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The effects of oestrogen and tamoxifen on purified normal and malignant human primary breast epithelial cells

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In order to investigate the effects of oestradiol (E2) and 4-hydroxy tamoxifen (4OHT) in epithelial cells from normal and malignant breast tissue, we have devised a system to purify these cells and study them in vitro.

Fresh pathological discard tissue from reduction mammoplasties and primary breast cancers were enzymatically digested and after appropriate filtration epithelial cells were isolated using the epithelial specific Ber-Ep4 immunolabelled beads (Dynal) and cultured. The purity of malignant cells from primary tumours was confirmed using conventional cytology and fluorescent in situ hybridization (FISH) and shown to be >95% and >90% respectively. Cytokeratin 8 and 18 immunostaining confirmed the epithelial nature of both, normal and malignant samples.

Oestrogen receptor alpha (ER) and progesterone receptor (PR) were examined by immunostaining and we have demonstrated that ER and PR expression were retained in malignant cells in culture. In contrast, ER expression was rapidly attenuated, in normal cells, resulting in loss of expression within the first 24-36 hours of culture. A similar reduction was noted in PR expression, although very low levels could be detected for upto 60 hours in culture. Further, contra-intuitively, E2 induced a proliferative response in normal cells and none in cancer cells. 4OHT had an inhibitory effect on proliferation in normal cells and decreased cell survival in some malignant cultures.

We conclude: (1) This novel system is an effective methodology for isolating malignant epithelial cells to a high degree of purity, (2) Oestrogenic responses are different in normal cells compared to cancer cells and (3) 4OHT treatment results in a decrease in cell numbers in some ER positive malignant cultures. This suggests that this can be a way of selecting tamoxifen sensitive patients from the ER+ group of patients.

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Multifluorescent labelling of immunmagnetic enriched circulating colon tumour cells and cell clusters

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Purpose: Detection and characterisation of circulating tumour cells may contribute to the better understanding of metastatic process in human. With a recently developed intracellular cytokeratin immunmagnetic microbead cell separation method intact cells and mixed cell clusters were detected in patients with different stage and duration of colorectal cancer. Development and evaluation of a fluorescent cytokeratin/haemopoetic labelling technique with immunmagnetic microbead cell separation method for the characterisation of enriched tumour cell and cell clusters in patients with colorectal cancer.

Methods: 16 colon cancer patients (stage TNM II, III, IV) and 20 healthy donor samples were evaluated. The colon cancer patients were evaluated before adjuvant chemotherapy. Immunmagnetic cell separation was performed from whole blood/ficoll separated cell blood fractions by the surface epithelial markers (HEA 125, Ber-EP4). The enriched cell fraction (400microliter from 5ml blood) was immuncytochemically labelled using an FITC labelled cytokeratin antibody (CAM 5.2) and a Texasred labelled CD45. Cell death was detected by Propidium Iodide nuclear staining. Spiking experiments were performed using the HT29 colon cancer cell line. The lowest detectable concentration was 1 cell/ml blood. A fluorescent inverse microscope was used to evaluate and manipulate the labelled cell fraction.

Results: Two of the twenty healthy donor samples contained CK+CD45+ cells (3 and 4 such cells in 5ml blood). In cancer patients the lowest cell number was 9 CK+CD45- cells/ml. Cell clusters containing CK+CD45-, CK-CD45+ and CK+CD45+ cells were detected only in colon cancer patients.

Conclusions: Immunmagnetic cell separation with immuncytochemical labelling is a useful method for detection and characterisation of circulating colon cancer cells and clusters. CD45+CK+ cells appear both in healthy and colon cancer patients in low number, as well. Their further characterisation and evaluation needs additional fluorescent markers and studies.

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ZD0473 exhibits marked in vitro anticancer activity in human tumor specimens

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ZD0473 is a new generation platinum drug that shows evidence of an extended spectrum of antitumor activity and overcomes platinum resistance mechanisms. ZD0473 has demonstrated in vivo activity against cisplatin-resistant human ovarian xenografts. The present study tested ZD0473 in a soft agar cloning assay to determine its in vitro activity against human tumor specimens taken directly from patients. One hundred and five patient tumor specimens were exposed to ZD0473 at 1, 4, and 16 μg/ml for 2 hours versus 24 hours, at 37°C. Approximately 35% of these specimens were evaluable, falling within negative and positive control parameters. Following exposure, cells were washed with medium and plated into a two-layer soft agar cloning system then incubated at 37°C for 14 days. Specimens were removed on Day 14 for colony counting. The number of colonies formed in the treated plates was compared with that from untreated control plates, and the percent colonies surviving at each concentration was calculated. Negative and positive controls were sterile saline (0.9% NaCl) and orthosodium vanadate (200 μg/ml), respectively. After 2-hour exposure, ZD0473 demonstrated activity in 15% (6/40), 30% (12/40), and 47% (15/32) of the specimens tested at 1, 4, and 16 μg/ml, respectively. After 24-hour exposure, ZD0473 demonstrated activity in 27% (10/37), 65% (24/37), and 87% (27/31) of the specimens tested at 1, 4, and 16 μg/ml, respectively. Notable responses to 2-hour ZD0473 16 μg/ml exposure were seen in breast (29%), ovarian (78%), colon (33%) and NSCL (60%) cancer specimens. Ongoing trials in these tumour types are designed to further investigate these results. Phase I studies have indicated that ZD0473 has a predictable and favorable toxicity profile, comparable to carboplatin (Trigo et al., Proc ASCO 1999; 18: 169a[abs 648]). ZD0473 is currently undergoing Phase II monotherapy and Phase I combination studies, Phase III studies are in planning.

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Immunmagnetic detection and characterisation of circulating tumour cells and cell clusters in patients with colorectal cancer

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Purpose: To produce metastases, tumour cells must enter into the circulation and survive the transport. Although most tumour cells are destroyed in the bloodstream, animal models seem to demonstrate that the greater number of cells and cell clusters and larger clusters released by a primary tumour, the greater the probability that some cells will survive and form metastases. The latest methods using RT-PCR techniques proved to be useful, but intact cells and cell clusters could not be detected. Our aim was the immunmagnetic (IM) detection and characterisation of circulating tumour cells and cell clusters in patients with different stages (TNM II, III, IV) of colorectal cancer.

Methods: 25 patients with colon cancer (test samples) and 7 healthy donors (control samples) were evaluated. Follow-up investigations were done in some cases. IM cell separation was performed from the buffy coat of peripheral blood samples by the Carcinoma Enrichment Kit (Miltenyi Biotech, Germany) avoiding any filtering steps. The enriched cell fraction was cytocentrifuged. The cytospins were immuncytochemically labelled using a pancytokeratin antibody (Dako MNF116) and biotin-streptavidin-peroxidase technique. Spiking experiments were performed using the HT29 colon cancer cell line.

Results: From the control samples only once did one of the samples contain a cytokeratin positive cell. However, from the 25 patients 20 showed cytokeratin positive cells. Besides single cytokeratin positive cells, tumour cell clusters (>2 cells), mixed cell doublets (one cytokeratin positive and one negative cell) and mixed cell clusters (>1 cytokeratin positive and negative cells) were detected in 15 of 25 patients. Most (74%) of the circulating cancer cells were found in clusters. The mean number of circulating tumour cells, single cells and the average size of the clusters correlated with the