carcinomas of colon, breast, lung, ovary and prostate while its low expression on normal tissue is restricted to the gastric mucosa, small intestine and pancreas. A calicheamicin conjugate that targets LeY could therefore provide a treatment option for a variety of cancers. The humanized IgG1 antibody, Hu3S193, that recognizes LeY was conjugated to a CM analog. FACS analysis showed that this conjugate (Hu3S193-CM) bound LeY-positive cells with similar selectivity and avidity as Hu3S193 indicating that conjugation did not influence these parameters. When tested on monolayer cultures of various human LeY-positive carcinoma cells, Hu3S193-CM was consistently more cytotoxic than a control conjugate. This difference in efficacy was not noted on LeY-negative cells. The efficacy of Hu3S193-CM depended qualitatively on the expression of LeY and on the sensitivity to CM. Quantitatively, the efficacy was neither directly proportional to the CM-sensitivity of tumor cells nor to their amount of LeY. Hence, other factors such as the nature of the surface proteins that carry LeY can co-determine the sensitivity to Hu3S193-CM. *In vivo*, Hu3S193-CM inhibited tumor growth of xenografted human gastric (N87), colon (LOVO) and prostate carcinomas (LNCaP) more effectively than control conjugates. When used against the N87, Hu3S193-CM (160 ug/kg, q4dx3) was curative. Our data show that Hu3S193-CM can specifically eliminate tumors that express LeY and therefore encourage development of this conjugate as an option for the treatment of carcinomas.

503

Inhibition of tumor metastasis by ING-1(heMab), a human-engineered(TM) monoclonal antibody targeting the epithelial cell adhesion molecule

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Tumor metastasis is one of the hallmarks of malignancy and remains the main cause of death in cancer patients. Despite this, very few treatment strategies are effective in preventing metastasis from primary tumors. Therapeutic monoclonal antibodies, either alone or conjugated with cytotoxic agents, have shown promise in inhibiting tumor invasion and metastasis. Here we demonstrate that a Human-Engineered(TM) monoclonal antibody, ING-1(heMAb), which recognizes a 40-KDa glycoprotein, Epithelial Cell Adhesion Molecule (Ep-CAM), significantly inhibits tumor metastasis in a murine model of human cancer. To investigate the efficacy of ING-1(heMAb), we established an *in vivo* adenocarcinoma-lung metastasis model, in which athymic nude mice received an intravenous (IV) injection of human HT-29 colon adenocarcinoma cells via their tail veins. All mice were pre-treated with human interleukin-1beta to augment tumor metastasis. In all studies, mice were observed for 8 weeks to allow for lung metastasis development. Mice (10 per group) received IV dosing of 1 mg/kg ING-1(heMAb) twice weekly for 3 weeks starting on day 2 or day 5. A negative control group received human IgG with the same dosing schedule starting on day 2. In the positive control group, 100 mg/kg 5-FU/leucovorin was injected intraperitoneally once weekly for 4 weeks. At the end of the study, the mice were sacrificed for gross necropsy and tissue collection. Three different methods were used for data analysis. First, visible tumor nodules (>3 mm) were counted throughout animal body cavities. Second, tumor nodules on lung surfaces were counted under a dissecting microscope. Last, micrometastatic foci in sections of lung tissues were counted after detection with hemotoxylin/eosin staining. ING-1(heMAb) treatment starting on day 2 significantly reduced the number of tumor nodules visible during necropsy ($P<0.01$, vs IgG group). When nodules on lung surfaces were examined, a significant reduction was again observed in the ING-1 (heMAb)/day 2-treated group ($P<0.005$, vs IgG group). Lastly, ING-1(heMAb) treatment starting on day 2 resulted in 91% reduction of micrometastases in lung tissues ($P<0.005$, vs IgG group). Delaying ING-1 treatment until day 5 still led to 54% reduction in micrometastases ($P<0.005$, vs IgG group). The above results have been confirmed in another independent study. In conclusion, ING-1(heMAb) effectively reduced tumor metastasis in a murine model of human cancer. While ING-1(heMAb) was particularly effective when administered early in the study on day 2, day 5 treatment was also effective in reducing micrometastases. These data suggest that ING-1 (heMAb) may show promise in treating metastatic diseases in humans.

504

Development of monoclonal antibodies targeting the uPA system for diagnosis and therapy

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The urokinase plasminogen activator system, comprised of the serine protease urokinase (uPA), its cell surface receptor uPAR and plasminogen activator inhibitor-1 (PAI-1), has been implicated in the neo-vascularization, invasion and metastasis of many solid tumors. uPAR plays an essential role in the regulated degradation and remodeling of the extracellular matrix by tumor cells and angiogenic endothelial cells. uPA-uPAR dependent cascades also result in the activation of proMMP-9 and the activation and release of growth factors and angiogenic factors including HGF, VEGF and TGFbeta. The important role of uPA-uPAR in tumor growth and its abundant expression within tumor, but not normal tissue, makes it an attractive diagnostic and therapeutic target. We previously described the production and characterization of a panel of monoclonal antibodies against the amino terminal fragment (ATF) of uPA. We have tested the ability of two of these antibodies, ATN-291 and ATN-292, to inhibit tumor growth *in vivo*. Briefly, 7x105 MDA MB 231 cells were injected into the mammary fat pad of Balb/c nu/nu mice. Tumors were staged to 35 mm3 before the start of treatment with anti-ATF or isotype matched control antibodies (10 mg/kg, 3 × week, IP). Treatment with ATN-291 or ATN-292 significantly inhibited tumor growth in this model. In addition, we have generated a panel of monoclonal antibodies against domains 2 and 3 (D2D3) of uPAR conjugated to KLH. Initial characterization of the antibodies indicates that they bind suPAR with affinities of 3-80 nM in direct binding assays and recognize suPAR, D2D3 but not domain 1 (D1) of uPAR, as determined by western blot. In functional assays a D2D3 antibody, as well as a polyclonal antibody against D2D3, effectively inhibited adhesion of integrin alpha5beta1 expressing CHO cells to suPAR. Furthermore, the antibody also inhibited uPA induced cell migration. Preliminary experiments indicate that the antibodies can be efficiently labeled with I-125 with no significant change in the binding characteristics of the antibodies. We will present results of internalization studies using these labeled antibodies and assess their potential to deliver cytotoxic agents to tumor cells.

505

Characterization of the intracellular mechanisms behind the cytotoxic effect of the immunotoxin, 425.3-PE in two breast cancer cell lines

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The aim of the study was to investigate the mechanisms behind immunotoxin (425.3-PE)-induced cell death. In addition, the combinational effect of 425.3-PE and cycloheximide (CHX) in cell culture was evaluated. Our immunotoxin consists of antibody 425.3, recognizing the EGF receptor, chemically conjugated to the bacterial toxin Pseudomonas exotoxin (PE). The two different breast cancer cell lines used, MA11 and MT1, respond differently to 425.3-PE treatment in several aspects, thereby giving us opportunity to evaluate mechanistic events in 425.3-PE induced cell death. The immunotoxin treatment of these two cell lines do not only inhibit protein synthesis (PS) (IC_{50}=0.5-2 ng/ml) by ADP-ribosylation of EF-2, it also induces caspase activation, cleavage of poly(ADP)-ribose polymerase (PARP) and DNA fragmentation, characteristic events of apoptosis. Western blot demonstrated immunotoxin-induced PARP cleavage, more rapidly induced in MA11 cells (maximal 5h) contra MT1 cells (maximal 10h). The broad-spectrum caspase inhibitor (z-VADfmk) totally inhibited PPAR cleavage and improved the cell viability (approx. 3-fold) without altering inhibition of PS in both MA11 and MT1 cells treated with 425.3-PE. The specific caspase-9 inhibitor (z-LEDHfmk) had no effect on 425.3-PE induced cell death in MT1 cells. However, in MA11 cells this specific inhibitor made them 1.6 times more resistant to 425.3-PE, suggesting activation of caspase-3 by caspase-9, mediated by mitochondria alterations. By combination of two PS inhibitors, CHX and 425.3-PE, the cell viability decreased in both MA11 and MT1 cells to a level corresponding to that of a 10 times higher concentration of 425.3-PE alone. The CHX concentration used gave by itself minimal effects on PS inhibition and cell viability. For MA11 cells the combination of CHX+425.3-PE increased the PS inhibition effect further, whereas MT1 cells were insensitive to the combination. Notably, 425.3-PE also decreased the p53 protein level in MA11 cells to 30% of control. The increased activity of 425.3-PE when combined with CHX will be further examined. In conclusion, the apoptotic program which 425.3-PE starts in MA11 and in MT1