Rhabdomyosarcoma undergoes apoptosis upon treatment with heat-killed *Saccharomyces cerevisiae in vitro*

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**ABSTRACT**

The current study was undertaken to investigate the ability of skeletal tumor cells to intake the heat-killed baker’s yeast and the possibility to induce apoptosis of these cells. Cytotoxicity assay was achieved for this purpose. Heat-killed *Saccharomyces cerevisiae*, was cultured with human rhabdomyosarcoma cancer cells (RD) and the percent of apoptotic cancer cells was determined by measuring the optical density of treated and untreated cancer cells. Upon phagocytosis of yeast, RD cancer cells underwent apoptosis. The highest concentration (0.2 mg/ml) of heat-killed yeast gives the highest killing effect on RD cells (56.5%) while the second suspension (0.04 mg/ml) showed inhibition rate (44.7%) as compared with the control cells. Phagocytic assay was accomplished to find out the ability of RD cancer cells to uptake and phagocytize killed yeasts after the co-culture of *S. cerevisiae* with the RD cells at a ratio of yeast to cancer cells 10:1 respectively, showed that RD cells demonstrate 41% of attachment to yeasts after 4 hours of exposure time while the percent of phagocytosis was 32% after 4 hours. Induction of apoptosis in RD cancer cells was dose-dependent.


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1. INTRODUCTION

Apoptosis, or programmed cell death, is a mechanism by which cells undergo death in response to unrepaired DNA damage, aging and abnormal cells [1]. The cancer cells are characterized by the capacity to evade apoptosis in addition to the unlimited proliferation and other characters like invasion [2]. Interest in microbe-based approaches for cancer therapy which is based on the use of microorganisms or their products for the treatment of cancer is not new [3].

William Coley began the first well documented use of bacteria and their toxins to treat end stage cancers [4]. Apoptosis-based approaches for cancer therapy through biotherapy is an important alternative way for the conventional anticancer therapies. It has been reported that some microorganisms display selective replication in tumor cells such as many viruses [5]. The phagocytosis of microorganisms and other materials by cells such as monocytes and macrophages which resulted in the induction of Apoptosis in these cells has been well studied [6].

Several studies indicated the increase of apoptosis in cells after phagocytosis of certain bacteria e.g. *Escherichia coli* [6], *Staphylococcus aureus* [7] and *Candida albicans* [8]. Another studies demonstrated that the breast cancer cell line cells able to phagocytise latex beads and fluorescent matrigel [9]. Further studies demonstrated phagocytosis in nonlymphatic tumor cells such as adenocarcinoma cells and epithelial carcinomas of the breast [10]. Malignant cells that are not phagocytic in origin in cervical cancer patients and *in vitro* showed a phagocytic activity against autologous lymphocytes [11], aged lymphocytes, bacteria and *C. albicans* [12]. Rhabdomyosarcoma (RMS) is a type of cancer which can be diagnosed in children and teens, it presents as a soft tissue mass commonly developed in head, neck, genital and urinary tract. The other type of (RMS) which affects people from all ages tend to arise in big muscles of legs, arms and trunk. Sarcomas in general is the cancer of connective
tissues of the body. Rhabdomyosarcoma accounts for over 50% of sarcomas in children and young People and it originally came from cells that normally develop into skeletal muscles; this type of cancer unfortunately can start at any point of the body [13-15]. It is worth mentioning that Rhabdomyosarcoma cell line was originally derived from a biopsy specimen obtained from a pelvic Rhabdomyosarcoma of a 7-year-old Caucasian girl [16]. The development and search for more effective and safe treatment modalities now is a very important part of the research studies, trying to avoid the serious side effects of radio and chemotherapy. The current study aims to examine whether RD cells undergo apoptosis post treatment with heat-killed *Saccharomyces cerevisiae* through phagocytosis.

2. MATERIALS AND METHODS

2.1. Tumor cell line

Rhabdomyosarcoma (RD) cell line was used in the current study; the cell line was thankfully supplied by the Iraqi Cancer Center for Medical and Genetic Research (ICCMGR). The tumor cells were maintained in RPMI medium supplemented with 10% fetal bovine serum and 100 µg/ml streptomycin and penicillin. Cells were maintained in a humidified incubator at 37 °C, and routinely sub-cultured whenever it reaches to a semi-confluent monolayer.

2.2. Preparation of *S.cerevisiae*

A commercially available baker’s yeast was used. Yeast suspensions were washed once in phosphate buffer saline (PBS) buffer and incubated in 90 °C for 1 h to kill the yeast by autolysis. The cells were quantified following washing using haemocytometer and the yeast was adjusted to 1 x 10^7 cells/ml. Yeast solution was prepared by dissolving 0.1 g of yeast in 100 ml D.W. to make the stock solution, then series dilutions were made by adding serum free medium.

2.3. Phagocytic assay

This assay was done according to Ghoneum and Gollapudi [17], with slight modification. Yeast cells were mixed with tumor cells at 10:1 ratio (yeast to tumor cells). For this purpose 0.5 ml of tumor cell culture containing 1 x 10^5 cells /ml was mixed with 0.5 ml of yeast suspension containing 1 x 10^7 cells/ml. The mixture was centrifuged for 5 min at 50 x g and incubated at 37 °C for 4 h. Then the mixture was re-suspended to detach the loosely attached cells. Slides were prepared by adding 200 µl of the cell suspension. Preparations were fixed in 100% methanol, air-dried, then stained with 4% Giemsa for 15 min and were examined for the attachment and uptake of yeast by tumor cells using oil immersion x100 in a light microscope. The estimation of attachment and uptake of yeast by tumor cells was as the following; the estimation of attachment of yeast by tumor cells was calculated as the percentage of 100 tumor cells that attached to one or more yeast on the other hand the estimation of uptake of yeast by tumor cells was calculated as the percentage of 100 tumor cells that ingested one or more yeast with the following formula:

\[
\text{AI} = \frac{\text{(% tumor cells attaching to yeast)} \times \text{(number of yeast per 100 tumor cells)}}{1000}
\]

The uptake of yeast by tumor cells was calculated as the following formula:

\[
\text{PI} = \frac{\text{(% tumor cells phagocytizing yeast)} \times \text{(number of yeast per 100 tumor cells)}}{1000}
\]

2.4 Apoptotic studies

2.4.1 Detection of the cytotoxic effect of yeast lysate on RD cells

A. RD cells were treated with the yeast suspensions of the prepared dilutions for 72 h. While controls cells were cultured in the absence of yeast cells. Then the apoptosis of the treated tumor cells was observed post staining with crystal violet stain. The results were obtained by measuring the optical density at 450 nm.

B. Detection apoptosis by morphological Analysis: Apoptosis is defined by the cell shrinkage, membrane blebbing and chromatin condensation due to DNA fragmentation these characteristics were used to identify the apoptotic cancer cells after staining.

2.4.2 Statistical analysis

The Student T-test was used to test the significant difference in the percent changes of apoptotic cancer cells which treated with yeast as compared to cancer cells alone, as well as the attachment and uptake of yeast cells by the cancer cells.

3. RESULTS AND DISCUSSION

Rhabdomyosarcoma cells were cultured with
heat-killed *Saccharomyces cerevisiae* (Baker’s yeast) in different concentrations for 72 h. The results depicted in Figure 1 show that the co-culture of cancer cells with yeast resulted in a significant increase in RD cells inhibition rate (death) with the increasing of yeast cells concentrations. The figure revealed that suspension 1 represents the highest concentration (0.2 mg/ml) of heat-killed yeast gives the highest killing effect on RD cells (56.47%) while the second suspension (0.04 mg/ml) showed inhibition rate (Cytotoxicity) (44.66%) as compared with the control cells on the other hand the last concentration has the minimum effect (15.4%) on RD cells.

![Figure 1](image1.png)

**Figure 1.** RD cancer cells treatment with heat-killed *Saccharomyces cerevisiae* suspensions

![Figure 2](image2.png)

**Figure 2.** Inhibition of RD cancer cells *in vitro* after treatment with heat-killed yeast cells; (A) and (B) treated with yeast concentrations 0.2 and 0.04 mg/ ml respectively.

Alteration in morphological characteristics of the cancer cell line was noticed as shown in Figure 2 and demonstrates the morphological change of cancer cells upon treatment with heat-killed yeast appeared rounded, shrunken with cell debris as a result of cell lysis and death by apoptosis, in comparison with the untreated RD cancer cells that seemed as a confluent monolayer as shown in Figure 3A and 3B).

Phagocytosis assay was achieved by mixing RD cells with yeast in percentage 1:10 respectively and the data shows that RD cells demonstrate 41% of attachment to yeasts after 4 h of exposure time while the percent of phagocytosis was 32% after 4 h of incubation, and the current result agreed with several studies reported that the level of attachment of tumor cells to yeasts is peaked at 2 h post treatment and it might either maintained or declined at 4 h.
Phagocytosis by different types of cancer cells such as leukemia [18], breast cancer [17], colon and tongue squamous cell carcinoma [19] and other types that exhibit the phagocytic activity against certain materials and microorganisms has been reported in several researches [20,21]. Phagocytosis by cancer cells is a phenomenon which was originally identified by the histological examination of human tumors which revealed the presence of intracytoplasmic leukocytes, erythrocytes and blood platelets in tumor cells [18]. It was hypothesized that the tumor cells acquire the ability of phagocytosis during the development of malignancy [17]. In the phagocytic activity of breast cancer cells (BCCs) using yeast as the test organism to trigger apoptosis of cancer cells, it was found that ergosterol which present in baker’s and brewer’s yeast inhibits the growth of human BCCs in vitro by a mechanism that might involve oxidation products of ergosterol [22].

The differences in the apoptosis percentage post-phagocytosis of baker’s yeast In vitro is depending on the type of cancer cell line and the capacity of cancer cells to uptake the yeast, moreover, it is suggested that the uptake of yeast by cancer cells, and not the attachment, may induce apoptosis of cancer cells [12].

RD cells have been shown to exhibit high phagocytic activity against yeast with an increased level of apoptosis. The mechanisms of apoptosis of RD cells post culture with yeast is not known, but it is suggested that the engulfed yeast could induce apoptosis by a mechanism associated with activation of caspases 8, 9 and 3 in the cancer cell line.

According to several findings, S. cerevisiae induces apoptosis in cancer cells also by a caspase-independent mechanism [23]. In response to an apoptotic stimulus, many mediators are released from mitochondria and transfer death signals to the nucleus in a caspase-independent manner. These mediators include mitochondrial proteins AIF, endonuclease G, NADH oxidoreductase and mitochondrial repair system enzymes [24]. Apoptosis could be triggered by the elevation of the intracellular levels of Ca2+ which is a very important factor in triggering apoptotic signals via mitochondria [12].
CONCLUSION

In summary, our results show that Rhabdomyosarcoma cancer cell line has the ability to uptake yeasts and undergo apoptosis in vitro. The effect was dose-dependent. This data may have a safe alternative way for targeting cancer cells using non-pathogenic yeast (S. cerevisiae) to induce apoptosis in cancer cells through phagocytosis process. We suggest establishing further studies in yeast-induced apoptosis as a cancer therapy that could have therapeutic implications and it is advisable to investigate the molecular mechanisms of cell killing as well as the enhancement of yeasts by other materials or extracts.

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