kinase activity and decreased the activity of S6 kinase, suggesting an involvement of mTOR pathway in the eEF-2 kinase regulation of autophagy. These results suggest that: (1) eEF-2 kinase plays a regulatory role in the autophagic process in tumor cells; (2) eEF-2 kinase is a downstream member of the mTOR signaling; (3) eEF-2 kinase may promote cancer cell survival under conditions of nutrient deprivation through regulating autophagy. Therefore, eEF-2 kinase may be a part of a survival mechanism in glioblastoma, and targeting this kinase may represent a novel approach to cancer treatment.

# Supportive care agents

**221** POSTER

**Leteprinim attenuates cisplatin-induced neuropathy**

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Sensory peripheral neuropathy is a dose-limiting toxicity of cisplatin chemotherapy. Cisplatin-induced neuropathy is generally associated with a reduction in the signal amplitude and velocity of sensory nerve, reflecting nerve fiber dysfunction. This dysfunction can be revealed in a rat model by behavioral sensory test such as hot plate test and by electrophysiological measures.
The aim of our study was to determine whether leteprinim (SPI-205) could improve changes and dysfunctions associated with cisplatin-induced neuropathy. In the present study, the neuroprotective effect of different formulations of SPI-205 was evaluated in a rat model of cisplatin-induced neuropathy.
Ten week-old female Dark Agouti rats were randomly distributed in 5 experimental groups: (a) a control group (n = 17), receiving sc treatment with the Placebo of SPI-205 suspension; (b) a control group (n = 17), receiving sc treatment with the Placebo of SPI-205 salt (0.9% NaCl); (c) a cisplatin-intoxicated group (n = 17), (d) a cisplatin-intoxicated group (n = 17) receiving sc treatment with SPI-205 suspension (50 mg/kg/d); (e) a cisplatin-intoxicated group (n = 17) receiving sc treatment with SPI-205 soluble salt solution (50 mg/kg/d). Cisplatin was given iv at 2 mg/kg biweekly during 4 weeks; SPI 205 was given at 50 mg/kg daily for 7 weeks. Body weight and survival rate were recorded daily. Animals were evaluated functionally by hot plate and EMG testing once a week for 7 weeks. Sciatic nerves were harvested from 5 animals per group at week 5 for histological analysis.
Results showed that treatment with SPI-205 markedly attenuates cisplatin-induced nerve dysfunction and accelerates the recovery from this disorder. These improvements were evident in most of studied parameters (H-wave amplitude and latency, SNCV and axonal degeneration) and seemed to be in good correlation with the improvement observed in the hot plate test. The results were similar with the two SPI-205 formulation
Histological results showed that the axonal diameter of cisplatin group is slightly increased. This might represent axonal degeneration, a phenomenon observed as a consequence of cisplatin intoxication in developing rat brain (Rzeski et al, 2004). SPI-205 treatment seemed to completely prevent this axonal swelling.
In summary, the present study showed that daily treatment with 50 mg/kg SPI-205 injected subcutaneously can improve cisplatin-related sensory neuropathy in rats.

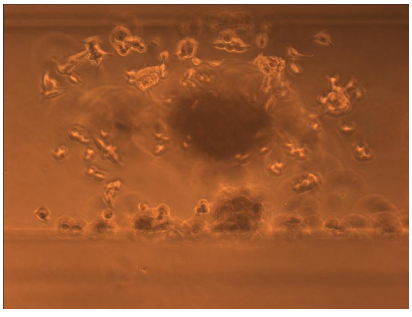
# Toxicology methods and models

**222** POSTER

**Study of in vitro tumor invasion and metastasis: the application of an innovative three dimensional tumor model**

H. Liu, W. Yang. *Min Hang Hospital, Rui Jing Hospital Group, Shanghai, China*

**Background:** As the biological characteristics of malignant tumors, invasion and metastasis are the most dangerous situations during the process of tumor growth and progression. About 60% of cancer patients are detected with metastasis at the time of first diagnosis, and 80% of them actually die from tumor invasion and metastasis. Since it is difficult to observe the process of cancer invasion and metastasis in a patient, and the spontaneous tumor in animal models rarely metastasizes in a short term, there is a pressing need to develop an in vitro three dimensional (3D) tumor model with the features that mimic the characteristics of in vivo solid tumor for the study of tumor invasion and metastasis.
**Materials and Methods:** Several tumor cell lines such as liver/colon/ovary/lung/breast/stomach cancer, and insulinoma were obtained from ATCC. These cells were seeded and cultured in an invented 3Dtissue culture device. Then the in vitro invasion and metastasis of the tumor were observed after the "primary" tumors were reestablished from these cell lines in this culture device.
**Results:** The biological characteristics of tumor invasion and metastasis were observed. For example, the rapid growth of tumor cells, the stationary and translocative motility, cellular structure of microvillus, lamellipodia and pilipodia, spread and adhesion of the tumor cells, the penetrative invasion, the dislodge and/or moving away of the tumor cells from the parent tumor, etc. For liver and lung cancers, many tumor cells were actively spread and moved to the surrounding and distant areas. These cells adhered and demonstrated a colonial dominance growth pattern and formed multiple metastasis tumors in the distant areas. The onset time, the frequency and the degree of tumor invasion and metastasis were different among different types of tumors. Two different types of liver cancers behaved quite differently in the biological characteristics of tumor invasion and metastasis. The process of tumor invasion and metastasis could be dynamically followed up for several months without destroying the specimens.



**Conclusions:** To our best knowledge, this is the first report of direct investigation of tumor invasion and metastasis in vitro after the 3D tumor models are rebuilt from tumor cell lines. Our results indicate that this innovative 3D tumor model can be used as an extremely valuable tool for the in vitro study of tumor invasion and metastasis, for the selection of subpopulations of the tumor cell with different potential of invasion and metastasis, and for the evaluation of potential tumor invasion and metastasis in individual cancer patient for the selection of proper treatment and the prediction of prognosis. Apply this 3D tumor models as an in vitro assay for the molecular targeted medicine study may help the discovery of therapeutic strategy specifically designed for the prevention and treatment of tumor invasion and metastasis.

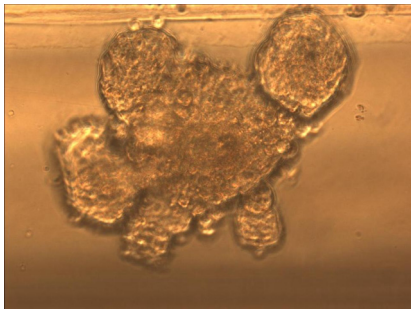
**223** POSTER

**An innovative three dimensional tumor model for in vitro study of tumor biology**

H. Liu, W. Yang. *Min Hang Hospital, Rui Jing Hospital Group, Shanghai, China*

**Background:** Malignant tumors in patients have different biological characteristics based on their intrinsic genetic diversity and the development of heterogeneous sub-clones with divergent phenotypes. Identifying the tumor malignancy behavior such as the proliferative ability and metastatic potential in vitro is critical to choice the appropriate treatment regimens and to evaluation the prognosis. Here we report an in vitro investigation of tumor biology by using an innovative three dimensional (3D) tumor model.
**Materials and Methods:** Various types of tumor cell lines such as liver, colon, ovary, lung, breast, stomach cancer and insulinoma were obtained from ATCC. These tumor cell lines were seeded and cultured in an innovative three dimensional tissue culture device. We observed the biological characteristics of each tumor such as the tumor morphology, the proliferative ability, in vitro invasion and metastasis, and apoptosis. For the comparison, normal stomach cells, hepatocytes and pancreas islet cells were cultured under same condition as control.
**Results:** The tumors rebuilt in vitro demonstrated the characteristics associated with solid tumor in vivo. Such as unlimited rapid growth of the cells and structurally arranged containing a necrotic core surrounded by an outer shell of proliferating viable cells.
Different types of tumors exhibited their unique morphology. For example, a round global shape for small cell type lung cancer; irregular nodular and cauliflower shapes for colon cancer. Some of the tumors expressed tumor associated antigen. For example, liver cancer expressed AFP, colon cancer secreted CEA and ovary cancer was associated with CA-125.
The biological characteristics of tumor invasion and metastasis were also observed: the stationary and translocative motility, cellular structure of microvillus, lamellipodia and pilipodia, spread and adhesion of the tumor cells, the penetrative invasion, dislodge and/or moving away of the tumor

cells from the parent tumor. These cells adhered to substratum and had a colonial dominance growth pattern that forming multiple metastasis tumors in the distant areas.
The biological characteristics were different among various types of tumors in terms of the onset time, the frequency and the degree of tumor invasion and metastasis.
The apoptosis of various types of tumors were also different. The ovary cancer had the most severe apoptosis among the tumor models investigated.



**Conclusions:** Our 3D tumor model better demonstrates tumor biology and brings significant improvement to the tumor model currently available. The studies based on this in vitro 3D tumor model will revolutionize our understanding of cell to cell interaction, tumor invasion and metastasis in the context of morphology, cell biology, biochemistry, and molecular biology. It opens up a broad spectrum in both basic scientific research and clinical application.

**224** POSTER

**Characterization of a murine EpCAM/CD3-bispecific BiTE molecule as a surrogate for preclinical development of the human EpCAM/CD3-bispecific BiTE molecule MT110**

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**Background:** Preclinical development of biologicals for human treatment is frequently hampered by a limited crossreactivity with the respective homologous proteins of test species as are typically used for safety and efficacy assessment. One example is MT110, an EpCAM/CD3-bispecific single-chain antibody construct of the BiTE class that is solely reactive with human and chimpanzee EpCAM and CD3 antigens, and is being developed for treatment of EpCAM-expressing adenocarcinoma. In order to assess the safety profile and therapeutic window of an EpCAM-specific BiTE molecule, we designed a mouse-reactive surrogate BiTE molecule, called muS110, starting from single-chain antibodies solely reactive with murine homology of EpCAM and CD3 proteins.
**Material and Methods:** MT110 and muS110 single chain bispecific antibody constructs were engineered by recombinant DNA technology and produced in Chinese hamster ovary cells. Expression and distribution of EpCAM and CD3 in humans and mice was determined by immunohistochemical analysis of a comprehensive panel of mouse and human tissues. Equilibrium dissociation constants ($K_D$) of anti-EpCAM and anti-CD3 binding domains of MT110 and muS110 were determined by saturation binding analyses, and in-vitro bioactivity was compared in FACS-based cytotoxicity assays. Finally, MT110 in-vivo bioactivity was determined in the SW480 human colon carcinoma xenograft model in NOD/SCID mice, whereas muS110 efficacy was assessed in the 4T1 orthotopic mamma carcinoma model in BALB/c mice.
**Results:** Tissue crossreactivity studies indicated that the distribution of EpCAM and CD3 in mouse was similar to that in humans. The in-vitro properties of muS110 and MT110 were not identical but considered to be sufficiently comparable. While both BiTE proteins bound their respective EpCAM target antigen with very similar affinity, muS110 bound the CD3 antigen with ca. 30-fold higher affinity. This however did not translate into a higher but a slightly lower efficacy of redirected lysis by muS110 of CHO cells transfected with murine EpCAM proteins when compared to redirected lysis by MT110 of CHO cells transfected with human EpCAM. Pharmacokinetic analysis of MT110 and muS110 in mice revealed comparable plasma half-lives in the range of 2–5 hours that are commonly found for antibody fragments with a molecular size of 55 kDa. Lastly, muS110 was found to have considerable anti-tumor activity in an orthotopic breast tumor model as was shown for MT110 in a human colon carcinoma xenograft model.
**Conclusions:** MuS110 is considered as an appropriate surrogate BiTE for safety and efficacy testing of a human-reactive BiTE molecule such as MT110. In-vivo, muS110 treatment induced anti-tumor activity at non-toxic dose levels obviously discriminating between EpCAM expressed on tumor and normal tissues. This is indicative for the existence of a therapeutic window for EpCAM-specific BiTE molecules.

**225** POSTER

**Endothelin signaling in osteoblastic metastases: molecular mechanisms and biomarkers**

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Osteoblastic metastases are common in advanced prostate cancer and some cases of breast cancer. It has been shown that uncontrolled bone formation at the site of metastasis is caused by a number of growth factors, in particular endothelin-1 (ET-1). Secretion of ET-1 by prostate cancer cells is a major factor in formation of osteoblastic metastases. A highly specific antagonist of the ETa receptor, ABT-627, has been developed by Abbott for the treatment of metastatic prostate cancer.
To elucidate the molecular mechanism of ET1-mediated bone formation, we established an *in vitro* osteoblast model system that was responsive to ET-1 treatment. A genomic screen of the ET-1-treated osteoblasts was followed by pathway analysis of the resulting gene expression signature. This analysis revealed three major themes: osteoblastic differentiation, survival, and invasion. We also observed coordinated induction of genes that constitute the calcineurin/NFAT pathway. Follow-up experiments demonstrated that ET-1 induces calcineurin activity in osteoblasts, followed by nuclear translocation of NFATc1 and NFAT-mediated transcription.
An independent set of experiments demonstrated that ET-1 protects osteoblasts from apoptosis induced by several known apoptosis inducers with different mechanisms of action (such as actinomycin D and TNF-alpha). This finding, combined with the previous observations of only weak mitogenic effects of ET-1 on osteoblasts, suggests that suppression of apoptosis is the main mechanism whereby ET-1 promotes osteoblastic metastasis in prostate cancer.
The ET-1 signature in osteoblasts contained several genes coding for secreted proteins previously implicated in invasion and metastasis. Their secretion was confirmed by ELISA. These proteins are currently being explored as biomarkers for osteoblastic metastasis and PD biomarkers for ABT-627.
Our results suggest that ET-1 stimulates bone growth at the metastatic sites by activating the calcineurin/NFAT pathway in osteoblasts and suppressing apoptosis in osteoblasts. These findings provide the foundation for further drug discovery efforts in metastatic prostate cancer. Additionally, we identified several candidate biomarkers that can be used to monitor the progression of osteoblastic metastases and the efficacy of antimetastatic therapies.

**226** POSTER

**Preclinical pharmacokinetic, tissue distribution, and metabolism studies of RTA 402 (CDDO-ME), a novel agent with anti-cancer and anti-inflammatory activities in phase 1 development**

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RTA 402 (CDDO-Me) is a novel, orally bioavailable synthetic triterpenoid in a phase 1 clinical study in patients with solid tumors and lymphoid malignancies at M.D. Anderson Cancer Center and the Dana-Farber Cancer Center. It has been previously reported that RTA 402 causes minimal toxicities in non-human primates at doses up to 1,800 mg/m$^2$/day, with a significantly smaller therapeutic window in rodent species (MTD in rats = 60 mg/m$^2$/day). A number of pharmacokinetic (PK), tissue distribution, and metabolism studies were performed to investigate this difference in tolerability. RTA 402 was administered PO QD by oral gavage to cynomologous monkeys and Sprague-Dawley rats in PK and tissue distribution studies. In the tissue distribution study, monkeys received 300, 900, or 1,800 mg/m$^2$/day for 28 consecutive days and were sacrificed 27−30 hours after the last dose. Rats received 60 mg/m$^2$/day for 4 days and were sacrificed 24 hours after the last dose. Tissues and blood were analyzed for RTA 402 concentrations using a validated LC/MS assay. For metabolism studies, RTA 402 was incubated with fresh hepatocytes and microsomes from rats, dogs, primates, and humans. PK studies demonstrated that peak plasma concentrations in primates and AUC values were significantly higher than in rodents. Tissue distribution studies revealed that mean tissue concentrations across most tissues were 20-fold higher in primates than in rodents, without any toxicity observed upon histopathological analysis. Metabolism studies demonstrated that the principal metabolites of RTA 402 in all species tested are mono- and di-hydroxy species. No other phase 1 metabolites or phase 2 metabolism was observed. The degree of metabolism across all species was similar. In summary, monkeys receiving the NOEL regimen of RTA 402 experienced