Cyclooxygenase-2 and estrogen receptor-β as possible therapeutic targets in desmoid tumors

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Received 20 January 2018
Accepted 13 February 2018

Kasr Al Ainy Medical Journal
2018, 24:47–52

Background and aim
Desmoid tumors are mainly treated by surgical excision and radiotherapy, but the failure to achieve complete response has given rise to the need for investigating the role of possible target therapy. The aim of this study was to evaluate the immunohistochemical detection of cyclooxygenase-2 (COX-2) and estrogen receptor-β (ERβ) in desmoid tumors and to assess their correlation with available clinicopathologic variables.
Materials and methods
A total of 17 desmoid tumor cases (11 abdominal, five extra-abdominal, and one intra-abdominal) were examined for immunohistochemical detection of COX-2 and ERβ using monoclonal antibodies. Toluidine blue staining was performed to confirm or exclude that COX-2 immunostained cells coincide with mast cells in co-localized sections. Correlation of results with available clinicopathologic variables was done and a *P* value less than 0.05 was considered significant.
Results
COX-2 was expressed in tumor cells in 92% of examined desmoid cases (16/17). Toluidine blue staining has shown that COX-2 immunostained cells do not coincide with the few metachromatically stained mast cells in co-localized sections. ERβ was expressed in 67.1% of tumor cells in desmoid cases (11/17); eight cases displayed high ERβ expression and three cases displayed low ERβ expression. No significant correlation was detected between ERβ or COX-2 immunohistochemical expression and patient's age, sex, tumor size, site, margins status, and recurrence history ($P>0.05$).
Conclusion
This study confirmed the immunohistochemical expression of COX-2 and ERβ in tumor cells of the majority of studied desmoid cases. These results introduce COX-2 and ERβ as potential therapeutic targets in desmoid tumors. Further studies with a large sample size and follow-up are recommended to validate the current results.

Keywords:
cyclooxygenase-2, desmoid, estrogen receptor-β, target

Kasr Al Ainy Med J 24:47–52

1687-4625

Introduction

Desmoid tumors are locally infiltrative soft tissue growth with high rate of local recurrence even after total surgical resection [1]. The current main treatment strategy of desmoid tumors, also known aggressive fibromatosis, is surgical excision accompanied by radiotherapy, but the failure to achieve complete response after surgery and the possible morbidity linked to radiotherapy in some cases has emerged the need for investigating the role of possible target pharmacological therapy [2,3].

The high incidence of desmoid tumors in women (about 80%) and the high rate of reported cases during or following pregnancy and spontaneous regression in postmenopause have raised the possibility of hormonal factors as major players in the pathogenesis of desmoid tumors [4].

The detection of estrogen receptors (ERs) in desmoid tumors was reported in early studies using ligand binding assays; however, more current studies using the immunohistochemical techniques yielded conflicting results regarding the expression of ERα and ERβ [5].

The wider distribution of ERβ than ERα in several tissues such as prostate, thyroid, and mesenchymal tissues and the recent availability of specific antibodies to ERβ directed researchers to investigate its role in the pathogenesis of desmoid tumors as most studies reported an absence of immunohistochemical expression of ERα [5].

 DOI: 10.4103/kamj.kamj_1_18

Cyclooxygenase (COX) enzyme family is involved in prostaglandins production from arachidonic acid. COX-2 is one of the inducible family members that is overexpressed in different types of cancer. It enhances tumorigenesis through inhibiting apoptosis, induction of angiogenesis, invasive potential, and stimulating growth factors such as platelet-derived growth factors. Accordingly, NSAIDs that inhibits COX-2 activity were introduced as target therapy that have beneficial effects in cancer prevention [2,6].

Multiple NSAIDs as indomethacin have been examined either single or in association with other hormonal treatments such as tamoxifen in the treatment of desmoid tumors [4], with reported partial or total response rates ranging from 37 to 57% in multiple studies [7].

We aimed in the current study to evaluate the immunohistochemical detection of COX-2 and ERβ in desmoid tumors and assess their correlation with available clinicopathologic variables.

Materials and methods

In this study, a total of 17 archived paraffin blocks of desmoid tumors were retrieved retrospectively from the Pathology Department, Faculty of Medicine, Cairo University in the period from January 2016 till June 2017. All samples included were obtained through total surgical excision. The margins were inadequate in eight cases. Twelve patients were women, whereas five were men. The cases were classified according to their site as abdominal, intra-abdominal, and extra-abdominal. Of note, the intra-abdominal desmoid tumor included in this study was a mesenteric mass associated with familial adenomatous polyposis. A total of 15 cases included were primary desmoids and two cases were recurrent.

The 17 retrieved formalin-fixed, paraffin-embedded tissue blocks were cut at 5 μm thickness, stained by hematoxylin and eosin and examined microscopically to confirm the diagnosis of desmoid tumor.

Immunohistochemical staining of cyclooxygenase-2 and estrogen receptor-β

Two sections were cut at 5 μm thickness from each of the 17 paraffin-embedded sections of desmoid cases on immunostaining slides. Applying the standard protocol of Dako, heat-mediated retrieval of antigen was performed by applying citrate buffer pH 6 in an automated water bath (Dako PT link, PT101; Santa Clara, CA, United States). The primary antibodies were COX-2 monoclonal antibody (SP21), #MA5-1456, manufactured by Thermo Fisher Scientific (Waltham, MA, USA) and monoclonal ERβ (PPG5/10), #MA1 81281, manufactured by Thermo Fisher Scientific. An autostainer (Dako Autostainer link 48) was used for immunostaining using a polymer-based detection system (Dako EnVision FLEX; Santa Clara, CA 95051, United States, K8000). Diaminobenzidine was applied as chromogen and counterstaining was done using Mayer's hematoxylin. Afterwards, the cover slips and DPX were used for mounting and preserving tissue sections. The positive control used for COX-2 and ERβ was urothelial carcinoma positive for COX-2 granulosa cells in normal ovary..

Another section was cut from the paraffin blocks at 5 μm thickness, stained with toluidine blue to highlight mast cells.

An Olympus BX51 (Melville, USA) light microscope equipped with a digital camera was used for examination and capturing of digital images.

Evaluation of immunostaining of both cyclooxygenase-2 and estrogen receptor-β

Immunostaining of COX-2 was reported as positive if more than 10% of tumor cells showed cytoplasmic positivity for COX-2 [8]. The ERβ nuclear immunostaining was reported as negative (<5% positive tumor cells), low expression (5–25% positive tumor cells), and high expression (>25% positive tumor cells) [9].

The results of COX-2 and ERβ immunostaining were correlated with multiple clinicopathologic factors (age, sex, tumor size, margin status, and recurrence status).

Evaluation of toluidine blue-stained sections

The COX-2 positive immunostained cells were compared with metachromatically stained mast cells in co-localized touldine blue stained sections to show if they coincide or not.

Statistical methods

Statistical analysis of all results and available variables were performed using the Statistical Package for Social Sciences version 15 (SPSS; SPSS Inc., Chicago, Illinois, USA). The χ^2-test was used to assess the difference between qualitative variables. *P* values of less than 0.05 levels were considered to be statistically significant.

The study took the approval of ethics committee, Faculty of Medicine, Cairo University.

Results

A total of 17 cases of desmoid tumors were studied (Fig. 1). The patient's age displayed a wide range from 7 up to 50 years with a median age of 33 years. Most patients were women (70.6%), whereas 29.4% were men. Tumors of more than 6 cm in greatest dimension constituted 64.7% of the cases (6/17). The localization of the tumors was classified as abdominal (11/17), extra-abdominal (5/17), and intra-abdominal (1/17). All desmoid tumors enrolled in this study were treated by surgical excision. All clinicopathologic data are summarized in Table 1.

The majority of the studied cases (16/17) showed positive cytoplasmic immunohistochemical staining for COX-2, whereas only one case showed negative staining.

The COX-2 positive immunostained cells were similar in morphology to tumor cells in hematoxylin and eosin stained sections. The toluidine blue-stained co-localized sections highlighted only few mast cells with specific metachromatic staining that did not coincide with COX-2 positive immunostained cells as shown in Fig. 2a and b.

ERβ nuclear staining was expressed in 64.7% (11/17) of cases; 47.1% (8/17) displayed high expression (Fig. 3) and 19.6% (3/17) displayed low expression (Fig. 4), whereas 35.3% (6/17) of cases showed negative staining.

No significant correlation was detected between COX-2 and ERβ expression with different clinicopathologic variables including patient's age, sex, tumor size, site, margins status, and history of recurrence as displayed in Tables 2 and 3, respectively.

COX-2 immunohistochemical expression was not significantly correlated with ERβ expression as shown in Table 4.

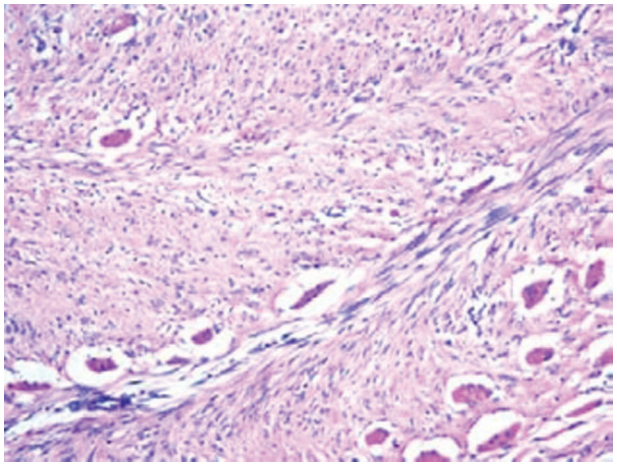
Discussion

Desmoid tumors are rare tumors with an incidence rate of two to four per million annually [10]. Although, the traditional treatment of desmoid tumors is surgery, the infiltrative borders of these tumors and the absence of tumor capsule make complete surgical resection with negative margins not always successful [11,12].

Table 1 Clinicopathologic variables of studied desmoid cases (*n*=17)

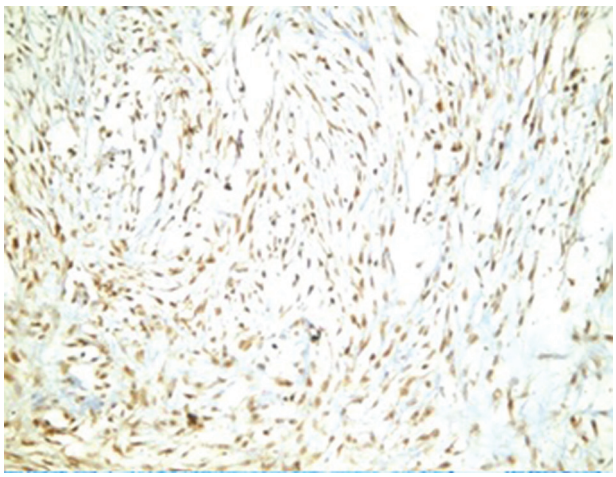
Clinicopathologic variables	Total=17 [*n* (%)]
Age (years)	
<33	10 (58.8)
≥33	7 (41.2)
Sex	
Male	5 (29.4)
Female	12 (70.6)
Tumor size (cm)	
<6	6 (35.3)
≥6	11 (64.7)
Tumor site	
Abdominal	11 (64.7)
Extra-abdominal	5 (29.4)
Intra-abdominal	1 (5.9)
Margin status	
Negative	9 (52.9)
Positive	8 (47.1)
Recurrence	
Not recurrent	15 (88.2)
Recurrent	2 (11.8)

Figure 1



Desmoid tumor (H&E ×200).

Figure 2



High ERβ expression in desmoid tumor (DAB ×200).

The introduction of systemic therapy as antihormonal and anti-inflammatory agents has been reported with various success rates in recurrent, unresectable, and locally advanced desmoid tumors [13].

Several studies reported that COX-2 inhibitors induced the shrinkage of desmoid tumors in clinical trials as COX-2 induces the growth of desmoid tumors through increased prostaglandin E2 production [14–16]. Moreover, complete regression of desmoid tumors with tamoxifen was reported in other studies [17,18], achieving a recurrence-free period of up to 9 years in a study performed by Maseelall *et al.* [19].

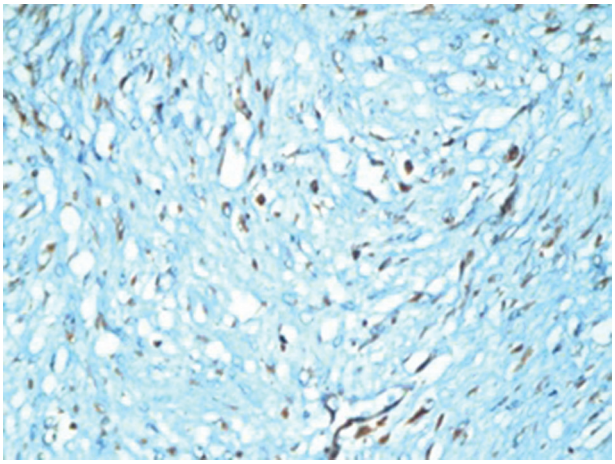
We aimed in this work to examine the expression of COX-2 and ERβ and in desmoid tumors using immunohistochemistry as it is a practical, accessible, and a cost-effective method available in almost all surgical pathology laboratories. To the best of our knowledge, several studies assessed ERβ detection in desmoid tumors by immunohistochemistry, but only two studies examined COX-2 expression and contradictory results were reported [14,16].

In this study, tumor cells expressed COX-2 protein in 92% (16/17 cases) of desmoid tumors without statistically significant correlation with all clinicopathologic variables including age, sex, tumor site, size, recurrence, and margin status ($P<0.05$). These results coincide with the results of Signoroni *et al.* [14] as all of their 14 studied desmoid tumors showed COX-2 positive immunohistochemical expression in tumor cells and their results were confirmed by the presence of COX-2 mRNA by means of RT-PCR.

In contrast, Emori *et al.* [16] reported that 56% of their 16 studied desmoid cases expressed COX-2 in mast cells only as clearly co-expressed by tryptase. This was against our findings which were confirmed by toluidine blue staining and proved that COX-2 immnnostained cells do not coincide with few mast cells in co-localized sections [16]. This controversy in results of COX-2 expression may be due to the different antibodies used and the small sample size enrolled in different studies.

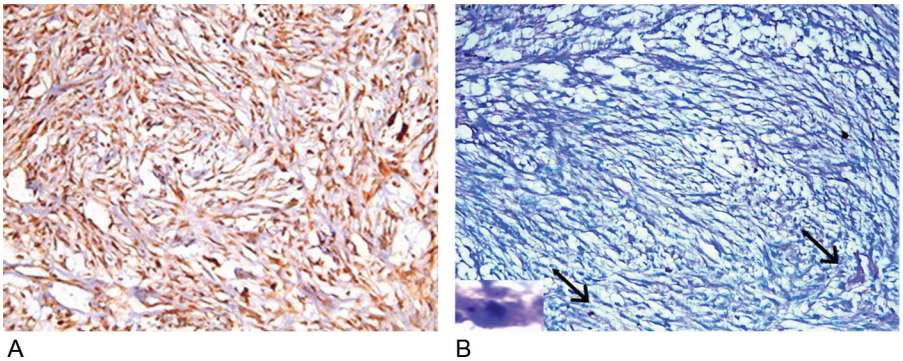
In addition, COX-2 expression was examined by Poon *et al.* [20] in cell cultures of 33 cases of aggressive fibromatosis and proved to be positive in 31 cases through RT-PCR and in only three out of 17 cases of normal tissue dissected from resection margins. Furthermore, the levels of PGE2 were higher in cell cultures of aggressive fibromatosis than examined normal margin fibroblasts ($P>0.05$) [20].ERβ was expressed in 64.7% of our studied desmoid tumor cases (11/17); high expression was reported in eight (47.1%) cases and low expression was reported in three (19.6%) cases. Six (35.3%) cases showed negative ERβ expression. The correlation between ERβ expression and all clinicopathologic variables including age, sex, tumor site, size, recurrence, margin status, and

Figure 3



Low ERβ expression in desmoid tumor (DAB ×400).

Figure 4



A B

A: COX-2 positive tumor cells in desmoid tumor (DAB ×200). B: Toluidine blue staining highlights only few mast cells (black arrows) that don't coincide with COX-2 positive tumor cells in desmoid tumor (Toluidine blue ×200, inset ×400).

Table 2 Correlation between cyclooxygenase-2 immunohistochemical expression and different clinicopathologic variables in studied desmoid cases

Clinicopathologic variables	*N* (%)	COX-2 [*n* (%)]		*P* value
		Negative	Positive	
Age (years)				
<33	10 (100)	0 (0)	10 (100)	0.071
≥33	7 (100)	1 (14.3)	6 (85.7)	
Sex				
Male	5 (100)	0 (0)	59 (100)	0.279
Female	12 (100)	1 (8.3)	11 (91.7)	
Tumor size (cm)				
<6	6 (100)	0 (0)	6 (100)	0.329
≥6	11 (100)	1 (9.1)	10 (90.9)	
Tumor site				
Abdominal	11 (100)	1 (9.1)	10 (90.9)	0.253
Extra-abdominal	5 (100)	0 (0)	5 (100)	
Intra-abdominal	1 (100)	0 (0)	1 (100)	
Margin status				
Negative	9 (100)	1 (11.1)	8 (88.9)	0.084
Positive	8 (100)	0 (0)	8 (100)	
Recurrence				
Not recurrent	15 (100)	1 (6.7)	14 (93.3)	0.125
Recurrent	2 (100)	0 (0)	2 (100)	

COX-2, cyclooxygenase-2.

Table 3 Correlation between estrogen receptor-β immunohistochemical expression and different clinicopathologic variables in studied desmoid cases

Clinicopathologic variables	*N* (%)	ERβ [*n* (%)]			*P* value
		Negative	Low expression	High expression	
Age (years)					
<33	10 (100)	4 (40)	0 (0)	6 (60)	0.071
≥33	7 (100)	2 (28.6)	3 (42.8)	2 (28.6)	
Sex					
Male	5 (100)	3 (60)	0 (0)	2 (40)	0.279
Female	12 (100)	3 (25)	3 (25)	6 (50)	
Tumor size (CM)					
<6	6 (100)	1 (16.7)	2 (33.3)	3 (50)	0.329
≥6	11 (100)	5 (45.5)	1 (9.0)	5 (45.5)	
Tumor site					
Abdominal	11 (100)	5 (45.5)	2 (18.2)	4 (36.3)	0.253
Extra-abdominal	5 (100)	0 (0)	1 (20)	4 (80)	
Intra-abdominal	1 (100)	1 (100)	0 (0)	0 (0)	
Margin status					
Negative	9 (100)	3 (33.3)	0 (0)	6 (66.7)	0.084
Positive	8 (100)	3 (37.5)	3 (37.5)	29 (25)	
Recurrence					
Not recurrent	15 (100)	4 (26.7)	3 (20)	8 (53.3)	0.125
Recurrent	2 (100)	2 (100)	0 (0)	0 (0)	

ERβ, estrogen receptor-β.

Table 4 Correlation between estrogen receptor-β and cyclooxygenase-2 immunohistochemical expression in studied desmoid cases

COX-2	*N* (%)	ERβ [*n* (%)]			*P* value
		Negative	Low expression	High expression	
Negative	1 (100)	0 (0)	0 (0)	1 (100)	0.550
Positive	16 (100)	6 (37.5)	3 (18.7)	7 (43.8)	

COX2, cyclooxygenase-2; ERβ, estrogen receptor-β.

COX-2 expression was nonsignificant ($P>0.05$). Similar figures were reported by Zhang *et al*. [9], as 80.5% of their studied desmoid tumors were positive for immunohistochemical expression of ERβ; most (67.5%) showed high-level expression, and 13% showed low-level expression.

Other investigators reported positive ERβ expression in almost majority of their cases as Deyrup *et al*. [5] showed that 100% of their studied extra-abdominal desmoid tumors were positive for ERβ. Santos *et al*. [21] also stated that 89% of their desmoid tumor cases showed positivity for ERβ without statistically significant correlation with all clinical parameters.

In contrast, Leithner *et al*. [22], reported low expression of ERβ in their desmoid tumors (7/80); four out of 46 cases in extra-abdominal, two out of 21 in abdominal, and one out of 13 in intra-abdominal cases. Ishizuka *et al*. [23] also reported a low percentage of ERβ expression in two out of 27 desmoid tumors (7.4%). Different antibodies used and the different sample size may contribute to this diversity in reported results.

Conclusion

To sum up, we reported a high percentage of COX-2 and ERβ immunohistochemical expression in tumor cells of the examined desmoid tumors. These observations, recommend antihormonal therapy and COX-2 inhibitors as possible therapeutic targets in the management of desmoid tumors. More prospective trials with a larger sample size and follow-up after treatment are needed to validate these results.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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