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Resveratrol inhibits human leiomyoma cell proliferation via crosstalk between integrin αvβ3 and IGF-1R

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ABSTRACT

Leiomyomas (myomas) are the most common benign smooth muscle cell tumor of the myometrium. Resveratrol, a stilbene, has been used as an anti-inflammatory and antitumor agent. In the current study, we investigated the inhibitory effect of resveratrol on the proliferation of primary human myoma cell cultures. Resveratrol arrested cell proliferation via integrin αvβ3. It also inhibited integrin αvβ3 expression and protein accumulation. Concurrently, constitutive AKT phosphorylation in myoma cells was inhibited by resveratrol. Expressions of proapoptotic genes, such as *cyclooxygenase (COX)-2*, *p21* and *CDKN2*, were induced by resveratrol in myoma cells. On the other hand, expressions of proliferative (anti-apoptotic) genes were either inhibited, as in BCL2, or unchanged, as in cyclin D1 and proliferating cell nuclear antigen (PCNA). The accumulation of insulin-like growth factor (IGF)-1 receptor (IGF-1R) was inhibited by resveratrol in primary myoma cells. IGF-1-induced cell proliferation was inhibited by co-incubation with resveratrol. Therefore, growth modulation of myoma cells occurs via mechanisms dependent on cross-talk between integrin αvβ3 and IGF-1R. Our findings suggest that resveratrol can be considered an alternative therapeutic agent for myomas.

1. Introduction

Myomas are benign tumors that arise from individual smooth muscle cells of the uterus, and they are the most common gynecologic neoplasm in reproductive-age women (Cheng et al., 2008; Islam et al., 2013). To date, several pathogenic factors such as genetic factors, epigenetic factors, estrogens, progesterone, growth factors, cytokines, chemokines, and extracellular matrix (ECM) components have been implicated in leiomyoma (myoma) development and growth (Cheng et al., 2008; Walker, 2002). Since the natural cause of myomas is unknown, a myomectomy or selected conditional hysterectomy has become the mainstream in managing the disease; however, these are not

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Table 1
Effect of resveratrol-induced anti-proliferation in human uterine fibroid primary cell cultures.

Normal			Moderate						Sensitive		
Case	Treatment	Value	Case	Treatment	Value	Case	Treatment	Value	Case	Treatment	Value
003	Control	61.1 ± 4.70	006	Control	73.8 ± 4.25	024	Control	95.7 ± 1.33	013	Control	63.4 ± 8.46
	RV	62.8 ± 6.81		RV	59.4 ± 0.94		RV	84.1 ± 2.16		RV	37.5 ± 1.56
004	Control	72.6 ± 7.12	009	Control	28.5 ± 3.37	025	Control	61.2 ± 1.30	014	Control	70.6 ± 2.30
	RV	73.5 ± 0.32		RV	24.2 ± 1.94		RV	54.7 ± 1.98		RV	50.1 ± 2.99
005	Control	35.0 ± 2.82	010	Control	81.5 ± 0.67	026	Control	47.7 ± 0.77	016	Control	79.6 ± 1.57
	RV	38.3 ± 0.29		RV	70.1 ± 6.12		RV	39.9 ± 0.11		RV	52.4 ± 2.72
007	Control	92.4 ± 2.38	015	Control	49.1 ± 4.20	027	Control	62.8 ± 1.78	018	Control	75.2 ± 0.95
	RV	95.8 ± 5.24		RV	43.9 ± 1.78		RV	56.2 ± 0.68		RV	55.7 ± 1.05
011	Control	111.5 ± 2.82	017	Control	39.7 ± 0.25	028	Control	107.0 ± 2.41	019	Control	92.2 ± 4.64
	RV	111.6 ± 3.59		RV	32.3 ± 0.50		RV	87.4 ± 1.93		RV	49.2 ± 1.20
			021	Control	79.1 ± 1.78	029	Control	108.6 ± 5.28	020	Control	111.1 ± 5.16
				RV	70.0 ± 2.90		RV	91.0 ± 4.11		RV	86.6 ± 5.58
			023	Control	75.1 ± 2.98						
				RV	64.1 ± 3.64						

Clinically collected uterine fibroid primary cells were tested for their sensitivity to 100 μM resveratrol treatment for 48 h by CyQUANT® NF Cell Proliferation Assay Kit. Growth inhibition shown less than 10% by treatment with 100 μM resveratrol were categorized as normal-, 10–20% were moderate and > 20% were sensitive.

attractive choices for many women, especially patients desiring to preserve their fertility potential (Walker, 2002). Insulin-like growth factor (IGF)-1 may be one of the growth factors that play an important role in the pathogenesis of myomas (Baird et al., 2009). Growth hormones can stimulate IGF-1 production, which is related to both the proliferation and inhibition of apoptosis. Boehm et al. (1990) reported that IGF-1 expression was elevated in myomas compared to normal myometrium. An animal model of Eker rats also showed upregulation of IGF-1 in myoma tissues (Burroughs et al., 2002). IGF-1 promotes myoma cell cycle progression in human uterine myoma cell lines, as demonstrated by estrogen-induced accumulation of IGF-I and the cell cycle-regulating transcriptional factor, A-myb (Swartz et al., 2005). All these lines of evidence describe the proliferative function of IGF-1 and its antiapoptotic effects on myomas.

It was shown that the proliferation of myomas is sensitive to gonadotropin-releasing hormone (GnRH) agonists (Garner, 1994; Sabry and Al-Hendy, 2012) and estrogen receptor antagonists. In addition, treatment with medicines such as tamoxifen also reduces myoma sizes (Sabry and Al-Hendy, 2012). Some patients can be treated with GnRH agonists, including Lupron, Synarel, and Zoladex, and/or aromatase inhibitors, such as anastrozole (Arimidex®), letrozole (Femara®), exemestane (Aromasin®), vorozole (Rivizor®), formestane (Lentaron®), fadrozole (Afema®), and testolactone (Teslac®). Reducing estrogen receptor (ER) levels by an adenovirus-expressing dominant-negative ER was shown to arrest myoma growth in a mouse model, which may be optional treatment (Al-Hendy and Salama, 2006). In addition, alternative medicine was shown to improve symptoms of myomas (Ohara et al., 2007; Su et al., 2012; Zeng et al., 2017). On the other hand, surgery was suggested, but it has serious consequences.

Polyphenols have attracted attention in the past few years due to their antioxidative effects on chronic human diseases such as cardiovascular diseases, diabetes mellitus, neurodegenerative diseases, and cancers (Costa et al., 2017). Resveratrol, a well-studied stilbene, induces p53-dependent apoptosis in several human cancer cell lines, including thyroid, prostate, and breast cancer cells (Cheng et al., 2017; Chin et al., 2014, 2015; Ho et al., 2017; Lin et al., 2017). It was suggested to be a multiple drug-resistant reversion molecule in breast cancer (Alamolhodaei et al., 2017). Resveratrol is also known as a chemopreventive, serving to suppress 9,10-dimethylbenz-A-anthracene (DMBA)-induced ductal breast carcinoma (Banerjee et al., 2002) and ultraviolet light (UV)-induced skin cancer (Jang et al., 1997) in mouse models. The possibility that the growth effect of IGF-1 can be reduced by resveratrol (Vanamala et al., 2010) and whether signals transduced through AKT phosphorylation are essential to its anti-proliferative actions remain to be elucidated.

In the current study, resveratrol was shown to induce anti-proliferative effects in human myoma primary cell cultures via crosstalk between integrin αvβ3 and the IGF-1 receptor (IGF-1R). Resveratrol inhibited proliferation of sensitive myoma primary cell cultures via integrin αvβ3. Expressions of proliferative genes were either inhibited or unaffected by resveratrol. In addition, the expression of integrin αvβ3 and accumulation of its protein were inhibited by resveratrol. Concurrently, constitutive phosphorylation of AKT in myoma cells was inhibited by resveratrol. On the other hand, resveratrol inhibited phosphorylated (p)IGF-1R accumulation and proliferation induced by IGF-1. These results indicate that the resveratrol-induced anti-proliferative effects occur via crosstalk between integrin αvβ3 and IGF-1R-sensitive signal transduction pathways. These findings also suggest a mechanism whereby IGF-1 might enhance the proliferation of myomas and thereby accelerate their progression.

2. Materials and methods

2.1. Cell lines

Myoma patients were admitted to the Department of Gynecology, Wan-Fang Hospital, Taipei Medical University, Taipei, Taiwan and were included in this study according to standardized diagnostic criteria. All patients provided informed consent to the protocol approved by the Institutional Review Board (TMU-JIRB no.: N201307019). Myoma primary cells were collected and tested by CyQUANTM® NF cell proliferation assay for their sensitivity to 100 μM resveratrol treatment for 72 h. Less than 10% of growth inhibition has been shown in treatment of 100 μM resveratrol which was categorized as normal/mildly sensitive. 10–20% were moderately and > 20% were highly sensitive. Cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), in a 5% CO_2 incubator at 37 °C unless other experimental conditions were required.

2.2. Cell viability assay

All established cell lines were plated at a density of 10^4 cells/well in 24 well plates. After starvation for 48 h, cells were treated with 100 μM resveratrol for 3 days and refreshed daily. Cell viability was determined by using the CyQUANT® NF Cell Proliferation Assay Kit (Molecular Probes, Eugene, OR, USA) at 72 h after treatment. Briefly, medium was removed, and cells were trypsinized, pelleted, and re-suspended in 1 ml PBS. 50 μL well-mixed cell suspension was incubated with CyQUANT® NF reagent for 1 h at 37 °C according to the manufacturer's instructions. Plates were then analyzed by using a microplate reader (Varioskan™