Research Article

Journal of Gynecology & Reproductive Medicine

Estrogen Receptor, Progesterone Receptor and Glucocorticoid Receptor Expression in Normal Myometrium and in Leomyoma

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Submitted: 27 Sep 2017; **Accepted**: 07 Oct 2017; **Published**: 15 Oct 2017

Abstract

Uterine leiomyoma is the most common benign smooth muscle tumour that originates from the myometrium. Ovarian sex steroids are responsible for the growth of such tumours, though estrogen and progesterone are not the only modulators involved in the tumourigenesis and growth of leiomyomata.

The activation of glucocorticoid receptor (GR) can induce the expression and activity of estrogen sulfotransferase, an enzyme that generally inactivates estrogen, because of the effects of sulfonated estrogen fails to activate the estrogen receptor, it is of interest to know whether GR is present or not in leiomyomata and in the normal myometrium. The aim of this study is to analyse the expression of GR in leiemyoma and myometrium.

***Method**: The immunohistochemistry of 47 patients was tested to find the expression status of estrogen receptor (ER), progesterone receptor (PR) and GR in leiemyoma and normal myometrium.*

***Results:** GR, ER and PR were expressed in both normal myometrium and in leiomyoma. There was a significant difference in the expression of GR found in normal myometrium compared with those expressed in leiomyoma (Allred Score, 256±29 versus 91 ±23). The GR expressed was significantly lower than the ER and PR expressed in leiomyoma.*

***Conclusion:** This presence of GR in the normal myometrium and in myomas is interesting and could be used for therapeutic action. But, first, the roles of G on myomas have to be understand.*

Keywords: ER PR GR Myometrium Leimyoma

Introduction

Ulterine leiomyomata (fibroids, myomas) are one of the most common gynecologic tumors in women of reproductive age. The incidence rates increased with age, and is around 30% of Caucasian and up to 50% in Black women aged >35years [1]. These tumors represent a significant public health problem, since they are responsible for approximately 200 000 hysterectomies per year and are also associated with clinical disease [2, 3]. Uterine leiomyomata tend to grow during the reproductive years, and regress after menopause, indicating ovarian steroid-dependent growth potential [4]. The ovarian steroid hormones, estrogen and progesterone, are believed to play an important role in the growth of uterine leiomyomata [4].

Steroid hormone levels in women with leiomyomata are similar to those in normal women [5]. Moreover, myomas are not observed more frequently in conditions such as polycystic ovarian syndrome in which there is chronic estrogen elevation. Finally, the heterogeneity of leiomyoma growth within the same uterus, despite the identical exposure to circulating sex steroid concentrations, suggests that sex steroids are not the only modulators of leiomyoma tumorigenesis and growth.

Glucocorticoids have been implicated in a variety of cellular processes, ranging from development to metabolism, immune response, and apoptosis. Endogenous glucocorticoids, such as cortisol in humans and corticosterone in rodents, are synthesized in the adrenal cortex under the control of the hypothalamic-pituitary-adrenal axis. In addition to their physiologic functions, glucocorticoids are among the most commonly prescribed drugs for their anti-inflammatory and immunosuppressive effect [6,7]. Glucocorticoids exert most of their functions by binding to the glucocorticoid receptor, a member of the nuclear hormone receptor superfamily. In addition, activation of glucocorticoid receptor (GR)

by dexamethasone induces the expression and activity of estrogen sulfotransferase, an enzyme important for the metabolic deactivation of estrogens, because sulfonated estrogens fail to activate the estrogen receptor [8]. This could be one of the explanations for the modulation of the estrogen-induced growth of fibroids.

The human GR is expressed predominantly in normal human myoepithelial cells but has not been studied in leimyomata. The present study was undertaken to analyze the expression of GR in human normal myometrium and in human leiomyomata.

Materials and methods

Uterus tissue samples (leiomyoma and surrounding myometrium) from patients undergoing hysterectomy for symptomatic leiomyoma were retrieved from the Department of Pathology, Erasme University Hospital, Brussels, Belgium, and consisted of 47 cases.

Immunohistochemistry

Four-micrometer sections were cut sequentially and mounted onto superfrost-treated slides (Menzel-Glasser, Braunschweig, Germany). The slides were dried overnight at 37°C before deparaffinization in xylene and rehydratation through graded ethanols. For epitope retrieval, the slides were immersed in a waterbath at 95 to 99°C for 90 min with an ethylenediaminetetraacetic acid buffer, pH 9.0 (S236, Dako Corp, Glostrup, Denmark). Then the slides were cooled in their buffer for 20 min at room temperature. H2O (O.3%) was added to the slides and incubated at room temperature for 30 min. The tissues were then incubated one hour at room temperature with a monoclonal antibody against the a estrogen receptor (ER) (ER clone 6F11, dilution 1/100, Novocastra Laboratories Ltd, Newcastle, UK), with a monoclonal antibody against the a and b progesterone receptor (PR) (PR clone 16-SAN27, dilution 1/100, Novocastra Laboratories Ltd, Newcastle, UK) and with a monoclonal antibody against the N-terminus of the glucocorticoid receptor (clone NCL-L-GCR, dilution 1/25, Novocastra Laboratories Ltd, Newcastle, UK).

2 pathologists performed the evaluation of ER, PR and GR independently by using the Allred score (Harvey). Briefly, a proportion score (PS) was assigned that represents the estimated proportion of positive tumor cells on the entire slide as follows: none = 0; 1 of 100 = 1; 1 of 10 = 2; 1 of 3 = 3; 2 of 3 = 4; and 1 of 1 (i.e., all of the cells are stained) = 5. An intensity score (IS) was assigned and estimates the average staining intensity of positive tumor cells as follows: negative = 0; weak = 1; intermediate = 2; and strong = 3. The PS and IS are added to obtain a total score (range, 0-8). We considered as positive result for ER, PR and GR when the total score was equal or superior to 3.

Statistical Analysis

The data were compared with Chi2 test and p value was considered as significant if <0.05.

Results

GR, like E and PR, were demonstrated both in normal myometrium and in leiomyoma **(Figure 1) (Table 1)**. A difference in GR expression was found to be significant (t-test for equality of means, p>0.02) between normal myometrium and leiomyoma (p=0.0003). GR expression is also significantly lower than ER (p=0.36) and than PR (p=0.008) in leiomyoma. There are no significant difference in the expression of GR, ER and PR in normal myometrium.

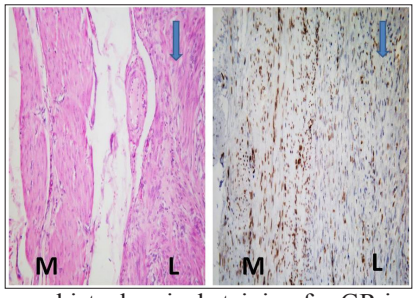

Figure 1: Immunohistochemical staining for GR in representative cases of (M) normal myometrium and (L) leimyoma.

Table 1: Glucocorticoid receptor (GR), estrogen receptor (ER) and progesterone receptor (PR) expression in normal myometrium and in leiomyoma (Allred Score).

	GR	ER	PR
Normal myometrium	256±29	275±42	291±20
Leiomyoma	91±23	283±25	297±8

Discussion

The accumulating evidence supports the concept that estrogen is closely related to the tumorigenesis and growth of myomas. Progesterone has both inhibitory and stimulatory effects on growth. There is also biological evidence for "cross talk" between the estrogen and progesterone hormone receptor [9].

In this study, we demonstrated that GR is present in myometrium and myomas but in lower concentrations in this benign tumor. The role of glucocorticoid on leiomyoma is unknown. Nevertheless, glucocorticoids antagonize estrogens by glucocorticoid receptor-mediated activation of estrogen sulfotransferase [8]. This lowering of GR expression could have a role in facilitating the growth of myomas by increasing the amount of nonsulfated estrogen.

Moreover, Whirledge et al. demonstrated also that glucocorticoids regulate cell proliferation and significantly reduce the percentage of S-phase cells either in the presence or absence of estrogen in leiomyomas but not smooth muscle cells [10].

That is why the administration of G for patients presenting such a pathology could be useful by reducing the amount of circulating active estrogen and / or therefore inhibit the growth of myomas? This hypothesis would of course require in vitro research before starting in vivo study. Furthermore, estradiol improves endothelial function and has a direct vasodilator action by means of endothelium-derived relaxing factor synthesis [11]. Ciccone et al. showed also that the administration of a single intranasal dose of 17-β-estradiol in healthy postmenopausal women increased cerebral perfusions [12]. By lowering non sulfonated estrogens, glucocortioids could thus perturb and/or decrease utérus and myomas perfusion.

Also, could the expression of GR and the role played by G on myomas be checked by an epidemiological study? For example, we know that life stresses can impair the immune, endocrine and nervous systems. Behavioural status are correlated with an increase in corticosterone and pro-inflammatory cytokines in both acute and chronic submissive groups [13]. Thus, with such patients, a

small amount of uterine myomas would confirm the protective role played by glucocorticoids. A easy population group to study would be patients receiving glucocorticoids as treatment for autoimmune diseases or for patients receiving glucocorticoids after receiving a transplant.

In this study, we demonstrated that GR are present in normal myometrium and, in lower concentration, in myomas. This lowering of GR expression could have a role in facilitating the growth of myomas by lowering the amount of sulfonated estrogen. However, by a similar process, the sensitivity of myomas to G could be used in therapeutic treatments.

This hypothesises needs looking into with an *in vitro* study with a view to setting up a clinical research study into the use of G in the treatment of myomas.

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