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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
We have completed task #2. Sigma 1 receptor (Sig 1 R) mRNA expression was examined in 49 human breast cancers using cDNA microarrays. The data indicates that Sig 1R mRNA expression is heterogeneous in breast cancer. A subset of tumors (20%) has high levels of expression compared to the median Sig 1 mRNA expression of the group as a whole. The cellular distribution of Sigma 1 protein was also assessed by immunohistochemistry (IHC) in 60 invasive breast cancer, 20 DCIS and 20 normal cases. A breast cancer pathologist currently conducts analysis of the IHC results. Preliminary results indicate strong cytoplasmic expression in a subset of breast cancers and absence of the receptor in normal breast epithelium.
We have also completed aims "a" and "b" of task #1. We have screened breast cancer cell lines for expression of Sig 1 R by RT PCR. MDA-MB-231, -435, and –361 cells express Sig 1 mRNA, in contrast MCF-7, BT-20 and MDA-MB-453 cells lack Sig 1 R expression. We are currently confirming these results with Western blots. We examined the effect of SKF 10047 (agonist) and reduced haloperidol (RH) (antagonist) on cell growth. SKF stimulated growth of Sig 1R + cells, RH inhibited cell growth.

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Introduction

Task # 1 of our proposal is to determine the affect of Sigma 1 receptor activity on breast cancer cell growth and tumorigenesis *in vitro* and *in vivo*. This work includes assessment of expression of Sigma 1 receptor in established breast cancer cell lines and normal breast epithelial cultures by RT-PCR. We also plan to study the effect of Sigma receptor activation or inhibition on cell proliferation, and apoptosis in vitro by MTT assay, colony formation assay and flow cytometry on different cell lines. To further evaluate the role of Sig 1 R we will generate stable transfectants of Sigma receptor and determine the biological effects of forced Sigma receptor expression.

Task # 2 of our proposal is to determine the expression pattern of Sigma1 1 receptor in human breast cancer. This work includes generation of a monoclonal antibody against Sigma receptor assessment of its staining characteristics in human tissues and immunohistochemistry staining of paraffin embedded sections of breast tumors and normal breast samples.

This progress report describes our results as they relate to these 2 specific tasks.

Body and Key Research accomplishments

We have completed task #2.

Sigma 1 receptor (Sig 1 R) mRNA expression was examined in 49 human breast cancers using cDNA microarrays. The data indicates that Sig 1R mRNA expression is heterogeneous in breast cancer (**Figure 1**). A subset of tumors (20%) has high levels of expression compared to the median Sig 1 mRNA expression of the group as a whole. The cellular distribution of Sigma 1 protein have been assessed by immunohistochemistry (IHC) on 100 human specimens including 60 invasive breast cancer, 20 DCIS and 20 normal cases. We used a commercially available Sig 1 R antibody (anti-Sig-1 R goat polyclonal antibody by Santa Cruz, catalog # sc16203). Briefly, antigen heat retrieval was applied and the primary antibody was used in 1:50 dilution, slides were incubated with the primary antibody overnight at RT. Staining Kit was obtained from Vector ABC Elite (Vector Laboratories), antigen retrieval solution A.R.S. II, was from Vel-Lab Research and DAB was used chromagen (20X, Zymed Laboratories). We tested the specificity of immunostaining by using Sig-1 blocking peptide also obtained Santa Cruz catalog # sc16203P and a non-specific peptide was used as control. Sig-1 peptide completely abolished staining whereas non-specific peptide at the same molar concentration did not affect cytoplasmic stating observed with the antibody. A breast cancer pathologist currently conducts analysis of the IHC results. Preliminary results indicate strong cytoplasmic expression in a subset of breast cancers and absence of the receptor in normal breast epithelium (**Figure 2**)

Figure 1. Expression of Sigma 1 receptor mRNA in 49 human breast cancer specimens. RNA was extracted from fine needle aspirations of newly diagnosed stage I-III breast cancer and transcriptional profiling performed with cDNA microarray proprietary to Millennium Pharmaceuticals.

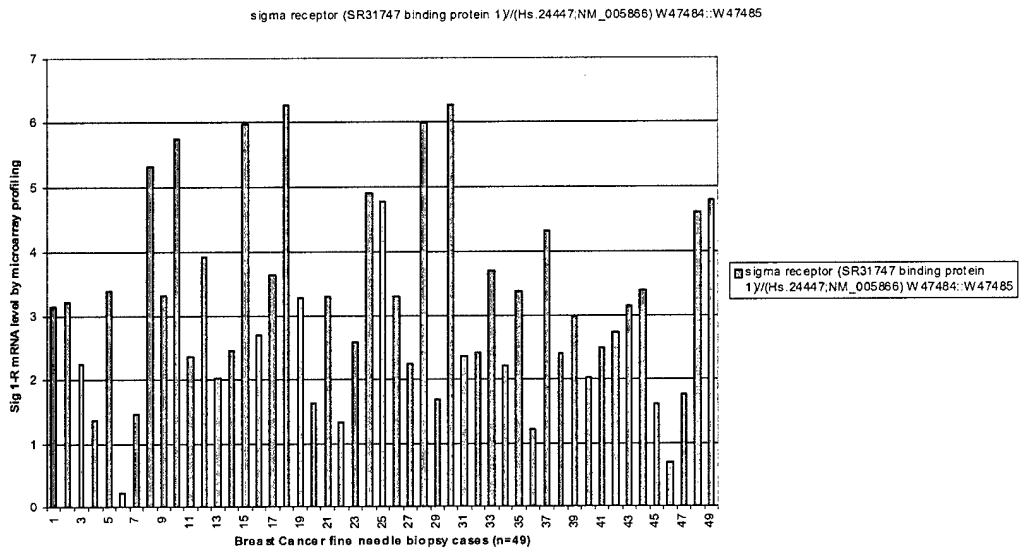
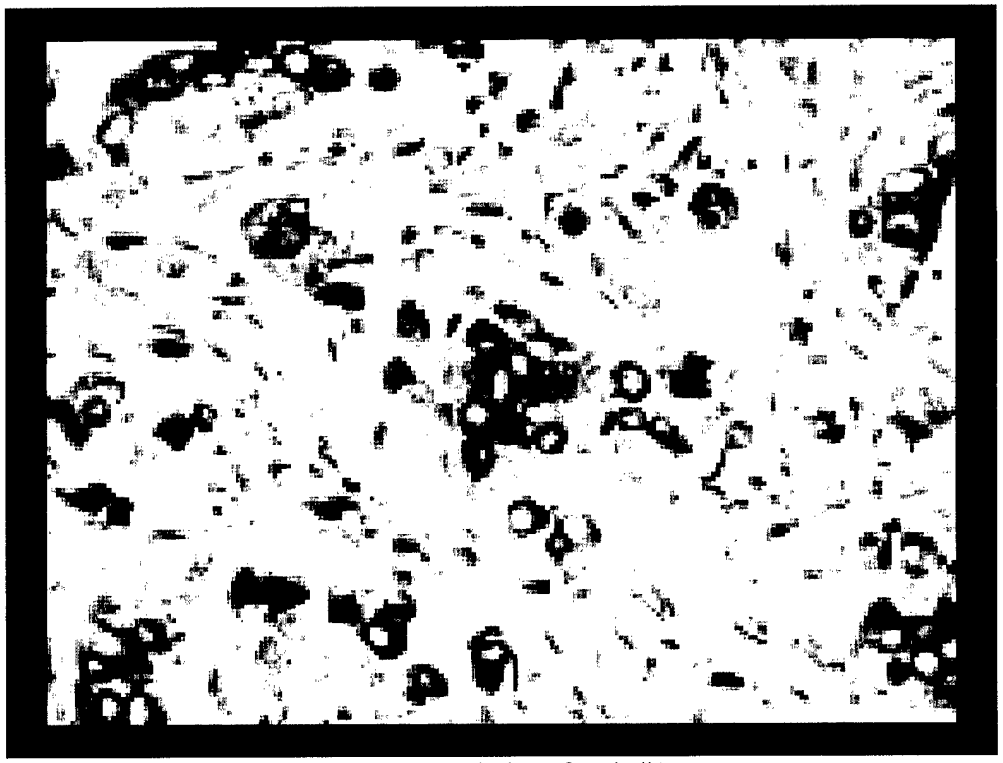


Figure 2. Expression of Sigma 1 receptor protein in invasive breast cancer cells

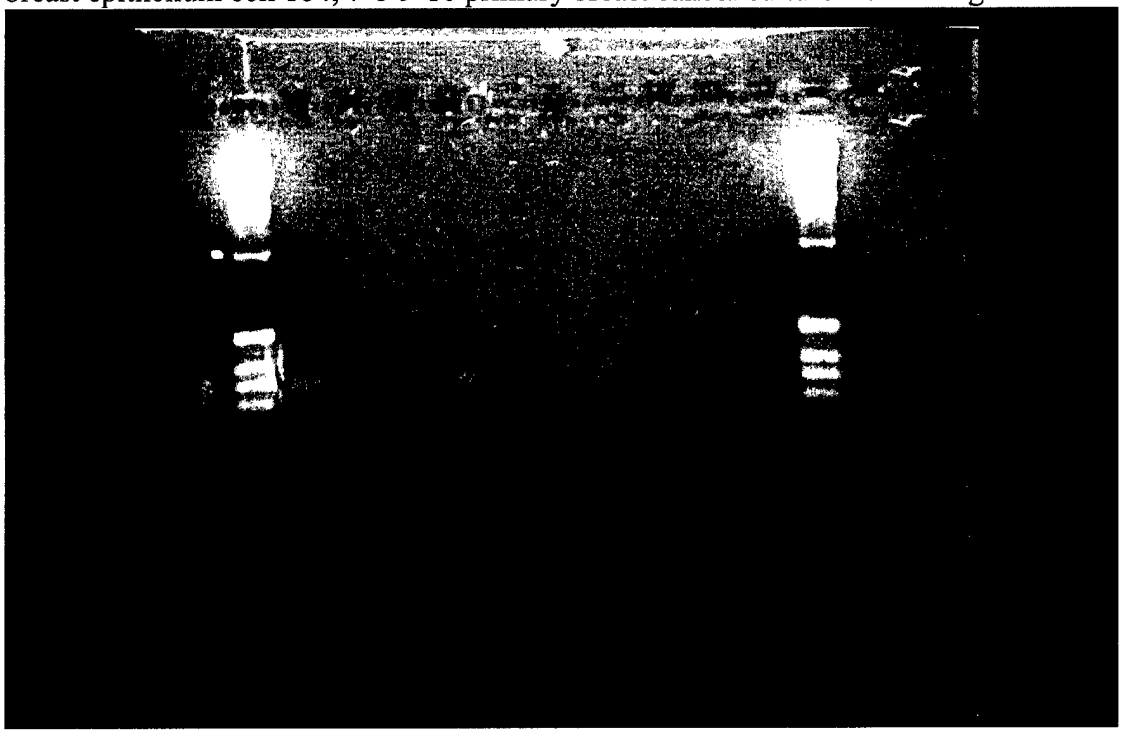


We have also completed aims "a" and "b" of task #1.

We have screened breast cancer cell lines for expression of Sig 1 R by RT PCR. MDA-MB-231, -435, and –361 cells express Sig 1 mRNA, in contrast MCF-7, BT-20 and MDA-MB-453 cells

lack Sig 1 R expression (**Figure 3**). We are currently confirming these results with Western blots.

Figure 3. Sig 1 R mRNA expression in breast cancer cells in vitro.
RT-PCR primers were described in the study proposal. The first column is a molecular weight marker, 2=MDA-MB-231, 3=MDA-MB-435, 4=MCF-7, 5=MCF-10, 6=primary immortalized breast epithelium cell 184, 7-8-9-10 primary breast cancer culture from malignant ascites fluid.



We also examined the effect of SKF 10047 (agonist) and reduced haloperidol (RH) (antagonist) on cell growth. SKF stimulated growth of Sig 1R + cells, RH inhibited cell growth.

Reportable outcome

None of the above results have been published yet. We are currently preparing a manuscript to describe Sigma 1 expression in breast cancer.

Conclusions

We have established that Sigma 1 receptor has variable expression in breast cancer. A subset of cancers shows strong expression of this receptor. We also demonstrated expression of this receptor in some but not all breast cancer cell lines. Sigma 1 R-positive cell lines show modest stimulation of cell growth after exposure to Sig 1 R agonist SKF 10047 and show inhibition of cell growth after exposure to Sig 1 R antagonist reduced haloperidol. Interestingly, cell lines that do not express Sig 1 R also show inhibition by haloperidol.

We have requested a Sig 1 R expression vector from Dr Vadivel Ganapathy (Dept Biochemistry and Molecular Biology, Medical College of Georgia, Agusta GA) to forcefully express Sig 1 R in sigma-negative cell lines. These cells together with parental cells will be used to study further the effect of Sig 1 R activation or inhibition on breast cancer cells.