Supplemental Methods

Purification and experiments using CSCs and NSCCs from human breast tissues

Five human invasive ductal carcinoma tissues (stage III) were purchased from AMS Biotechnology and Biochain Inc. All these tissues were negative for ER, PR and HER2 expression (triple negative). Immunomagnetic purification of CSCs and NSCCs was performed according to Shipitsin et al. (Shipitsin M et al., Cancer Cell, 2007). Briefly, the breast tissues were minced into small pieces (1mm) using a sterile razor blade. The tissues were digested with 2mg/ml collagenase I (C0130, Sigma) and 2mg/ml hyalurinidase (H3506, Sigma) in 37^0C for 3h. Cells were filtered, washed with PBS and followed by Percoll gradient centrifugation. The first purification step was to remove the immune cells by immunomagnetic purification using an equal mix of CD45 (leukocytes), CD15 (granulocytes), CD14 (monocytes) and CD19 (B cells) Dynabeads (Invitrogen). The second purification step was to isolate fibroblasts from the cell population by using CD10 beads for magnetic purification. The third step was to isolate the endothelial cells by using an "endothelial cocktail" beads (CD31 BD Pharmingen cat no. 555444, CD146 P1H12 MCAM BD Pharmingen cat no. 550314, CD105 Abcam cat no. Ab2529, Cadherin 5 Immunotech cat no. 1597, and CD34 BD Pharmingen cat no. 555820). In the final step from remaining cell population only the $CD44^{high}$ cells were purified using CD44 beads. These cells were sorted for $CD44^{high}/CD24^{low}$ (CSC) cells. On the other hand, $CD24^{high}$ cells were purified using CD24 beads. These cells were sorted for $CD44^{low}/CD24^{high}$ (NSCCs) cells. These CSC and NSCC populations were sorted again in order to increase their purity (>99.2% in all cases). The overall strategy was to use magnetic bead separation to purify epithelial cells fsrom the stromal and immune cells present in the tumors and then to use flow cytometry to sort CSCs, and NSCCs according to their CD44 and CD24 profiles. The magnetic bead separation is less stressful to the tumor cells in comparison to multiple rounds of cell sorting, thereby allowing the use of these cells in cell culture experiments.