Genistein Shows Promise in Inhibiting Prostate Cancer Cells In Vitro Although Key Questions Remain

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Abstract

Cancer comes in many forms and from various etiologies and sites. Prostate cancer is the most common form of cancer in men. The pure soybean shows promise as an anticarcinogen or carcinopreventive agent through its bioactive derivative, genistein. In this research study, genistein displayed inhibitory effects on LNCaP prostate cancer cells in vitro. However, contrary effects were also observed. Thus, genistein may have a biphasic effect on prostate cancer cells in vitro. Alternatively, there may be other cofactors at play. Can the cancer-inhibitory phase of genistein be captured (isolated) and applied as an anticancer or carcinopreventive agent? Regarding prostate cancer cells in vitro, genistein shows promise, but key questions remain.

Keywords: 3-Bromopyruvate; Cancer; Genistin; Genistein; Natural Anticarcinogen; Prostate Cancer; Soy; Tumor

Abbreviations

CNS: Central Nervous System; FBS: Fetal Bovine Serum; WBC: White Blood Cell

Introduction

A brief review of cancer

Cancer is a disease in which abnormal cells proliferate uncontrollably destroying healthy body tissue [1]. Cancer cells form a mass of tissue, termed a tumor (Figure 1). Cancer can occur anywhere in the body, with breast cancer being most common in women and prostate cancer in men. Some cancers can spread or metastasize to other parts of the body (Figure 2) [1].

Categories of cancer

There are five main categories of cancer formation [2]:

1. Carcinoma
2. Sarcoma
3. Leukemia
4. Lymphoma
5. Central Nervous System

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**Figure 1:** A depiction of tumor formation.
*Note: Figure in the public domain.*

**Figure 2:** A depiction of metastasis from an unknown origin.
*Note: Figure in the public domain.*

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Carcinoma

Carcinomas arise in the skin or tissues that line the internal organs, such as in the kidneys or liver. They rarely metastasize to other parts of the body. The types of carcinomas [3] are as follows:

- Types of Carcinoma:
  - Basal Cell
  - Squamous Cell
  - Renal Cell
  - Ductal Carcinoma in situ
  - Invasive Ductal
  - Adenocarcinoma

Sarcoma

Sarcomas are a rare form of cancer (malignant). They grow in connective tissue and are found most commonly in bones, muscles, tendons, cartilage, nerves, fats, and blood vessels (Figure 3) [4].

![Types of Sarcoma](image)

**Figure 3:** A depiction of various sarcomas and their sites of origin.

Note: Figure in the public domain.

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Leukemia

Leukemia is a cancer of the blood cells, which affects the body's blood-forming tissue, such as bone marrow and the lymphatic system (Figure 4). White blood cells are the most common type of blood cells to be affected [5].

![Normal Blood vs Leukemia](image)

*Figure 4: A depiction of the microscopic differences in healthy cells and cells in leukemia. Note: Figure in the public domain.*

Lymphoma

Lymphomas are cancer of the lymphatic system [6]. They are solid tumors formed in the immune system and affect primarily the immune cells, called lymphocytes or WBC (Figure 5).

![Lymphomas and Enlarged Lymph Nodes](image)

*Figure 5: A depiction of lymphomas and enlarged lymph nodes. Note: Figure in the public domain.*
Central nervous system tumor

Central nervous system (CNS) tumors are cancer cells that form in the lymph tissue of the brain and spinal cord. Typically, they develop in other parts of the body, then spread to the central nervous system (i.e. metastatic brain tumor). Specific genetic syndromes may increase the risk of CNS tumors; such as Li-Fraumeni syndrome, neurofibromatosis, nevoid basal cell carcinoma syndrome, tuberous sclerosis, Turcot syndrome and von Hippel-Lindau disease (Figure 6) [6].

Study Purpose

The purpose of the study was to perform a preliminary investigation into the possible anticarcinogenic effects of genistein and 3-bromopyruvate on prostate cancer cells.

Hypothesis

As the concentration levels of genistein increase, there will be a corresponding decrease in the proliferation of prostate cancer cells (the cancer cells will be inhibited).

Prostate gland

The prostate gland is an exocrine gland of the male reproductive system, located directly under the bladder and anterior to the rectum. The primary function of the prostate gland is to secrete prostatic fluid, one of the components of the semen. The muscles of the prostate gland aid the seminal fluid through the urethra during ejaculation (Figure 7) [7].

Prostate cancer

Prostate cancer is a disease in which cells of the prostate gland proliferate uncontrollably (Figure 8 and 9). Most prostate cancers are adenocarcinomas (cancers that develop from the gland cells that make prostate fluid); other types include sarcomas, small cell carcinomas, neuroendocrine tumors, and transitional cell carcinomas.
Figure 7: A depiction of the location of the prostate gland in the human male; lateral view.
Note: Figure in the public domain.

Figure 8: A depiction of the four stages of prostate cancer.
Note: Figure in public domain.

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Etiology of prostate cancer

Prostate cancer is the most common cancer among men, affecting one in seven men. In 2017, it was estimated that there were 161,360 new cases of prostate cancer in the U.S. and 26,730 deaths from prostate cancer. Prostate cancer is the third leading cause of cancer death in American men. About one man in thirty-nine will die of prostate cancer [8]. Prostate cancer has one of the highest survival rates with a 5-year relative survival rate at almost 100% [7].

Current treatments for prostate cancer

Treatment options for prostate tumors are case-dependent, and may include:

- Watchful waiting or active surveillance
- Surgery
- Radiation therapy
- Cryotherapy (cryosurgery)
- Hormone therapy
- Chemotherapy
- Vaccine treatment
- Bone-directed treatment [8].

Genistein

Genistein is a soy-derived isoflavone and phytoestrogen. It can be found in soybeans and soy products [9]. Genistein acts as a chemotherapeutic agent against different types of cancer [9], mainly by altering apoptosis, the cell cycle, angiogenesis, and inhibiting...
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Genistein and Genistein

Genistin, when ingested in the diet, is readily converted to its aglycone form, genistein (Figure 10) [10]. Genistin, when metabolized in the body, is hydrolyzed by removing the covalently-bound glucose to form genistein [10]. Genistein is absorbed in the intestine and is responsible for the biological activities of the isoflavone.

Figure 10: A depiction of the molecular structure of genistein, contrasted with daidzein and estradiol. 
*Note: Figure in public domain.*

Materials

- Prostate cancer cells (LNCaP)
- Genistein
- Trypsin
- Electrophoresis reagents (TAE buffer, agarose, EtBr).

Methods

Gel electrophoresis was used to separate DNA molecules according to fragment size (Figure 11). Additional materials used in the lab technique: DI water, agarose, electrophoresis reagents, and ethidium bromide.

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Cell culture of LNCaP (prostate cancer cells) was grown in RPMI 1640 media containing FBS (fetal bovine serum) for an incubation period of two days. The cells were transferred into a 48-well plate. The fourth column was the control. Half of the plate was treated with genistein in triplicate concentration levels (20, 30, 40, 50, 70, 90). The other half of the plate was treated with 3-bromopyruvate in various concentrations (ranging from 60, 80, 100, 120, 140, 160). Fluorescent staining and MTT assay were then conducted (Figure 11).

**Figure 11:** Images of the lab equipment and evaluation used in this investigation.

*Note: Images by Fernand Jean-Baptiste.*
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MTT assay (cell viability assay) was used to see if the cells were viable and living. Cells actively metabolizing will convert MTT into a formazan purple product. Cells that are not living or damaged will remain a yellow color. MTT assay was utilized to determine if drug concentrations were effective and limited or destroyed the cancerous cells.

Fluorescence was employed to determine the form of cell death. A cocktail of 750 mL ethidium bromide and 750 mL acridine orange was used. The cells were then placed under a fluorescence microscope to detect if any cells fluoresced (Figure 12).

**Figure 12:** View through the fluorescence microscope to assess cancer cell death.

*Note: Image by Fernand Jean-Baptiste.*

**Results**

MTT assay viability numbers were: 55.737, 2653.27, 49.18, 27.86, 46.72, 49.18 (Figure 13).

**Figure 13:** A graph of cell viability over concentration.

*Note: Graph by Fernand Jean-Baptiste.*

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**Discussion**

The purpose of this experiment was to determine the role of genistein in inhibiting the growth of prostate cancer cells. LNCaP cells were treated with genistein and incubated for 48 hours.

MTT assay was expected to show a decrease in cancer cell viability as genistein concentrations increased. However, initially (from 20–40 micrometers), cancer cell viability increased. At 40 micrometers, there was a corresponding decrease in cancer cell viability. From 50–90 micrometers, cancer cell viability increased slightly, then remained somewhat constant.

**Summary**

Was it possible that the 50–90 micrometer cell viability count was due to the LNCaP cells developing resistance to genistein and or other factors? Future research should focus on why the viability of prostate cancers cells initially increased then decreased at relatively lower concentrations of genistein, yet their viability increased slightly in relatively higher concentrations. In light of these results, it is fundamental to investigate what caused this initial surge in viability followed by a corresponding drop and then slight rise—a “rebound” effect? As a footnote, gel electrophoresis on the first attempt resulted in no bands. In the second attempt, bands appeared (but the size of the bands were indeterminable).

**Conclusion**

Prostate cancer is the most common cancer among men. Finding remedies to prevent, treat, and cure prostate cancer is of paramount importance; particularly treatments that have no or minimal adverse side effects. Remedies derived from nature and natural sources often have minimal or no side effects; however, not all are effective. One such natural source, genistin derived from the soybean and in its bioactive form, genistein, is theoretically promising in the prevention or treatment of cancer.

In the exposure of prostate cancer cells (LNCaP) to genistein, genistein expressed characteristics of being "biphasic": having a phase in which the cancer cells appeared to proliferate in the presence of genistein and having another phase in which the cancer cells appeared to diminish. Thus, future research should focus on what factors are contributing to this biphasic phenomenon.

Do prostate cancer cells gain resistance rapidly to genistein, or are there other factors at play? If genistein does prove biphasic in specific applications, can the antagonistic phase be “captured”(isolated) and utilized as a treatment or co-treatment? This preliminary research was introductory, a starting point for a more in-depth analysis of genistein and its interaction with and effects on cancer cells *in vitro*. Future research may seek and unlock the anticancer and carcinopreventive potential of the soybean, through its bioactive component, genistein, to prevent, treat, or cure prostate and other forms of cancer.

**Conflict of Interest Statement**

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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