Materials and methods: Fresh tissue samples were obtained from 55 breast cancer patients undergoing mastectomy or breast conserving surgery. Total RNAs were isolated from 55 surgical specimens of breast cancer tissue and 16 non-cancer breast tissue. The relative mRNA abundance of CxCR4 and CCR7 was measured by real time reverse transcription-PCR analysis based on TaqMan method, and the results were standardized with b-globin mRNA expressions. Statistical analyses were performed using Mann-Whitney test and Kruskal-Wallis test, and the statistical significance was defined as $p<0.05$.

Results: CxCR4 mRNA expression was significantly enhanced in breast cancer tissues compared to non-cancer tissues ($p<0.01$). CCR7 mRNA expression was also significantly enhanced in breast cancer tissues compared to non-cancer tissues ($p<0.01$). However, neither mRNA expression of CxCR4 or CCR7 correlated with any clinicopathological fact ors such as lymph node status, lymphatic invasion, venous invasion, hormone status, distant metastasis or tumor stage.

Conclusions: These results suggest that both CxCR4 and CCR7 mRNA expressions, significantly up-regulated in tumor specimens comparing to non-cancer breast tissue, might have an association with carcinogenesis in breast cancer. However, because the metastasis will be formed by not only chemokine receptor but also its ligand at site, it seemed to be difficult to predict cancer metastasis only by measuring the mRNA expression level of CxCR4 or CCR7.

188 POSTER

The rates of growth of breast cancers

R. Blamey, M. Brooks, S. Pinder, M. Mitchell. *Nottingham City Hospital, Breast Institute, UK*

The diagnostic rate at prevalent screening, the excess of cancers diagnosed at screening over expected presentation, the natural rate of symptomatic presentation and the diameter of screen detected and symptomatic tumours have been used to calculate the diameter doubling time during the observable phase of primary breast cancers (10 mm to 30 mm). These are for grade I 5 years, grade II 2 years and grade III around 3 months. The relative rates match the ratios of mitotic counts for each grade. If growth was by volume doubling, from inception to presentation this would take 150 years in grade I tumours! We have previously (Connor, 1988) shown that mitoses are concentrated in the outer 2 mm shell. We suggest that growth initially is by volume doubling but from 4 mm is by doubling of the outer shell only. Cell doubling rate remains constant. Screen detected tumours average 12 and symptomatic 22 mm diameter. This represents 15 cell doublings in the outer shell and cell doubling rates may be calculated. From the cell doubling rate the time from inception to presentation may be calculated (25 doublings).

Grade	15 cell doublings (months)	Cell doubling time (months)	Mean time from inception to 12 mm (months)
I	62	4.1	102
II	24	2.6	40
III	6	0.4	10

The length of time from inception to presentation in many tumours casts doubt on the role of tamoxifen in 'prevention', more easily explained by inducing responses in undiagnosed cancers. The observation also explains the predominance of grade III tumours at presentation in young women, allowing inception at the same time in good grades but longer to diagnosis.

References

[1] Connor, AJM, et al. Intratumoural heterogeneity of proliferation in invasive breast carcinoma evaluated with MIB1 antibody. The Breast 1997: 6: 171–176.

189 POSTER

Overexpression of eukaryotic elongation factor-1 subunits in breast carcinoma

M. Al-Maghrebi[1], L. Temmim[2], A.A. Olalu[3]. *[1]Faculty of Medicine-Kuwait University, Biochemistry, Kuwait, Kuwait; [2]Hussein M. Al-Jumma Cancer Center, Clinical Laboratories, Kuwait, Kuwait; [3]Hussein M. Al-Jumma Cancer Center, Pathology Laoboratory, Kuwait, Kuwait*

Background: Wide evidence suggests the involvement of translation elongation factors (EFs) at the onset of oncogenesis. To investigate the potential role of the EF-1 subunits (alpha, beta, and gamma) in formation and progression of breast cancer, we compared their expression in breast cancers with that in non-cancerous tissues.

Materials and Methods: Total RNA was isolated from fifty eight frozen specimens including 20 primary tumors, 9 fibroadenomas, and matched normal adjacent tissue. The expression of the EF-1 subunits (alpha, beta, and gamma) mRNA in breast cancer tissues was determined using RT-PCR. The mRNA expression was also examined in three breast cancer cell lines and one normal breast cell line using northen blot analysis.

Results: EF-1 alpha, beta and gamma mRNA expression was significantly higher in cancerous over normal tissues ($p<0.05$). However, there was no significant difference in the expression of the three EF-1 subunits between grades I, II, and III tumors. A 2–3 fold increase was observed in mRNA expression in breast cancer cell lines (MCF-7, T47D, and MDA-231) when compared to a normal cell line (MCF-10A).

Conclusion: Overexpression of the three EF-1 subunits was observed in malignant but not in normal breast tissues. Similar results were obtained in cell lines of breast tissue. The elevated levels of these translation factors are indicative of a possible role in the pathogenesis of breast cancer.

190 POSTER

Tartrate-resistant acid phosphatase is expressed by breast cancer cell lines

L.M. Adams[1], M.J. Warburton[2], A.R. Hayman[1]. *[1]University of Bristol, Department of Clinical Veterinary Science, Bristol BS40 4DU, UK; [2]St George's Hospital Medical School, Department of Cellular Pathology, London SW17 0RE, UK*

Tartrate-resistant acid phosphatase (TRAP, EC 3.1.3.2) is a histochemical marker of osteoclasts, however its biological function is not fully understood. TRAP is also expressed by macrophages and dendritic cells and occurs in a wide variety of tissues including spleen, lung, thymus, skin, the linings of the gastrointestinal tract, tissues in the nervous system, as well as the skeleton. Studies using mice lacking TRAP as a result of targeted gene disruption have demonstrated that TRAP is essential for the normal mineralisation of cartilage in developing bones and the maintenance of the adult skeleton. Macrophages and dendritic cells lacking TRAP displayed an abnormal immunomodulatory response and cytokine profile. It has been suggested that TRAP may be involved in cell recruitment in bone and the immune system.

Osteopontin, identical to the T-cell cytokine Eta-1, is a substrate for TRAP. It is a highly phosphorylated protein with a wide tissue distribution like TRAP. A variety of functions are associated with it some of which are known to be phosphorylation dependent. This cytokine contributes substantially to metastasis formation by various cancers. Breast cancer, one of the principal neoplasms that metastasise to bone causing extensive destruction by osteoclasts, is associated with an abundant secretion of osteopontin. However the mechanism by which cancer cells interact with osteoclasts is not fully understood. Serum TRAP is a marker of metastatic bone disease in breast cancer patients and can be used to monitor its response to treatment.

Our aim in this study was to investigate TRAP in breast cancer, to determine if TRAP is expressed by breast cancer cells. Breast cancer cell lines MCF-7, T47-D, MDA-MB-435 were used for experiments. Cell line Hb4a derived from normal human mammary epithelial cells was used as a control. Cells were cultured and lysates assayed for TRAP activity using p-nitrophenyl phosphate as the substrate. The MDA-MB-435 cell line had an activity of 114 nmoles/mg/min, which was 2 fold higher than the other cell lines. Immunohistochemistry using an antibody that specifically recognises TRAP showed positive staining in all cell lines compared with non-immune controls. We conclude that breast cancer cells do express TRAP and initial studies show that activity is increased in cells that are more tumourigenic.

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191 POSTER

Analysis of cell growth inhibitory effects of antineoplaston through MAPK in human breast cancer cell line SKBR-3

T. Fujii[1,2], A. Nakamura[2], H. Yanaga[1], G. Yokoyama[1,2], M. Mishima[1,2], E. Ogo[3], T. Koga[4], H. Yamana[5], K. Shirouzu[1], H. Tsuda[6]. *[1]Kurume University, Surgery, Kurume, Japan; [2]Kurume University, Research Center for Innovative Cancer Therapy, Kurume, Japan; [3]Kurume University, Radiology, Kurume, Japan; [4]Hirose Hospital, Surgery, Fukuoka, Japan; [5]Kurume University, Multidisciplinary Treatment Center, Kurume, Japan; [6]Kurume University, Anesthesiology, Kurume, Japan*

We have investigated the cell growth inhibitory effects of antineoplaston that are naturally occurring peptides and amino acid derivatives on the human breast cancer cell line SKBR-3, and the mechanism of its action, with emphasis on the cell cycle and mitogen-activated protein kinases (MAPK). A significant dosage-dependently growth inhibition was observed after treatment with antineoplaston. At 48 hours after the

addition of antineoplaston, cells at the G_1 phase were increased by 15%, compared with the control. Analysis of the expression of cell cycle-related proteins after the addition of antineoplaston showed that the p21 and p16 proteins were increased after administration, however, that the expression of the cyclin D1 and cyclin E proteins was unchanged. At 24 hours after the administration of antineoplaston, the phosphorylation of retinoblastoma (Rb) was inhibited. Furthermore, the analysis of the MAPK expression showed that the phosphorylated ERK MAPK protein began to decrease at 3 hours after antineoplaston administration. To further confirm a role of ERK MAPK in SKBR-3 cell growth, we used PD98059, an inhibitor of mitogen-activated protein kinase kinase (MEK) 1, the kinase responsible for ERK MAPK activation. PD98059 significantly reduced the levels of phosphorylated ERK MAPK, without noticeable changes in total ERK MAPK. Analysis of cell proliferation revealed that PD98059 dose-dependently inhibited cell growth compared to control (vehicle-treated cells), thereby confirming the importance of the ERK MAPK pathway in the control of cell proliferation in SKBR-3 cells. From these results, we have speculated that, in the breast cancer cell line SKBR-3, antineoplaston dephosphorylated ERK MAPK, and that the dephosphorylated ERK MAPK regulated the expression of p21, p16 proteins, inhibited the phosphorylation of Rb, and thereby causing G_1 arrest. The results suggested that antineoplaston may be an effective adjuvant therapy after breast cancer surgery.

192 POSTER

The role of SERMs on the induction of apoptosis of human breast cancer cells in vitro

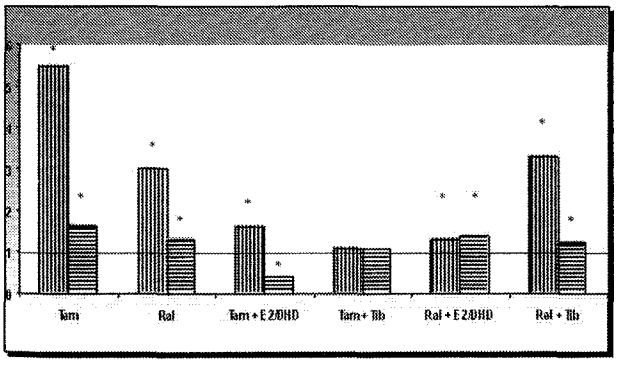
H.R. Franke[1], H.M.J. Werner[2], F. Wolbers[3], I. Vermes[3]. [1]*Medisch Spectrum Twente Hospital Group, Obstetrics and Gynecology, Enschede, The Netherlands;* [2]*Medisch Spectrum Twente, Obstetrics and Gynecology, Enschede, The Netherlands;* [3]*Medisch Spectrum Twente, Clinical Chemistry, Enschede, The Netherlands*

Introduction: In vitro research demonstrated that selective estrogen receptor modulators (SERMs) induce apoptosis (programmed cell death) in human mammary carcinoma cells. However the use of SERMs in vivo can cause severe climacteric complaints sometimes necessitating interrupting treatment. The combined use of tamoxifen and continuous combined HRT did not increase the risk of recurrences in breast cancer survivors (Dew et al., 2002).

Materials and methods: The SERMs tamoxifen (Tam) and raloxifene (Ral) alone or combined with estradiol (E2) plus dihydrodydrogesterone (DHD) as well as tibolone (Tib) were administered to MCF-7 cells, estrogen receptor (ER) positive and MDA-MB-231, ER negative human breast cancer cell lines, in a concentration of 10^{-6} M for 144 hours in vitro.

Proliferation was determined by measuring the expression of Cyclin D1 and apoptosis by using the DNA fragmentation assay and both performed in duplicate. The mean ratios apoptosis versus proliferation were calculated and the 95% confidence intervals assessed.

Results: Tam and Ral alone induced apoptosis in ER positive and negative breast cancer cells (figure 1). Tam and Ral combined with E2 plus DHD did induce apoptosis in ER positive breast cancer cells, however Tam plus Tib neither induced nor stimulated ER positive and negative breast cancer cells. Ral combined with Tib induced apoptosis in both cell lines.



Apoptosis/proliferation versus controls after 144 hours

Ratio>1 means induction of apoptosis. * $P < 0.05$ versus controls.

Conclusion: We demonstrated that our laboratory data mirror cell biological behaviour in vivo and we therefore suggest that ER positive breast cancer survivors using Tam or Ral may simultaneously start with E2 plus DHD or Tib to reduce side effects without compromising its efficacy.

193 POSTER

Antiproliferative activity of tamoxifen on MCF-7 breast cancer cells is modulated by weak electromagnetic field exposure

V. Hanf[1], H. Schimming[2], R. Kreienberg[3], R. Girgert[3]. [1]*Universitäts-Frauenklinik Göttingen, Germany;* [2]*Wissenschaftliche Werkstatt der Universität Ulm, Germany;* [3]*Frauenklinik der Universität Ulm, Germany*

Breast Cancer (BC) incidence has been rising ever since the second world war in industrialized countries, a trend paralleled by increasing electrification. Usage of electrical power is intricately associated with electromagnetic field (EMF) exposure. Tamoxifen, a partial estrogen receptor antagonist, is the most frequently used BC medication.

Aim of this study was to substantiate sporadic experimental communication that the anti-proliferative activity of Tamoxifen in MCF-7 BC cells can be modulated by extremely low frequency (ELF) EMF exposure.

Materials and Methods: In order to expose BC cells to reproducibly homogeneous sinusoidal 50 Hz ELF-EMF of defined electromagnetic flux density, we specifically designed tissue culture incubators delivering flux densities of either 0 μT, 1.2 μT, 10 μT or 100 μT at 37°C, 5% CO_2. MCF-7 estrogen receptor positive BC cells were incubated in MEM supplemented with 5% fetal calf serum and treated with increasing Tamoxifen (Tam) concentrations at a given magnetic flux density. After 7 days of culture resulting cell concentrations were measured using a colorimetric test, dose–response curves for Tam were recorded and EC_{50}-concentrations for Tam were calculated at each flux density.

Results: In control experiments without measurable EMF-exposure low Tam-concentrations ($<2\times10^{-7}$ M) expectedly exerted an agonistic proliferative effect on MCF-7 cells. With increasing Tam-concentration the well-known anti-proliferative effect was seen (half maximal effect at 2.2×10^{-6} M). EMF at 1.2 μT flux density shifted Tam dose–response curves to higher concentrations, resulting in a maximal proliferative effect at 1.8×10^{-6} M. In comparison to control experiments at 0 μT flux density, 2 times higher Tam-concentrations were needed to induce a half maximal anti-proliferative effect at 4.4×10^{-6} M when a 1.2μT field was applied. While 0.2μT fields exhibited a similar, if weaker curve shifting effect, higher flux densities at 10 μT or 100 μT exhibited a much less pronounced activity ("window effect").

Conclusion: Clearly, sinusoidal alternating EMF-exposure at environmental flux densities to MCF-7 BC cells modulated anti-proliferative activity of Tam. Clinically, it is interesting to note that 1.2 μT EMF induced near maximal proliferation at a Tam concentration (1 μM) that is usually achieved in serum of patients under Tam-therapy for primary or recurrent BC.

Wednesday, 17 March 2004 16:00–17:15

PROFFERED PAPERS

Psychosocial aspects

194 ORAL

Expectations for breast treatment – complex biopsychosocial determinants

C. Campbell[1], P. Durning[2]. [1]*University of Teesside, School of Social Sciences and Law, Middlesbrough, UK;* [2]*James Cook University Hopsital, General Surgery, Middlesbrough, UK*

Expectations regarding the health care experience, treatment process and treatment outcome are key determinants in how the individual will view that health care experience. Within the health literature the study of expectations is often linked to measures of patient satisfaction. There is a need however, for expectations to be considered in relation to specific attributes or aspects of care, rather than being limited to generalisations about the totality of a service which tends to conflate many issues and fails to provide useful information for service improvement (Thompson & Sunol, 1995). In relation to breast care services what is often not considered is that despite an increase in the numbers of patients being diagnosed with breast cancer, many patients referred via a rapid referral system are found to have benign disease. Allied to this, the fast track referral to a breast clinic of a patient who does not have cancer can have adverse intra-personal effects due to a heightening of anxiety (Durning, Morris, Gash & Gray, 1998) and related distress (Nosarti, Roberts, Crayford, McKenzie & David, 2002). Furthermore, such expeditious referral can impact greatly on the expectations held by those individuals presenting with a breast problem.