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The role of angiogenesis, inflammation and estrogen receptors in breast implant capsules development and remodeling

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Summary *Background:* Capsular contracture is the most common complication following breast implant placement. The multiple factors unbalancing the physiological response to the foreign body have not been fully elucidated. The aim of this study was to investigate the role of neo-angiogenesis, inflammation and estrogen receptors in peri-prosthetic tissue development and remodeling.
Methods: The study enrolled 31 women who underwent expander substitution with definitive implant. Specimens were stained with hematoxylin/eosin, Masson trichrome, immunohistochemistry and immunofluorescence for alpha-smooth muscle actin, estrogen receptor-α (ER-α), estrogen receptor-β (ER-β), Collagen type I and III, CD31 (as a marker of neo-angiogenesis) and vascular endothelial growth factor (VEGF). Inflammatory infiltration was quantified and analyzed. Transmission electron microscopy was performed for ultrastructural evaluation.
Results: Myofibroblasts, mainly localized in the middle layer of capsular tissue, expressed VEGF, ER-α and ER-β. ER-β expression positively correlated with Collagen type I deposition ($p = 0.025$). Neo-angiogenesis was predominant in the middle layer. CD31 expression positively

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correlated with Collagen type I expression ($p = 0.009$) and inflammatory infiltration grade ($p = 0.004$). The degree of inflammatory infiltration negatively correlated with the time from implantation ($p = 0.022$).
Discussion: The middle layer is key in the development and remodeling of capsular tissue. Myofibroblasts produce VEGF, that induces neo-angiogenesis. New vessels formation is also correlated to the inflammatory response. Collagen deposition is associated with ER-β expression and neo-angiogenesis. These findings may prelude to targeted pharmacologic therapies able to control such interactions, thus hampering the self-sustaining loop promoting the progression of physiologic fibrosis toward pathologic contracture.

Introduction

Capsular contracture (CC) is the most common complication following breast implant placement, with a reported incidence ranging from 1.3 to 30%.[1] It is the leading cause for reoperation in these patients.[2] On the other hand, capsular tissue is also a versatile and reliable source of flaps and grafts to address complications, such as implant malposition and contour deformities.[3] In spite of worldwide efforts, to date, the genetic, molecular, immunological and environmental factors involved in and often imbalancing the physiological response to the foreign body are not fully understood.

The reaction against the prosthesis starts with the recruitment of polymorphonuclear cells and macrophages, which promote the inflammatory process by secreting cytokines and growth factors, such as transforming growth factor-β (TGF-β). These proteins are among the main stimuli promoting the differentiation of fibroblasts into contractile myofibroblasts. Myofibroblasts produce collagen and provide a sustained force to decrease the surface area of the capsule as the collagen matrix remodels and matures.[4] They also secrete pro-inflammatory and pro-fibrotic cytokines, thus inducing a self-amplifying autocrine and paracrine loop ultimately promoting myofibroblast differentiation and collagen production.[5] The hormonal status was recently linked to these processes. Dancey and colleagues found post-operative pregnancy to be a risk factor for capsular contracture.[6] In 2014, Persichetti et al. reported lower contracture severity in reconstructive patients undergoing anti-estrogenic therapy. Moreover, they described that estrogen receptors-alpha (ER-α) and -beta (ER-β) were expressed by myofibroblasts of capsular tissue.[7] In-depth analysis of the interplays among the multiple factors involved in capsule formation and remodeling would be paramount to address contracture as well as to better use capsular tissue in revision procedures. The aim of this study was to investigate the role of neo-angiogenesis and estrogen receptors expression in peri-prosthetic tissue remodeling.

Materials and methods

The study enrolled 31 patients (32 capsules) treated at our Institution between January 2012 and December 2014. Mean age of the patients was 56 years. Contracture severity was evaluated according to Baker's classification.[8] The time from implantation was expressed in months. All the specimens were harvested during surgery for expander substitution with definitive implant. Inclusion criteria were previous radical mastectomy with sparing of the pectoralis major muscle, submuscular implant placement and texturized silicone surface. Exclusion criteria were implant rupture, infection and radiotherapy. No patient underwent chemotherapy. The study was approved by the local ethic committee and a written consent was signed by every patient.

Histopathology, immunohistochemistry and immunofluorescence

Tissue specimens for hematoxylin 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.[9] The following antibodies were used: mouse monoclonal antibody for the alpha subunit of smooth muscle actin (α-SMA, 1A4 clone; Dako, Glostrup, Denmark), rabbit monoclonal antibody for ER-α (SP1 clone; Dako, Glostrup, Denmark), rabbit polyclonal antibody for ER-β (ab3577; Abcam, Cambridge, USA), rabbit polyclonal antibody for human Collagen type I (Collagen I, Polyclonal, AbD Serotec), rabbit polyclonal antibody for human Collagen type III (Collagen III, Polyclonal, AbD Serotec), and mouse monoclonal antibody for CD31 or PECAM-1 (CD31, BC2 clone, Biocare Medical).

Microscopic images were captured by a Videocam (SPOT Insight; Diagnostic Instrument, Inc., Sterling Heights, MI) connected to an Olympus BX-51 light microscopy (Olympus, Tokyo, Japan) and processed with an Image Analysis System (Delta Sistemi, Rome, Italy). Immunofluorescence and double-labeling experiments were performed using the same primary antibodies, a polyclonal goat antibody for ER-β (1:50 dilution; L20 clone, Santa Cruz Biotechnology Inc., Santa Cruz, CA), as previously reported,[9] and a polyclonal rabbit antibody for vascular endothelial growth factor (VEGF) (A-20, 1:100 dilution; sc-152 clone, Santa Cruz Biotechnology Inc., Santa Cruz, CA). Incubation with primary antibodies was followed by Alexa Fluor 488 (green; dilution 1:50; goat anti-mouse; Invitrogen, USA) and by Alexa Fluor 568 (red; dilution 1:50; donkey anti-rabbit/goat Invitrogen, USA). Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA) containing 4,6-diamidino-2-phenylindole (Molecular Probes, Eugene, OR, USA) was used for nuclear contrasting. Images were acquired with Leica Leitz DMRB microscope (Leica Microsystems GmbH, Wetzlar, Germany) and the software Simple PCI (Hamamatsu Corporation, Sewickley, PA, USA). Capsular thickness was measured on