Novel Anti-Cancer Agents Targeting Leptin Receptors

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Introduction

Triple negative breast cancer (TNBC) has the characteristics of lacking expression of estrogen receptor, progesterone receptor and the human epidermal growth factor receptor type 2 (her2). Despite its relative sensitivity to chemotherapy, patients usually have poor outcome. Recently, leptin receptor (LR) has been proposed as one of the promising targets to combat triple negative breast cancer (TNBC). Increase in LR expression and serum leptin levels have been associated with BC, especially in TNBC, but the mechanism underlying the association has yet to be identified. Based on computational analyses we hypothesized that the small peptides designed based on sequence LR binding proteins to provide structural and mechanistic clues towards the generation of peptide antagonists of LR. The high affinity positions of leptin sequence in the active binding site of LR are localized based on High Ambiguity Driven Biomolecular Docking (HADDOCK) scores to determine the effect of potential anti-cancer agents. Discovery of novel druggable LR pockets and lead molecules targeting these alternative binding pockets have provided structural clues towards the development of new generation of small molecule therapeutics that could be used in TNBC and as complementary treatments to the already existent therapies.

Background

Breast cancer is the second leading cause of deaths caused by cancer. The aggressiveness and late diagnosis make the cancer difficult to treat. In presence of hormone receptors, estrogen receptor (ER), progesterone receptor (PR) and epithelial growth factor receptor (HER2) have been targets for drug treatment and development. TNBC, however, does not express the genes for any of the three receptor types, and requires different therapeutic approach to treat the cancer. LR is a single glycoprotein 130 (gp130) domain of cytokine receptor. Upon ligand binding one pair of two domains of LR dimerizes and exhibits signals to induce downstream pathway of cellular activity. Although previous studies have identified three different active ligand binding sites in Pancreatic cancer cells, the full mechanism of ligand signaled LR activity in TNBC remains unclear and future study is yet to be required.

Method

In our study we examined LR binding ligand 9F8 Fab fragment antibody (PDB: 3V6O) and native human Leptin (PDB: 1AX8) as our model sequence. Total of 62 point mutated peptides were created using molecular visualization software called Chimera, and highest affinity scores were predicted by HADDOCK. It was found that, despite the high affinity of native leptin to LR, peptide based on Fab antibody sequence, peptide11, showed higher affinity score of -106.9. The point mutated peptide based on sequence of peptide 11, MUT33, also depicted higher affinity to LR than the point mutated peptide based on sequence of native leptin, MUT52, showed (Table 1). MUT33 peptide with sequence 102GHETMDYWGQGTG114 was then synthesized through Solid Phase Protein Synthesis method (Figure 1).

Experimental

Figure 2: Summary of Experimental Method

Figure 3: Crystal Structural model of LR-antibody complex (top), Peptide11 (middle) and Peptide52 (bottom). Protein sequence of Peptide 11 is cut into three different pieces based on shapes and clusters of high affinity residues using Chimera program

Results

Figure 5: Fluorescence image of peptide 33 inhibition of MDA-MB-231 cell proliferation. Based on close structured peptide Pep33 Mimic experiment demonstrated significant inhibition of IGF1 induced survival. The image was captured and analyzed by Zeiss Axio Observer Inverted Microscope and BioTEK Synergy fluorescent plate reader.

Conclusion

Leptin receptor is a novel therapeutic target in Triple Negative Breast Cancer (TNBC) cells. Successful antagonistic peptide binding of leptin receptor shows significant role of leptin receptor in TNBC cell. With expanding health concern and association with obesity, next generation of therapeutic drug development on LR exhibits promising future in treating TNBC cells.

References


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