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Developing Functional Monoclonal Antibodies for Beta-3 Adrenergic Receptor

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Abstract

G protein-coupled receptors (GPCRs), one of the most commonly used and successful targets for drugs, are a large family of multi-transmembrane proteins and an important class of receptors. Over 40% of all modern medicines interact with this protein group. These cell surface receptors are acted on by a wide variety of ligands, including small molecules and soluble proteins. Monoclonal antibody (mAb) therapy has major advantages over small molecule therapy in that mAbs are more selective and therefore tend to have fewer non-specific or off-target toxicity issues, while having a longer duration of action than small molecule drugs. Unfortunately, it is extremely difficult to create antibodies against GPCRs using traditional approaches, especially for clinical applications. Multispan combines its proprietary immunization technology using its patented GPCR high expression system and in-depth expertise in developing well-designed and validated GPCR functional assays to select mAb leads that perturb disease-relevant signaling pathways. In this poster, we detail the development of Beta-3 adrenergic receptor (β3-AR) mAbs. Our preliminary data showed that several mAb clones specifically bound to the receptor while increasing the receptor function by acting as agonists.

Rationale for β3-AR Monoclonal Antibody Therapeutics

β3-AR as cardiovascular disease target:

- catecholamines activated, negative inotropic cardiac protective adrenergic receptor, acting like nature's own β-blocker.
- expression up-regulated in disease conditions such as ischemia in both rats and humans.
- mediate the activation of endothelial nitric oxide synthase (eNOS) and increase of nitric oxide (NO) levels that in turn protect the heart against ischemia-perfusion injury and improve survival after heart attack in animals and humans.
- The potential therapeutic indications of β3-AR agonists are early heart failure, ventricular hypertrophy, ischemic heart disease in addition to hypertension.

Problem: β3-AR small molecule agonists cross-reacted with β1-AR and β2-AR, causing significant side effects.

Solution: mAb approach is an attractive alternative due to mAbs' known superior specificity.

Polyclonal Abs Raised in Mice Bound to the Extracellular Domain of β3-AR

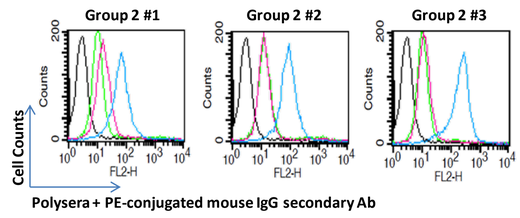

Figure 1. Multispan's polyclonal Abs raised in mice bound to the extracellular domain of β3-AR. HEK293T cell line stably expressing β3-AR was used to evaluate the immune response of immunized mice. Surface expression of the β3-AR was measured by flow cytometry (FACSort, Becton Dickinson) using a PE-conjugated anti-mouse IgG secondary antibody staining alone (black lines), or mice polysera before immunization (green lines), after the 1st immunization (pink lines) and after the 3rd immunization (blue lines) in primary staining prior to using a PE-conjugated anti-mouse IgG antibody in secondary staining. Positive results from 3 independent mice are shown (#1, #2, and #3) with #3 mouse showing the strongest immune response. Multiple GPCRs have been tried and showed similar immune responses (data not shown).

Polyclonal Antibody Bound to β3-AR Selectively

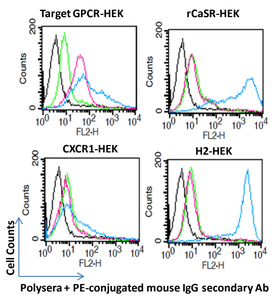

Figure 2. Multispan's polyclonal antibody raised in mice selectively bound to β3-AR's extracellular domain, not other 3 randomly selected GPCRs expressed in the same HEK293T mammalian cell background. HEK293T cell lines stably expressing β3-AR, rat CaSR, CXCR1 and H2 were used to evaluate the polysera specificity. Anti-FLAG antibody (blue lines), mice polysera before immunization (green lines), after the 3rd immunization (pink lines) were used in primary staining of the stable cell lines followed by secondary staining with PE-conjugated anti-mouse IgG antibody for detection. Anti-Flag antibody staining confirmed that all GPCRs are expressed on the cell surface at varying levels and polysera from immunized mice only bound to cells expressing the β3-AR .

mAbs Bound to β3-AR with nM Affinity

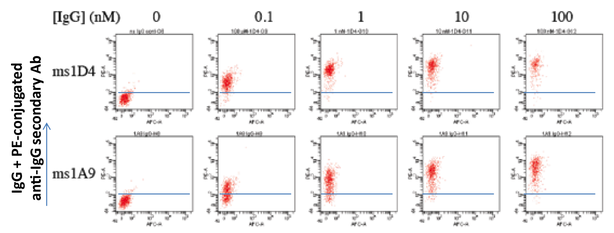

Figure 3. Recombinant IgG mAbs ms1D4 and ms1A9 bound to Multispan's β3-AR-over-expression cells with 0.9nM and 10.6nM affinity as shown by flow cytometry using serial dilution of the mAbs (FACSort, Becton Dickinson). The mAbs were engineered from scFv (single chain variable fragment) genes isolated from phage-display scFv library constructed from spleen of a GPCR-immunized mouse at Multispan that demonstrated specific target GPCR binding shown in **Figure 1 and 2**. This work was done in collaboration with Dr. James Marks' lab in UCSF.

Preliminary Functional Data Showing β3-AR Agonist Activity

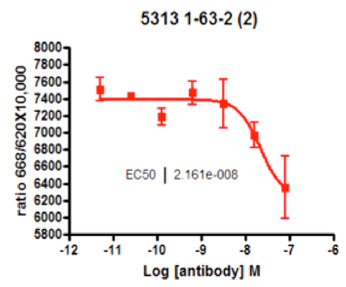

Figure 4. An example of functional agonist β3-AR mAb. Spleen from an immunized mouse showing positive titer to the target was harvested and fused to SP2/0 (*Sigma Aldrich*) myeloma fusion partner cells to make hyridoma. Purified mAb derived from a hybridoma subclone demonstrated agonist activity to increase cAMP response (*Cisbio cAMP HiRange 62AM6PEC*). The assay was performed using Multispan's stable cell line expressing the target GPCR selected using its natural agonist ligand (*data not shown*).

Work in Progress

1. mAbs will be humanized, affinity matured, produced recombinantly and scaled up.

2. *In vitro* characterization: 1) functional specificity over β1-AR and β2-AR; 2) Gαs agonist signaling and hypertrophy protection in cardiomyocytes in vitro models.

3. *In vivo* characterization in wild-type and genetic-deficient β3-AR-/- control mice : 1) hypertrophy protection 2) cardiac remodeling.

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