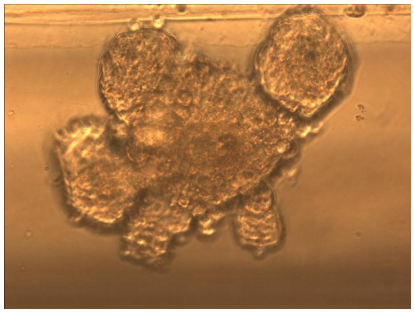
cells from the parent tumor. These cells adhered to substratum and had a colonial dominance growth pattern that forming multiple metastasis tumors in the distant areas.
The biological characteristics were different among various types of tumors in terms of the onset time, the frequency and the degree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