Carcinoembryonic antigen: an adhesion molecule controlling cell–cell and cell–matrix interactions

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Carcinoembryonic antigen (CEA) is a member of a family of at least 10 related gene products which share structural and sequence homology and are found on a variety of malignant and normal tissues. cDNA sequencing of CEA has revealed a highly significant homology with members of the immunoglobulin (Ig) gene family which play an important role in cell recognition. We have recently identified a collagen receptor on a human colon carcinoma cell line (SW1222) which recognises the arginine-glycine-aspartic acid (RGD) sequence present in type I collagen. This receptor mediates not only the binding of colon carcinoma cells to collagen but also the binding of colon carcinoma cells to collagen gels. In order to characterise further this and other cell adhesion molecules mediating epithelial cell–collagen interactions, we screened a panel of monoclonal antibodies (mAbs) for their ability to inhibit the collagen binding and the glandular differentiation of SW1222 cells. We found that four different mAbs recognising the 180 kDa CEA glycoprotein and other members of the CEA family, have similar inhibitory activity on the collagen binding and glandular differentiation of SW1222 cells in 3D collagen gel. In addition we showed that CEA is not a collagen binding protein itself but is likely to be associated with the RGD-directed collagen receptor complex expressed by the SW1222 human colon carcinoma cell line. This suggests that CEA functions as an adhesion molecule mediating cell–matrix as well as cell–cell interactions which are two fundamental processes controlling the morphological differentiation of colorectal tumour cells.

The activation by endogenous enzymes of a nitrogen mustard prodrug designed for use in antibody-directed enzyme prodrug therapy (ADEPT)

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The novel prodrug 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid has been synthesised for use in ADEPT. The bacterial enzyme carboxypeptidase G2(CPG2) specifically cleaves the prodrug to a cytotoxic benzoic acid nitrogen mustard. When the enzyme is conjugated to a monoclonal antibody and pre-administered to mice bearing human xenografts, the conjugate localises in tumours where it activates subsequently administered prodrug (Bagshawe *et al.*, *Br J Cancer* 1988).

The activation of the prodrug by endogenous enzymes has been studied in nude mice bearing human xenografts without prior administration of antibody–enzyme conjugate. Prodrug was activated by endogenous enzymes to yield low concentrations of active drug, but this conversion took greater than 2 h *in vivo*. By contrast activation of prodrug occurs in less than 5 min to yield high concentrations of active drug when animals have previously received antibody–enzyme conjugate. Since there is no known mammalian homologue of CPG2, further studies were aimed at determining whether the endogenous enzymes responsible were of bacterial origin. Accordingly mice were treated with benzylpenicillin and streptomycin. Contents of the caeca from these mice were incubated with the prodrug and compared to untreated controls. The contents of caeca from untreated animals activated prodrug with a total conversion of 4% over the 50 h incubation study. Caecal contents from antibiotic-treated mice were unable to convert prodrug. No active drug was detected in the tissues of antibiotic-treated animals after administration of prodrug *in vivo*. Control animals had measurable levels of active drug, but not before 2 h post prodrug injection.

These studies indicate that low levels of activation of the prodrug *in vivo* is caused by bacterial enzymes present in the gut of mice, but that pre-treatment of the animals with an antibiotic regime can prevent this conversion to active drug.

Improved delivery of radiolabelled pan B-cell antibodies to lymphoma xenografts by pre-dosing with unlabelled antibody

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Pre-administration of unlabelled (cold) antibody prior to injection of radiolabelled MoAb may be a way to improve delivery of the radiolabelled antibody to tumour. Two pan B-cell MoAbs (B1 anti-CD20 IgG2a and MB-1 anti-CD37 IgG1) have been studied in a human B-cell lymphoma xenograft athymic nude mouse model to test this hypothesis. Groups of 9–10 mice bearing sc human Raji Burkitt lymphoma xenografts weighing 0.5–1.8 g were injected with a single intraperitoneal injection of 100 μg cold B1 or MB-1 antibody 2 h prior to the injection of 1 uCi ^{125}I-labelled B1 or MB-1, respectively. The biodistribution results at 4 days after injection were compared to those from animals not given the cold antibody pre-dose. For the animals injected with ^{125}I-labelled B1 alone, the mean %ID/g±s.e. in tumour was 8.8 ± 1.0 compared to 12.7 ± 1.2 for the animals receiving the cold B1 pre-dose. These values were significantly different, $P = 0.02$. The uptake in most normal tissues was unchanged. For the animals injected with ^{125}I-labelled MB-1 alone, the mean %ID/g in tumour was 2.6 ± 0.1 compared to 3.5 ± 0.4 with unlabelled MB-1 pre-dosing ($P = 0.02$). In both studies, the blood levels of the radiolabelled MoAbs tended to increase following cold antibody pre-dosing, and the tumour/non-tumour ratios obtained following cold antibody pre-dosing were not changed. This study demonstrates that cold antibody pre-dosing can lead to increased tumour delivery of radiolabelled pan B-cell MoAbs. This phenomenon may be due to the increased blood levels of radiolabelled MoAbs following cold antibody pre-dosing, which may be a consequence of binding of the cold antibody to non-specific binding sites in normal tissues or to circulating antigen and tumour cells.

Imaging and therapy of human malignant gliomas utilising the monoclonal antibody MUC 2-63

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The monoclonal antibody MUC 2-63 identifies an antigen present on malignant gliomas (MG) with the molecular weight of 32 kD. 16 patients with biopsy verified MG received 0.4 mg of the MUC 2-63 monoclonal antibody labelled with 69 to 205 MBq DTPA-^{111}In. 11 of 16 patients had an uptake within the tumour area of 0.01 to 0.25% of the injected dose. All 10 patients with high grade MG had positive images, while only 1 with low grade MG had a positive image. The maximal uptake in the tumour was observed between 46 and 67 h. Half-life was 38–110 h. 1 patient also received a "non-specific" monoclonal antibody against human milk fat globule (HMFG1). The uptake was 0.02% and the maximal uptake was observed after 24 h. The corresponding figures with MUC 2-63 were 0.04%. No side effects were recorded. From 5 patients we had frozen tumour material. The 2 patients with high grade MG had an intense positive staining with MUC 2-63, while the HMFG1 controls were negative. 1 out of 3 patients with low grade MG had a positive immunohistochemical result with MUC 2-63. 7 patients received intravenous therapy doses with 146 to 830 MBq ^{90}Y coupled to DTPA-MUC 2-63. In 2 patients who received up to 4 doses of ^{90}Y, or who had grade 2 thrombocytopenia due to previous CCNU therapy, we observed grade 4 thrombocyte toxicity. The other 5 patients were without major adverse effects. 2 patients had clinical improvements and CT-verified signs of tumour necrosis which may be related to antibody therapy.