Targeting Aggressive African American Triple Negative Breast Cancers: Exploring Novel Therapeutic Avenues

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I have Nothing to Disclose
Breast Cancer Facts for African Americans

Breast cancer is the most commonly diagnosed cancer among African American women. Additionally, African American women with breast cancer are more likely to die from it and at a younger age as compared to white women.

[ KNOW THE FACTS ]

- **41%**
  - Death Rate
  - Nationally, African American women have a 41% higher death rate from breast cancer than white women

- **1 in 8**
  - Minority Groups
  - 1 in 8 deaths were from minority-populations such as African American, Hispanic, and American Indian

- **1 in 3**
  - Ages 40-64
  - More than one-third of local women who die from breast cancer are between ages 40-64

- **31%**
  - Access to Care
  - 31% of local women ages 40-64 could not afford health care due to poverty or lack of insurance

- **37%**
  - Late Detection
  - 37% of local African American women were diagnosed after the disease had reached an advanced stage
White Women Are Diagnosed Earlier

Percent of breast cancer cases by stage at diagnosis

Source: American Association for Cancer Research

THE HUFFINGTON POST
Research Project: International Breast Cancer Registry

Overarching Goal: To evaluate association between African ancestry & high-risk breast cancer subtypes

- Step 3: Explore novel aspects of tumor biology
  - Breast cancer stem cells; ALDH1
  - Are there differences in the oncogenic potential of mammary tissue that are associated with ancestry?

“ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome” Ginestier, Wicha Cell, 2007
Studies utilizing African American and Caucasian Established Breast Cancer Cell Lines

Differences in biology between cells obtained from various ethnic populations

Singh et al., Cancer Res 2000
Pervin et al., PNAS 2000
Pervin et al., Cancer Res 2001
Singh et al., JBC 2002
Pervin et al., Cancer Res, June 2003
Pervin et al., Cancer Res, Dec 2003
Pervin et al., Cancer Res 2007
Pervin et al., Cancer Res 2008
Pervin et al., BJC 2011
Increased sensitivity of African American triple negative breast cancer cells to nitric oxide-induced mitochondria-mediated apoptosis

Luis Martinez1, Easter Thames2, Jinna Kim3, Gautam Chauchuri4,5, Rajan Singh4,5 and Shehl Perev5,6

Abstract

Background: Breast cancer is a complex heterogeneous disease where many distinct subtypes are found. Younger African American (AA) women often present themselves with aggressive form of breast cancer with unique biology which is very difficult to treat. Better understanding the biology of AA breast tumors could lead to development of effective treatment strategies. Our previous studies indicate that AA but not Caucasian (CA) triple negative (TN) breast cancer cells were sensitive to nitrosative stress-induced cell death. In this study, we elucidate possible mechanisms that contribute to nitric oxide (NO) induced apoptosis in AA TN breast cancer cells.

Methods: Breast cancer cells were treated with various concentrations of long-acting NO donor, DETA-NONOate and cell viability was determined by trypan blue exclusion assay. Apoptosis was determined by TUNEL and caspase 3 activity as well as changes in mitochondrial membrane potential. Caspase 3 and Bax cleavage, levels of Cu/Zn superoxide dismutase (SOD) and Mn SOD was assessed by immunoblot analysis. Inhibition of Bax cleavage by Caspase inhibitor, and levels of reactive oxygen species (ROS) as well as SOD activity was measured in NO-induced apoptosis. In vitro and in vivo effect of NO treatment on mammary cancer stem cells (MSCCs) was assessed.

Results and discussion: NO induced mitochondria-mediated apoptosis in all AA but not in CA TN breast cancer cells. We found significant TUNEL-positive cells, cleavage of Bax and caspase-3 activation as well as depolarization mitochondrial membrane potential only in AA TN breast cancer cells exposed to NO. Inhibition of Bax cleavage and quenching of ROS partially inhibited NO-induced apoptosis in AA TN cells. Increase in ROS coincided with reduction in SOD activity in AA TN breast cancer cells. Furthermore, NO treatment of AA TN breast cancer cells dramatically reduced aldehyde dehydrogenase (ALDH1) expressing MSCCs and xenograft formation but not in breast cancer cells from CA origin.

Conclusions: Ethnic differences in breast tumors dictate a need for tailoring treatment options more suited to the unique biology of the disease.

Keywords: Breast cancer, health disparity, African American, Unique biology
Oxidative Stress induces downregulation of survivin and xenograft growth of African American Breast Tumors

Pervin et al., British Journal of Cancer 2013
Approaches to understand African American breast tumors

Diet and Life Style: Nicotine and High Fat

Translational Research: Veronica Amygdalina

Microenvironment: Adipocytes

Cancer Stem Cell: Targeting

Autophagy: Targeting
Transplantable (T) xenografts express beige/brown adipose specific markers

Microenvironment: Adipocytes

Pervin et. al., Mol Cancer Res 2016
**Increased expression of beige adipose markers in AA TN breast tumors**

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**IHC: TMEM 26**

**MDA-MB-468**

**MDA-MB-231**
Increased expression of beige adipocyte markers in AA TN breast cancer cell lines
Tissue resident macrophages and mammary cancer stem cell lineages determine engraftment of breast cancer cells in immunodeficient mice

Microenvironment: Adipocytes

- CD163
- ALDH1
- Psoriasin
- β-actin

HCC 70

High attachment plate
Low attachment plate

HA- High attachment plate
LA- Low attachment plate
Increased expression of beige markers in xenografts is associated with high inflammation and macrophage infiltration.
Chemotherapeutic Vulnerability of Triple-negative Breast Cancer Cell-derived Tumors to Pretreatment with Vernonia amygdalina Aqueous Extracts

Howard et al; Anticancer Research: 2016
In Vivo Exposure to Nicotine and HFD increase MCSCs, tissue-resident and infiltrating macrophages

Diet and Life Style: Nicotine and High Fat
In Vitro Exposure to Nicotine and Palmitate increase MCSCs, tissue-resident and infiltrating macrophages

Diet and Life Style: Nicotine and High Fat
Treatment with mecamylamine and MAPK inhibitor SB 203580 reduced xenograft growth in nicotine and high fat exposed mice.

Diet and Life Style: Nicotine and High Fat
MEK independent inhibition of pERK1/2 in xenografts from African American (AA) Triple Negative (TN) Breast Cancer cell lines
Increased expression of DUSP9 and embryonic stem cells in xenografts from African American (AA) Triple Negative (TN) breast cancer cell lines
Increased addiction of AA TN breast cancer cells to autophagy: 
Emphasis on apoptosis inhibition
Autophagy inhibition induced apoptosis in AA TN breast cancer cells
Treating aggressive AA TN breast tumors has been extremely challenging.

These breast cancer cells are dependent on macrophages, cancer stem cells, adipocytes, autophagy etc. for survival.

Our studies strengthen the concept that dependency of these breast cancer cells on MCSCs, macrophages and autophagy could be exploited to induce apoptosis in these cells.
Case-based Question:

1) A 51-year-old obese woman who has smoked for 20-years is diagnosed with breast cancer. The breast cancer was found to be estrogen-receptor negative with limited nodal involvement. She underwent surgery, radiation and chemotherapy but after a brief period of remission her cancer comes back. She has been unable to quit smoking during the period of her treatment.

What is the reasonable course of action for this patient?

   a) Surgery and chemotherapy + aromatase inhibitor  
   b) Surgery, chemotherapy and radiation  
   c) Surgery, chemotherapy + nicotinic acetylcholine receptor blockers  
   d) Surgery, chemotherapy + nicotinic acetylcholine receptor blockers + MAPK inhibitors

Answer: (d)

2) A 60-year-old lean woman who neither smoked nor drank alcohol is diagnosed with triple negative breast cancer with nodal involvement. She underwent surgery, radiation and chemotherapy but after a brief period of remission her cancer comes back.

What is the reasonable course of action for this patient?

   a) Surgery, chemotherapy and radiation  
   b) Surgery, chemotherapy + autophagy inhibitors  
   c) Surgery, chemotherapy + aromatase inhibitor + autophagy inhibitor

Answer: (b)
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