Tumor Biol.
DOI 10.1007/s13277-016-5268-7

ORIGINAL ARTICLE

Interleukin-6 receptor in spindle-shaped stromal cells, a prognostic determinant of early breast cancer

Labovsky Vivian[1] · Martinez Leandro Marcelo[1] · Calcagno María de Luján[2] · Davies Kevin Mauro[3] · García-Rivello Hernán[3] · Wernicke Alejandra[3] · Feldman Leonardo[4] · Giorello María Belén[1] · Matas Ayelén[1] · Francisco Raúl Borzone[1] · C. Howard Scott[5] · Chasseing Norma Alejandra[1]

Received: 18 March 2016 / Accepted: 15 July 2016

Abstract Spindle-shaped stromal cells, like carcinoma-associated fibroblasts and mesenchymal stem cells, influence tumor behavior and can serve as parameters in the clinical diagnosis, therapy, and prognosis of early breast cancer. Therefore, the aim of this study is to explore the clinicopathological significance of tumor necrosis factor-related apoptosis-induced ligand (TRAIL) receptors (Rs) 2 and 4 (TRAIL-R2 and R4), and interleukin-6 R (IL-6R) in spindle-shaped stromal cells, not associated with the vasculature, as prognostic determinants of early breast cancer patients. Receptors are able to trigger the migratory activity, among other functions, of these stromal cells. We conducted immunohistochemical analysis for the expression of these receptors in spindle-shaped stromal cells, not associated with the vasculature, of primary tumors from early invasive breast cancer patients, and analyzed their association with clinicopathological characteristics. Here, we demonstrate that the elevated levels of TRAIL-R2, TRAIL-R4, and IL-6R in these stromal cells were significantly associated with a higher risk of metastatic occurrence (p = 0.034, 0.026, and 0.006; respectively). Moreover, high expression of TRAIL-R4 was associated with shorter disease-free survival and metastasis-free survival (p = 0.013 and 0.019; respectively). Also, high expression of IL-6R was associated with shorter disease-free survival, metastasis-free survival, and overall survival (p = 0.003, 0.001, and 0.003; respectively). Multivariate analysis showed that IL-6R expression was an independent prognostic factor for disease-free survival and metastasis-free survival (p = 0.035). This study is the first to demonstrate that high levels of IL-6R expression in spindle-shaped stromal cells, not associated with the vasculature, could be used to identify early breast cancer patients with poor outcomes.

Keywords Breast cancer · Cancer-associated fibroblasts · Interleukin-6 receptor · Mesenchymal stem cells · Spindle-shaped stromal cells

✉ Chasseing Norma Alejandra
achasseing@ibyme.conicet.gov.ar; alejachase@gmail.com

[1] Laboratorio de Inmunohematología, Instituto de Biología y Medicina Experimental (IBYME) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Vuelta de Obligado 2490, CP 1428, Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

[2] Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, CP 1113, Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

[3] Departamento de Anatomía Patológica, Hospital Italiano, Juan Domingo Perón 4190, CP 1181, Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

[4] Departamento de Trasplante de Medula Ósea, Fundación Favaloro, Solís 443, CP 1428, Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

[5] University of Memphis, 3720 Alumni Ave, 38152, Memphis, TN, USA

Introduction

Breast epithelium genetic changes can induce cancer. However, the components of stromal microenvironment can favor tumor progression [1]. The stromal compartment is composed of stem cells, cancer-associated fibroblasts (CAFs), endothelial cells, immune cells, adipocytes, cytokines, and various types of macromolecules comprising the extracellular matrix [2]. Breast cancer cells release factors that act on stromal cells, changing the composition and function of normal tissue, resulting in stromal reactions such as angiogenesis, bone marrow mesenchymal

Published online: 27 July 2016

stem cell recruitment, and CAF development, among other inflammatory responses [1, 3–5].

CAFs are spindle-shaped stromal cells, CD34(−), that represent the major component of this inflammatory tumor microenvironment. They modify the composition of the extracellular matrix by increasing collagen concentration, an event called desmoplastic reaction [1–3, 6, 7]. CAFs and mesenchymal stem cells (MSCs, one source of CAFs), among others, promote tumor progression inducing self-renewal of cancer stem cells, as well as epithelial-mesenchymal transition, epigenetic changes, proliferation, migration, and invasion of tumor cells [1, 2, 7–12]. Therefore, the role of both types of splindle-shaped stromal cells can lead to a chemo/endocrine and target resistance in early breast cancer patients (BCPs) [12–16].

Recently, studies showed that CAFs and MSCs intra vasate and extravasate blood or lymphatic vessels along with cancer cells, favor the evolution of metastatic cascade [2, 7, 17–20]. Monteiro AC et al. [21] suggested the idea that "engraftment of breast tumor cells is supposed to be preceded by changes in the metastatic target tissue to create a permissive microenvironment, the pre-metastatic niche, for the establishment of the metastatic foci." It is known that the primary tumor cells orchestrate pre-metastatic niche formation through secretion of a variety of cytokines and growth factors that promote mobilization and recruitment of bone marrow-derived cells (macrophage, endothelial progenitors, hematopoietic cells, MSCs) as well as other stromal cells, like CAFs, to future metastatic sites [22].

In previous studies, we found that spindle-shaped stromal cells, not associated with the vasculature, sampled from patients with breast cancer in early clinicopathological stages (I–II), express tumor necrosis factor-related apoptosis-induced ligand (TRAIL) receptors (Rs) 2 and 4 (TRAIL-R2 and R4), and interleukin-6 R (IL-6R) at significantly higher levels compared with values of non-neoplastic breast tissues [23]. Furthermore, IL-6R expression in these stromal cells had a significant positive association with stromal cell-derived factor 1 (SDF-1) and IL-6 expression in tumor breast cells of primary tumors from these patients [23]. It was described that these evaluated receptors are able to increase the migratory activity of CAFs as well as MSCs [20, 24, 25]. Consequently, as MSCs and CAFs preferentially migrate to tumors and sites of tissue injury, it is likely that they may prepare the sites for subsequent cancer cell colonization [26]. This perspective could explain the evidence that both spindle-shaped stromal cells behave in a pro-metastatic manner.

Taking into consideration all these findings, we explored the clinicopathological significance of these receptors in spindle-shaped stromal cells, not associated to the vasculature, as prognostic determinant of early BCPs.

Materials and methods

Patients

We conducted a retrospective study of 63 consecutive patients (age range 42–80 years) with breast cancer who underwent initial surgery at the Italian Hospital, Buenos Aires, Argentina. Key inclusion criteria were women with breast infiltrative ductal carcinoma, (I and II stages, following International Union Against Cancer TNM classification) and a minimum post-surgery period of 10 years (the cases were diagnosed between 2001 and 2004). Exclusion criteria included neoadjuvant therapies, lack of tissue, and another primary tumor development. After surgery, all patients were treated with the indicated therapy, depending on their clinical status and the histopathological characteristics of their tumor, which were determined according to the recommendations of the European Society for Medical Oncology [27]. The Ethics Committee of the Institute of Biology and Experimental Medicine and Italian Hospital approved this study, and informed consent was obtained from patients or the relatives of deceased patients. This work was performed in accordance with the principles of the Helsinki Declaration.

The following clinicopathological characteristics were included in patient records: age, tumor size, histological grade, HER2/neu status, expression of estrogen receptors (ERs) and progesterone receptors (PRs), regional lymph node status (*classical prognostic markers*), as well as metastatic recurrence, local relapse, disease-free survival (DFS), metastasis-free survival (MFS), and overall survival (OS).

Analysis of the classical prognostic markers was determined as previously described [28]. Briefly, in the case of hormonal receptor and HER2/neu, expression was considered to be negative or positive according to Hammond et al. and Wolff et al., respectively (Table 1) [29, 30]. The determination of DFS, MFS, and OS was made as previously described by Martinez et al. [28].

Analysis of protein expression

Breast tissues were processed according to Labovsky et al. [23]. Immunohistochemistry protocol was used to determine the levels of TRAIL-R2, TRAIL-R4, and IL-6R in spindle-shaped stromal cells not associated with the vasculature and it was completed as described in a previous work [23]. The immunohistochemical signal was scored based on Allred score [29]. In particular, the percentages of positive cells were assigned scores as the following: 0 (<10 %), 1 (10–30 %), 2 (31–60 %), 3 (61–90 %), and 4 (>90 %). Staining intensity was scored as 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong), according to relative intensity of cytokeratin AE1-AE3 expression [23]. The final staining score was calculated using the sum of the percentage of positive cells and the

Table 1 Clinicopathological characteristics of 63 patients with early invasive ductal breast cancer

Clinicopathological characteristics	Patients (*n*)	Patients (%)
Age (years)		
<50	10	15.9
≥50	53	84.1
Unknown	–	–
Tumor size (cm)		
<2	45	71.4
≥2	17	27.0
Unknown	1	1.6
Histological grade		
G1	15	23.8
G2	22	34.9
G3	24	38.1
Unknown	2	3.2
HER2/neu status		
Negative	43	68.2
Positive	19	30.2
Unknown	1	1.6
ER status		
Negative	13	20.6
Positive	49	77.8
Unknown	1	1.6
PR status		
Negative	13	20.6
Positive	49	77.8
Unknown	1	1.6
Regional lymph nodes		
Negative	44	69.8
Positive	16	25.4
Unknown	3	4.8
Local relapse		
Negative	50	79.4
Positive	6	9.5
Unknown	7	11.1
Metastatic event		
Non-metastasis	45	71.4
Metastasis	11	17.5
Unknown	7	11.1

HER2/neu human epidermal growth factor receptor 2, *ER* estrogen receptor, *PR* progesterone receptor

staining intensity score, which ranged from 0 to 7. Stromal cells included in this study presented spindle shape and were not associated with vasculature. CD34 expression was undetectable in this type of stromal cells as previously demonstrated [28].

The agreement in immunohistochemical evaluation between two observers was 91.77 % (Kappa value = 0.895).

Statistical analysis

The statistical analysis of the associations between receptor expression and clinicopathological characteristics, as well as the determination of the optimal cutoff value, was made as previously described by Martinez LM et al. [28]. The cutoff value was used to assign an expression in samples as negative/low or high. To determine the optimal cutoff value, the first quartile (Q1), median, and the third quartile (Q3) values were used for the binomial classification of samples. We then individually tested the association between different categorized receptor expressions and OS of patients in a univariate analysis. The cutoff value with the lowest *p* value was chosen. The optimal cutoff values for receptor expression were as follows: TRAIL-R2 = 5 (Q3), TRAIL-R4 = 5 (median), and IL-6R = 6 (Q3). We used Fisher's exact test to evaluate the association of the expression of these receptors with classical prognostic markers as well as local relapse and metastatic occurrence.

Moreover, the association between the receptor expression and metastatic occurrence is displayed as a heat map prepared using Excel.

The Cox proportional hazards model to the multivariate survival analysis was applied using backward stepwise selection (likelihood ratio) incorporating only the significant variables in the univariate analysis.

Results

Association of TRAIL-R2, TRAIL-R4, and IL-6R expression in spindle-shaped stromal cells with patients' clinicopathological characteristics

The expression of IL-6R was significantly associated with age ($p = 0.032$; Table 2). Specifically, IL-6R expression was high in 3/8 patients <50 years of age and 3/48 patients ≥50 years of age (Table 2). Moreover, IL-6R expression was significantly associated with HER2/neu status ($p = 0.009$; Table 2). High levels of IL-6R expression were detected in 5/17 patients with positive HER2/neu and 1/38 patient with negative Her2/neu (Table 2). Furthermore, IL-6R expression was associated with ER status ($p = 0.023$; Table 2). High IL-6R expression was observed in 4/13 patients with negative ER and 2/42 patients with positive ER (Table 2).

The patients with a high expression of TRAIL-R2, TRAIL-R4, and IL-6R in spindle-shaped stromal cells were at significantly higher risk for metastatic occurrence than patients with low expression ($p = 0.034$, 0.026, and 0.006; respectively; Table 2, Fig. 1). A high expression of TRAIL-R2 was observed in 5/11 patients with metastatic tumors and 6/43 patients with non-metastatic tumors (Table 2). In patients with metastatic or non-metastatic tumors, 7/11 and 11/45 expressed high levels of TRAIL-R4, respectively (Table 2). High levels

Table 2 Association of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor (R)-2 (TRAIL-R2), TRAIL-R4, and interleukin-6 R (IL-6R) expression in spindle-shaped stromal cells, not associated with the vasculature, of primary tumor with clinicopathological characteristics in early breast cancer patients

Clinicopathological characteristics		Receptors in spindle-shaped stromal cells								
		TRAIL-R2			TRAIL-R4			IL-6R		
		n	High expression (%)	*p*	*n*	High expression (%)	*p*	*n*	High expression (%)	*p*
Age (years)	<50	9	0 (0.0)	0.102	10	2 (20.0)	0.302	8	3 (37.5)	0.032*
	≥50	52	14 (2.9)		53	21 (39.6)		48	3 (6.3)	
Tumor size (cm)	<2	43	10 (23.3)	>0.999	45	17 (37.8)	>0.999	38	3 (7.9)	0.359
	≥2	17	4 (23.5)		17	6 (35.3)		17	3 (17.6)	
Histological grade	G1	15	4 (26.7)	0.508	15	5 (33.3)	0.840	13	2 (15.4)	0.150
	G2	20	3 (15.0)		22	9 (40.9)		18	0 (0.0)	
	G3	24	7 (29.2)		24	8 (33.3)		24	4 (16.7)	
HER2/neu status	Negative	41	8 (19.5)	0.338	43	15 (34.9)	0.776	38	1 (2.6)	0.009*
	Positive	19	6 (31.5)		19	8 (42.1)		17	5 (29.4)	
ER status	Negative	13	5 (38.5)	0.159	13	5 (38.5)	>0.999	13	4 (30.8)	0.023*
	Positive	47	9 (23.3)		49	18 (36.7)		42	2 (4.8)	
PR status	Negative	13	5 (35.7)	0.159	13	4 (30.8)	0.751	13	2 (15.4)	0.619
	Positive	47	9 (19.6)		49	19 (38.8)		42	4 (9.5)	
Regional lymph nodes	Negative	43	11 (25.6)	0.742	44	16 (36.4)	>0.999	40	5 (12.5)	0.678
	Positive	15	3 (20.0)		16	6 (37.5)		14	1 (7.1)	
Local relapse	Negative	49	10 (20.4)	>0.999	51	15 (29.4)	0.314	45	3 (6.7)	0.072
	Positive	5	1 (20.0)		5	3 (60.0)		5	2 (40.0)	
Metastatic occurrence	Negative	43	6 (13.9)	0.034*	45	11 (24.4)	0.026*	39	1 (4.6)	0.006*
	Positive	11	5 (45.4)		11	7 (63.6)		11	4 (36.4)	

The association between variables was performed using the Fisher exact test

HER2/neu human epidermal growth factor receptor 2, *ER* estrogen receptor, *PR* progesterone receptor

*Statistically different, $p < 0.05$

of IL-6R were expressed in 4/11 B.P. with metastasis and 1/39 patient with non-metastatic tumors (Table 2).

There was an association of high TRAIL-R4 expression in spindle-shaped stromal cells with shorter DFS and MFS ($p = 0.013$ and 0.019; respectively; Table 3 and Fig. 2). The values of patients' DFS and MFS for high and low TRAIL-R4 expression were (X+/−SE, in months) as follows: DFS = 94.13 ± 14.15 vs. 135.13 ± 7.87; and MFS = 101.46 ± 13.23 vs. 139.08 ± 7.06; respectively.

Moreover, there was an association of high IL-6R expression in spindle-shaped stromal cells with shorter DFS, MFS, and OS ($p = 0.003$, 0.001, and 0.003; respectively; Table 3, Fig. 3). The values of DFS, MFS, and OS of patients with high and low IL-6R expression were (X+/−SE, in months) as follows: DFS = 56.80 ± 20.11 vs. 127.85 ± 8.02; MFS = 63.40 ± 19.06 vs. 134.29 ± 7.06; and OS = 84.20 ± 22.62 vs. 143.64 ± 5.11; respectively.

Univariate analysis of the association of classical prognostic markers with DFS, MFS, and OS

Of all the clinicopathological characteristics analyzed, only tumor size was associated with MFS ($p = 0.020$; Table 3). Patients with tumors >2 cm had earlier metastasis compared

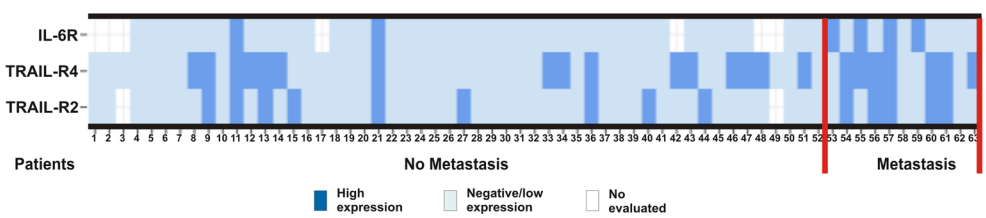

Fig. 1 Heat map graph showing the association of receptor expression in spindle-shaped stromal cells, not associated with the vasculature, of primary tumors with metastatic occurrence in early breast cancer patients. *TRAIL-R2* and *4* tumor necrosis factor-related apoptosis-inducing ligand receptor types 2 and 4, *IL-6R* interleukin-6 receptor

Table 3 Univariate analysis of classical prognostic markers and receptor expression in spindle-shaped stromal cells, not associated with the vasculature, of primary tumors with disease-free survival, metastasis-free survival, and overall survival in early breast cancer patients

Univariate analysis	Disease-free survival *p*	Metastasis-free survival *p*	Overall survival *p*
Age	0.598	0.448	0.500
Tumor size	0.113	0.020*	0.069
Histological grade	0.178	0.291	0.207
HER2/neu status	0.966	0.799	0.590
ER status	0.549	0.376	0.454
PR status	0.851	0.884	0.408
Regional lymph nodes	0.595	0.805	0.620
TRAIL-R2/stromal cells	0.189	0.070	0.221
TRAIL-R4/stromal cells	0.013*	0.019*	0.475
IL-6R/stromal cells	0.003*	0.001*	0.003*

The association between variables was performed using log-rank (Mantel-Cox)-test

TRAIL-R2 and 4 tumor necrosis factor-related apoptosis-inducing ligand receptor types 2 and 4, *IL-6R* interleukin-6 receptor

*Statistically different, $p < 0.05$

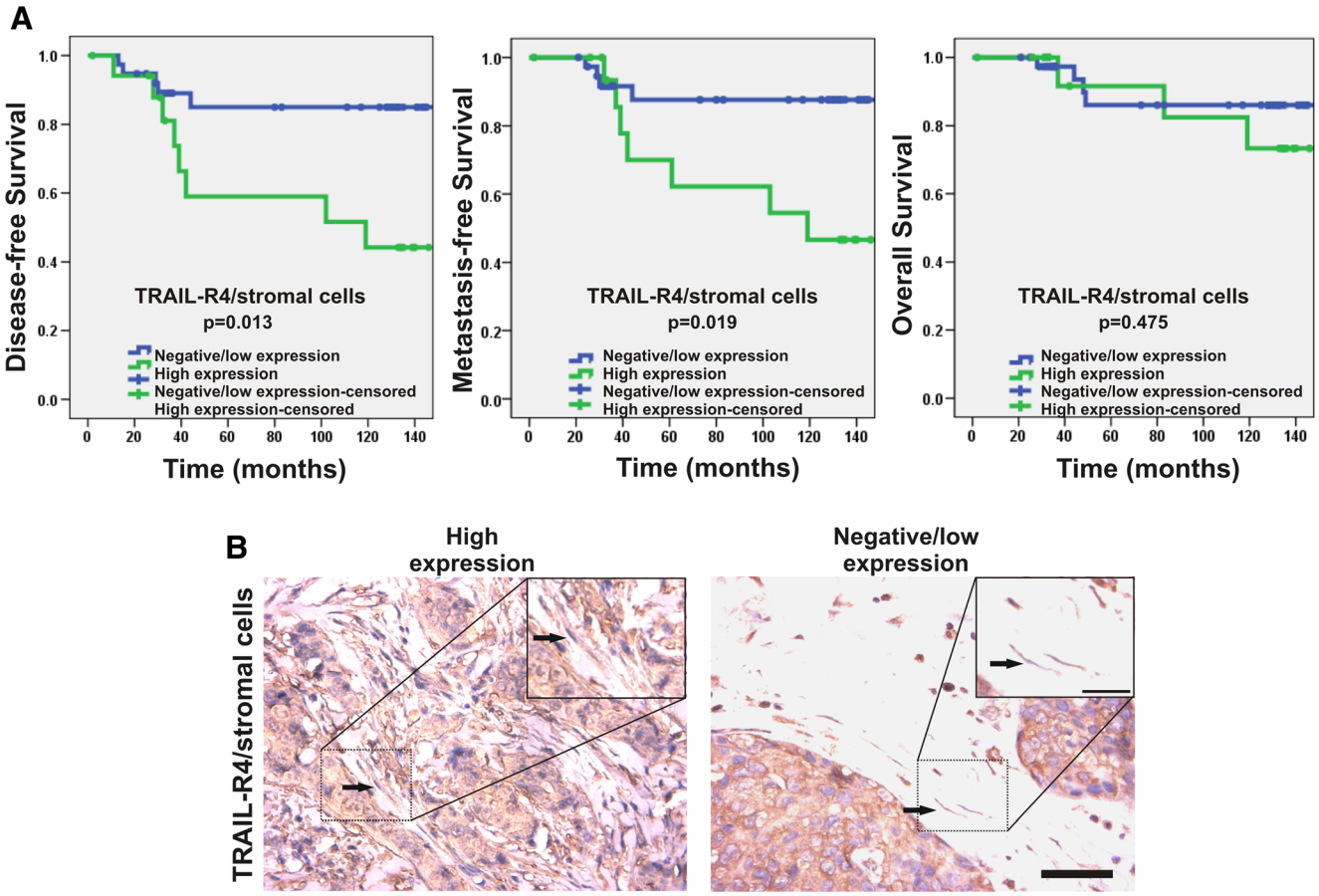

Fig. 2 Association of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor (R) 4 (TRAIL-R4) expression in spindle-shaped stromal cells, not associated with the vasculature, of primary tumors with disease-free survival, metastasis-free survival, and overall survival in early breast cancer patients. **a** Kaplan-Meier curves show that high stromal cell expression of TRAIL-R4 is associated with a shorter disease-free survival and metastasis-free survival [$p = 0.013$ and 0.019, respectively, by log-rank (Mantel-Cox) test]. **b** Photographs show a representative immunohistochemistry staining for tumor samples with high and negative/low expression of TRAIL-R4. The *arrows* show positive staining of evaluated stromal cells. *Inset* shows positive expression of TRAIL-R4 in stromal cells. Original magnification: ×400. The *scale bar* represents 50 and 20 μm in the *inset*

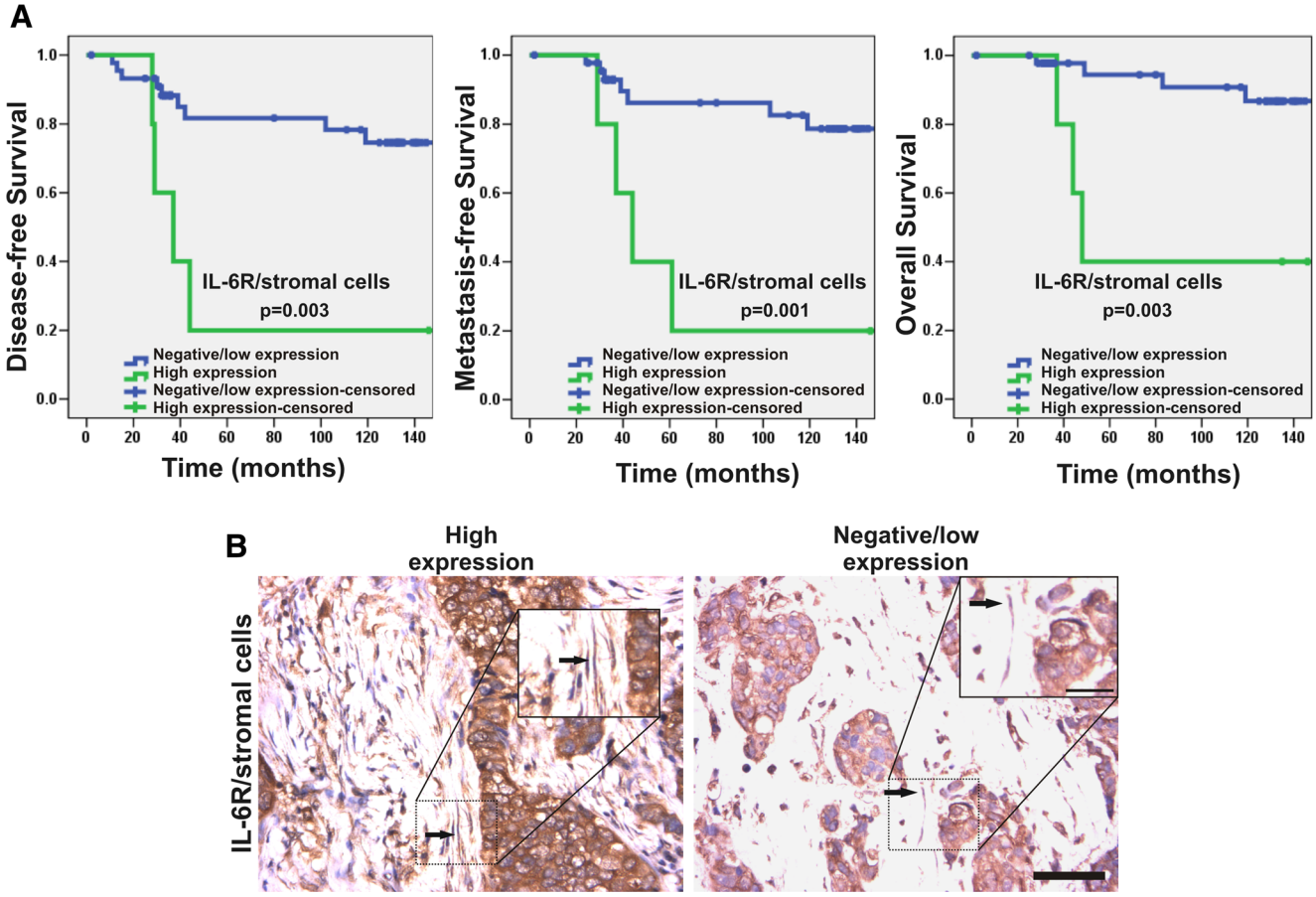

Fig. 3 Association of interleukin-6 R (IL-6R) expression in spindle-shaped stromal cells, not associated with the vasculature, of primary tumors with disease-free survival, metastasis-free survival, and overall survival in early breast cancer patients. **a** Kaplan-Meier curves show that high stromal cell expression of IL-6R is associated with a shorter disease-free survival, metastasis-free survival, and overall survival [p = 0.003, 0.001, and 0.003, respectively, by log-rank (Mantel-Cox) test]. **b** Photographs show a representative immunohistochemistry staining for tumor samples with high and negative/low expression of IL-6R. The *arrows* show positive staining of evaluated stromal cells. *Inset* shows positive expression of IL-6R in stromal cells. Original magnification: ×400. The *scale bar* represents 50 and 20 μm in the *inset*

with those with tumors ≤2 cm (X+/−SE, months): 93.00 ± 15.59 vs. 139.02 ± 6.47; respectively.

Multivariate analysis

L-6R expression in spindle-shaped stromal cells was an independent prognostic factor for DSF and MFS (p = 0.035 in both cases) (Table 4).

Discussion

In the last decade or so, with the progression of breast cancer studies, the importance of the tumor microenvironment is becoming clearer. In this type of solid cancer, the stroma plays an important function in tumor development, the progression from ductal carcinoma in situ to invasive carcinoma, as well as in the evolution of the metastatic cascade [9, 31, 32]. It is

Table 4 Multivariate analysis of disease-free survival and metastasis-free survival in early breast cancer patients

	Variables	HR	95 % CI	p
Disease-free survival	TRAIL-R4/stromal cell	2.615	0.819–8.351	0.105
	IL-6R/stromal cell	3.760	1.098–12.889	0.035*
Metastasis-free survival	Tumor size	3.955	1.124–13.910	0.032*
	TRAIL-R4/stromal cell	3.252	0.886–11.936	0.076
	IL-6R/stromal cell	4.016	1.106–14.586	0.035*

The Cox proportional hazards model [backward stepwise selection (likelihood ratio)] was applied to the multivariate survival

CI confidence interval, *HR* hazard ratio

*Statistically different, $p < 0.05$

known that the tumor/stroma ratio and stroma type are associated with the local relapse, distance metastasis, and survival of BCPs, indicating in particular the importance of the stromal cells and extracellular matrix [33–35].

Recently, it was described that tumor spindle-shaped stromal cells, like CAFs and MSCs, can serve as parameters in the clinical diagnosis, therapy, and prognosis of breast cancer. For example, Yamashita M and coauthors found that α-SMA expression in myofibroblasts or CAFs is an independent predictor of metastasis and poor prognosis in invasive BCPs [7, 36].

Our results showed that high expression of TRAIL-R2 and TRAIL-R4 in spindle-shaped stromal cells, not associated with the vasculature, was significantly associated with a higher risk of metastatic occurrence in early invasive BCPs. Moreover, patients with high TRAIL-R4 expression in these stromal cells presented shorter DFS and MFS. As we described before, these TRAIL receptors are able to induce the migratory capacity of mesenchymal stromal cells [24, 37], properties that could favor intravasation and extravasation processes of these stromal cells for the evolution of breast cancer pre-metastatic niches. Findings that indicate the importance of evaluating TRAIL-R2 and TRAIL-R4 expression in spindle-shaped stromal cells, not associated with the vasculature, prevent the use of TRAIL treatment in antiestrogen-resistant BCPs [38].

On the other hand, high IL-6R expression in these spindle-shaped stromal cells was significantly associated with positive HER2/neu and negative ER in patients <50 years of age. Moreover, patients that expressed high levels of IL-6R presented metastatic occurrence and shorter DFS, MFS, and OS. Furthermore, we found that IL-6R expression was an independent prognostic factor for DFS and MFS. In relation to the IL-6/IL-6R axis, Chang et al. have demonstrated that "the IL-6/Janus kinase (JAK)/signal transducer and activator of transcription 3 (Stat3) pathways drives tumor progression through the stroma and metastatic niche" [25]. The alteration of this ligand-receptor signaling modified the interaction between tumor and stromal cells in vivo, thus reducing fibroblastic infiltration and activity in both the primary tumor and the pre-metastatic niche [25, 39]. In addition, it is important to highlight that IL-6 is a chemoattractant for mesenchymal stromal cells, like MSCs and CAFs, which enhances their migratory activity [20, 25]. Therefore, these last data indicate the importance of evaluating IL-6R expression in this type of stromal cells since its expression plays a critical role as a pharmacological target that inhibits the evolution of metastatic cascade in early invasive BCPs.

In summary, this study is the first to demonstrate that high expressions of TRAIL-R2, TRAIL-R4, and IL-6R in spindle-shaped stromal cells, not associated to the vasculature, serve as prognostic determinants of metastatic occurrence in women with early invasive ductal breast cancer. In particular, IL-6R serves as a prognostic determinant of shorted DFS, MFS, and OS. These new findings provide a rationale for further studies designed to primarily target IL-6R signaling pathways to facilitate the diagnosis, prevention, and treatment of early invasive ductal breast cancers.

Acknowledgments The authors thank the Pathology Department of Italian Hospital for the help with tumor sample collection. This research was supported by grants from the National Agency of Scientific and Technological Promotion (FONCYT PICT 2006-01915), Argentina; Grant PIP2011 (166) from the National Council of Scientific and Technical Research CONICET (PIP 2011/2013), Argentina; Roemmers Foundation (2009–2011), Argentina, and Florencio Fiorini Foundation (2013), Argentina, and René Barón Foundation (2015), Argentina.

Compliance with ethical standards

Ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Moreover, we have obtained a statement of informed consent to publish from the participant (or legal parent or guardian for children) to report individual patient data.

Conflicts of interest None.

References

1. Kharaishvili G, Simkova D, Bouchalova K, Gachechiladze M, Narsia N, Bouchal J. The role of cancer-associated fibroblasts, solid stress and other microenvironmental factors in tumor progression and therapy resistance. Cancer Cell Int. 2014;14:41.
2. Cammarota F, Laukkanen MO. Mesenchymal stem/stromal cells in stromal evolution and cancer progression. Stem Cells Int. 2016;2016:4824573.
3. Mueller MM, Fusenig NE. Friends or foes—bipolar effects of the tumour stroma in cancer. Nat Rev Cancer. 2004;4:839–49.
4. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol. 2009;19:329–37.
5. Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K, et al. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol. 2004;2:E7.
6. Dudley AC, Shih SC, Cliffe AR, Hida K, Klagsbrun M. Attenuated p53 activation in tumour-associated stromal cells accompanies decreased sensitivity to etoposide and vincristine. Br J Cancer. 2008;99:118–25.
7. Qiao A, Gu F, Guo X, Zhang X, Fu L. Breast cancer-associated fibroblasts: their roles in tumor initiation, progression and clinical applications. Front Med. 2016. doi:10.1007/s11684-016-0431-5.
8. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6:392–401.
9. Jung YY, Kim HM, Koo JS. The role of cancer-associated fibroblasts in breast cancer pathobiology. Histol Histopathol. 2015. doi:10.14670/HH-11-700.
10. Luo H, Tu G, Liu Z, Liu M. Cancer-associated fibroblasts: a multifaceted driver of breast cancer progression. Cancer Lett. 2015;361:155–63.
11. Xouri G, Christian S. Origin and function of tumor stroma fibroblasts. Semin Cell Dev Biol. 2010;21:40–6.

12. Sun X, Mao Y, Wang J, Zu L, Hao M, Cheng G, et al. IL-6 secreted by cancer associated fibroblasts induces tamoxifen resistance in luminal breast cancer. Oncogene. 2014. doi:10.1038/onc.2014.158.
13. Pontiggia O, Sampayo R, Raffo D, Motter A, Xu R, Bissell MJ, et al. The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through β1 integrin. Breast Cancer Res Treat. 2012;133:459–71.
14. Mueller KL, Madden JM, Zoratti GL, Kuperwasser C, List K, Boerner JL. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 2012;14:R104.
15. Paulsson J, Micke P. Prognostic relevance of cancer-associated fibroblasts in human cancer. Semin Cancer Biol. 2014;25:61–8.
16. Recouvreux S, Sampayo R, Bessone MI, Simian M. Microenvironment and endocrine resistance in breast cancer: friend or foe? World J Clin Oncol. 2015;6:207–11.
17. Ao Z, Shah SH, Machlin LM, Parajuli R, Miller PC, Rawal S, et al. Identification of cancer-associated fibroblasts in circulating blood from patients with metastatic breast cancer. Cancer Res. 2015;75:4681–7.
18. Schor SL, Ellis IR, Jones SJ, Baillie R, Seneviratne K, Clausen J, et al. Migration-stimulating factor: a genetically truncated oncofetal fibronectin isoform expressed by carcinoma and tumor associated stromal cells. Cancer Res. 2003;63:8827–36.
19. Wels J, Kaplan RN, Rafii S, Lyden D. Migratory neighbors and distant invaders: tumor-associated niche cells. Genes Dev. 2008;22:559–74.
20. Barcellos-de-Souza P, Gori V, Bambi F, Chiarugi P. Tumor microenvironment: bone marrow-mesenchymal stem cells as key players. Biochim Biophys Acta. 2013;1836:321–35.
21. Monteiro AC, Leal AC, Gonçalves-Silva T, Mercadante AC, Kestelman F, Chaves SB, et al. T cells induce pre-metastatic osteolytic disease and help bone metastases establishment in a mouse model of metastatic breast cancer. PLoS One. 2013;8:e68171.
22. Peinado H, Lavotshkin S, Lyden D. The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts. Semin Cancer Biol. 2011;21:139–46.
23. Labovsky V, Martinez LM, Davies KM, García-Rivello H, Calcagno Mde L, Matas A, et al. Association between ligands and receptors related to the progression of early breast cancer in tumor epithelial and stromal cells. Clin Breast Cancer. 2015;15:e13–21.
24. Secchiero P, Melloni E, Corallini F, Beltrami AP, Alviano F, Milani D, et al. Tumor necrosis factor-related apoptosis-inducing ligand promotes migration of human bone marrow multipotent stromal cells. Stem Cells. 2008;26:2955–63.
25. Chang Q, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, et al. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. Neoplasia. 2013;15:848–62.
26. Hurst DR, Welch DR. A MSC-ing link in metastasis? Nat Med. 2007;13:1289–91.
27. Pestalozzi BC, Luporsi-Gely E, Jost LM, Bergh J, ESMO Guidelines Task Force. ESMO Minimum Clinical Recommendations for diagnosis, adjuvant treatment and follow-up of primary breast cancer. Ann Oncol. 2005;16(Suppl 1):i7–9.
28. Martinez LM, Labovsky V, Calcagno M d L, Davies KM, Garcia Rivello H, Bianchi MS, et al. CD105 expression on CD34-negative spindle-shaped stromal cells of primary tumor is an unfavorable prognostic marker in early breast cancer patients. PLoS One. 2015;10:e0121421.
29. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28:2784–95.
30. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.
31. Hu M, Yao J, Carroll DK, Weremowicz S, Chen H, Carrasco D, et al. Regulation of in situ to invasive breast carcinoma transition. Cancer Cell. 2008;13:394–406.
32. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev. 2013;32:303–15.
33. de Kruijf EM, van Nes JG, van de Velde CJ, Putter H, Smit VT, Liefers GJ, et al. Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple negative carcinoma patients. Breast Cancer Res Treat. 2011;125:687–96.
34. Qian N, Ueno T, Kawaguchi-Sakita N, Kawashima M, Yoshida N, Mikami Y, et al. Prognostic significance of tumor/stromal caveolin-1 expression in breast cancer patients. Cancer Sci. 2011;102:1590–6.
35. Gujam FJ, Edwards J, Mohammed ZM, Going JJ, McMillan DC. The relationship between the tumour stroma percentage, clinicopathological characteristics and outcome in patients with operable ductal breast cancer. Br J Cancer. 2014;111:157–65.
36. Yamashita M, Ogawa T, Zhang X, Hanamura N, Kashikura Y, Takamura M, et al. Role of stromal myofibroblasts in invasive breast cancer: stromal expression of α-smooth muscle actin correlates with worse clinical outcome. Breast Cancer. 2012;19:170–6.
37. Corallini F, Celeghini C, Rimondi E, di Iasio MG, Gonelli A, Secchiero P, et al. Trail down-regulates the release of osteoprotegerin (OPG) by primary stromal cells. J Cell Physiol. 2011;226:2279–86.
38. Yin S, Rishi AK, Reddy KB. Anti-estrogen-resistant breast cancer cells are sensitive to cisplatin plus TRAIL treatment. Oncol Rep. 2015;33:1475–80.
39. Tawara K, Oxford JT, Jorcyk CL. Clinical significance of interleukin (IL)-6 in cancer metastasis to bone: potential of anti-IL-6 therapies. Cancer Manag Res. 2011;3:177–89.