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RNAi mediated downregulation of bcl-2 and xIAP may have therapeutical potential in human breast adenocarcinoma

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Purpose: Resistance to cytotoxic drugs may be partly due to resistance to apoptosis, which may be conferred by genes such as bcl-2 or xIAP. Therefore, we investigated if downregulation of bcl-2 or xIAP gene expression with siRNAs sensitised MCF-7 human breast adenocarcinoma cells to etoposide and doxorubicin.

Methods: Cells were transfected either with control siRNAs or with siRNAs designed against bcl-2 mRNA or against xIAP mRNA. For the chemosensitisation studies, cells were treated with the IC50 dose of etoposide or doxorubicin. Uptake of the FITC-siRNAs was studied by fluorescent microscopy and bcl-2 or xIAP downregulation was verified by Western blotting. Cellular proliferation studies were carried out with the BrdU incorporation assay and apoptosis was verified with the TUNEL assay. The number of viable cells following treatment with siRNAs and/or chemotherapeutical drugs was verified with the Trypan blue exclusion assay.

Results: Both siRNAs were taken up by the MCF-7 cells. RNA interference was confirmed, protein downregulation being stronger at 48 hours following transfection. Both siRNAs caused an inhibition of cellular proliferation and an increase in apoptosis. RNA interference of bcl-2 sensitised cells to etoposide and doxorubicin. However, siRNAs for xIAP did not have a significant effect on sensitisation of cells to either of these drugs.

Conclusion: RNA interference was possible in human MCF-7 breast adenocarcinoma cells. Downregulation of bcl-2 or xIAP inhibited cellular proliferation and induced apoptosis. Downregulation of bcl-2 sensitised MCF-7 cells to etoposide and doxorubicin.

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Which complement regulatory proteins could be a good target for a breast cancer vaccine?

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Introduction: To avoid self-attack by complement, cells express complement regulatory proteins CD59, CD55 and CD46. Invasive ductal breast carcinomas showed variable expression of these proteins. Targeting complement regulatory proteins is potentially a very attractive approach to tumour therapy, as these vaccines can eliminate any cell over-expressing a complement inhibitor.

Aim and methods: We have investigated the correlation between CD55 and CD59 expression and tumour characteristics, patient features and outcome in a series of primary operable breast cancers diagnosed between 1987 and 1992. 500 tumour samples were stained using an anti-CD55 monoclonal antibody RM1 (developed in the department) and anti-CD59 (clone MEM-43). As there are no commercially available anti-CD46 antibodies which react on paraffin sections, we have recently developed a monoclonal antibody specific to CD46 that looks promising on material processed in this way.

Results: 95% of the tumours showed positive immunoreactivity for both CD55 (RM1) and CD59. High expression of CD55 and CD59 was significantly associated with low histological grade ($p<0.001$) and good prognosis tumours (Nottingham prognostic Index <3.4) ($p<0.001$). There was a significant relationship between CD55 and CD59 expression and overall survival showing that loss of CD55 and CD59 in breast tumours correlates with poor survival ($p<0.001$, $p=0.006$ respectively). The anti-CD46 antibody is now being used to screen the same breast tumour tissue arrays as assessed with anti-CD59 and anti-CD55 (RM1) antibodies to complete the picture of the role of these complement inhibitory proteins in tumour prognosis.

Conclusion: These data indicate unexpectedly that loss of CD55 and CD59 is associated with aggressive breast tumours. There appears, however, to be an inverse association between loss of CD55 and increased expression of CD46 in malignant breast tumours and over-expression of CD46 breast tumours could potentially be a good target for a breast cancer vaccine.

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Additive cytotoxic and proapoptotic effects of external radiation and Rituximab on B cell lymphoma cell lines in vitro.

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Background: The combination of the anti-CD20 antibody Rituximab with chemotherapy including anthracylin and steroids has been shown to be effective against B cell lymphoma cells in vitro and in vivo. However, little is known about combinations of Rituximab and external radiation.

Material and Methods: The cytotoxic effect of rituximab (1 microg/ml and 10 microg/ml, respectively) given 20 h after radiation (10, 20 and 40 Gy, respectively) was investigated in transformed lymphoma cell lines: 2 follicular lymphoma (SU-DHL4 and Karpas 422), 2 Burkitt lymphoma (Daudi and Ramos) and 1 diffuse large cell lymphoma (Balm3). Endpoints were: antibody-dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and direct apoptotic effects. ADCC and CDC were assessed by by propidium jodid (PI) staining in a flowcytometric analysis; direct cytotoxic effects were determined by Annexin V externalization.

Results: Although all cell lines expressed high levels of CD20 antigen, the susceptibility to Rituximab differed significantly between cell lines. In general, if a CD20+ B-NHL cell line showed susceptibility to a certain effect of Rituximab, this effect added to the cytotoxic effect of radiation. Supra-additive (synergistic) effects were not observed in these experiments. For example, SU-DHL4 cells were susceptible to the direct apoptotic effect of Rituximab with 29% of the cells being Annexin V positive. After 10, 20, and 40 Gy of radiation, 11%, 12%, and 19% of the cells stained positive for Annexin V, respectively. After combined treatment, (10 microg/ml Rituximab + 10, 20, or 40 Gy) 36%, 37%, and 38% of the cells became apoptotic, respectively. Daudi cells were grossly resistant to direct apoptotic effects of Rituximab but susceptible to ADCC exerted by the antibody. These effects added to radiation induced cell damage: 34% of the cells were PI-positive after incubation with NK cells and Rituximab solely, radiation with 10, 20, and 40 Gy resulted in 15%, 18%, and 27% PI-positive cells, respectively. After combined treatment, 46%, 50%, and 53% of the cells became PI-positive. Ramos cells were typical targets for CDC obtained with Rituximab. 22% of the cells were PI-positive after incubation with complement and Rituximab. Radiation with 10, 20, and 40 Gy resulted in 13%, 18%, and 28% PI-positive cells, respectively. After combined treatment, 47%, 50%, and 58% of cells became PI-positive. The reaction of Karpas 422 cells and Balm3 cells was comparable to Daudi cells.

Conclusion: Incubation with Rituximab twenty hours after irradiation resulted in an additive interaction in all cell lines and at all end points tested. In current experiments, different time scheduling and the mechanisms of interaction are under investigation.

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A phase I/II single arm trial to determine the safety, tolerability, and biological activity of intrahepatic delivery of doxorubicin hydrochloride adsorbed to magnetic targeted carriers (MTC-DOX) in patients with metastatic tumors in the liver

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Purpose: To test the safety and metabolic tumor activity following a single selective arterial infusion of doxorubicin adsorbed to Magnetic Targeted Carriers (MTC-DOX) under magnetic guidance in patients with metastatic disease in the liver from various primary tumor types.

Materials and Methods: A phase I/II dose escalation study was undertaken in up to 20 patients. MTC-DOX (a combination of doxorubicin bound to magnetic targeted carrier 1:8.3 w:w) was delivered regionally to the tumor via arterial catheterization. An external magnet (field strength of 5 KG) was positioned over the tumor to both guide the MTC-DOX into the proper location and to extravasate the material into the tumor parenchyma. Tumor localization of MTC-DOX was confirmed by MRI post administration. A