Understanding the Signaling Pathway of Triple Negative Breast Cancer in African American Women to Address Cancer Disparities

La'Mayah Hodges[1,2], Jorge Hernandez[1,3], Chris Reid, Alexandria Tucker [1], Rajan Singh, PhD [1], Shehla Pervin, PhD [1]

Charles Drew Univerisity of Medicine and Science, David Geffen School of Medicine at UCLA, California State University, Dominguez Hills

Urban Health Institute
Charles R. Drew University of Medicine and Science
A private university with a public mission.

Abstract

BACKGROUND: Breast cancer is the most common cancer in women and the second most common cause of cancer deaths in the same population. Of all of subtypes, triple negative breast cancer (TNBC) is the most aggressive and is most prevalent in African American women. Due to vast heterogeneity, treatment options are limited. Characterization of key players in the signaling pathway may help identify potential targets for therapeutic intervention.

METHODS: Western Blots were performed to confirm results of preliminary RNA-Seq data. Quantitative PCRs were then performed to determine if the origin of proteins expressed were that of tumor cells or of the host environment.

RESULTS: Western blots demonstrated increased expression of beige adipocytes, collagen, macrophages, and mammary cancer stem cell markers. Quantitative PCR confirmed increases in expression seen in Western assay. Further, it was shown that beige adipocytes differentiated from, both, tumor cells and the host environment while collagen, ALDH1, and macrophage cell markers were of only host origin.

DISCUSSION: Our findings confirm the results of the RNA-Seq analysis and identified potential key players in tumorigenesis and disease progression. These key players may serve as targets for novel treatments and ultimately decrease the disparity of breast cancer deaths among African American women.

BACKGROUND

Breast cancer subtypes are classified by the hormone receptors located on the cells' surface [1]. Activation of said receptors initiate specific signaling pathways that promote tumor growth. Estrogen, progesterone, and Her2 Neu receptors—and their respective signaling pathways—are well characterized in the literature and serve as targets for effective drug therapies [1,2]. Triple negative breast cancer (TNBC) is a subtype that is characterized by the absence of estrogen, progesterone, or Her2 Neu receptors. This subtype is the most aggressive with the least treatment options[3]. TNBC most commonly affects younger African American (AA) women resulting in a much higher mortality rate in this population. Even after adjustments for socio-economic factors, AA breast tumors appear to exhibit specific aggressive characteristics suggesting existence of unique biology contributed both by tumor cells and host microenvironment [3].

In attempt to understand the complex signaling pathways of TNBC, a RNA-Seq analysis was performed using AA TNBC tumor xenografts of the MDA-MB 468 cell line. The results of the RNA-Seq analysis showed increases in expression of collagen and macrophage cell markers in xenografts. The data also revealed significant increases in ALDH1, cytokines, cyclins, skeletal muscle and beige adipocyte markers in xenografts. Western Blot Assays were performed to confirm the results of the RNA-Seq analysis and compare the expression of the slower growing MDA-MB 468 tumors to those of the faster growing HCC 70 cell line. Lastly, a quantitative PCR was performed to determine whether the increase in these gene products were contributed by the tumor cells or host environment.

RESULTS

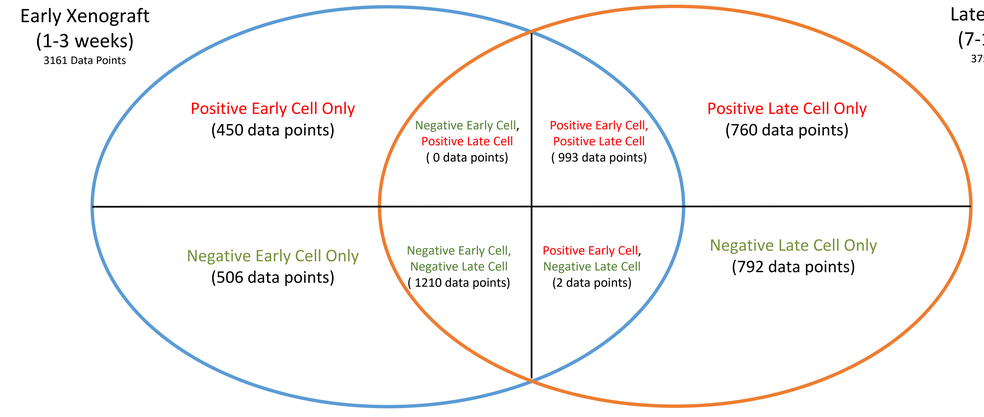

Figure 1: RNA Seq Data Analysis

RNA Seq performed using xenograft from AA TNBC cell line MDA-MB 468

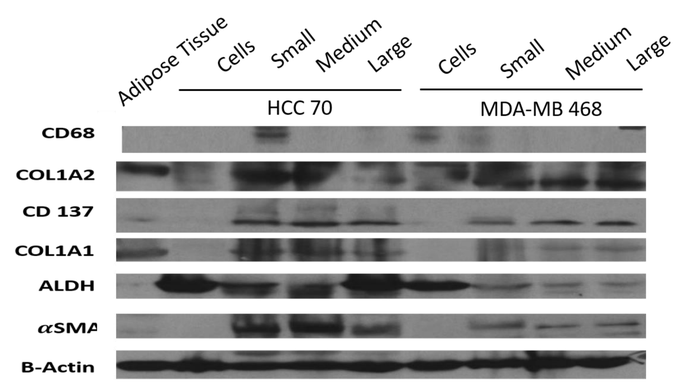

Figure 2: Western Blot Assay

Western Blot performed using HCC 70 and MDA-MB 468 cells and xenografts

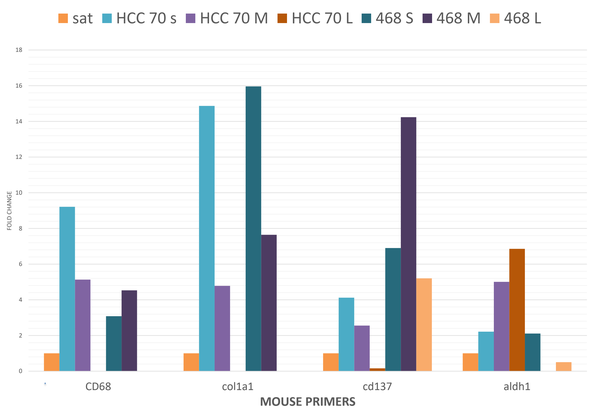

Table 1: qPCR Data Mouse Primers

qPCR performed to determine changes in the expression within the host environment

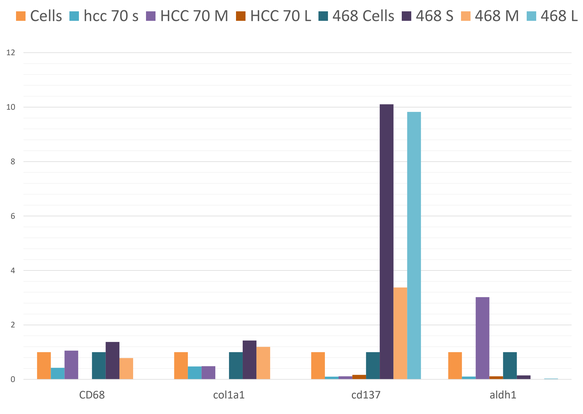

Table 1: qPCR Data Mouse Primers

qPCR performed to evaluate changes in expression of tumor cells

METHODS

Preparation of Xenografts

All human breast cancer cell lines were obtained from American Type Culture Collection in 2013. Six to eight week old nude mice were used for xenograft engraftment. MDA-MB 468 and HCC-70 cells were mixed with Matrigel and implanted subcutaneously in nude mice [4]. Small, medium, and large sized tumors of the HCC 70 cell line were monitored over a period of 2, 5, and 9 weeks respectively. Small, medium, and large tumors of the MDA-MB 468 cell line were grown for a period of 2 weeks, 8weeks, and 5mos respectively.

Western Blot

Cells and xenografts were lysed and incubated at 4 °C for 30 min. Protein concentrations were measured using Bio-Rad protein assay dye concentrate. Lysates (30 µg) were resolved electrophoretically on 10 % SDS-polyacrylamide gel and electrotransferred to a Polyvinylidine difluoride membrane (Bio-Rad). The membranes were incubated with the following primary antibodies: CD137, COL1A1, ALDH1, AND CD68 for 1 h. Immunoreactive bands were visualized by enhanced chemiluminescence detection system [4].

Real-Time Quantitative PCR

Total RNA was extracted by using Trizol reagent, and equal amounts (2 µg) of RNA were reverse transcribed. Power Sybr Green PCR master mix was used with 7500 fast real-time PCR system to quantify gene amplification. Samples were analyzed in duplicate with GAPDH and Beta Actin controls [5].

DISCUSSION AND CONCLUSION

Our findings show increased expression in collagen, ALDH1, beige adipocyte cell markers as well as macrophage cell markers in xenografts. These findings confirm the results of the RNA-Seq analysis and suggest potential key players in the tumorigenesis and progression of triple negative breast caner in African American women. Further, the qPCR shows that interaction of tumor cells with the host environment induces increased expression of beige adipocyte cell markers (CD137) of host origin. While their role in the AA TNBC signaling pathway is still unclear, they may potentially serve as targets for novel treatments and ultimately decrease the disparity of breast cancer deaths among African American women.

RESOURCES

1. Cadoo KA, Fornier MN, Morris PG. Biological subtypes of breast cancer: current concepts and implications for recurrence patterns. Q J Nucl Med Mol Imaging. 2013;57:312–21.
2. 4. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edminston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295:2492–502. doi: 10.1001/jama.295.21.2492.
3. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clin Breast Cancer. 2009;9(Suppl 2):S73–81. doi: 10.3816/CBC.2009.s.008.
4. Luis Martinez, Easter Thames, Jinna Kim, Gautam Chaudhuri, Rajan Singh, Shehla Pervin. BMC Cancer. 2016; 16: 559. Published online 2016 Jul 29. doi: 10.1186/s12885-016-2547-z
5. Singh R, Braga M, Reddy ST, et al. Follistatin Targets Distinct Pathways To Promote Brown Adipocyte Characteristics in Brown and White Adipose Tissues. Endocrinology. 2017;158(5):1217–1230. doi:10.1210/en.2016-1607