Ceramide and azacytidine in combination as an alternative treatment for triple-negative breast cancer

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Premise

This study looks into the possibility to test and compare the effectiveness of two different chemotherapeutic agents, ceramide and azacytidine, in inducing apoptosis in triple negative breast cancer cells (cell line MDA-MB-231) and reactivating silenced tumor suppressor genes, with minimal or no effect on the normal mammalian cells (cell line MCF10A).

Rationale

- Breast cancer is the second leading cause of death in women worldwide.
  - TNBC represents approximately 10-15% of all diagnosed breast cancers (TNBC) cells lack estrogen and progesterone receptors, as well as a protein receptor called HER2.
  - TNBC is more aggressive than other forms of breast cancer.
  - The pattern of metastatic spread in TNBC is different from the other breast cancer subtypes with a higher likelihood of brain and lung involvement and less frequent bone lesions.[4]
- TNBC has the poorest prognosis among all breast cancer subtypes.
- Limited treatments because TNBC is not responsive to hormone therapy or treatments directed to HER2, emphasizing the need for new targeted treatments for TNBC.[4]
- This literature study involves treatments with ceramide (CER) and azacytidine (AzaC) to observe the effect on TNBC which may suggest the possibility of a new targeted therapy combining Cer and AzaC.

Hypothesis

- AzaC and CER in combination could induce higher rates of cancer cell apoptosis than either compound alone with minimal negative effect on the healthy cells.

Background

- *Breast cancer* is a disease in which abnormal cells in the breast grow out of control.[5]
- Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer.[6]
- TNBC cells are estrogen receptor (ER) negative, progesterone receptor (PR) negative and human epidermal growth factor receptor 2 (HER2) negative. (Figure 1)[1]
- TNBC is characterized by its unique molecular profile, aggressive nature, distinct metastatic patterns and lack of targeted therapies.[6]

Background (Cont.)

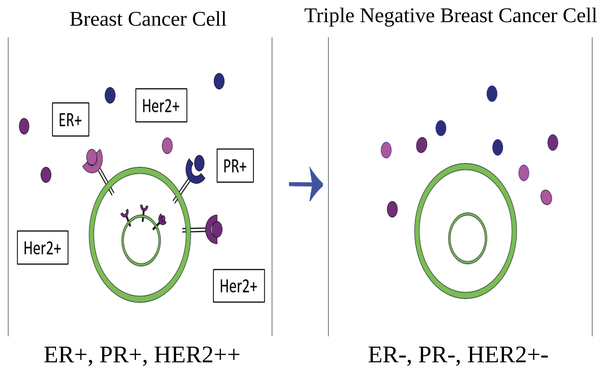

Fig 1[1]. A comparison graph of TNBC and other breast cancers.

- Chemotherapy is the standard of care for TNBC and no agent has been specifically approved for this breast cancer subtype. Chemotherapies approved for metastatic breast cancer (MBC) are used for TNBC.[6]

Ceramide

- Regulatory lipids that modulate cell growth, survival, senescence, and apoptosis in cancer.
- Central role in cell membrane integrity, cellular stress response, inflammatory signaling and apoptosis.[7] (Figure 2)

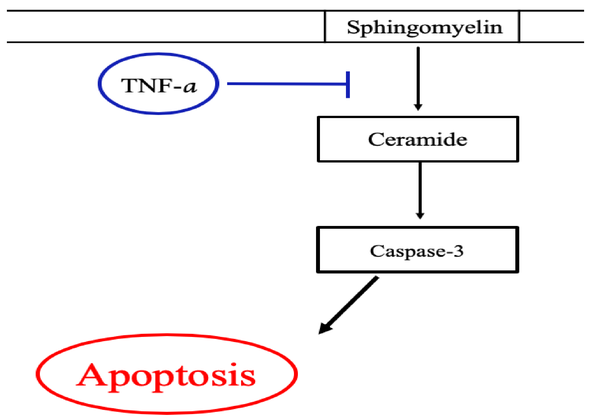

Fig 2. [3]Schematic representation showing Ceramide activate caspase-3 which activates apoptosis.

Azacytidine

- Aberrant DNA methylation is an epigenetic event that can play a key role in the etiology of cancer.
- AzaC is a DNA methyltransferase inhibitor.
- The inhibition of DNA methylation by AzaC leads to the reactivation of tumor suppressor genes that were silenced by aberrant DNA methylation, which in turn leads to apoptosis.[8] (Figure 3)

Background (Cont.)

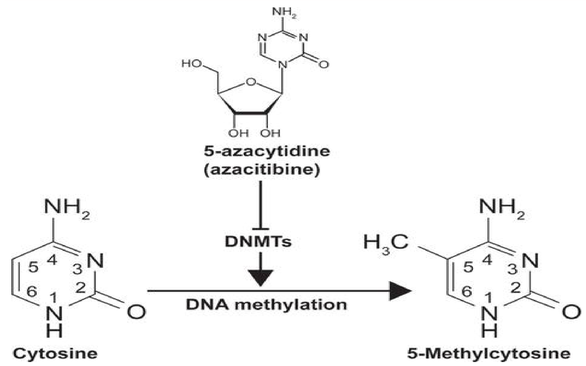

Fig 3.[2] Inhibition of DNMTs by 5-azacytidine.

Cell lines

MCF-10As carry a near-diploid karyotype;
- lost p16 locus , an important regulator of cell cycle progression.
- Express normal p53, estrogen and progesterone-receptor-negative but express HER2 at low levels.

- ***MDA-MB-231*** is an immortalized human metastatic cell line
- MDA-MB-231 cells are highly malignant, invasive and aggressive.
- Have a lack of normal chromosome 8 and chromosome 15.
- Carrier the gene of a single, mutant copy of TP53
- TP53 encodes the tumor suppressor p53
  - deletes both copies of CDKN2A, whose product p16 is important in regulating cell cycle progression.
- estrogen receptor negative (ER-), progesterone receptor negative (PR-), and human epidermal growth factor negative (HER2-).

Anticipated Methods

Cell Culture

- MCF10A cells grow in DMEM and Ham's F12 medium supplemented with 5% FBS, 20 ng/ml epidermal growth factor, insulin 10µg/ml (Sigma), hydrocortisone 0.5 mg/ml (Sigma), cholera toxin 100 ng/ml (Sigma), 100 units/ml penicillin and 100 µg/ml streptomycin.
- MDA-MB-231 cein DMEM (Dulbecco's modified Eagle's medium) cell culture media supplemented with 10% FBS(fetal bovine serum) at 37°C and 5% CO_2.

Anticipated Methods (Continued)

Treatment

- A dosage determination test of each chemotherapeutic agent would be needed to determine a drug-response dosage for the combined treatment.

MTT assay

- A colorimetric assay for cell viability
- MTT reacts with living cells to produce a deep purple compound
- The absorbance of the sample is read at 600 nm; the higher the absorbance, the greater the number of healthy living cells.

The Caspase-3/CPP32

- A colorimetric assay for cell apoptosis
- It provides a simple and convenient means for assaying the activity of caspases that recognize the sequence DEVD.
- The assay uses the spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate DEVD-pNA.

Significance

- Were this research to be done, the findings would suggest further study of CER and AzaC combined, to improve current treatments for TBNC.

Anticipated Outcomes

Based on the function of AzaC and CER and their current findings in treating breast cancers while used as a separate treatment, would suggest that the research carried on in the future would expect the combination treatment to induce higher rates of cancer cell apoptosis while having minimal negative effect on the healthy cells.

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