# The Effect of Resveratrol on Cellular Proliferation and Apoptosis in Human Leiomyoma Cells

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## ABSTRACT

**Purpose.** To determine whether resveratrol can inhibit the proliferation of human, uterine leiomyomas (fibroid tumors) *in vitro* as effectively as previous studies using rat (ELT3) cell lines and whether that inhibition is due to apoptosis.

**Methods**. Normal human myometrium, human leiomyoma, and ELT-3 cells were obtained and cultured in DMEM or estrogen-free DF8 media containing 10% FBS, respectively. Cells were plated and treated with various concentrations of resveratrol ranging from 10 – 200 μM or vehicle (0.15% DMSO). A non-treated control was also cultured. Cell growth was determined at 1 - 5 days by sulfarhodamine B assay and a plate reader. Percent apoptosis was determined using flow cytometry.

**Results.** The results of sulforhodamine B assay indicated that resveratrol, at increasing concentrations, significantly inhibited cell growth and contributed to cell death (apoptosis). Resveratrol had its maximal effects at the 200 μM concentration level starting at day 1 and peaking 5 days later.

**Conclusion**. The growth of human leiomyomas and ELT-3 rat-leiomyoma cells *in vitro* was inhibited by resveratrol. Experimental inquiries into the mechanisms of cell inhibition and/or cell death posed by resveratrol in uterine leiomyoma cells are ongoing. Currently, *in vivo* studies of resveratrol using an appropriate animal model are under development.

## INTRODUCTION

Uterine leiomyomas (fibroids) are benign smooth muscle tumors of the uterine myometrium. The estimated prevalence of fibroids maybe as high as 77%; however, they are of clinical concern in about 25% of women[1], and they are the most common cause for hysterectomy in the U.S[2]. Symptoms include pelvic pain and pressure, abnormal bleeding and reproductive dysfunction. Leiomyomas tend to exhibit higher estrogen and progesterone receptors relative to matched normal myometrial tissue[4]. Consequently, leiomyomas typically grow under elevated estrogen and progesterone conditions and regress when these hormones are withdrawn; such as post-menopause. Treatment for leiomyomas can vary from hysterectomy and myomectomy to uterine artery embolization, endometrial ablation, and GnRH agonist treatment. These methodologies tend to have numerous adverse side effects or are undesirable; thus, alternative treatment options are needed. The phytoestrogen resveratrol has recently been shown to be an effective inhibitor of tumor cell proliferation in several hormonally-responsive tumors, acting as either an agonist or antagonist, depending on cell type[5-8]. Previous studies in our laboratory indicated that Resveratrol was effective in inhibiting rat (ELT-3) leiomyoma cells *in vitro*. Hypothetically, resveratrol may be equally effective in treating human leiomyomas.

## MATERIALS & METHODS

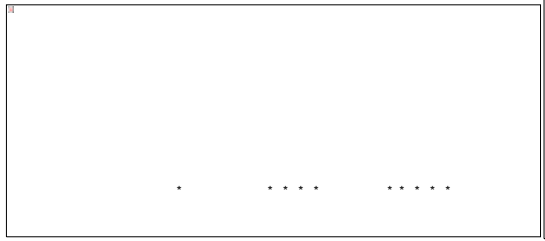
Human leiomyoma and normal myometrial smooth muscle cells (SMC) were cultured in DMEM (Gibco/BRL) supplemented with 10% FBS. ELT-3, rat leiomyoma cells were provided by Dr. Cheryl Walker's laboratory (Anderson Cancer Center, Smithville, TX) and cultured in DF8 media containing 10% fetal bovine serum (FBS).

Cell viability was determined using a Sulforhodamine B assay (Sigma). Cells were seeded at 5000 cells/well in 96-well microplates. Each assay was performed in triplicate. After 24 hours, cells were treated with varying concentrations of Resveratrol (10, 25, 50, 100, 150, 200μM) or DMSO for 1-5 days. Sulforhodamine B Solution (0.4%) was added to each well and the appropriate protocol for rinsing and treating was followed. Plates were read at 565 nm on plate reader.

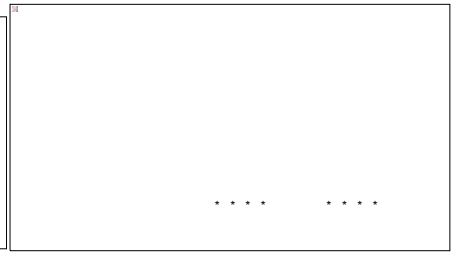
To assess apoptosis, cells were plated at 1 x $10^5$ cells/well in 6-well plates, and allowed to adhere overnight. After 24 hours, Human leiomyoma, myometrium and ELT-3 cells were treated as indicated above. Both adherent and floating cells were harvested on days 1, 2, and 3 post-treatment. The cells were fixed and stained with propidium iodide solution (PI). A minimum of 1 x $10^5$ stained cells were analyzed for the Sub-G1 profile, indicating apoptotic cells, on a FACScan Flow Cytometer (Becton Dickinson, San Jose, CA). Cells undergoing apoptosis were determined as a percentage of cells with sub-G1 DNA content compared with the total number of cells present using the FACScan system.

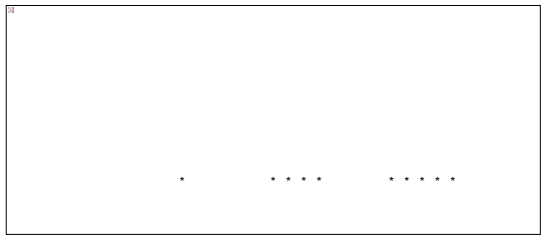
## RESULTS

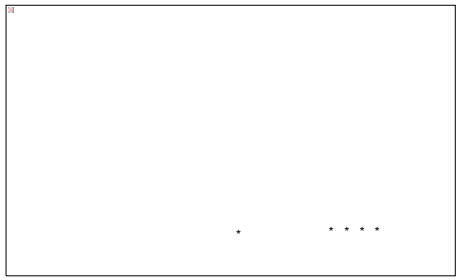
**Cell Viability with & without Resveratrol Treatment over a 5-Day Period**

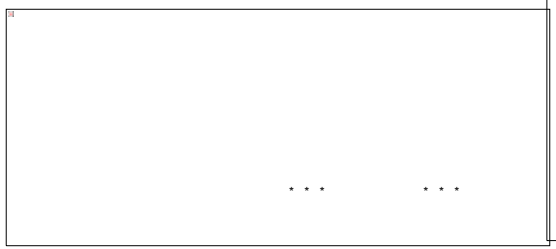


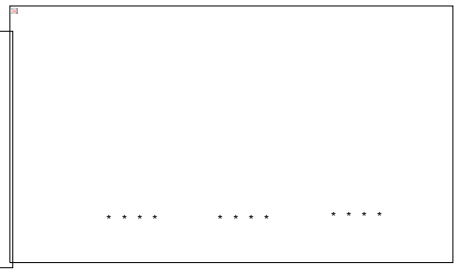
**Percent Apoptosis with & without Resveratrol Treatment over a 72-hour period.**





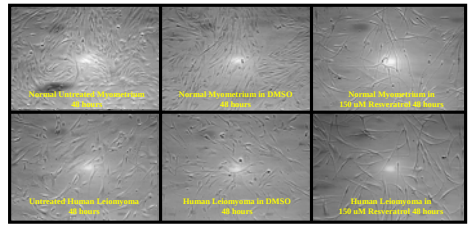






* Significantly different than No-Treatment and DMSO controls; Tukey's Post Hoc (p<0.05)

## RESULTS




## CONCLUSIONS

**Resveratrol** has been shown to be an effective **inhibitor** of tumor **cell proliferation** in several hormonally-responsive tumors, and it has been shown to act **through estrogen and progesterone receptors**[5-8], both of which are overexpressed in leiomyomas relative to normal myometrium[11]. Furthermore, the expression of $ER_\alpha$ tended to be greater than the expression of $ER_\beta$ in both leiomyomas and adjacent myometrium[14]. **Resveratrol** was shown to be an **effective estrogen antagonist in** mammary **tissues containing $ER_\beta$** and an estrogen agonist in mammary tissues containing $ER_\alpha$[12]. *In vivo* **studies** of immature rat uterine tissue **demonstrated** that **resveratrol reduced** the expression of **$ER_\alpha$** protein in a dose-dependent manner **in** epithelial, stromal and **myometrial cells**; however, it did not change the expression of PR[7]. Therefore, resveratrol may possess anti-tumorigenic properties in leiomyomas.

In this study, the ***in vitro*** proliferations of **Human Leiomyoma** and ELT-3 **rat** uterine **leiomyoma cells** were **inhibited by resveratrol**. Resveratrol exhibited a dose dependent inhibitory effect on tumor cells from both species beginning at the 25 uM concentration, and these effects were especially pronounced at the 5-day interval. Flow cytometry data suggests that **resveratrol** acts at the G1 phase of the cell cycle by **inhibiting** growth of **primary-human** and **ELT-3 rat**, **tumor-cell lines**. Apparently, **resveratrol** also **acts on normal human myometrium** as well, inhibiting cell growth and viability beginning at the 25 μM level of resveratrol. These results suggest that the biochemical pathways affected by resveratrol are common to both leiomyomas and normal myometrium and would not be unexpected given Resveratrol's affinity for estradiol receptors. **Further studies** using human tissues are **ongoing** in an effort to determine the exact mechanisms by which resveratrol acts to inhibit growth and development of these tumors. **Future studies** using an appropriate **animal model** are under development.

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