A High-Throughput Screening Process for Partial Agonists Targeted at the ErbB4 Receptor Tyrosine Kinase

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ErbB4 (HER4) is a member of the ErbB family of receptor tyrosine kinases, a family that also includes the epidermal growth factor receptor (EGFR/ErbB1), ErbB2 (HER2/Neu), and ErbB3 (HER3). Conflicting reports in the literature suggest that ErbB4 may function either as an oncogene or as a tumor suppressor in human malignancies. Thus, our long-term goal is to develop ErbB4 ligands that can be used to elucidate ErbB4 function and ErbB4 antagonists that could be used to treat ErbB4-dependent tumors. Our strategy is based on the observation that the Q43L mutant of the naturally-occurring ErbB4 agonist Neuregulin 2beta (NRG2β) functions as a partial agonist at ErbB4; NRG2β/Q43L stimulates ErbB4 tyrosine phosphorylation, but does not stimulate ErbB4 coupling to cell proliferation and competitively antagonizes agonist stimulation of ErbB4 coupling to cell proliferation. Therefore, we have developed a 3-step high-throughput screening process that identifies molecules that stimulate ErbB4 tyrosine phosphorylation, fail to stimulate ErbB4 coupling, and inhibit agonist induced ErbB4 coupling. Molecules that stimulate ErbB4 tyrosine phosphorylation but do not stimulate ErbB4 coupling to cell proliferation are highly likely to function as ErbB4 antagonists.

For the primary screen, a 96-well sandwich ELISA (enzyme-linked immunosorbent assay) has been developed to identify molecules that stimulate ErbB4 tyrosine phosphorylation. This ELISA uses an anti-ErbB4 capture antibody, HRP-conjugated anti-phosphotyrosine detection antibody, and TMB substrate to analyze lysate samples from CEM/ErbB4 cells that have been previously stimulated with potential ErbB4 ligands, NRG2β (agonist positive control), NRG2β/Q43L (partial agonist positive control), and mock (negative control). The ErbB4 tyrosine phosphorylation ELISA has been optimized such that it produces a Z-factor of 0.730 for a full agonist (NRG2β) and 0.664 for a partial agonist (NRG2β/Q43L).

The secondary screen was developed using an MTT proliferation assay to detect molecules that stimulate ErbB4 coupling to cell proliferation. This assay utilizes a BaF3 cell line that expresses ErbB2 and ErbB4. These cells are seeded into a 96-well plate before being stimulated with NRG1β (agonist positive control), potential ErbB4 ligands, and mock (negative control). After stimulation, the cells are incubated for 120 hours before being analyzed with the MTT assay. The MTT proliferation assay has been optimized so that it can detect as little at 10 nM of agonistic activity. This assay produces a Z-factor of 0.572 for 10 nM of agonistic activity.

Our tertiary screen is being developed using an MTT proliferation assay as well. This assay will be used to identify molecules that inhibit agonist induced ErbB4 coupling to cell proliferation. For this assay, the cells are seeded into a 96-well plate and first stimulated with potential ErbB4 ligands, NRG2β/Q43L, and mock. The cells are then stimulated with 10 nM NRG1β. After the stimulations, the cells are incubated for 120 hours before being analyzed with the MTT assay. From the results of the development of the secondary screen, we have determined that if we are able to reduce the effective agonistic activity from 10 nM to 4 nM, a 60% reduction in activity, we would achieve a Z-factor of 0.593 for the tertiary screen.

Once the optimization of the tertiary screen has been completed, libraries of potential ErbB4 ligands such as a library of monoclonal antibodies could be efficiently screened in hope of discovering ligands that could be candidates for chemotherapeutics to treat ErbB4 dependent tumors.