Cancer stem cells (CSCs) provide a more tumorigenic and metastatic breast cancer phenotype through their ability to long-term self-renew and repopulate the tumor. They are believed to be responsible for chemoresistance, metastasis and relapses. However, identification of CSCs with biomarkers revealed to be complex. Putative CSCs markers have been identified, but none of them, alone or in combinations, were found to be specific and reliable to recognize these cells. One main limitation to this approach is the fact that CSC have a high degree of phenotypic plasticity. The purpose of this work was to characterize the properties of Sca-1 expressing cells. We provide evidence that cells expressing the putative CSC marker Stem cell antigen-1 (Sca-1) are more resistant to chemotherapy drug treatment and have a better ability to colonize distant sites. In addition, the Sca-1+ subpopulation was isolated from the 4T1 metastatic murine breast cancer cell line to gain insights into the properties of Sca-1 expressing cells. Once injected in vivo, the immune response to Sca-1+ cells was enhanced compared to the immune response to Sca-1- cells. We also observed that Sca-1 expression on 4T1 cells in vitro was not stable but rather modulable as it was induced by chemotherapy drug treatment, culture confluency and hypoxia. In contrast, high and stable Sca-1 expression was observed in a cell line (MR13) obtained from 4T1 cells after treatment with chemotherapy. MR13 cells were more resistant to chemotherapy, had an increased migration capacity, a weaker interaction with the extracellular matrix (ECM), as well as a better anchorage-free survival compared to the parental 4T1 cells, consistent with their increased metastatic ability. From these data we conclude that Sca-1 expression is enhanced by chemotherapy and Sca-1 expressing cells play a critical role in both chemoresistance and metastatic capacity of breast cancer cell lines.