Engineering a Novel Receptor for Molecular Imaging

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Introduction

The ability to longitudinally detect and evaluate gene expression in living animals can provide investigators with an understanding of the ontogeny of its biological function(s). Non-invasive imaging techniques such as MRI, PET, SPECT, or optical are well suited to this purpose (1-5). Currently mouse models are being used to develop and optimize molecular imaging strategies that can be used to obtain information about tumor functional status.

Many of these strategies have focused on the use of endogenous receptor/ligand-reporter systems. Several endogenously expressed plasma membrane receptors; e.g., the somatostatin type-2 receptor and dopamine type-2 receptor (1-5), are being studied. However, the targeting of endogenous receptor expression potentially is limited by a number of confounding drawbacks including: 1) Loss of absolute specificity, due to uptake of labeled ligand outside the tumor site. Several non-specific sites may be involved, which lowers the effective concentration of the labeled ligand. Moreover this can give rise to false positives and a masking of the signal as well as subjecting normal tissues to toxic labeled ligands; 2) Competition with endogenous ligand results in the use of high amounts of labeled ligand as well as signal attenuation at the targeted site; 3) Labeled ligands binding endogenous receptors induce signal transduction cascades that bring about changes in gene expression, which disrupts the normal physiology that is the focus of the measurements; and 4) Diminished or loss of receptor expression during tumor progression gives rise to false negatives.

Our goal is to develop a non-mammalian receptor/ligand imaging system, to overcome these potential difficulties. We are evaluating fusion constructs of plasma membrane receptors. These receptors have been engineered to have both a native mammalian transmembrane type II receptor signal/anchor domain and the ligand binding-site of a non-mammalian transmembrane type II receptor Er-1mem. Thus, this type of receptor will be unique to the mammalian cells in which it is expressed and will only interact with its ligand Er-1. Er-1mem is from the marine protozoan cilate *Euplotes raikovi* (6). Er-1mem has no mammalian homologues and Er-1 is a small (5 kDa) ligand, which can be readily labeled for any imaging modality.

Methods

Using standard molecular biology methodologies we have placed the transmembrane type II fusion gene constructs under the control of the human EF-1α promoter for strong constitutive expression. A fusion gene of Er-1 and glutathione S-transferase (GST) has been prepared to facilitate the preparation of pure Er-1 (ligand).

Results

We have evidence that FLAG tagged constructs of these receptors can be expressed at the surface of breast cancer cells (Fig.1). Figure 2 preliminary data that indicates that preparation of the Er-1 ligand from GST-Er-1 fusion protein can be achieved.

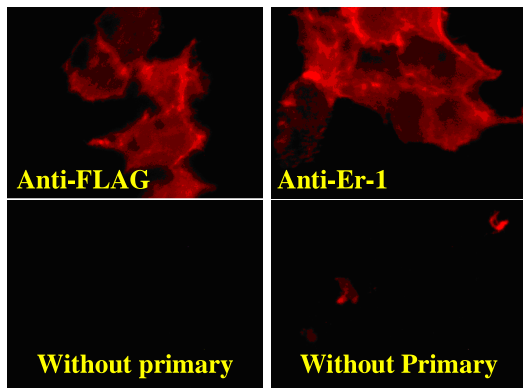

Figure 1. Immunohistochemistry showing plasma membrane expression of type II transmembrane fusion proteins on MCF-7 breast cancer cells. Top left panel shows anti-FLAG staining and top right panel shows anti-Er-1 staining. Both bottom panels shows processing without the use of primary antibody.

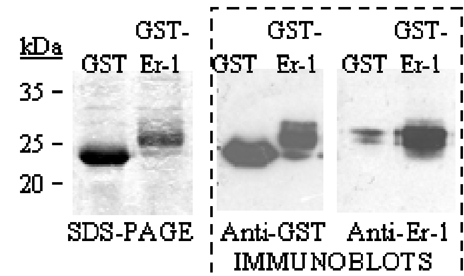

Figure 2. SDS-PAGE and immunoblots showing GST protein and GST-Er-1 fusion protein. Molecular weight (M_w) markers (kDa) are indicated at the left show that GST has the appropriate M_w of approximately 22kDa and that the fusion protein GST-Er-1 has the expected mw of about 27 kDa

Disscussion

The Er-1mem/Er-1 system will be useful for wide-ranging applications in detecting gene expression, or gene expression driven by microenvironmental conditions such as hypoxia. In combination with the functional imaging capabilities of PET and MRI, the Er-1mem/Er-1 system will allow a molecular/functional imaging approach to understanding the impact of a gene of interest.

References

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Acknowledgements

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