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ORIGINAL ARTICLE

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Anatomical variation of the oestrogen receptor in the non-neoplastic myometrium of fibromyomatous uteri

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Abstract Myometrial tissues from a total of 30 normal and 30 fibromyomatous uteri were compared in order to assess whether the oestrogen receptor distribution is similar for both types. All patients concerned were premenopausal with no history of exogenous hormone usage. Material taken from the subserosal, midmyometrial and subendometrial regions of both the fundus and the lower segment was stained by immunocytochemistry for the oestrogen receptor. No significant difference in the oestrogen receptor content was noted between the fundus and the lower segment in either the normal or the fibromyomatous myometria. Similarly, the phase of the menstrual cycle did not affect the total receptor content of either group of tissue. The oestrogen receptor content in the non-neoplastic portion of the fibromyomatous myometria was highest in the subendometrial and lowest in the subserosal region. The differences in receptor content between normal and fibromyomatous myometria were minimal in the subendometrial region but marked in the subserosal region. The myometrium of fibromyomatous uteri thus expresses significantly increased levels of oestrogen receptor, and the pathogenesis of fibromyomata may be related to an inherent abnormality in the myometrium.

Key words Immunocytochemistry · Fibromyoma · Myometrium · Oestrogen receptors · Uterus

Introduction

Previous investigators of the oestrogen receptor content of fibromyomata have, in general, assumed that the host myometrium in which these tumours occur is inherently normal. They have therefore tended to use the adjacent myometrium as a source of normal control samples [1, 2, 4, 6, 7, 9, 13, 16]. There is a dearth of studies specifically comparing the content and distribution of the oestrogen receptor in fibromyomatous myometrium with that in normal myometrium.

A recent study of normal myometrium from proliferative phase uteri has demonstrated the existence of a significant distribution gradient for the oestrogen receptor through the depth of the myometrial wall [10]. We noted significantly greater levels of the receptor in the subendometrial region than in the midmyometrial and subserosal regions. No difference was detectable between the fundal and lower segment regions.

The present study is aimed at establishing, first, whether such a gradient exists in the myometrium of normal secretory phase uteri, and secondly, whether the values for the receptor differ between the proliferative and the secretory phase, in order to establish the presence or absence of a menstrual cycle effect. Finally, the oestrogen receptor content of the non-neoplastic myometrium of fibromyomatous uteri is assessed and compared with that of normal uteri to test whether the assumptions of the past hold true.

Materials and methods

All the samples of myometrial tissue used in this study were obtained from hysterectomy specimens from patients with no history of exogenous reproductive steroid hormone ingestion. The patients ranged in age from 31 to 50 years and underwent hysterectomy for menorrhagia or dysmenorrhoea. In total, 30 normal secretory phase uteri, 10 fibromyomatous proliferative phase uteri and 20 fibromyomatous secretory phase uteri were collected.

Within 10 min of surgical excision, each uterus was sectioned in the sagittal plane and a transmural block of tissue dissected out from each of the fundal and lower segment regions. The blocks of tissue were fixed in 10% formalin and processed to wax.

Endometrial cycle phase and anatomical position of dissection were confirmed by bright-field microscopy of 1 μm HE-stained sections. The avidin-biotin technique [3] was employed for immunocytochemical localisation of the oestrogen receptors in the par-

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affin-embedded tissue. In summary, blocked dewaxed sections were microwaved in citrate buffer (pH 6.0) in a 600-W household microwave oven at 75% power for two 10-min periods. Sections were then treated with normal rabbit serum (1:20 dilution) followed by the monoclonal oestrogen receptor antibody (Dako-ER M7407 1:50 dilution) prior to the application of the biotinylated rabbit anti-mouse. Streptavidin at a 1:500 dilution was then applied, followed by diaminobenzidine-H2O2 (DAB). Each antibody application was followed by a 3-min wash in TRIS buffer (0.01 M tris-HCl, 1.5 mM ethylenediaminetetraacetic acid, pH 7.4). Sections were lightly counterstained with haematoxylin prior to dehydration, mounting and viewing by Hoffman modulation contrast microscopy [11].

Cell counting was done manually by a single operator as described in detail elsewhere [10]. In summary, each stained myometrial section, whether from the fundal or the lower segment, was divided for counting purposes into subendometrial, midmyometrial and subserosal regions. Cells of the endometrium and serosa were not included in the counting process. The total cell populations, the number of DAB-positive cells and the percentage nuclear staining were assessed for each demarcated region.

Results

Bright-field microscopy confirmed that the dissected blocks of myometrium from both the normal and fibromyomatous uteri had been removed from the correct anatomical regions and that the tissue was in either the proliferative or secretory phase of the menstrual cycle.

Myometrium from both normal and fibromyomatous uteri stain for the oestrogen receptor in a similar manner, with the DAB reaction product isolated to the nucleus of immunopositive cells. No reaction product is seen in any of the endothelial nuclei.

The results of the cell counts for normal fundal and lower segment myometria, in secretory phase are presented in Tables 1 and 2, respectively. In the subendometrial regions of both the fundal and lower segment samples, the percentage of oestrogen receptor-positive cells is significantly greater than that in the midmyometrial or subserosal regions. Analysis of variance testing shows no significant difference in either the cellularity or the oestrogen receptor positivity between the fundal and lower segment regions. A comparison of the data obtained from the normal secretory phase myometria with those obtained for normal proliferative phase myometria [10] shows no significant variation between these two phases of the menstrual cycle.

The results of the cell counts for fundal and lower segment fibromyomatous myometria in proliferative phase are presented in Tables 3 and 4, respectively. Tables 5 and 6 outline the results obtained for fundal and lower segment fibromyomatous myometria in the secretory phase of the endometrial cycle.

For all the myometrial samples extracted from fibromyomatous uteri, the subendometrial region is consistently the most cellular, with an average of 100 nuclei per high-power field. This region also predominates with

Table 1 Oestrogen receptor staining of normal fundal myometrium[a] in secretory phase (*Total cells/HPF* total cells per high-power field, *Positive/HPF* total oestrogen receptor-positive cells counted per high-power field)

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=30)	Positive/HPF (n=30)	Total cells/HPF (n=30)	Positive/HPF (n=30)	Total cells/HPF (n=30)	Positive/HPF (n=30)
Mean	108.9	92.4	65.23	44.77	40.47	20.77
Max.	159	132	100	75	72	40
Min.	68	57	39	9	21	8
SD	23.12	19.29	16.22	16.76	12.01	8.22
SEM	4.22	3.52	2.96	3.06	2.19	1.5

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

Table 2 Oestrogen receptor staining of lower segment myometrium[a] in secretory phase

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=25)	Positive/HPF (n=25)	Total cells/HPF (n=25)	Positive/HPF (n=25)	Total cells/HPF (n=25)	Positive/HPF (n=25)
Mean	101.96	87.68	65.56	43.52	38.16	18.72
Max.	156	121	99	69	57	39
Min.	67	59	38	22	21	6
SD	24.08	18.27	19.05	13.88	10.46	7.90
SEM	4.82	3.65	3.81	2.78	2.09	1.58

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

respect to oestrogen positivity, on average 86% of all nuclei counted being oestrogen receptor positive. The midmyometrium is approximately half as cellular as the subendometrium. Nuclear positivity does remain high in this region, however, with more than 77% of the total nuclei demonstrating DAB reaction product. With only 34 nuclei per high-power field the subserosa is the least densely populated of the three regions. Positivity in this outlying region is only marginally less than that of the midmyometrium and averages 72% of counted nuclei.

Analysis of variance testing fails to show any significant difference in the positivity between the fundus and lower segment regions ($P<0.6549$) for fibromyomatous uteri. Variation in the counts for positivity for the three regions from the fibromyomatous myometria may appear to be small, but are in fact significant ($P<0.001$). As with normal myometrium, the phase of the endometrial cycle does not significantly affect the immunopositivity of the different regions of the fibromyomatous myometrium ($P<0.5593$).

The data from normal and fibromyomatous myometria can be compared, as the cell counting for both was conducted by the same operator with intermingling of the samples to ensure that all samples were treated in the

Table 3 Oestrogen receptor staining of fundal fibromyomatous myometrium[a] in proliferative phase

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=10)	Positive/HPF (n=10)	Total cells/HPF (n=10)	Positive/HPF (n=10)	Total cells/HPF (n=10)	Positive/HPF (n=10)
Mean	102.20	87.2	49.40	37.90	26.60	19.40
Max.	124	98	75	54	40	31
Min.	80	68	33	20	11	9
SD	13.57	10.15	12.59	12.19	10.88	10.14
SEM	4.29	3.21	3.98	3.85	4.86	4.53

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

Table 4 Oestrogen receptor staining of lower segment fibromyomatous myometrium[a] in proliferative phase

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=10)	Positive/HPF (n=10)	Total cells/HPF (n=10)	Positive/HPF (n=10)	Total cells/HPF (n=10)	Positive/HPF (n=10)
Mean	108.9	96.50	71.50	53.90	48	34.20
Max.	141	128	85	75	82	54
Min.	71	62	45	10	23	18
SD	19.25	17.48	11.94	18.19	17.36	11.59
SEM	6.08	5.53	3.78	5.75	5.49	3.66

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

Table 5 Oestrogen receptor staining of normal fundal fibromyomatous myometrium[a] in secretory phase

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=15)	Positive/HPF (n=15)	Total cells/HPF (n=15)	Positive/HPF (n=15)	Total cells/HPF (n=15)	Positive/HPF (n=15)
Mean	97.33	82.80	52.40	42.93	32.00	22.47
Max.	119	103	74	58	49	36
Min.	74	57	36	25	21	14
SD	14.76	13.33	11.58	10.05	8.49	6.82
SEM	4.58	3.44	2.98	2.59	2.19	1.76

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

Table 6 Oestrogen receptor staining of lower segment fibromyomatous myometrium[a] in secretory phase

	Region					
	Subendometrial		Midmyometrial		Subserosal	
	Total cells/HPF (n=20)	Positive/HPF (n=20)	Total cells/HPF (n=20)	Positive/HPF (n=20)	Total cells/HPF (n=20)	Positive/HPF (n=20)
Mean	90.85	79.30	45.45	35	27.70	20.50
Max.	134	117	62	58	51	41
Min.	68	53	26	2	4	4
SD	15.87	15.30	10.45	12.32	10.09	8.27
SEM	3.55	3.42	2.33	2.75	2.26	1.85

[a] Total positive cells per high-power field in the subendometrial region are significantly greater than in the subserosal and midmyometrial regions

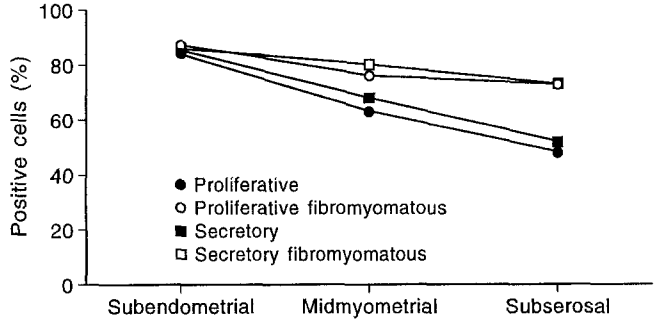

Fig. 1 Graphical representation of the oestrogen receptor content of normal and fibromyomatous myometria during the proliferative and secretory phases of the endometrial cycle

same manner. While little difference is found between normal and fibromyomatous myometria with regard to the total cellularity for each respective region counted, significant differences are noted with respect to the relative positivity of the midmyometrial and subserosal regions. Figure 1 illustrates the differences in the positivity between normal and fibromyomatous myometria in terms of region and endometrial cycle phase where the myometrium of tumour-bearing uteri have a significantly greater total population of oestrogen receptor-positive nuclei (P<0.0001).

The percentage area of the nucleus obscured by reaction product is similar for both normal and fibromyomatous myometria. In the subendometrium of normal myometria over 60% of oestrogen receptor-positive nuclei are greater than two thirds obscured by reaction product, while only 5% of nuclei in the subserosal region are similarly obscured. A similar picture emerges for fibromyomatous myometria, with 59% of subendometrial and 12% of subserosal nuclei two thirds obscured.

Discussion

The results of the present study indicate that both the content and the distribution of oestrogen receptors differ significantly between the myometria of normal and fibromyomatous uteri. The marked differences in receptor concentrations obtained by different groups of researchers [6, 14] makes comparison of their data difficult. There is also little agreement the literature as to whether or not the oestrogen receptor content of the myometrium is affected by the normal cyclical variations in the serum levels of the reproductive steroid hormones. Marugo et al. [5] note higher receptor concentrations during the proliferative phase, attributing these to the higher levels of circulating oestrogen. They suggest that as a result, tissue levels of the hormone increase, with a subsequent rise in the cytoplasmic production of the receptors. This is followed by an increase in the transfer of the hormone receptor complex into the nucleus. During the secretory phase, endogenous progesterone blocks the nuclear uptake of oestrogen hormone complex, and hence receptor levels decline. Similar reductions in the quantity and staining of the receptor during the secretory phase have also been reported [4, 7, 12]. However, as the oestrogen receptor is now thought to be resident solely in nuclei [15] hypotheses such as these do not provide an explanation for the cyclical oestrogen receptor content discrepancies described by these authors.

In the present study, neither normal nor fibromyomatous myometria have been shown to undergo any significant alterations in their oestrogen receptor status during the normal menstrual cycle. Similarly, Chrapusta et al. [1] were also unable to demonstrate any significant menstrual cycle effect.

Normal uteri have been shown to demonstrate a significant oestrogen receptor concentration gradient through the depth of the myometrial wall [10]. The transmural oestrogen receptor concentration gradient of the non-neoplastic myometrium of a fibromyomatous uterus is, however, markedly flattened compared with that of normal myometrium (Fig. 1). Both the midmyometrial and subserosal areas of fibromyomatous myometria are far more heavily populated with oestrogen receptor-positive cells than their normal counterparts. As a result, the overall receptor content of the non-neoplastic myometrium of a fibromyomatous uterus is increased.

It is not possible from these samples to assess whether or not the presence of fibromyomata is responsible for the overall increase in the oestrogen receptor content of

the surrounding host myometrium. However, the facts that fibromyomata are rarely solitary and that they tend to recur subsequent to myomectomy suggest a primary abnormality within the host myometrium. The increase in the total amount of oestrogen receptors within the myometrium possibly produces a state of heightened irritability and responsiveness that is sufficient to act as a major predisposing factor for fibromyoma formation. Furthermore, the majority of fibromyomata are known to arise in the midmyometrial and subserosal areas of affected uteri, where the oestrogen receptor concentration is markedly raised, lending support to the theory of irritability.

The pathogenesis of fibromyomata can perhaps be further explained in terms of the oestrogen receptor concentration ratios between normal and affected myometria for each of the three transmural regions. Within the subendometrium a ratio of almost 1:1 for nuclear positivity exists between normal and abnormal myometria, and thus the difference is insufficient to act as a major factor in tumour formation. In the remaining two thirds of the myometrial wall the ratio changes to 1:1.3 and 1:1.5 for the middle and outer regions, respectively. Thus, the positivity is 30–50% greater than normal in these regions. Such an increase may well aid in providing the necessary nidus for tumour formation.

In conclusion, even though the non-neoplastic myometrium of fibromyomatous uteri demonstrates a similar differential pattern of oestrogen receptor distribution to that of normal myometrium, the distribution curve is flattened. No significant difference in oestrogen receptor content is noted between fundus and lower segment in either normal or abnormal myometria. The myometria of fibromyomatous uteri may be considered to be abnormal, by virtue of their higher levels of oestrogen receptor positivity than in a normal uterus. Despite the differences in the total oestrogen receptor content between normal and abnormal myometria, neither demonstrates receptor variation during the menstrual cycle. Finally, as the non-neoplastic myometrium of a fibromyomatous uterus differs with respect to its oestrogen receptor status from that taken from a normal uterus, myometrial control samples should always be taken from a separate but equivalent population of normal uteri.

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