312 PUBLICATION

Development, characterisation and gene expression profiling of a novel more invasive variant established from the human breast cancer cell line Hs 578T

L. Hughes[1], K. Malone[1], S. O'Brien[2], W. Gallagher[2], S. McDonnell[1].
[1]*University College Dublin, Chemical and Biochemical Engineering, Dublin, Ireland;* [2]*University College Dublin, Department of Pharmacology, Conway Institute of Biomolecular and Biomedical Rese, Dublin, Ireland*

Background: The purpose of this study was to identify invasion-specific genes in breast cancer cell lines using Affymetrix microarray technology. Since an isogenic cell line model (i.e. different cell lines with the same genetic background) with different *in vitro* invasiveness was not available a new more invasive variant was generated from the Hs 578T breast cancer cell line.
Methods: To generate invasive variants from the Hs 578T, 1×10^6 cells were allowed to invade for five hours through six well matrigel invasion chambers, invading cells were removed from the bottom of the membrane and designated Hs 578Ts(i)$_1$ and this selection procedure was repeated eight times. Invasion and migration assays were done using matrigel 24 well invasion chambers and falcon 24 well inserts respectively, assays were stained using crystal violet. All cells were cultured under standard conditions in DMEM supplemented with 10% foetal calf serum, 10% L-glutamine and 0.1 unit/ml bovine insulin. Growth, detachment and adhesion assays were carried out in 24 well plates and quantified using CellTiter 96® AQueous One Solution Reagent (MTS), absorbance was read at 492 nm. Gene expression profiles were identified using the Affymetrix Human Genome U133A gene chip. Data analysis was done using GeneSpring 6.0 software. Real-time PCR analysis was completed using SYBR green.
Results: A distinct difference in invasiveness and other properties emerged between the Hs 578T and the new variant Hs 578Ts(i)$_8$. The new cell line was three fold more invasive than Hs 578T and grew significantly faster (up to four times faster). Hs 578Ts(i)$_8$ detached faster on trypsinisation (four minutes for s(i)$_8$ against sixteen minutes for Hs 578T) and there was a small increase (no more than two fold) in migratory ability. In soft agar, Hs 578Ts(i)$_8$ formed four times more colonies than the parental cell line. Microarray analysis of the two cell lines showed that 508 genes were differentially expressed. Twenty of these genes were chosen for validation and further studies. To date seven genes have been validated using Real-Time PCR analysis. Cathepsin Z was five fold up-regulated and Lumican was 14 fold down regulated, correlating with array data.
Conclusion: We have generated and extensively characterised a novel more invasive variant from the Hs 578T breast cancer cell line. We have also identified a panel of genes associated with invasive breast cancer.

313 PUBLICATION

Plasma kinetics and uptake of a cholesterol-rich microemulsion (lde) associated to a derivative paclitaxel by neoplastic breast tissue

L.A. Pires[2], D.G. Rodrigues[1], R. Hegg[2], S.R. Graziani[2], R.C. Maranhão[1,3].
[1]*Institute of Heart, USP, Metabolism Lipids Laboratory, São Paulo, Brazil;*
[2]*Medical School, USP, Gynecology Departament, São Paulo, Brazil;*
[3]*Faculty of Pharmaceutical Sciences, USP, Biochemistry, São Paulo, Brazil*

Background: Previously we described the association of paclitaxel to a cholesterol-rich nanoemulsion (LDE) that binds to low-density lipoprotein (LDL) receptors and concentrates in neoplastic tissues. The association of the drug is stable, preserves the anti-proliferative activity of the drug and reduces the toxicity to animals. The present study was designed to determine the plasma kinetics of the association LDE: paclitaxel oleate and to verify whether the complex has the ability to concentrate in malignant breast cancer.
Material and methods: To facilitate the association to LDE, paclitaxel is derivatized with oleic acid. [^{3}H]paclitaxel oleate associated to LDE labeled with [^{14}C]-Cholesteryl oleate was intravenously injected into 3 patients with breast cancer (60±7 yr.) 24 h before surgery. Blood samples were collected over the 24 h period to determine the plasma decay curves of the radioactive labels. Radioactivity present in plasma aliquots was quantified in a scintillation solution and the pharmacokinetic parameters were calculated by compartmental analysis. Specimens of tumors and normal breast excised during the surgery were collected for lipid extraction, separation by thin layer chromatography and radioactive counting. The experimental protocol was approved by the Ethics Committee of the hospital and an informed consent was obtained from each participant.
Results: Fractional clearance rate (FCR) of LDE and of the drug were similar (0.030±0.026 e 0.018±0.018, respectively, P = 0.5742). The uptake of both [^{14}C]-LDE and [^{3}H]-paclitaxel oleate by breast malignant tissue was two and three fold greater than that of the normal breast tissue, respectively (LDE uptake = 680±481 and 290±247 and paclitaxel oleate uptake = 1134±1549 c.p.m./g and 469±695, respectively). Paclitaxel oleate has a $T_{1/2}$ = 19 h, AUC = 1.4 mg/h/L, VSS = 41.8 L and CL = 1.5 L/h.
Conclusions: Our results indicate that most of the drug is retained in the microemulsion particles until its removal from the circulation and internalization by the cells. In addition, we showed that when paclitaxel is associated with LDE, the drug can be concentrated in malignant breast tissues while deviating from the normal tissue. Therefore, LDE can be used to target paclitaxel against malignant breast cells.

314 PUBLICATION

Leptin expression on breast cancer patients and the effects of leptin expression on survival in obese postmenopausal women with antiestrogen-treated breast cancer

Y.N. Kim[1], S.-Y. Kim[1], J.J. Lee[1], J.H. Seo[1], Y.H. Kim[3], S.H. Koh[4], H.J. Yoon[1], K.S. Cho[1]. [1]*Kyung Hee University, Department of Internal Medicine, Seoul, Korea;* [3]*Kyung Hee University, Department of Anatomical Pathology, Seoul, Korea;* [4]*Kyung Hee University, Department of General Surgery, Seoul, Korea*

Background: Leptin is an adipocyte-derived cytokine that acts through its receptor and is related to obesity. Obesity in postmenopausal women is associated with an increased risk of breast cancer. Recent trials suggest that functional cross talk between leptin and estrogen systems exists. We tried to evaluate whether leptin & leptin receptor (ObR) effect on the prognosis of the obese postmenopausal breast cancer patients who were treated with antiestrogen, tamoxifen in adjuvant setting and to investigate leptin and ObR expression in breast cancer patients.
Material and methods: From 1994 to 2004, 91 patients who were diagnosed as stage I or II breast cancer after a curative resection were analyzed. The expression levels of leptin and ObR were measured using immunohistochemical (IHC) staining with rabbit polyclonal anti-human leptin and mouse monoclonal anti-mouse ObR (Santa Cruz Biotechnology, Santa Cruz, CA, USA) from paraffin-embedded primary tumor specimens. Compared with the intensity of IHC staining of adipocyte, that of tumor cells was divided into two groups (negative, positive).
Results: Among a total of 91 patients (stage I/II = 23/68 pts), 82 pts were hormone receptor positive breast cancers and the median age was 48 years. 43 pts (52.4%) were menopause status and BMI (body mass index) were 23 or higher in sixty-one pts (67.0%). 69 pts received adjuvant chemotherapy. All pts with hormone receptor-positive tumors received tamoxifen. The median follow-up duration was 41 months. Leptin expression was observed in 77 pts (84.6%) of total pts. Among 82 pts with hormone receptor-positive tumors, 40 pts (48.8%) were menopausal status, 54 pts (65.9%) were 23 or higher BMI and 28 pts (70%) of 40 menopausal pts were obese. Leptin expression was positive in 70 pts (85.4%) of 82 pts, in 34 pts (85.0%) of 40 menopausal pts, in 46 pts (85.2%) of 54 obese pts and in 25 pts (89.3%) of 28 obese postmenopausal pts among 82 hormone receptor-positive pts. 14 pts (17.1%) of 82 pts experienced relapse. No significant difference of disease-free survival (DFS) was shown between leptin expression and obese postmenopausal breast cancer (p = 0.42). But DFS in leptin expression of obese postmenopausal patients was shown to tend to be higher than that of no expression. The result of ObR expression will be presented at the meeting.
Conclusions: This current study is the one among a few data to evaluate the relation of leptin with breast cancer patients and analyzed survival according to leptin expression of obese postmenopausal patients with tamoxifen-treated breast cancer. Present negative result must be continuously observed to overcome short median follow-up duration.

315 PUBLICATION

Cytochromes P450IID6 genotypes and its influence on the behavior of breast cancer in women under forty years

C. Rodrigues[1], D. Pinto[1], S. Sousa[2], R. Catarino[1], J. Leal da Silva[2], C. Lopes[1], H. Rodrigues[2], R. Medeiros[1]. [1]*Portuguese Institute of Oncology, Molecular Oncology – CI, Porto, Portugal;* [2]*Portuguese Institute of Oncology, Medical Oncology I, Porto, Portugal*

Background: CYP2D6, a member of the Cytchromes P450 (CYP) family, is a phase I metabolic enzyme involved in the oxidative metabolism of numerous endogenous and exogenous molecules, including cytotoxic agents. The CYP2D6*4 polymorphism has been reported to be a major cause of CYP2D6 poor metaboliser phenotype, leading to the absence or decrease in the amount and activity of its protein. The aim of this study was to understand the role of *CYP2D6* genotypes on the behavior of breast cancer in women under forty years.
Methods: This study included 112 patients diagnosed with breast cancer under the age of forty in the Portuguese Institute of Oncology –