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Human scFv against the epithelial tumour marker MUC-1

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New anti-cancer agents are being developed which specifically recognise tumour cells. Where recognition being dependent on the enhanced expression of antigenic determinants on the surface of tumour cells. The tumour exposure and the extracellular accessibility of the mucin MUC-1 makes this marker a suitable target for directed tumour therapy. So far mainly murine antibodies have been described for the targeting of MUC-1 expressing tumours, but they do have their limitations for the use in human immunotargeting and immmnotherapy. Targeting of tumours with human single chain Fv (scFv) antibody fragments may overcome some of the limitations of murine antibodies. We isolated and characterised a panel of human scFv which bind to the MUC-1 core protein using phage display technology. These scFv have been selected directly on a MUC-1 expressing breast carcinoma cell line for binding to the tandem repeat core protein of MUC-1. The binding characteristics have been studied by ELISA, FACS and indirect immunofluorescence. The human scFv are very specific and their binding is inhibited by soluble antigen. Four human scFv recognise the hydrophilic region PDTR of the MUC-1 core protein, which is thought to be an immunodominant region. For imaging or targeting to tumours expressing MUC-1 it might be feasible to use these human scFv as vehicles to deliver anti-cancer agents.

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Induction of ADCC,ADMC,Complement-fixation,Anoikis and Apoptosis in chemoresistant NSCLC overexpressing HER-2/neu after treatment with chimeric Abs linked on pegylated SUVs with encapsulated vinorelbine (ILV) targeted against the SH2 domain of Grb2 and p85A of PI3-K.

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NSCLC cells with high HER-2/neu expression use Grb2 to transduce signals to MAPK and AKT via binding of pTyr to SH2 domain propagating mitogenic signals.NSCLC cells characterized by overexpression of HER-2,Ras and Akt were obtained by FNA biopsy from a patient who has developed chemoresistance.Chimeric antibodies against the SH2 domain of Grb2 and PI3-K were conjugated onto the pegylated liposomal surface of SUVs loaded with vinorelbine molecules in their aqueous phase forming a compound called immunoliposomal vinorelbine (ILV).After treatment of NSCLC cells with ILV,we observe binding inhibition of pTyr to SH2 domain by ELISA,SPR and isothermal calorimetry. Downregulation of Ras,MAPK,PI3-K and Akt was detected by Western-blotting. Immunology assays have shown inhibition of ADCC,ADMC and complement-fixation.Transmission electron microscopy has exhibited inhibition of membrane ruffles and lamellipodia due to inhibition of actin polymerisation due to Grb2 SH2 inhibition. Additionally,there was inhibition of mitotic microtubule formation due to vinorelbine's depolymerising action.Also,there was forced detachment of adherent tumour cells indicating anoikis which was correlated with repressed Akt protein levels.Finally,TEM exhibited irreversible apoptotic signs of D2 stage in treated tumour cells forming apoptotic bodies which were phagocytosed by adjacent tumour cells which subsequently were eradicated indicating a bystander killing effect.Growth inhibition was confirmed by diminished [3H] thymidine uptake of treated NSCLC cells.Furthermore,metabolic activity by MTT and DNA synthesis by BrdU were greatly reduced compared to controls.Concluding,these results demonstrate great antimitotic,immunogenic and antimotogenic activity of potential antitumour agent ILV which inhibits specific intracellular pathways leading to eradication by apoptosis of chemoresistant NSCLC.