Indole-3-Carbinol Induces Apoptosis in a Breast Cancer Stem Cell Model System

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Abstract
The cancer stem cell hypothesis dictates that cancers are heterogeneous and arise from tumor-initiating cells, commonly referred to as cancer stem cells. The discovery of tumor cells that behave like stem cells offers a possible explanation as to why breast cancer is so difficult to eradicate. Conventional therapies effectively eliminate the bulk of tumor cells, but fail to eradicate cancer stem cells leading to a relapse. Her2 overexpression is correlated with aggressive metastasis has been implicated in regulating breast cancer stem cells. Overexpression of Her2 has been shown to increase the population of breast cancer stem cells and the drug Indole-3-carbinol (I3C) is reportedly able to modulate Her2 signaling in breast cancer. Taken together, Her2 induced aberrant stem cells may provide the necessary targets of I3C for the development of breast cancer prevention strategies. One of these stem cell markers, nucleostemin, has been identified as a molecular target of I3C. In stem cells, nucleostemin is concentrated in the nucleolus and functions to modulate the oncogene MDM2 and tumor suppressor p53. I have shown that upon I3C treatment, nucleostemin is downregulated and stabilized with I3C treatment. By elucidating this signaling pathway, the use of I3C as a treatment for breast cancer stem cells will lead to a better understanding of new preventative measures against breast cancer.

Breast Cancer and Breast Cancer Stem Cells
According to the American Cancer Society, breast cancer is the second most diagnosed cancer in American women with 1 in 7 American females developing breast cancer during their lifetime. Furthermore, breast cancer is the second most leading cause of cancer death. To eradicate breast cancer tumor cells, some treatment options include surgery, hormone targeted therapies, immuno-therapies, radiation, and chemotherapy, which can unfortunately be invasive and produce harmful side effects. Therefore, the interaction between the negative regulator p16INK4a and nucleostemin is destabilized with I3C treatment. By elucidating this signaling pathway, the use of I3C as a treatment for breast cancer stem cells will lead to a better understanding of new preventative measures against breast cancer.

Indole-3-Carbinol

Indole-3-Carbinol, or I3C, has been shown to demonstrate anti-cancerous properties by inducing anti-proliferative signaling and apoptosis in breast cancer cells. As a naturally produced phychochemical in cruciferous vegetables with minimal side effects, it is an optimal drug candidate of interest.

I3C Mediates an Apoptotic Response
Profiles of 10AT Neo and Her2 cells treated with I3C demonstrate a dramatic increase in sub-G1 DNA content, indicative of I3C mediated apoptosis. Cells treated with or without I3C show no change in cell cycle control.

I3C Treatment Activates Nucleostemin Sequestration of MDM2 into Nucleolus
Western blots of cells treated with I3C for 48 hours show PARP cleavage in I3C sensitive 10AT Her2 cells, indicative of I3C induced apoptosis.

Future Objectives and Experiments
The treatment of MCF-10AT Her2 cells with I3C shows regulation of apoptotic signaling in a breast cancer stem cell model. Downstream of the Her2 receptor, Akt1 and MDM2 serve as modulated targets of I3C, which induces anti-proliferative and apoptosis signaling through a p53 pathway. In particular, nucleostemin, with I3C treatment, can sequester away active MDM2 into the nucleoli allowing for p53 activation and increased function. Furthermore, interactions between p16INK4a and nucleostemin are disrupted, allowing for nucleostemin and p14ARF to individually and negatively regulate MDM2, effectively promoting apoptosis in a breast cancer stem cell model.

Summary and Proposed Model
The treatment of MCF-10AT Her2 cells with I3C shows regulation of apoptotic signaling in a breast cancer stem cell model. Downstream of the Her2 receptor, Akt1 and MDM2 serve as modulated targets of I3C, which induces anti-proliferative and apoptosis signaling through a p53 pathway. In particular, nucleostemin, with I3C treatment, can sequester away active MDM2 into the nucleoli allowing for p53 activation and increased function. Furthermore, interactions between p16INK4a and nucleostemin are disrupted, allowing for nucleostemin and p14ARF to individually and negatively regulate MDM2, effectively promoting apoptosis in a breast cancer stem cell model.

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