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Interleukin-2 and its receptor complex (α, β and γ chains) in *in situ* and infiltrative human breast cancer: an immunohistochemical comparative study

Ignacio García-Tuñón[1], Mónica Ricote[1], Antonio Ruiz[2], Benito Fraile[1], Ricardo Paniagua[1] and Mar Royuela[1]

[1]Department of Cell Biology and Genetics, University of Alcalá, Alcalá de Henares, Madrid, Spain
[2]Department of Pathology, Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain

Corresponding author: Mar Royuela (e-mail: mar.royuela@uah.es)

Abstract

Introduction. The presence and distribution of interleukin-2 (IL-2) and its receptor complex (Rα, Rβ, Rγ) were studied in 52 women who were clinically and histopathologically diagnosed with breast tumours (17 *in situ* and 35 infiltrating), and in 13 women with benign fibrocystic lesions in the breast.

Methods. Immunohistochemistry with antibodies against IL-2, IL-2Rα, IL-2Rβ and IL-2Rγ was used. A comparative semiquantitative immunohistochemical study between the three breast groups (fibrocystic lesions, *in situ* tumours and infiltrating tumours) was performed.

Results. IL-2 and its three receptor chains were immunodetected in the cytoplasm of epithelial cells. The three receptor chains were also detected on the cell surface. In fibrocystic lesions, immunoreactions to IL-2 (38.5% of cases),

IL-2Rα (53.8%) and IL-2Rβ (30.8%) were very weak, whereas immunoreaction to IL-2Rγ (46.1%) was somewhat more intense. In *in situ* tumours, the percentages of cases that immunostained positively for IL-2 and its three receptor chains were similar to those observed in fibrocystic lesions, but immunostainings of the four antibodies were more intense. In infiltrative tumours, the percentages of positively stained cases and also immunostaining intensities were approximately twice that found for *in situ* tumours. Within infiltrating tumours, the percentage of cases showing immunoreaction to IL-2 and their three receptor chains was higher in the patients with lymph node infiltration at the time of surgery.

Conclusion. The development of breast tumour is associated with an increased expression of IL-2 and its three receptor chains, and this expression also seems to be associated with the malignancy of the tumour.

Keywords: breast cancer, interleukin-2, interleukin-2 receptor

Introduction

Interleukin 2 (IL-2) is a lymphocytotrophic cytokine that is involved in the growth and differentiation of T and B cells and enhances the cytolytic functions of natural killer (NK) cells; it is also known to have some function in the proliferation of several non-lymphoid cells [1,2]. IL-2 receptor complex (IL-2R) comprises three subunits (α, β and γ), encoded by unrelated genes [3]. The affinity of IL-2R depends on which subunits are expressed at the cell surface [4,5]: β and γ chains together form the intermedi-ate-affinity receptor, whereas the high-affinity receptor requires the additional presence of the α subunit [6].

IL-2 has been detected in normal tissues including the endothelium, the intestinal epithelium and nerve cells [7]. Increased expression of IL-2 and IL-2R has been reported in actively proliferating tumours such as stomach, renal and spinocellular cancers [8], squamous cell carcinomas of the head and neck [9], melanomas, neuroblastic tumours [10] and prostate cancer [11].

IL = interleukin; IL-2R = interleukin-2 receptor; TBS = Tris-buffered saline.

In normal tissues [7] and several tumours, including stomach, renal and spinocellular cancer [8] and squamous cell carcinomas of the head and neck [9], IL-2 seems to stimulate cell proliferation and is more abundant in mitotic cells than in cells in the other phases of the cell cycle [8,9]. Experimental studies of squamous cell carcinomas of head and neck have shown that antisense IL-2 treatment or blocking of IL-2Rβ results in growth inhibition [12].

Nevertheless, it has also been shown that systemic administration of IL-2 induces the regression of metastatic murine renal carcinoma [13], possibly through the induction of interferon-γ, tumour necrosis factor-α and anti-angiogenic products such as IP-10 [14]. This finding has led to some authors to conclude that whereas endogenous IL-2 stimulates cell proliferation, exogenous IL-2 inhibits such a proliferation [7,15].

Studies on IL-2 and its receptors in breast cancer are few. Katano and colleagues [16] cultured two breast carcinoma cell lines (M and M25-SF lines), and found IL-2 expression in the latter line only. *In vivo* immunohistochemical studies of IL-2Rα and IL-2Rβ in ductal infiltrating breast cancer by Ridings and colleagues [10] revealed that only the β receptor chain was present in these tumours.

The aim of the present study was to characterise the expression patterns of IL-2 and its three receptor chains (α, β and γ) by immunohistochemistry, in both *in situ* and infiltrating breast tumours, to elucidate the role of these proteins in breast cancer progression and metastasis.

Materials and methods

Total or partial mastectomy specimens obtained from 52 women, who were clinically and histopathologically diagnosed with breast adenocarcinoma during 1998 in our hospital, were used for the study. Seventeen of these women (aged from 47 to 75 years) presented *in situ* carcinoma (8 lobular and 9 ductal); 1 of these 17 women also showed lymph node infiltration at the time of surgery. Thirty-five women (aged from 51 to 77 years) had infiltrating carcinoma (15 lobular and 20 ductal); 13 of these 35 women showed lymph node infiltration at the time of surgery, and 7 of these 13 developed metastasis 7–24 months after surgery. At present (January 2003), neither the remaining 22 women with infiltrating tumours nor the 17 women with *in situ* tumours have developed metastasis.

Tumour samples were compared with breast biopsies from 13 women (aged from 16 to 59 years) with benign fibrocystic lesions. Removal of tissues and the study of samples were approved by the Hospital's Ethics Committee and were performed with the consent of the patients' relatives.

All samples were processed for immunohistochemical study by light microscopy. The primary antibodies used were goat anti-human IL-2, rabbit anti-human IL-2Rα, rabbit anti-human IL-2Rβ and rabbit anti-human IL-2Rγ (Santa Cruz Biotechnology, California, CA, USA).

Tissues were fixed for 24 hours at room temperature in 0.1 M phosphate-buffered 10% formaldehyde, then dehydrated and embedded in paraffin. Sections (5 μm thick) were processed with the avidin–biotin–peroxidase complex (ABC) method. Following deparaffination, sections were hydrated and then incubated for 30 minutes in 0.3% H_2O_2 diluted in methanol to reduce endogenous activity. After rinsing in Tris-buffered saline (TBS), the slides were incubated with normal donkey serum at 10% in TBS for 30 minutes, to prevent non-specific binding of the first antibody. Thereafter, the primary antibodies were applied at a dilution of 1:100 for the four antibodies in TBS overnight at room temperature. The sections were then washed twice in TBS and incubated with rabbit anti-(goat IL-2), or pig anti-(rabbit IL-2Rα, IL-2Rβ and IL-2Rγ) biotinylated immunoglobulin (Dako, Barcelona, Spain) at 1:500 in TBS. After incubation for 1 hour with secondary antibody, the sections were incubated with a standard streptavidin–biotin complex (Vector Laboratories, Burlingame, CA, USA) and developed with 3,3′-diaminobenzidine (DAB), using the glucose oxidase–DAB–nickel intensification method.

Immunochemical procedure specificity was checked with negative and positive controls. For negative controls of immunoreactions, tissues of each type were incubated with preimmune serum at the same immunoglobulin concentration as that used for each antibody, or with blocking peptides (Santa Cruz Biotechnology). As positive controls, histological sections (immunohistochemistry) of thymus samples were incubated with the same antibodies.

A comparative histological quantification of immunolabelling among the different groups of breast samples (*in situ* adenocarcinomas, infiltrating adenocarcinomas and benign fibrocystic lesions) was performed for each of the four antibodies. For each breast sample, six histological sections were selected at random. In each section, the staining intensity (optical density) per unit surface area was measured with an automatic image analyser (MIP4 version 4.4; Consulting Image Digital, Barcelona, Spain) in five light microscopic fields per section, using the 40× objective. Delimitation of stained surface areas was performed manually with the mouse of the image analyser. For each positively immunostained section, one negative control section (the next in a series of consecutive sections) was also used, and the optical density of this control section was subtracted from that of the stained section. From the average values obtained for each breast, the means ± SD for each breast group were calculated. The statistical significance between means of the different
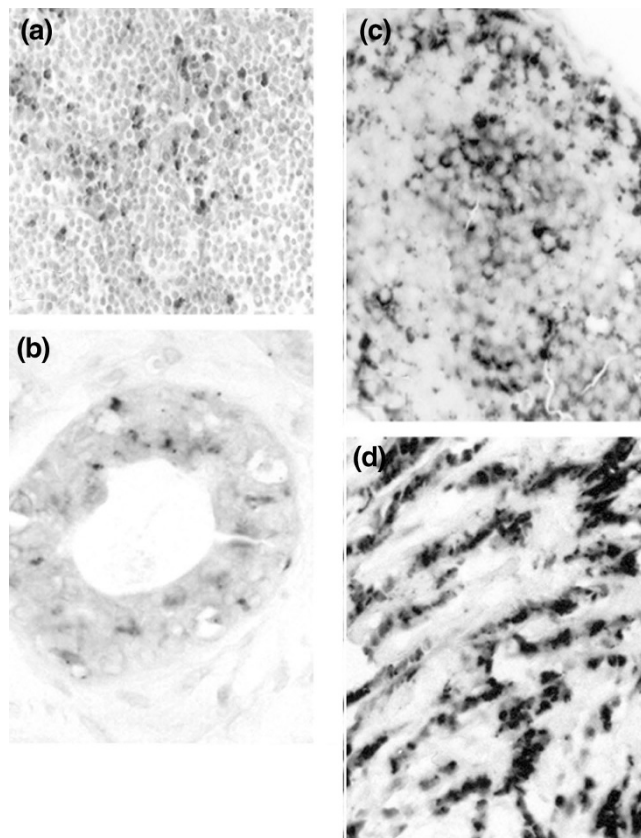
Figure 1
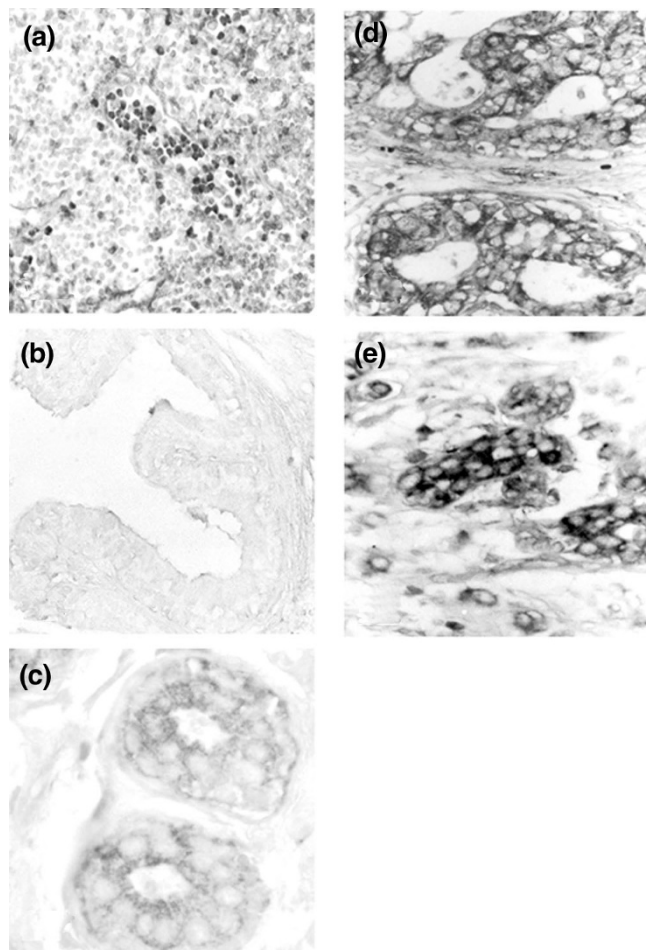
IL-2 immunostaining. **(a)** Thymus sections were used as a positive control. Fibrocystic lesions show either no immunoreaction **(b)** or a weak immunoreaction limited to a circumscribed supranuclear region **(c)**. In ductal *in situ* **(d)** and ductal infiltrating **(e)** carcinomas, most of the cytoplasm was labelled. Original magnification ×375.

Figure 2

Immunostaining of IL-2Rα. **(a)** Moderate staining of thymus section. **(b)** Fibrocystic lesions showing a weak immunoreaction. **(c)** Lobular *in situ* carcinoma showing moderate immunostaining. **(d)** Lobular infiltrating carcinoma showing a more intense immunostaining. Original magnification ×375.

breast group samples was assessed by the Fisher and Behrens test at $P \leq 0.05$, by multiple pairwise comparisons of all the values for each breast zone, for each specific antibody separately.

To test whether the increase in IL-2 immunoexpression intensity (optical density) was correlated with the increase in expression of each IL-2 receptor chain, Pearson's coefficient of correlation was calculated for each of the following pair of values (IL-2 and IL-2Rα, IL-2 and IL-2Rβ, IL-2 and IL-2Rγ).

Results

No immunoreaction was observed in the negative controls incubated with preimmune serum or with the use of the antibodies preabsorbed with an excess of purified antigen.

Staining of thymus sections (positive controls) was always positive for all antibodies used (Figs 1a, 2a, 3a and 4a).

In the three groups of specimens (fibrocystic lesions, *in situ* tumours and infiltrating tumours), immunostaining of IL-2 appeared in the cytoplasm of epithelial cells, whereas immunostaining of the three types of IL-2R (α, β and γ) appeared in both the cell surface and cytoplasm of epithelial cells.

For each antibody assayed, the percentage of positive cases and immunostaining intensities are shown in Table 1. The immunostaining intensities (optical density) are given as means ± SD. Significance was determined by multiple pairwise comparisons by the Fisher and Behrens test at $P \leq 0.05$. All the values for each breast zone and for each specific antibody were compared separately.

Immunoreaction to IL-2 was found in 38.5% of benign fibrocystic lesions (Fig. 1b,c), in 41.2% of *in situ* tumours (Fig. 1d) and in 82.9% of infiltrating tumours (Fig. 1e).

Table 1

Comparison of immunostaining intensities between groups of breast samples

Group (no. of cases)	IL-2		IL-2Rα		Il-2Rβ		IL-2Rγ	
	Positive samples	Optical density	Positive samples	Optical density	Positive samples	Optical density	Positive samples	Optical density
Human thymus (4)	4 (100%)	12.1±1.2	4 (100%)	10.07±1.47	4 (100%)	14.33±2.1	4 (100%)	13.21±0.89
Fibrocystic benign lesions (13)	5 (38.5%)	4.02±0.37[a]	7 (53.8%)	7.13±0.19[a]	4 (30.8%)	2.05±0.35[a]	6 (46.1%)	15.40±2.4[a]
In situ carcinomas (17)	7 (41.2%)	20.47±2.42[b]	6 (35.3%)	20.30±1.29[b]	6 (35.3%)	28.90±1.15[b]	7 (41.2%)	25.05±2.5[b]
Infiltrating carcinomas (35)	29 (82.9%)	26.93±4.82[c]	21 (60.0%)	52.92±4.77[c]	28 (80.0%)	31.22±6.80[b]	28 (80.0%)	40.82±5.78[c]
Lymph node infiltration (13)	11 (84.6%)	28.24±4.9[c]	11 (84.6%)	54.41±4.8[c]	10 (76.9%)	28.94±5.66[b]	12 (92.3%)	38. 8±6.12[c]
Neither lymph node infiltration nor metastasis (22)	18 (81.8%)	26.06±4.89[c]	10 (45.4%)	51.55±4.79[c]	18 (81.8%)	32.28±6.80[b]	16 (72.7%)	42.16±6.93[c]

Optical densities are means±SD. Values denoted by different superscripts are significantly different from each other. Those values sharing the same superscript are not statistically different from each other. Statistical analysis refers to each antibody separately. Significance was determined by multiple comparisons by the Fisher and Behrens test at $P \leq 0.05$.

Immunostaining was very weak in benign fibrocystic lesions, and more intense in both tumour types.

Immunoreaction to IL-2Rα was present in 53.8% of fibrocystic lesions (Fig. 2b), in 35.3% of *in situ* tumours (Fig. 2c) and in 60.0% of infiltrating tumours (Fig. 2d). Immunostaining was very weak in benign fibrocystic lesions, more intense in *in situ* tumours, and even more intense in infiltrating tumours.

Immunoreaction to IL-2Rβ was observed in 30.8% of fibrocystic lesions and was very weak (Fig. 3b). An intense immunoreaction was detected in 35.3% of *in situ* tumours (Fig. 3c) and in 80.0% of infiltrating tumours (Fig. 3d).

Immunoreaction to IL-2Rγ appeared in 46.1% of fibrocystic lesions. This was the only IL-2 receptor that showed a certain degree of staining intensity in fibrocystic lesions (Fig. 4b). Immunoreaction to this receptor was observed in 41.2% of *in situ* tumours (Fig. 4c), in which staining was more intense. The most intense immunoreaction appeared in 80.0% of infiltrating tumours (Fig. 4d).

Within the group of infiltrating carcinomas with lymph node infiltration (13 women), 10 of them (76.9%) presented a positive immunoreaction to IL-2 and its three receptor chains. In the group without lymph node infiltration (22 women), only 10 cases (45.5%) presented a positive immunoreaction to IL-2 and its receptors.

The correlation (*r*) between the immunoexpression intensity of IL-2 and each receptor chain was as follows: IL-2/IL-2Rα, 0.88; IL-2/IL-2Rβ, 0.97; IL-2/IL-2Rγ, 0.85.

Discussion

Immunohistochemical studies of IL-2 and IL-2R have been performed in several normal tissues such as human and murine fibroblasts [17,18], oral mucosae [9] and epithelial basal cells of the prostate [11], and it has been suggested that IL-2 is involved in the control of the equilibrium between proliferation and apoptosis [11]. In all these normal tissues the expression of IL-2 and IL-2R was weak. These data agree with present findings in fibrocystic lesions of the breast. In these specimens, breast epithelial cells showed a characteristic staining pattern: a weak immunoreaction to IL-2, limited to a circumscribed cytoplasmic area corresponding to the Golgi zone. These observations suggest that, in breast tissue, this interleukin is processed and secreted in the same way as in haematopoietic cells, in agreement with the report by Sander and colleagues [19].

The functional location of IL-2 receptor complex is the plasma membrane. Each of the three chains has a cytoplasmic domain [20], which binds to intracellular signalling molecules and thereby relays specific intracellular signals [21]. In the present study, the three IL-2R chains were immunodetected not only at the cell surface but also in the cytoplasm. This intracellular immunoreaction might correspond to neosynthesised receptor chains that have not yet reached the cell membrane.

During the past decade, consistent results have demonstrated the occurrence and functional implication of IL-2 in tumour cells [7,9]. In the present study, in contrast with the weak immunolabelling observed in benign fibrocystic lesions, breast tumour cells (*in situ* tumours, and even
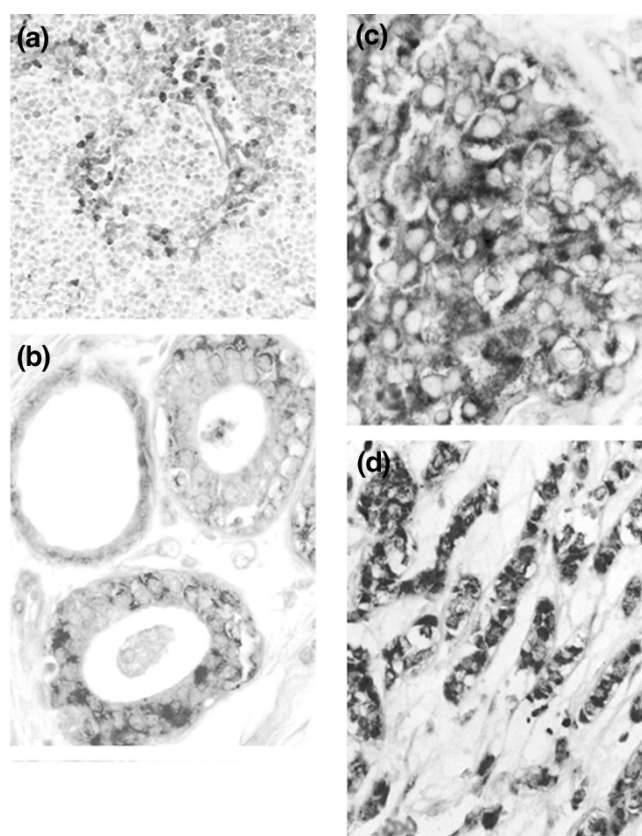
Figure 3
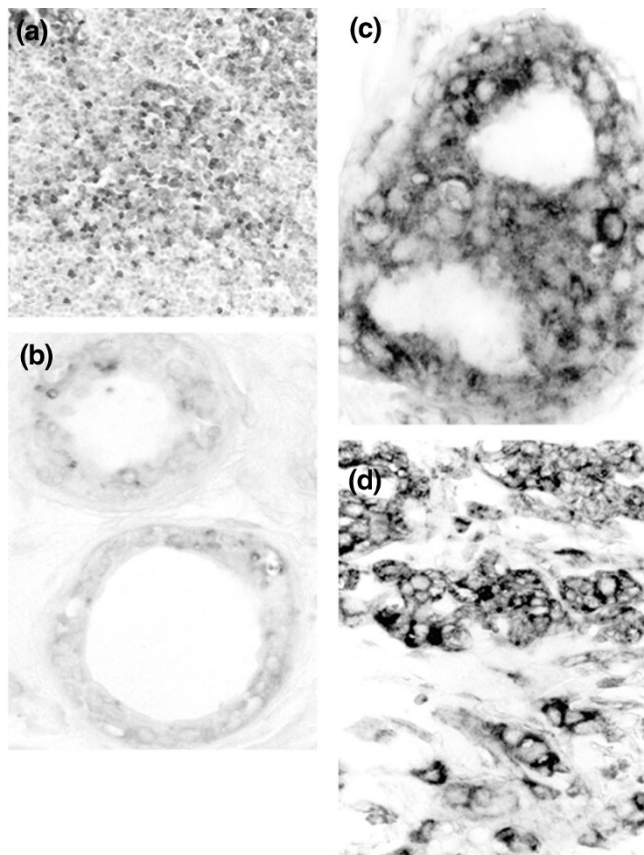
Immunostaining of IL-2Rβ. **(a)** Thymus sections were always positive. **(b)** Fibrocystic lesions showing a weak immunoreaction. Ductal *in situ* carcinoma **(c)** and ductal infiltrating carcinoma **(d)** showing intense immunostaining. Original magnification ×375.

Figure 4

Immunostaining of IL-2Rγ. **(a)** Presence of positive immunoreaction in thymus sections. **(b)** Fibrocystic lesions showing moderate immunoreaction. **(c)** Lobular *in situ* carcinoma in which immunostaining was more intense. **(d)** Even more intense immunostaining in lobular infiltrating carcinoma than in *in situ* carcinoma. Original magnification ×375.

more in infiltrating tumours) showed a large part of the cytoplasm immunostained (from moderately to intensely) to IL-2 and its receptor. According to the staining distribution there are no specific subpopulations within individual patients, because IL-2/IL-2R expression was uniform throughout the whole epithelium. This suggests that IL-2 secretion acts throughout an autocrine mechanism. There also was a positive correlation between the increased expression of IL-2 and that of its three receptor chains: IL-2Rα ($r=0.88$), IL-2Rβ ($r=0.97$) and IL-2Rγ ($r=0.85$). Nevertheless, more than half of *in situ* carcinoma patients, and about 20% of patients with infiltrating carcinomas, did not immunoreact to IL-2 and IL-2R. The association between IL-2 and breast cancer might therefore not be generalised.

The effect of this high expression of IL-2 and IL-2R in breast cancer cells might be a contribution to the development of a tumour by enhancing cell proliferation and/or inhibiting apoptosis, together with other factors including anti-apoptotic factors of the Bcl-2 family gene products,

as has been reported in other tumour tissues [11]. Lelle [22] reported an increase in the proliferation index from breast benign lesion to tumour tissue. Reicher and colleagues [9] described, in tumour cell lines of squamous cell carcinomas of the head and neck, that tumour cells in G_0, G_1 and S phases showed a juxtanuclear localisation of IL-2, whereas tumour cells in G_2 and mitosis showed an increased IL-2 expression, distributed throughout the whole cytoplasm [15]. Azzarone and colleagues [7] reported similar findings in several breast cancer cell lines, as did Reicher and colleagues [15] in squamous cell carcinomas of head and neck.

The expression of IL-2 and its receptors was higher in breast infiltrating tumours than in *in situ* tumour samples. This agrees with the above-mentioned role of IL-2 in tumour cell proliferation because infiltrating tumours are more aggressive than *in situ* tumours. In these latter, tissue keeps its glandular structure, whereas infiltrating tumour epithelial cells lose their normal structure and

invade the adjacent stromal tissue [23]. In this context, Querzoli and colleagues [24] reported that infiltrating breast cancers have a higher proliferation index than *in situ* tumours.

Using immunofluorescence, Ridings and colleagues [10] found that most infiltrating ductal breast carcinomas were positive for IL-2Rβ, but only 10% were positive for IL-2Rα. In contrast, the present study of infiltrating ductal breast carcinomas revealed an intense expression of β and γ chains in 80% of cases, and of α chains in 60% of cases. Because IL-2Rβ and IL-2Rγ are necessary to form the active receptor [25], these results suggest that infiltrating breast tumours expressed functional receptors, able to respond to IL-2 stimuli.

The increased expression of the γ chain is not exclusively related to the response to IL-2 stimuli. Because this chain is also a component of receptor complexes for other interleukins, including IL-4, IL-7 and IL-9 [26,27], the function of these interleukins might also be enhanced. Several authors have demonstrated the overexpression of receptors for these interleukins in such different tumours as lung, renal, melanoma, colorectal and breast [28,29].

Several interleukins, including IL-2, IL-4, IL-5 and IL-10, have been shown to induce IL-2Rα expression in inflammatory processes and viral infections [21]. Because this chain is necessary to form the high-affinity IL-2R [30], which enhances the IL-2 signal, the increased expression of IL-2Rα in breast-infiltrating tumours suggests that the IL-2 signal is more active in this tumour group.

Conclusion

Although immunohistochemical studies cannot provide any information about the function of these molecules, the present data suggest that the development of breast tumour is associated with an increased expression of IL-2 and its three receptor chains, and this expression also seems to be associated with the malignancy of the tumour.

Competing interests

None declared.

Acknowledgements

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Correspondence

Mar Royuela, Department of Cell Biology and Genetics, University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain. Tel: +34 91 8854798; fax: +34 91 8854799; e-mail: mar.royuela@uah.es