Transcription regulation of chemokine receptor CXCR4 by nuclear respiratory factor 1 (NRF1) controls estrogen-malignant transformation of breast epithelial cells to breast cancer stem cells

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Introduction: Treatment of metastatic breast cancer is still unsolved due to development of breast cancer stem cells (BCSCs) which are resistant to chemotherapy. The role of nuclear respiratory factor 1 (NRF1) as a transcription factor has been found to be a prognostic factor in various types of cancer. The objective of our study is to find out whether NRF1 regulates phenotypes of breast cancer stem cells (BCSCs) which are considered as poor prognosis in breast cancer patients.

Results: E2 increased the proportion of CD44+CD24−/CD133+/ALDH2+ cells.

Abstract: Chemokine receptor CXCR4 is involved in the maintenance of stemness and drug resistance of cancer cells. NRF1 is one of the major transcription factors that control transcription of CXCR4. In this study, we investigated whether transcription regulation of CXCR4 by NRF1 controls estrogen-induced malignant transformation of breast epithelial cells. The functional regulation of transcription of NRF1 target genes has not been assessed in breast cancer. We have previously shown that NRF1 might influence estrogen-induced malignant transformation of breast epithelial cells; however, the mechanisms of transcriptional regulation of the NRF1 target genes, such as CXCR4, remain unknown. In this study, we show that NRF1 and estrogen jointly contribute in regulating expression of breast epithelial cells to breast cancer stem cells (BCSCs).

NRF1 might influence estrogen induced malignant transformation of breast epithelial cells, including anchorage-independent growth, and invasive properties. Our findings are important to the development of new therapeutic strategies for breast cancer stem cells. These data support NRF1 as a therapeutic target for therapeutic intervention against breast cancer.

Summary of Key Findings:

- NRF1 might influence estrogen-induced malignant transformation of breast epithelial cells.
- Anchorage-independent growth, and invasive properties are increased through ROS signaling.
- NRF1 expression is increased by estrogen and ROS-dependent signaling.

E2 also increased the proportion of CD44+/CD24−/CD133+/ALDH2+ cells.

E2 induced expression of O-succ-G, CXCR4 & BNIP3 as a result of reactive oxygen species (ROS) production with the treatment of E2. (E2) Vector / MCF10AWT + MCF10A Vector / MCF10A NRF1 / MCF10A Vector / MCF10A Vector / MCF10A WT **p<0.01, vector vs NRF1

Both E2 and NRF1 jointly increased the expression of mRNA levels of CXCR4 & BNIP3 and ROS scavenger (NAC, 10mM) reduced the expression of CXCR4 & BNIP3 in E2 and NRF1-induced the mRNA levels of CNCR4 in MCF10A.

Figure 1. The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

References:


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Image 2: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 3: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 4: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 5: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 6: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 7: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 8: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 9: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 10: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.

Image 11: The representative flow sorted data of the cells for CSC markers (CD44+/CD24−/CD133+/ALDH2+) in E2 treated breast epithelial cells compared to control group of E2. Error bars represent the mean ± sd of 3 different replicates and n=10000 cells. Both E2 (100nM) and NRF1 jointly increased spherical sizes and decreased MCF10A WT induced the spherical formation.