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Oestrogen receptor-alpha and -beta expression in breast implant capsules: Experimental findings and clinical correlates

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Summary Myofibroblasts provide a force to decrease the surface area of breast implant capsules as the collagen matrix matures. 17-β-Oestradiol promotes myofibroblast differentiation and contraction. The aim of the study was to investigate the expression of oestrogen receptors α and β in capsular tissue. The study enrolled 70 women (80 capsules) who underwent expander or implant removal, following breast reconstruction. Specimens were stained with haematoxylin/eosin, Masson trichrome and immunohistochemistry and immunofluorescence stainings for alpha-smooth muscle actin (α-SMA), oestrogen receptor-alpha (ER-α) and oestrogen receptor-beta (ER-β). The relationship between anti-oestrogenic therapy and capsular severity was evaluated. A retrospective analysis of 233 cases of breast reconstruction was conducted. Myofibroblasts expressed ER-α, ER-β or both. In the whole sample, α-SMA score positively correlated with ER-α ($p = 0.022$) and ER-β expression ($p < 0.004$). ER-β expression negatively correlated with capsular thickness ($p < 0.019$). In capsules surrounding expanders α-SMA and ER-α, expressions negatively correlated with time from implantation ($p = 0.002$ and $p = 0.016$, respectively). The incidence of grade III–IV contracture was higher in patients who did not have anti-oestrogenic therapy ($p < 0.036$); retrospective analysis of 233 cases confirmed this finding ($p < 0.0001$). This study demonstrates the expression of oestrogen receptors in myofibroblasts of capsular tissue. A lower contracture severity was found in patients who underwent anti-oestrogenic therapy.

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Breast implant placement is among the most common procedures performed by plastic surgeons. In 2012, more than 214,000 women underwent breast augmentation in Europe[1]; in the same year, more than 457,000 breast implants were placed for both reconstructive and aesthetic purposes (unpublished data, from Ernst & Young breast implants statistics). Capsular contracture is the most common complication of breast heterologous reconstruction and augmentation, with a reported incidence ranging from 1.3% to 30% in the largest studies.[2] Moreover, it is the leading cause of reintervention in these patients.[3] Although several risk factors have been identified, actually, the exact nature and contribution of molecular, immunological and microbiological factors remain unclear. Risk factors for capsular contracture are: radiotherapy[2]; implantation for reconstructive purposes[4,5]; smooth implants[6–11]; silicone shell, when compared to the polyurethane one[12]; subglandular placement[13] and implant rupture, smoking, collagenopathies and postoperative haematomas.[2] There are contrasting pieces of evidence about the hypothesis of an infectious aetiology.[14–19] Furthermore, recently, a study by Dancey et al.[20] retrospectively analysed 1400 cases of breast augmentation and reported pregnancy to be a risk factor for capsular contracture.

Histologically, capsular tissue presents as a three-layered structure,[21] with the middle layer being the most important in the pathogenesis of contracture.[22] Myofibroblasts are contractile fibroblasts that provide a sustained force to decrease the surface area of the capsule as the collagen matrix remodels and matures, thus stabilising contracture.[23] 17-β-Oestradiol has been shown to increase the contraction of myofibroblasts and the production of type 1 collagen, fibronectin and transforming growth factor-β (TGF-β).[24,25] TGF-β is largely present in capsular tissue.[26] TGF-β as well as mechanical stress and the ED-A splicing variant of fibronectin are among the main stimuli involved in the transition from fibroblast to myofibroblast.[27] However, tamoxifen, a selective oestrogen receptor modulator (SERM), has been demonstrated to reduce TGF-β production and myofibroblast contraction.[28,29] The main receptors for 17-β-oestradiol, as well as the targets of tamoxifen, are oestrogen receptor-alpha (ER-α) and oestrogen receptor-beta (ER-β).[25] The aims of the study were to investigate the expression of ER-α and ER-β in capsular tissue and to evaluate their possible significance in the pathogenesis of capsular contracture.

Methods

Patients

The study enrolled 70 patients (80 capsules) treated at our institution between January 2010 and November 2011. The mean age of the patients was 53 years; 57 patients suffered from ductal breast cancer and 13 had lobular breast cancer. Specimens were taken during surgery for expander substitution with definitive implant (53 specimens) or definitive implant removal (27 specimens). Inclusion criteria were previous radical mastectomy according to Madden's technique,[30] submuscular placement and texturised surface of the prosthesis. Exclusion criteria were implant rupture, infection and radiotherapy. Contracture severity was evaluated according to Baker's classification[31]: four specimens were taken from grade I contractures, 32 from grade II, 38 from grade III and six from grade IV. A total of 48 capsules were taken from patients who had anti-oestrogenic therapy (tamoxifen, aromatase inhibitors and/or gonadotropin-releasing hormone (GnRH) analogues), while 32 capsules were taken from patients who did not. Specimens were grouped into two categories: uncontracted capsules (Baker's grades I and II) and contracted capsules (Baker's grades III and IV). The time from implantation was expressed in months and ranged from 4 to 312 months (mean time was 40.8 months). A retrospective analysis of 233 cases of breast reconstruction that met the inclusion criteria was conducted in regard to anti-oestrogenic therapy and contracture severity. The study was approved by the ethics committee of "Campus Bio-Medico of Rome" University and written informed consent was obtained from every patient.

Histopathology, immunohistochemistry and immunofluorescence

Tissue specimens for haematoxylin and eosin and Masson's trichrome stainings were routinely processed. Immunohistochemical and immunofluorescence stainings were performed on 3–5-μm tissue sections as previously described; the indirect technique was used.[32–34] The following antibodies were used: mouse monoclonal antibody for the alpha subunit of smooth muscle actin (α-SMA, 1:50 titre, 1A4 clone; Dako, Glostrup, Denmark), rabbit monoclonal antibody for ER-α (1:100 titre, SP1 clone; Dako, Glostrup, Denmark) and rabbit polyclonal antibody for ER-β (1:50 titre, ab3577; Abcam, Cambridge, MA, USA). Sections were then incubated with the appropriate secondary biotinylated antibody labelled with the avidin–biotin complex (LSAB, Dako, Glostrup, Denmark). Light microscopy images were captured by a videocam (SPOT Insight; Diagnostic Instrument, Inc., Sterling Heights, MI, USA) connected to an Olympus BX-51 light microscope (Olympus, Tokyo, Japan) and processed with an image analysis system (Delta Sistemi, Rome, Italy). Immunofluorescence was performed using the same primary antibodies except for the evaluation of the co-expression of ER-α/ER-β, where we used a polyclonal goat antibody for ER-β (1:50 titre; L20, Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). Sections were then incubated with anti-rabbit and anti-mouse secondary antibodies conjugated to IRIS 3.5 and IRIS 2, respectively (with anti-goat IRIS 5 and anti-mouse IRIS 2 for the co-expression of ER-α/ER-β; 1:200 titre; Cyanine Technologies, Turin, Italy). Images were acquired with a Leica Leitz DMRB microscope (Leica Microsystems GmbH, Wetzlar, Germany) and the software SimplePCI (Hamamatsu Corporation, Sewickley, PA, USA). Negative controls were processed without primary antibody for every staining. Capsular thickness was measured on specimens stained with haematoxylin and eosin or Masson trichrome at 40× magnification in five randomly chosen fields, then averaged. Immunohistochemical positivity of α-SMA, ER-β and ER-α was evaluated at 200× magnification in five randomly chosen fields, averaged and scored as follows: 0 (≤5% of positive cells), 1 (6–25% of positive cells), 2 (26–50% of