Identification of Target Antigens on Breast Cancer Stem Cells Using Phage-Developed scFv Antibodies: *A Proteomic Approach*

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ABSTRACT

The term 'cancer stem cell' has only recently emerged, and is defined as a cancer cell that has the ability to self-renew and give rise to another malignant stem cell, as well as differentiate to give rise to the phenotypically diverse non-tumorigenic cancer cells. The 'cancer stem cell' hypothesis emerged from the fact that tumors contain a heterogeneous mixture of cells and that not all the cells within a tumor contribute to tumor growth. Using phage display technology, our previous studies have identified two single chain variable fragment (scFv) antibodies (clones 207, 208) that were found to specifically bind human SUM159 breast cancer stem cells (BCSC). We have also shown in a murine xenograft tumor model prepared from the implanted SUM159 cells that these scFv antibodies predominantly bind to the cells located at the periphery of the tumors. Taking in light of these discoveries, the next logical question to be investigated was, "What cellular-antigens are these scFvs binding to?" In order to answer this in the present investigation, we used proteomic approaches based on a small scale immunoprecipitation, followed by purification and mass-spectrometry-based identification of the binding target antigens. A pure population of BCSC was acquired by flow cytometry, using ALDEFLUOR®-based sorting of the SUM159 cell line. Cell lysates of the sorted BCSC were prepared and the target antigens were immunoprecipitated with scFv-207 and scFv-208 antibodies using a solid-state immunoprecipitation assay. The immunoprecipitated target antigens were purified and separated. Mass-spectrometric techniques (LQT-MS/ESI mass-fingerprinting) were employed to compare the proteomic data to protein databases. The identification of BCSC-specific targets will be a crucial step in designing and developing better and more specific antibody-based therapies against breast cancer.