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

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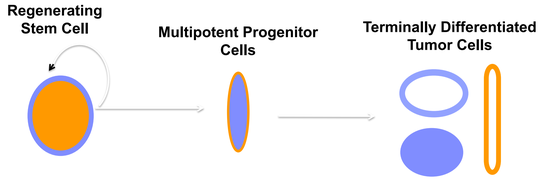
Abstract

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 activated to sequester and inhibit MDM2, freeing p53 to induce apoptosis. Furthermore, the interaction between the negative regulator $p14^{ARF}$ 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.

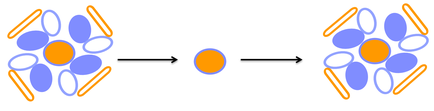
Breast Cancer and Breast Cancer Stem Cells

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, a recent goal of research has been to identify novel compounds with fewer side effects. Our lab has shown that a natural phytochemical, indole-3-carbinol (I3C), a hydrolysis product of glucobrassicin in cruciferous vegetables such as broccoli, cabbage, and brussel sprouts, can induce apoptosis of human breast cancer cells.

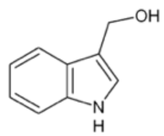
Breast cancer stem cells are self-renewing cells capable of initiating new tumor development. Derived from either deregulated normal or stem cells in mammary tissue, they are posited to produce multipotent progenitor cells, which rapidly proliferate into a heterogeneous population of tumor cells.
Since they generate new heterogeneous cancer populations with distinct phenotypes, a novel and efficacious approach towards cancer stem cell treatment is necessary.



Traditional chemotherapies or radio-therapeutic methods cannot target breast cancer stem cells since they possess the unique ability to self-renew and are capable of initiating new tumor development.

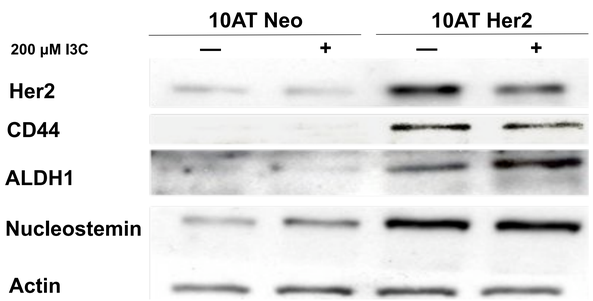


Indole-3-Carbinol



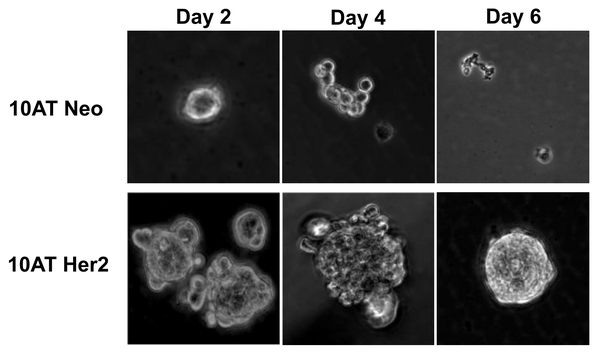
Indole-3-Carbinol, or I3C, has been shown to demonstrate anti-cancerous properties by inducing anti-proliferative signaling and apoptosis in breast cancer cells. As a naturally produced phytochemical in cruciferous vegetables with minimal side effects, it is an optimal drug candidate of interest.

Breast Cancer Stem Cell Induction by Her2 Overexpression

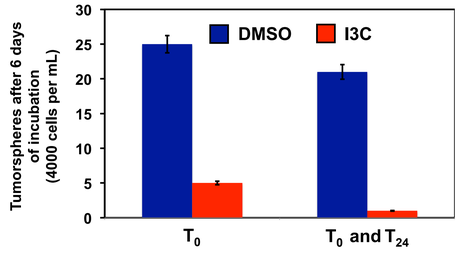


Overexpression of Her2 in the MCF-10AT cell line is sufficient to induce expression of stem cell markers CD-44, ALDH-1, and nucleostemin. Neither cell lines expresses CD-24 (data not shown).

Her2 Overexpressing 10AT Cells Exhibit Tumorsphere Formation

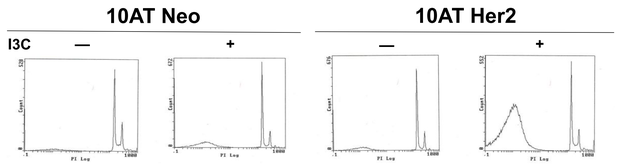


Her2 overexpressing cells are capable of tumorsphere formation, which is a unique characteristic of a stem cells.

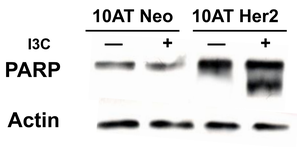


When Her2 overexpressing 10AT cells are treated with I3C, tumorsphere formation decreases as seen by the diminishing number of tumorspheres produced. A second treatment at T_{24} hours more drastically reduces the count of tumorspheres.

I3C Mediates an Apoptotic Response

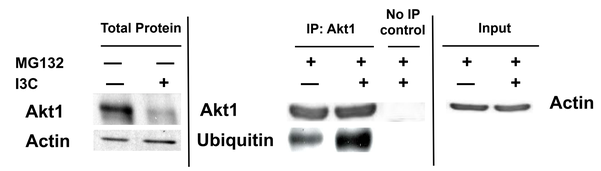


Profiles of 10AT Neo and Her2 cells treated with I3C demonstrate a dramatic increase in sub-G1 DNA content, indicative of I3C mediated apoptosis. Cells treated with or without I3C show no change in cell cycle control.



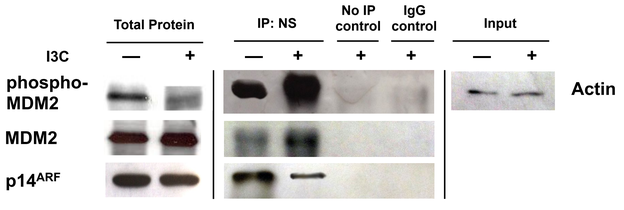
Western blots of cells treated with I3C for 48 hours show PARP cleavage in I3C sensitive 10AT Her2 cells, indicative of I3C induced apoptosis.

I3C Destabilizes Akt1 at the Protein Level

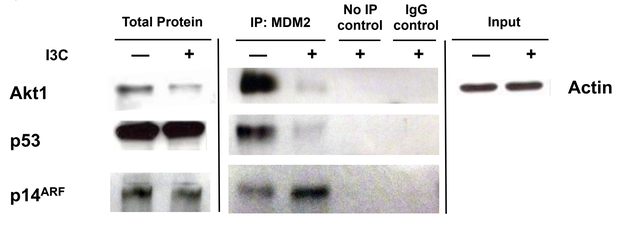


Cells treated with I3C downregulate Akt1 protein levels. When co-treated with I3C and MG132, a proteasome inhibitor, Akt1 protein levels are rescued and stabilized.

I3C Modulates Selective Nucleostemin and MDM2 Interactions

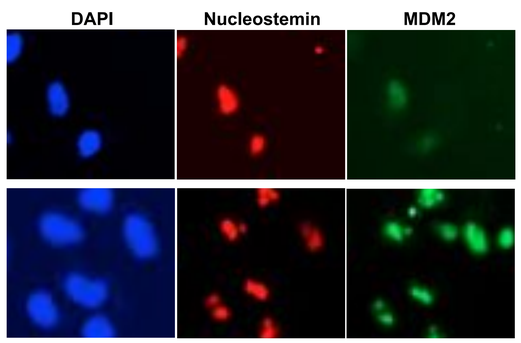


Coimmunoprecipitation of nucleostemin indicates increased sequestering of MDM2 and active phospho-MDM2 with I3C treatment. Furthermore, nucleostemin exhibits decreased interaction with $p14^{ARF}$, a positive regulator of p53.



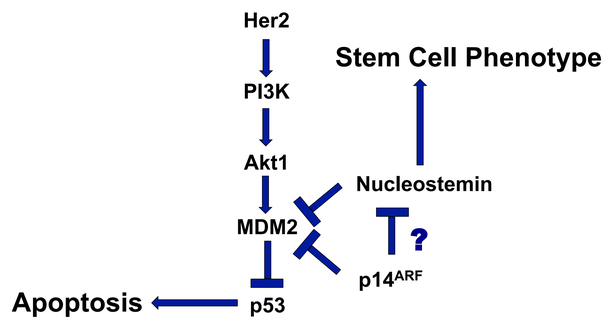
Coimmunoprecipitation of MDM2 shows decreased binding of Akt1 as well as p53, a positive mediator of apoptosis.

I3C Treatment Activates Nucleostemin Sequesteration of MDM2 into Nucleolus



MCF-10AT Her 2 cells were treated with 200 uM I3C for 48 hours, fixed with formaldehyde, permeabilized with Triton-X-100, and stained with DAPI and antibodies against nucleostemin (Texas Red) and MDM2 (Alexa Fluor 488).

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 nucleolus allowing for p53 activation and increased function. Furthermore, interactions between $p14^{ARF}$ and nucleostemin are disrupted, allowing for nucleostemin and $p14^{ARF}$ to individually and negatively regulate MDM2, effectively promoting apoptosis in a breast cancer stem cell model.

Future Objectives and Experiments

Characterize the negative regulation and interaction between nucleostemin, MDM2, and $p14^{ARF}$

Determine the functionality and mediation of a p53 dependent response by transfecting dominant negative p53 into 10AT Her2 cells

Identify Akt1's ability to override I3C induced apoptosis by transfecting constitutively active Akt1

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