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We completed specific tasks #1 and #2 and have published the results (Breast Cancer Res Treat 87:205-214, 2004). We also extended the S1R mRNA correlative studies to gene expression data obtained from 600 breast cancers. There continued to be no consistent correlation between S1R mRNA expression and prognosis or lymph node or estrogen receptor status. However the data confirmed frequent mRNA expression of S1R in breast cancer.

Progress has been made in task #3. Eleven breast cancer cell lines were screened for S1R expression with Western blot and one cell line T47D were negative. This cell line was successfully transfected with S1R using PcDNA 3.1 construct. No change in cell proliferation, apoptosis or sensitivity to chemotherapy was seen at baseline or after S1R ligand (SKF 10047) exposure. This was consistent with the negative results previously observed in S1R-positive cell lines treated with SKF 10047.

S1R appears to play a role in regulating cell migration. SKF 10047 inhibits migration of MDA 231 and MDA 435 cells in vitro. Gene expression analysis after exposure to SKF 10047 suggests that this compound inhibits small GTPase-mediated signaling pathways. We are currently examining the molecular mechanism of S1R mediated inhibition of cell migration by measuring the activity of Rho, Rac and CDC42 before and after SKF 10047 exposure. We are also in the process of developing S1R knock down cells by using siRNA technology.

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Introduction

The goal of this research project is to evaluate Sigma 1 receptor (S1R) expression in human breast cancer and determine its biological role in the pathomechanism of breast cancer. We used immunohistochemistry to determine S1R expression in normal breast, hyperplasia, in situ cancer and invasive cancer. We also analyzed a gene expression data generated by Affymetrix U133A genechips from 600 breast cancers to assess correlation between S1R mRNA expression and clinical variables (grade, ER and HER2 status, lymph node involvement) and prognosis. To address the biology of S1R, we studied the effect of S1R ligands on the proliferation, chemotherapy sensitivity and migration of breast cancer cell lines in vitro. We also generated a S1R transfected variant of the T47D cell line and are currently in the process of developing S1R knock down cells using siRNA technology

Body and Key Research accomplishments

1. S1R mRNA overexpression was detected in 64 % of invasive breast cancers compared to normal breast tissue. Immunohistochemistry (IHC) also showed positive staining of neoplastic cells for S1R protein in 60% of invasive cancers (Figure 1). S1R expression was neither prognostic nor predictive marker for efficacy of adjuvant chemotherapy in the 58 cancer patients included in the IHC study. We extended the S1R mRNA correlative studies to gene expression data obtained from 600 breast cancers. There continued to be no correlation between S1R mRNA expression and prognosis (Figure 2 & 3) or lymph node or estrogen receptor status. There was also no correlation with response to preoperative chemotherapy with paclitaxel and anthracycline. However the data confirmed frequent expression of S1R in breast cancer.
2. S1R has no sequence homology to other known receptors and its signal transduction mechanism remains unknown. Ten of 11 breast cancer cell lines express S1R mRNA and protein (Figure 4). We examined the effect of non-specific S1R ligands (haloperidol, reduced haloperidol and progesterone) and S1R specific ligand SKF 10047 on cell growth, sensitivity to chemotherapy and cell migration in vitro. Non-specific ligands inhibited cell growth at supra-pharmacological (>10 μ M) concentrations and reduced haloperidol also showed additive cytotoxic effects when combined with doxorubicin, vinorelbine, paclitaxel and docetaxel in vitro. The S1R-specific ligand, SKF 10047 demonstrated minimal growth inhibitory activity and showed no interaction with chemotherapy. Next, we transfected T47D cells (S1R-negative cell line) with S1R receptor. No change in cell proliferation, apoptosis or sensitivity to chemotherapy was seen at baseline or after SKF 10047 exposure (Figure 5). This was consistent with the negative results previously observed in S1R-positive cell lines treated with SKF 10047. These results suggest that S1R does not play a major role in regulating cell proliferation or sensitivity to chemotherapy.
3. SKF 10047 significantly inhibited migration of MDA 231 and MDA 435 cells in vitro (Figure 6). T47D cells do not migrate in the in vitro migration assay (Biocoat, Cell environments, cat#354578). Next, we examined transcriptional changes in response to SKF 10047 exposure in these cells using Affymetrix U133A gene chips. Gene expression analysis suggests that this compound inhibits small GTPase-mediated signaling pathways (Figure 7).

We are currently examining the molecular mechanism of S1R mediated inhibition of cell migration by measuring the activity of Rho, Rac and CDC42 before and after SKF 10047 exposure. We are also in the process of developing S1R knock down cells by using siRNA technology.

Reportable outcome

1. Wang B, Rouzier R, Albarracin CT, Sahin AA, Wagner P, Yang Y, Smith TL, Meric-Bernstam F, Aldaz CM, Hortobagyi GN, Pusztai L. Expression of sigma 1 receptor in human breast cancer. *Breast Cancer Res Treat* 87:205-214, 2004.

Conclusions

S1R receptor is frequently expressed in breast cancer cells both in vitro and in vivo. It does not appear to be a clinically useful prognostic marker. S1R expression or S1R ligands do not alter proliferation rate or sensitivity to chemotherapy in vitro. However, S1R appears to play a role in regulating cell migration. SKF 10047 inhibits migration of MDA 231 and MDA 435 cells in vitro. Gene expression analysis after exposure to SKF10047 suggests that small GTP-ase-mediated signaling pathways (Rho and Rac) are inhibited by this compound.

Figure 1. S1R immunohistochemistry

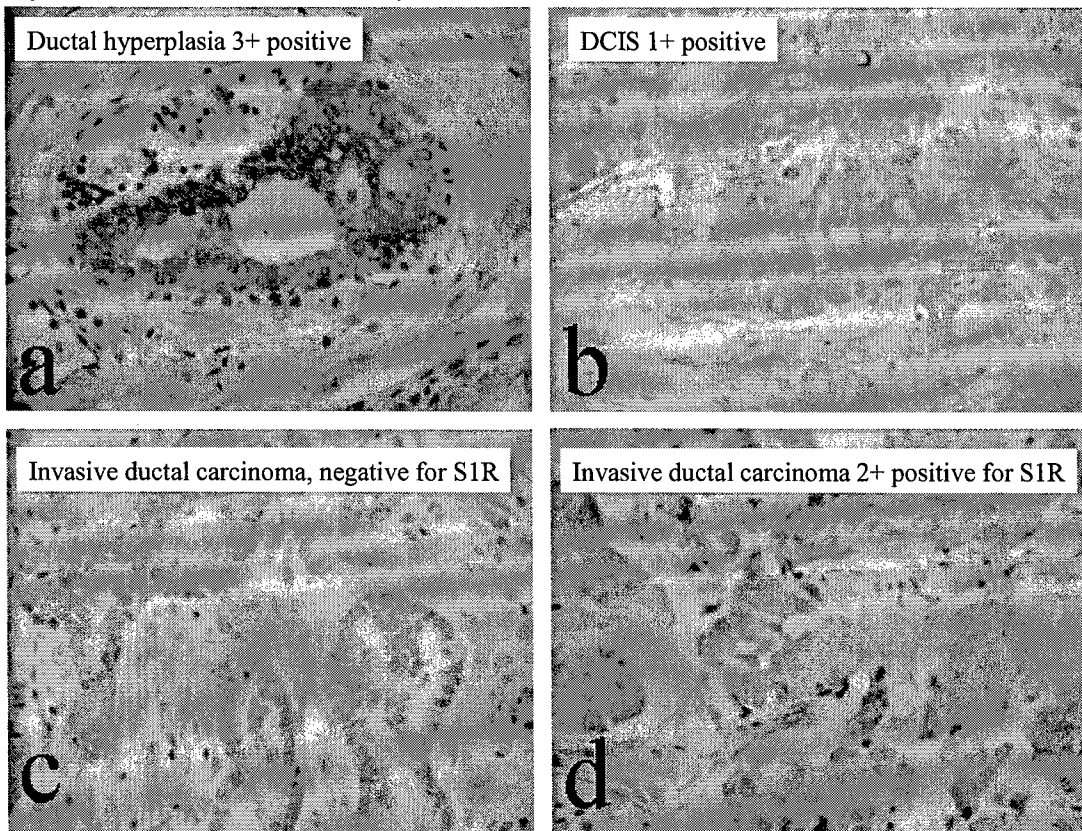


Figure 2 Correlation between S1R mRNA expression and distant metastasis (n=286 cases, Stage I-II no adjuvant therapy, Affymetrix U133A genechip probe 201692)

Lancet286: Distant Metastasis vs. OPSR1: 201692

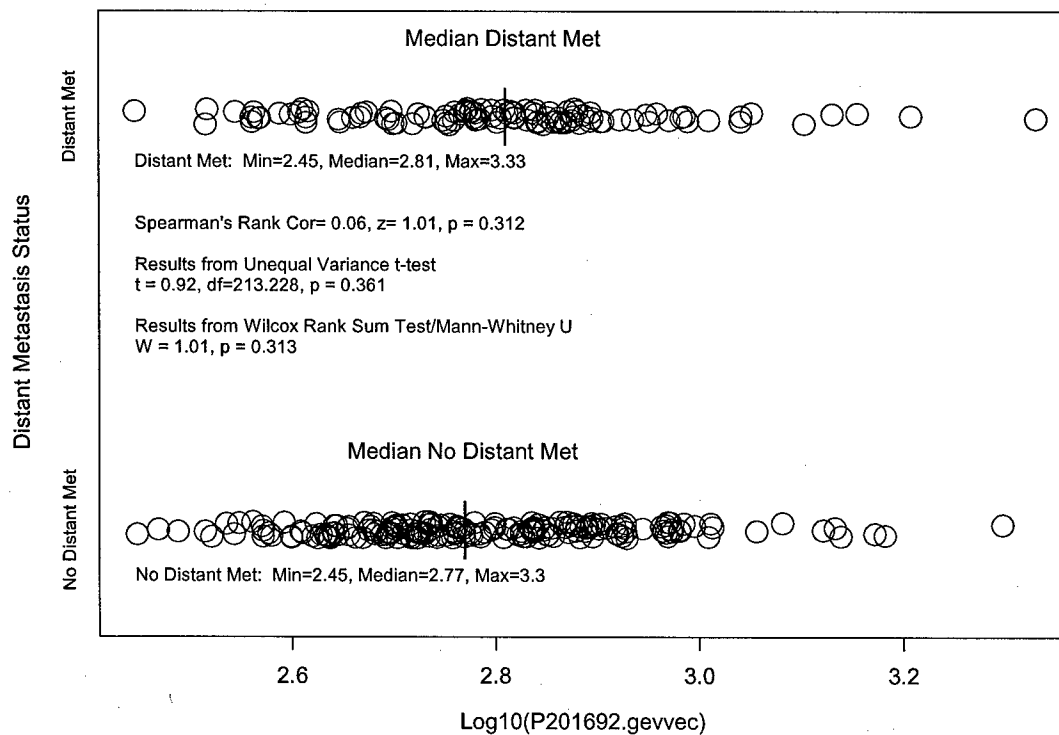


Figure 3. Correlation between S1R mRNA expression and distant metastasis
(n=202 cases, Stage I-II no adjuvant therapy, Affymetrix U133A genechip probe 201692)

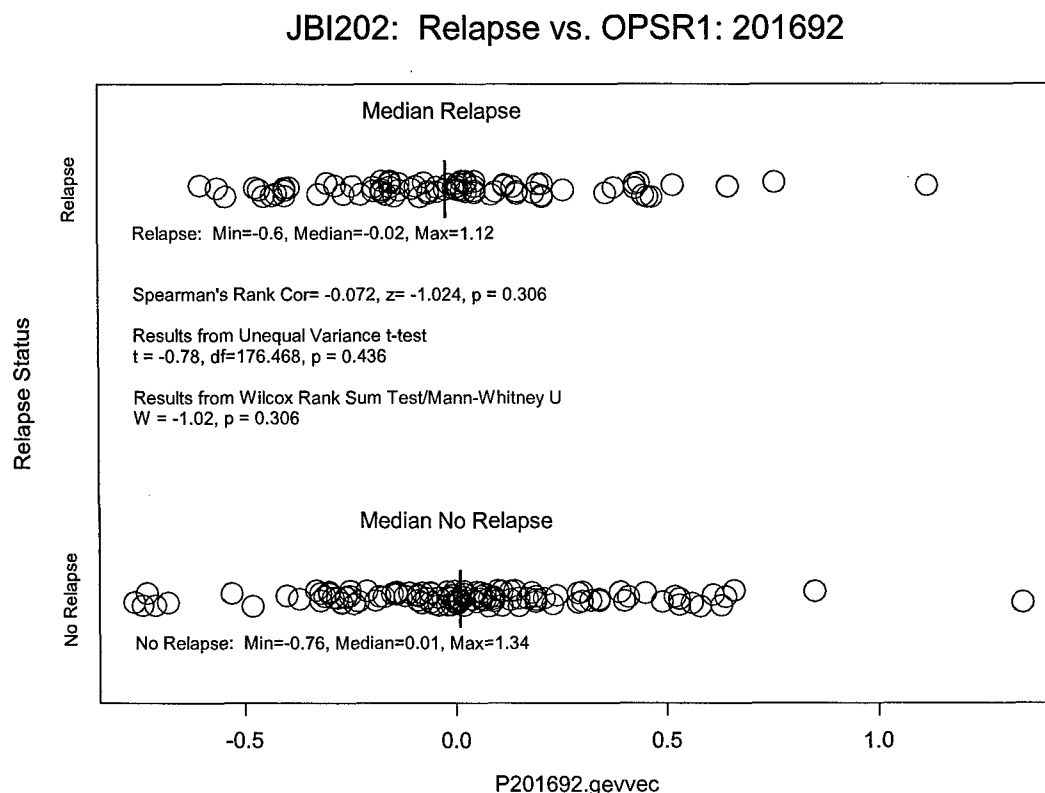
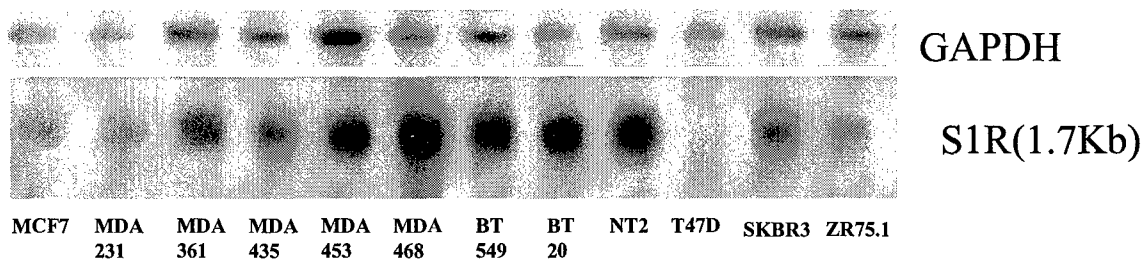


Figure 4

S1R mRNA expression breast cancer cell lines



S1R protein expression breast cancer cell lines

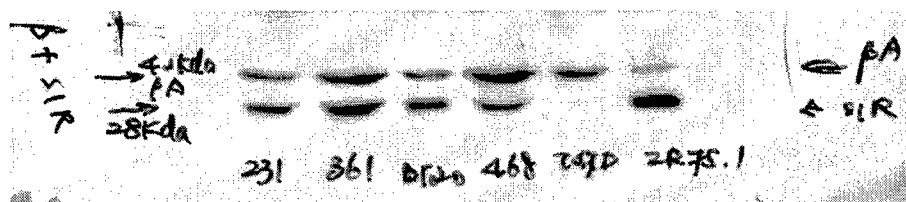
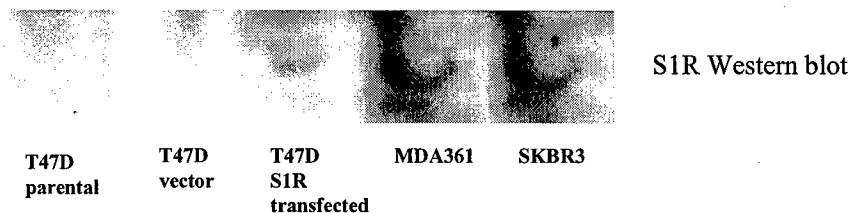


Figure 5

Forced Expression of S1R in T47D cells



S1R transfection does not affect sensitivity to paclitaxel or epirubicin chemotherapy of T47 D cells

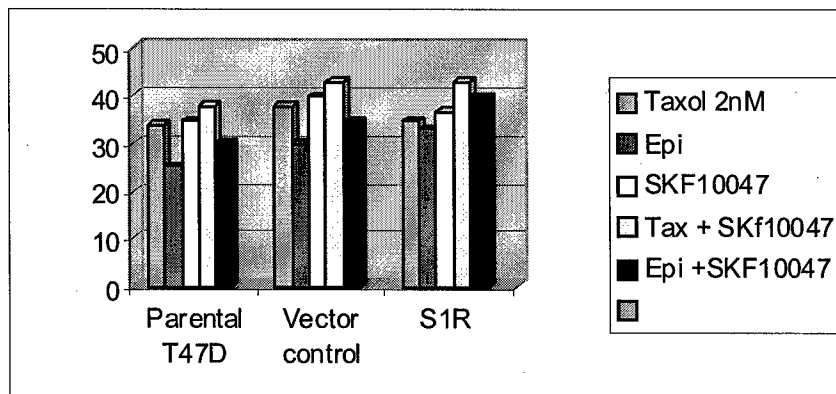
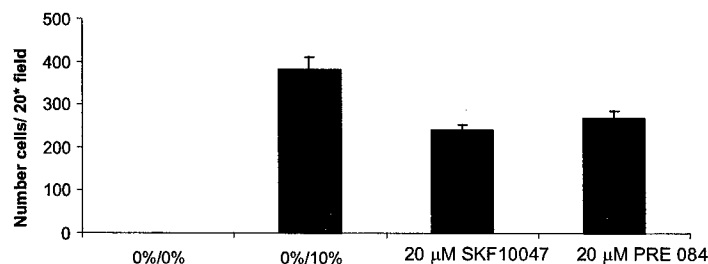


Figure 6

S1R ligand SKF 10047 inhibits migration of breast cancer cells in vitro

Migration of MDA-MB-231 cells in the presence or absence of SKF 10047



Migration of MDA-435 cells in the presence or absence of SKF 10047

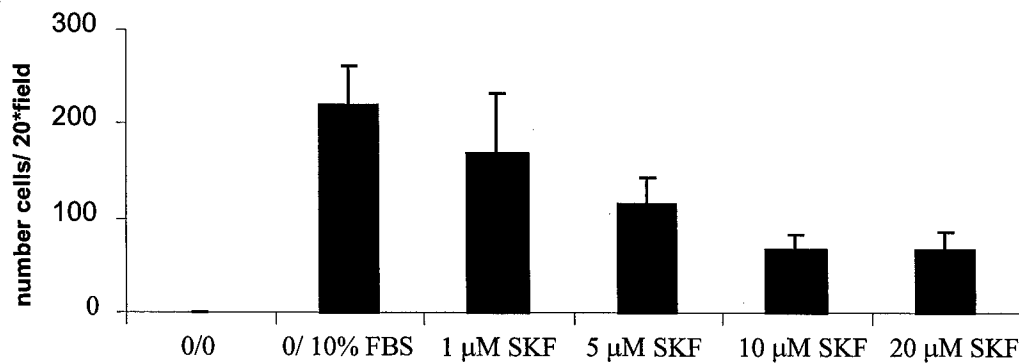


Figure 7. Transcriptional profiling of MDA435 cells after 24h exposure to SKF 10047 shows 6 genes down-regulated > fold and 1 gene up-regulated > 2 fold.

