number of Tregs. And it suggests that adoptive T cell therapy influences immunoescape mechanism in patients with cancer. It will be necessary to clarify the mechanism of the effect and to develop an adoptive immunotherapy which has more beneficial clinical effect.

1112 POSTER

Imbalance in VEGF-A/sFLT-1 Enables Malignant Ascites to Resist Dendritic Cell-based Immunotherapy

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Background: Malignant ascites (MA) is an intractable and immunotherapy-resistant state of advanced gastrointestinal and ovarian cancers. Dendritic cells (DCs) have a potential for DC-based immunotherapy as a new therapeutic modality for cancers. We recently proposed a unique and powerful method to activate DCs for cancer immunotherapy, 'immunostimulatory virotherapy', using a new DC-activating modality, the replication-competent, as well as fusion (F)-gene-deleted, nontransmissible recombinant Sendai viruses (rSeVs). The objective of this study was to explore the validity of immunostimulatory virotherapy for MA.
Material and Methods: An immunocompetent murine model of MA was generated using CT26 colon cancer cells, and DCs were generated from mouse bone marrow.
Results: Although we found a significant prolongation in the survival of the tumour-bearing mice by DC-rSeV/dF-GFP treatment, the outcome was nevertheless unsatisfactory. We determined that the imbalance between the vascular endothelial growth factor-A/vascular permeability factor (VEGF-A/VPF) and its decoy receptor, soluble *fms*-like tyrosine kinase receptor-1 (sFLT-1), was a major cause of the resistance to dendritic cell (DC)-based immunotherapy in the murine model of MA. We found that the ratio of VEGF-A/sFLT-1 was increased not only in murine, but also in human MA, and rSeV/dF-mediated secretion of human sFLT-1 by DCs dramatically improved the survival of tumour-bearing animals and inhibited the increase in their body weight. The improvement of survival was associated with enhanced CTL activity and the infiltration of these cells into peritoneal tumours. These findings were not seen in the immunodeficient mice. In vitro, while rSeV/dF-GFP infection did not affect DC expression of the typical co-stimulatory molecules, DC-rSeV/dF-hsFLT1 showed significant increases in positive cell numbers of, at least, CD40, CD83, and CD86 cells. Furthermore, the mIL-1b, mIL-6 and JE/mMCP-1 restoration of proinflammatory cytokine expression was observed in the mice treated with DC-rSeV/dF-hsFLT1.
Conclusions: The imbalance between VEGF-A/VPF and its soluble decoy receptor, sFLT-1, is responsible for the resistance of MA to DC-based immunotherapy, and the correction of this ratio by gene transfer of hsFLT-1 into DCs dramatically augmented not only DC function itself, but also the tumour-specific immune response. Therefore, this new concept, 'targeting VEGF-A/VPF activity during intraperitoneal DC vaccination', could represent a significant strategy to treat MA in the clinical setting.

1113 POSTER

Activation of Checkpoint Kinase 2 (Chk2) Contributes to the Antitumour Synergy Between IGF1 Receptor Kinase Inhibitor NVP-AEW541 and Sunitinib in Hepatocellular Carcinoma

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Background: Insulin-like growth factor (IGF) signaling pathway has been demonstrated an important regulatory mechanism of tumorigenesis and drug resistance in many cancers. Previous studies have shown that inhibition of IGF signaling may induce apoptosis and reverse resistance to cytotoxic agents in HCC cells. The present study explored whether the efficacy of sunitinib or sorafenib can be improved by IGF receptor kinase inhibitor NVP-AEW541 (Novartis) in HCC cells and human umbilical venous endothelial cells (HUVECs).
Materials and Methods: HCC cell lines tested included Hep3B, PLC5, and SK-Hep1. The potential synergistic growth inhibitory effects were measured by MTT and median dose effect analysis. Apoptosis was measured by flow cytometry. The activity of pertinent signaling pathways and expression of apoptosis-related proteins were measured by Western blotting.
Results: IGF can activate IGF receptor and downstream AKT and ERK signaling activities in all the HCC cells and HUVECs. Addition of IGF increased resistance of HUVECs to the multi-kinase inhibitors sorafenib and sunitinib. Resistance of HCC cells to sunitinib, but not sorafenib, was also increased with the addition of IGF. The IGF1 receptor inhibitor NVP-AEW541 (Novartis) significantly enhanced the apoptosis-inducing effects of sunitinib, but not sorafenib, of HCC cells both *in vitro* and *in vivo*. The synergistic effects between sunitinib and NVP-AEW541 were independent of inhibition of IGF receptor, AKT, and ERK activities by NVP-AEW541. Activation of Chk2, which played important roles in regulation of DNA damage response, was found when NVP-AEW541 was combined with sunitinib but not with sorafenib. Knockdown of Chk2 expression by small interfering RNA partially abrogated the synergistic apoptosis-inducing effects of sunitinib and NVP-AEW541.
Conclusions: IGF in tumour microenvironment may increase resistance of HCC to molecular targeted therapy. The apoptosis-enhancing effects of IGF1 receptor inhibitors in HCC cells may be drug-specific, and Chk2 activation may be one important downstream mediator of the anti-cancer synergy between IGF1 receptor inhibitors and molecular targeted agents.
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1114 POSTER

The Anticancer MTOR-inhibitor Temsirolimus Induces Cardiotoxicity in a Mouse Model

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Background: Cardiotoxicity is a major drawback and social problem linked to many anticancer treatments. Early identification of signs of this adversity would certainly benefit the management of oncologic patients. The mTOR-inhibitor temsirolimus is currently being evaluated for anticancer efficacy in hundreds of clinical trials and is approved for treatment of advanced renal cell carcinoma. However, the PI3K/Akt pathway converges on mTOR, which is a central regulator of cell growth, including cardiomyocyte growth. Here, we aim at evaluating the cardiac effects of the anticancer mTOR-inhibitor temsirolimus in a mouse model in vivo.
Materials and Methods: Left Ventricular (LV) fractional shortening (FS) was assessed by M-mode echocardiography in sedated C57BL/6 mice (2−4 mo. old) at day 0, and after 2, 7, 14, 21 days from a single i.p. injection of temsirolimus (0.1 mg/kg, a dose comparable to the one used to treat cancer in humans) or vehicle. Doxorubicin (Doxo, 2.17 mg/kg/day for 7 days) was used as a positive control. With Speckle Tracking echocardiography (ST) we also evaluated radial myocardial strain (%), a very sensitive parameter which can detect subtle changes in cardiac function.
Results: After 2 days, there was no change in FS with temsirolimus, but FS was already reduced with Doxo: 52±0.2%, $p = 0.0000001$ vs sham (60±0.4%). With temsirolimus, FS was reduced only after 21 days: 50±3%, $p = 0.009$ vs sham. Interestingly, with Speckle Tracking echocardiography we found that in the temsirolimus group radial strain was already decreased at 7 days: 42±5%, $p = 0.01$ vs sham (59±1%).
Conclusions: The antineoplastic mTOR-inhibitor temsirolimus induces LV dysfunction in mice. Such dysfunction occurs later than the one observed with Doxo, but speckle tracking echocardiography is more sensitive than conventional echocardiography and can detect early signs of myocardial alteration that may prelude to overt LV dysfunction. The clear mechanisms of temsirolimus cardiotoxicity are to be elucidated in further experimental studies. We also plan to apply speckle tracking echocardiography to clinical studies, in order to evaluate the impact of early identification of temsirolimus cardiotoxicity in the treatment of renal cell carcinoma.

1115 POSTER

Impaired Autophagy Contributes to Resistance to Metronomic Cyclophosphamide Chemotherapy

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Background: Autophagy is a cellular stress response that is emerging as an important determinant of response to a wide range of anticancer therapies. Specifically, autophagy is usually thought to contribute to tumour cell survival, and thus therapeutic resistance, in tumours subjected to conventional chemotherapy (i.e., intermittent cytotoxic drug administration at maximum tolerated doses). Conversely, the role of autophagy during chronic anticancer therapy such as low-dose metronomic (i.e., antiangiogenic) chemotherapy is unknown.