distant metastasis.

Results: A significant correlation was found between level of expression of Bcl-2 and tumour grade of breast tumours ($p<0.001$), and tumour size ($P=0.001$). There was a strong association between the presence of Bcl-2 and the presence of ER and PR ($p=0.042$). Moreover, a positive association was found between prevalence of CD44+ cells and Bcl2 expression ($p=0.004$); i.e. CD44+ cells express higher levels of anti-apoptotic protein Bcl2, therefore can resist apoptosis.

Conclusion: Anticancer therapy including chemotherapy normally target non-tumorigenic cells in tumour, while cancer stem cells are still survive and leading to tumour recurrence. Therefore, targeting this population of cells which are more resistance to apoptosis in combination with current treatments is expected to be more effective in breast cancer patients.

495 Poster

Melatonin Receptor (MT1) and Nestin Coexpression: an indicator of more advanced breast cancer?

O. Rögelsperger[1], C. Ekmekcioglu[1], M. Svoboda[1], R. Königsberg[2], M. Klimpfinger[3], W. Jäger[4], T. Thalhammer[1]
[1]Medical University Vienna, Center for Physiology and Pathophysiology, Wien, Austria; [2] Kaiser Franz Josef Spital, 3rd Medical Department Center for Oncology And Hematology, Wien, Austria; [3] Kaiser Franz Josef Spital, Institute of Bacteriology and Pathology, Wien, Austria; [4] University of Vienna, Department of Clinical Pharmacy and Diagnostics, Wien, Austria

Aim: The G-protein coupled receptor MT1 was found to regulate proliferation and differentiation of malignant and non-malignant cells and their progenitors. As a recent study showed that the neuronal intermediate filament protein nestin is upregulated in infiltrating tumor cells with stem cell properties in basal epithelial breast cancer, we investigated whether nestin expressing breast cancer cells would also contain MT1.

Methods: Carcinomatous (Tu) and adjacent non-tumorous (NTu) tissue samples were available from 42 untreated patients with primary breast cancer of different grading and staging. We performed indirect double-immunofluorescence staining with antibodies against nestin and MT1. Tissue sections were analyzed for nestin and MT1 IF-staining with a Zeiss AxioplanII fluorescence microscope and quantitative image analysis was done with the TissueQuest® software.

Results: MT1 was detected in 93% of Tu and 95% of NTu specimens. In the latter, MT1 staining was restricted to luminal and myoepithelial cells. MT1 levels in the Tu were higher than in the adjacent NTu tissue in 59% and lower in 29% of cases. Nestin was not detectable in luminal epithelial cells but clearly present in myoepithelial cells where it was found together with MT1 in 88% of NTu and 67% of Tu samples (in areas with still intact milk ducts). Nestin was also often seen in single cells spread in the Tu stroma. In 17% of all Tu samples, MT1 and nestin costaining was observed in >10% of these infiltrating cells. Other patients had high MT1 (≤46%) and nestin (≤18%) levels in invading cells spread in the Tu stroma. Nestin was also, occasionally, seen in small blood vessels. Colocalization of MT1 and nestin in specimens from patients with higher MT1 levels in Tu>NTu was associated with higher tumor stage (86% in IIA-IIIC) while lower stage patients (75% I) had MT1 and nestin in different cells and higher MT1 scores in NTu vs.Tu tissue. This difference was not associated with the menopausal status of the patients (mean age 55±15 yrs vs. 49±9 yrs).

Conclusion: We showed that higher MT1 levels in Tu vs. NTu specimens and coexpression of MT1 and nestin in breast cancer cells are associated with a more advanced Tu stage, suggesting an implication of MT1 in cancer progression. Therefore, MT1-mediated signaling pathways in advanced breast cancer have to be further investigated, particularly, as melatonin is often used by cancer patients for e.g. insomnia.