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RESEARCH ARTICLE

IDO is highly expressed in breast cancer and breast cancer-derived circulating microvesicles and associated to aggressive types of tumors by in silico analysis

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Abstract Indoleamine-2,3-dioxygenase (IDO) has been established as a normal mechanism of peripheral tolerance and immunosuppression. Besides, malignant tumors release microvesicles (MV) related with tumor dissemination. The aims of this study were to determine the expression of IDO in breast cancer and circulating microvesicles from breast cancer patients and to perform an in silico analysis to find genes co-expressed to IDO. One hundred and twenty-two tissue and serum breast samples (91 malignant, 21 benign, and 10 normal), and MCF7, MDA-MB-231, and T47D breast cancer cell lines were included. Standard immunohistochemistry (IHC), immunocytochemistry (ICC), Western blot (WB), and RT-PCR were employed. Microvesicle isolation from plasma samples was obtained by serial centrifugation and ultracentrifugation. By IHC, 60 % breast cancer, 43 % benign, and 20 % normal samples were positive. Significant differences were found among normal, benign, and malignant samples. Breast cancer stages I, II, and III expressed IDO in 42, 66, and 71 % of samples, respectively, while breast cancer cell lines also reacted; by WB, 9/25 microvesicles fractions showed bands at 42 kD. In silico analysis of IDO 1 gene expression in breast cancer showed its association with several genes related to immune response and apoptosis. Moreover, IDO and co-expressed genes were found predominately in basal and erbB2 subtypes. The cumulative data indicate a high expression of IDO in breast cancer which increased with higher stages. Furthermore, IDO was found in association with circulating breast cancer MV, while experimental and in silico gene expression revealed that IDO was mainly expressed in a triple-negative subgroup.

Keywords Breast cancer · IDO · Microvesicles · Gene expression · Immune tolerance

Introduction

Indoleamine-2,3-dioxygenase (IDO) is a 42- to 45-kD enzyme which catalyzes the rate-limiting step of tryptophan (Trp) degradation along the kynurenine pathway. IDO is expressed in a variety of cells and tissues [1–3], and it was originally described in placenta as contributing to maternal tolerance towards the fetus [4]. IDO has also been implicated in mechanisms of protection to graft rejection and in autoimmune disorder treatments [5].

Evidence suggests that IDO has a role in tumor survival, and it restricts the host immunosurveillance through blocking the initial response to tumor antigens. IDO may be related to immune regulation via local metabolic changes in the immediate microenviroment and local tissue milieu, and these local changes may ultimately impact on the development of systemic immune tolerance. Kynurenine release and Trp consumption by accessory cells expressing IDO generate signals via aryl hydrocarbon receptor (AhR) and aminoacid sensors (GCN2, mTOR), respectively, that have profound effects on T cells and Treg responses to inflammatory and antigenic signals. IDO activity in antigenic presenting cells (APCs) also enhances Treg differentiation from naïve CD4 T cells via these metabolic pathways. GCN2 appears to enhance Treg activity and inhibit T effector cells [6]. Overexpression of IDO in tumor cells inevitably results in deficiency of Trp and T cells in the mid-G1 phase very sensitive to Trp deficiency. As a result, T cells can neither effectively proliferate nor amplify via clonal expansion. Moreover, T cell proliferation is difficult to be re-activated once it is inhibited [7].

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Natural killer (NK) cells are an important component of innate immunity that can eliminate transformed cells, and it has been reported that malfunction and significantly reduced number of NK cells contribute to immune escape of cancer cells in cancer patients [8, 9]. Song et al. [10] found that L-kynurenine, a Trp catabolyte of IDO, induces apoptosis via generation of reactive oxygen species in human NK cells.

Besides, malignant tumors release exosomes or microvesicles which were related to tolerogenic mechanisms promoting immune evasion [11]. Among these microvesicles, exosomes are estimated to be 50 to 100 nm in diameter and have been linked to cancer progression, largely because of their abundance in body fluids of cancer patients with advanced disease.

The present research was performed to develop the hypothesis that IDO may be implicated in tumor immune tolerance. We have studied IDO expression of breast cancer patients circulating microvesicles (MV), breast cancer samples, and breast cancer cell lines. IDO expression was also assessed in benign and normal tissue samples. Finally, we have investigated IDO 1 co-expressed genes by an in silico analysis.

Materials and methods

Samples

One hundred and twenty-two tissue and serum samples from 91 breast cancer patients, 21 benign disease patients, and 10 normal controls derived from cosmetic mammoplastic specimens were analyzed. Breast cancer type distribution was as follows: 84 % DCNOS, 9 % LC, and 7 % other types. Pathological staging was established according to UICC TNM classification system established: 34, 40, and 26 % corresponding to stages I, II, and III, respectively. Receptor status: 78 % of samples were positive for estrogen receptor (ER), 71 % for progesterone receptor while 18 % for HER2-neu.

Samples and clinical data were obtained from hospitals related to the Faculty of Medical Sciences, National University of La Plata, Argentina. Procedures followed the World Medical Association Declaration of Helsinki (Finland, 1964) and further modifications.

Informed consent was obtained from all patients included in this study. This research was approved by the Medical Bioethics Committee, Faculty of Medical Sciences, National University of La Plata, Argentina.

Monoclonal antibodies (MAbs)

Anti-IDO MAb (Millipore, USA) was used in immunohistochemistry (IHC), while anti-CD9 (Santa Cruz, USA) and anti-CD63 (Santa Cruz, USA) were employed in Western blot analysis.

Cell lines and culture

MCF7, T47D, and MDA-MB-231 breast cancer cell lines were employed. Cells were seeded in flasks (Gibco-BRL, Gaithersburg, MD, USA) and grown in RPMI 1640 medium, supplemented with 10 % fetal bovine serum (PAA, Austria) and antibiotics. Cells were cultured in 5 % CO_2 humidified atmosphere at 37 °C.

Immunohistochemical analysis

The technique was performed following standard procedures: paraffin-embedded specimens were treated with 10 mM sodium citrate buffer pH 6.0 at 100 °C for 10 min for antigenic retrieval and incubated overnight at 4 °C with MAb [12]. Negative controls were incubated with PBS instead of MAb. Reaction was developed with the LSAB+biotin-avidin peroxidase-conjugated anti-mouse immunoglobulins kit (DAKO, USA) following manufacturer's instructions. The chromogen employed was 3,3′-diaminodiazobenzidine (DAKO, USA).

Sections were examined by light microscopy, and the antibody staining patterns were scored in a semiquantitative manner. Staining intensity was graded as negative (−), low (+), moderate (++), or strong (+++). The number of optical fields in a specimen that were positively stained was expressed as a percentage of the total number of optical fields containing tissue. The staining of cytoplasm, plasma membrane, and nucleus was evaluated; cells were considered positive when at least one of these components was stained. The pattern of reaction was classified as linear (membrane reaction), cytoplasmic, or mixed (cytoplasmic and membrane), and the positive reaction in gland lumen content was identified as cellular debris or secretion.

Immunocytochemistry (ICC)

For ICC, cells were grown for 24 h on glass coverslips and then fixed with 4 % formaldehyde in PBS. ICC was performed following the above-mentioned IHC standard technique without antigenic retrieval.

Immunofluorescence (IF)

Cells were grown for 24 h on glass coverslips and then fixed with 4 % formaldehyde in PBS. IF was performed following the above-mentioned IHC standard technique without antigenic retrieval, and the reaction was detected employing streptavidin conjugated FITC (DAKO, USA). Photographic register was obtained with a BX51 Olympus microscope.

RT-PCR

For IDO reverse- transcription PCR (RT-PCR), total cellular RNA was extracted from tumor tissue and cell lines using Trizol (Invitrogen, USA) following the manufacturer's instructions. First strand complementary DNA (cDNA) was synthesized from total RNA using Superscript II (Invitrogen, USA) and oligo(dT) 12–18. PCR was performed in a 20-μl reaction mixture with 5 μl cDNA and 100 pmoles/μl each of the following primers: sense, 5′-TGCAAATGCAAGAACG GGAC-3′, and antisense, 5′-CTGAAAGACGCTGCTTTG GC-3′. Primers pairs span exon–exon junctions were designed using Primer-blast (NCBI-NIH, USA) and Unipro UGENE softwares. PCR amplification was done for 40 cycles to detect IDO for 30 s at 94 °C, 40 s at 64 °C, and 40 s at 72 °C. Agarose and polyacrylamide gels were used to detect bands.

Microvesicles isolation

Microvesicles were isolated by differential centrifugation. Six milliliters of plasma from 25 patients, 500 ml of conditioned medium (24-h harvest in serum-free medium) from MCF7 and T47D cell lines, and 25 mL of ascitic fluid from a patient diagnosed with ovarian cystoadenocarcinoma were centrifuged at 1,500×*g* at 4 °C for 30 min. The supernatant was centrifuged at 15,000×*g* at 4 °C for 30 min, and the supernatant filtered through 0.22- and 0.1-μm pore filters. Then, filtered plasma or medium was centrifuged at 100,000×*g* for 2 h at 4 °C. The pellet was dissolved in PBS and centrifuged at 100,000×*g* for 2 h at 4 °C. This final pellet was re-suspended in 200 μl of PBS, aliquoted, and frozen at −70 °C. Protein content was determined by the Qubit assay (Invitrogen, USA).

Electron microcopy

Five microliters of the MV fractions were placed onto copper grids, incubated for 5 min, and washed with PBS. Samples were then counterstained with 2 % phosphotungstic acid for 40 s and then observed in a JEM 1200 EXII electron microscope (JEOL) at the Electron Microcopy Central Service, UNLP.

SDS-PAGE and Western blotting

MV-enriched fractions (20 μl) were analyzed under reducing conditions in SDS-PAGE in a discontinuous (4–10 %) system, and Western blot (WB) was performed following Towbin et al. [13]. After electrophoresis, gels were electrophoretically transferred to nitrocellulose membranes (Whatman, Germany) which were blocked with 10 mM PBS/5 % skimmed milk (blocking buffer). After washing with PBS-Tween 20 (PBST), sheets were incubated with MAb diluted in blocking buffer. IDO MAb was diluted 1/1,000, while CD9 MAb was diluted 1/500. Sheets were incubated overnight at 4 °C and rinsed with PBST. A final incubation with 1/400 peroxidase-conjugated anti-mouse immunoglobulins was performed according to the manufacturer's instructions (DAKO, USA). Nitrocellulose sheets were developed by enhanced chemoluminescence.

After transfer, gels were stained with Coomassie blue (SIGMA, USA).

Molecular analysis of IDO 1

To analyze *IDO 1* co-expressed genes in breast cancer platform-specific microarray collections, the web-based bioinformatics tool Multiexperiment Matrix (MEM) was employed, http://biit.cs.ut.ee/mem/. A total of 76 microarray studies (n=4,822 samples) were analyzed to obtain the 50 best correlated genes. One of the microarray studies (GSE21653) was chosen to analyze these genes, in which samples are grouped according to breast cancer molecular classification: Luminal A, Luminal B, erbB2, Basal-like, and Normal-like. In order to identify the molecular pathways that were mainly affected by these 50 genes, protein/gene interaction networks in the common core of overlapped genes were studied. The protein–protein interaction network was generated using the STRING database ("Search Tool for the Retrieval of Interacting Genes/Proteins," http://string.embl.de/). We also employed the bioinformatics tool TNBCtype [14] to classify those samples labeled as Basal in the GSE21653 dataset.

Statistical analysis

Differences between groups were assessed employing the χ^2 test, Mann–Whitney U test while differences in trends among ordinal variables were evaluated by the Kendall τ test.

All statistical analysis was performed with SPSS statistics (v17) software. In all cases, null hypothesis rejection and statistically significant differences were considered when $p<0.05$.

Results

IDO expression in breast cancer samples and benign breast disease' samples by IHC

One hundred and twenty two breast samples were studied: 60 % of breast cancer, 43 % of benign diseases, and 20 % of normal controls showed IDO expression. Table 1 shows immunohistochemical results according to diagnosis and stages of breast cancer. By χ^2 analysis, differences among groups were found, being greater the expression in breast cancer ($p<0.05$) (Fig. 1a) with a positive correlation by Kendall ($p<0.05$). Significant differences were found between breast cancer and normal samples ($p<0.05$). Moreover, the intensity of the reaction was significantly higher in cancer samples than

Table 1 Immunohistochemical results according to diagnosis and breast cancer stages

Tissue sample		IDO positive/total (%)
Normal		2/10 (20)
Benign		9/21 (43)
Breast cancer	Stage I	13/31 (42)
	Stage II	24/36 (66)
	Stage III	17/24 (71)
	Total	55/91 (60)

in benign and normal specimens (Mann–Whitney U test, $p<0.05$).

The percentage of positivity increased in relation to the increment of disease stage: 42 %, 66 %, and 71 % for stage I, II, and III, respectively (Table 1, Fig. 1b). Furthermore, a positive correlation by Kendall was found among disease stage and IHC positivity as well as intensity of the staining ($p<0.05$). When IDO reactivity and intensity were compared with nuclear grade, a positive correlation ($p<0.05$) was also found.

Figure 2 shows microphotographs of IDO expression in invasive ductal carcinoma samples, a predominant cytoplasmic pattern is observed (Fig. 2a–c) while a strong nuclear reaction is also depicted. Figure 2d shows a negative reaction in a benign breast specimen.

Table 2 shows the immunohistochemical results of IDO expression among the different subgroups based on the receptor status. IDO was expressed in 42 % of breast cancer specimens which were ER and/or PR positive and negative for HER2-neu, and they were also positive in 80 % of triple-negative subgroup. Interestingly, a significant negative correlation between IDO and ER expression was obtained while non significant correlations were found when histological differentiation, PR and HER2-neu status were considered.

IDO expression in breast cancer cell lines by ICC

In order to analyze IDO expression in breast cancer cell lines, ICC was performed in T47D, MDA-MB-231, and MCF7 cell lines. In all cases, IDO was expressed with a cytoplasmic pattern (Fig. 2e, f); similar results were obtained by immunofluorescence (Fig. 2g, h).

Detection of IDO by RT-PCR

By RT-PCR, messenger RNA (mRNA) of 11 breast cancer samples as well as breast cancer cell lines were analyzed. Amplicons were run in agarose and/or polyacrylamide gel and bands at 251 bp were detected in 4 samples which were IDO positive by immunohistochemistry. Seven samples which were negative to IDO by immunohistochemistry did not show any band by RT-PCR (Fig. 3).

Analysis of microvesicles enriched fractions

Mean protein concentration of MV fractions was 7.86 μg/ml of plasma with a maximum of 28.78 μg/mL and median of 3.58 μg/ml. By WB, the expression of IDO, CD63, and CD9 was analyzed in microvesicles fractions isolated from breast cancer plasma patients. Bands of 42 kD were obtained in 9 out of 25 (36 %) samples when anti-IDO MAb was employed (Fig. 4a). Anti-CD9 and anti-CD63 were used as exosome markers and 10 out of 25 (40 %) samples showed positivity for at least one marker. Five out of nine (56 %) samples showed both IDO and exosome marker reaction. No statistically significant association was found between exosome markers detection and age, clinical stage, or receptor status.

Electron microscopy analysis of MV fractions showed the presence of small (50 – 200 nm) rounded vesicles that occasionally showed a cup shaped form (Fig. 4b).

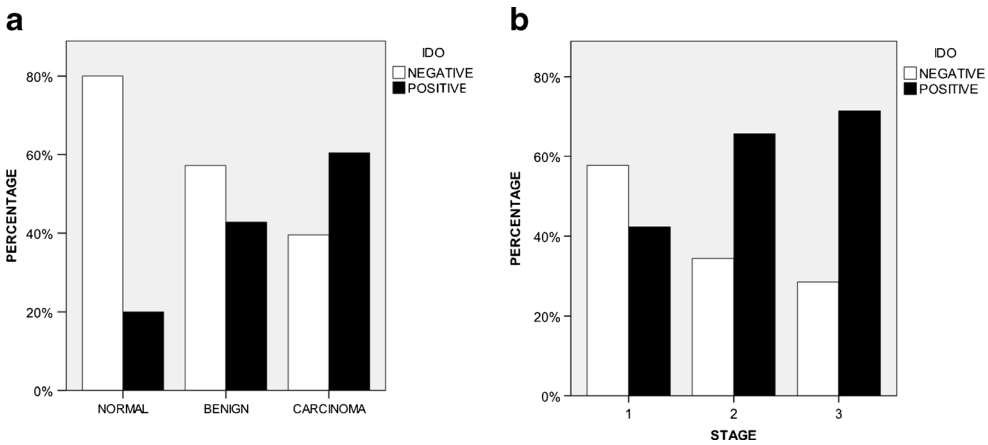

Fig. 1 Immunohistochemical IDO expression in breast cancer, benign breast disease, and normals. **a** *Bars* show the percentage of IDO positive (*black*) and negative (*white*) samples vs. diagnosis. **b** *Bars* show the percentage of IDO positive (*black*) and negative (*white*) samples vs. stages (1, 2, and 3 represent stage I, II, and III, respectively)

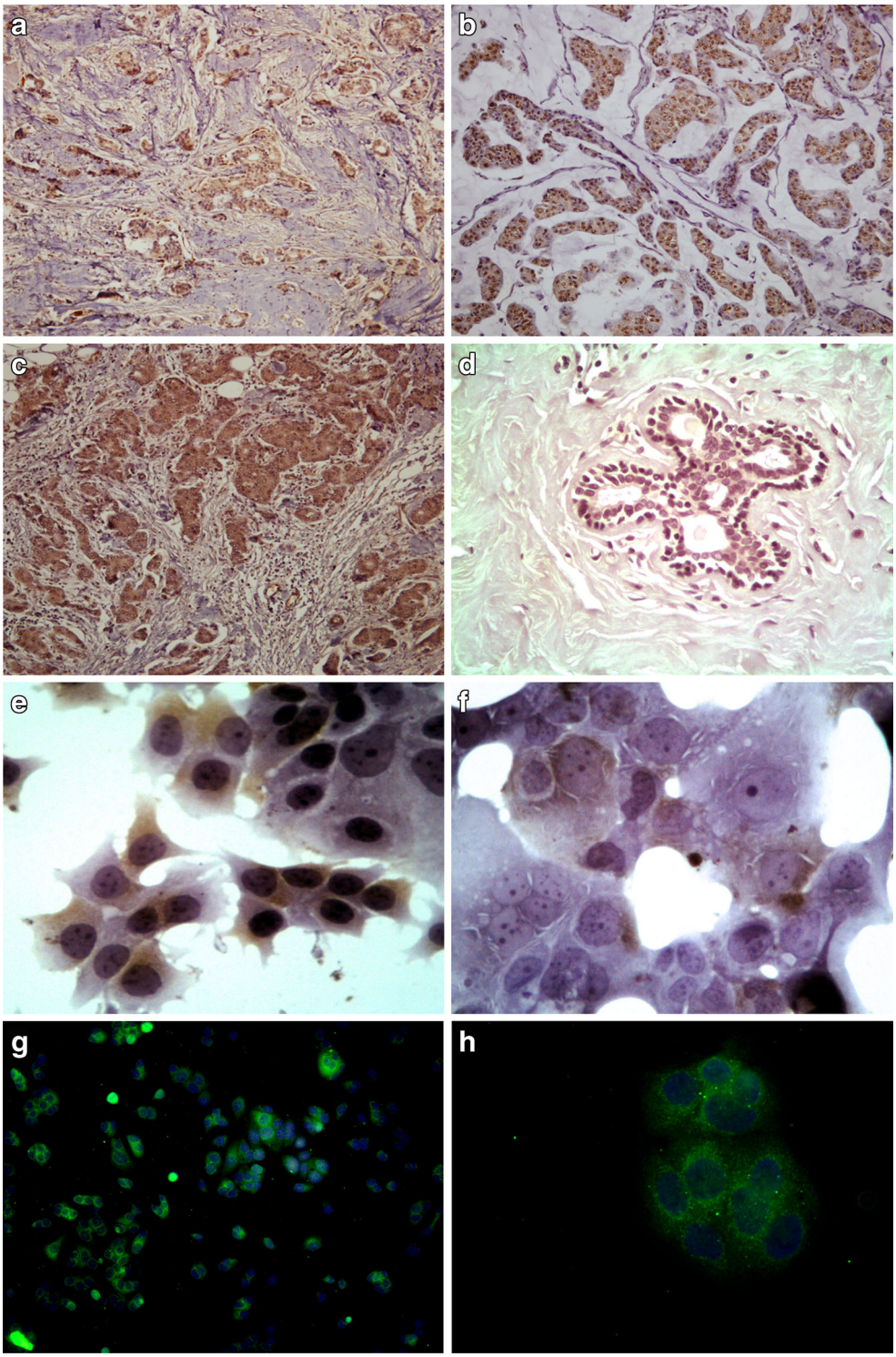

Fig. 2 Microphotographs of immunoperoxidase staining of breast cancer, benign breast disease samples, and breast cancer cell lines stained with MAb anti-IDO (200×). **a** IHC of a breast cancer specimen shows a strong reaction with a cytoplasmic pattern and nuclear reaction. **b** IHC of DCNOS sample with a strong nuclear staining and reaction at cytoplasmic level. **c** The microphotograph shows a section of a ductal carcinoma with a homogeneous cytoplasmic reaction. Nuclei stained strongly. **d** IHC of a benign disease breast sample showing a negative anti-IDO reaction. **e** A group of MCF7 cells show a positive reaction. **f** A positive ICC reaction of T47D cell line is depicted (200×). **g** Microphotograph of immunofluorescence staining of MCF7 cell line with anti-IDO monoclonal antibody; a cytoplasmic and perinuclear reaction is observed (100×) (**h**) and (**g**). Microphotographs of immunofluorescence staining of MCF7 cell line with anti-IDO monoclonal antibody; a cytoplasmic and perinuclear reaction is observed at 100× (**h**) and 1,000× (**g**)

IDO 1 molecular analysis

To analyze *IDO 1* co-expressed genes in breast cancer platform-specific microarray collections, the web-based bioinformatics tool Multiexperiment Matrix (MEM) was employed, http://biit.cs.ut.ee/mem/. The 50-best positive correlated genes, displayed as rows, across a total of 76 microarray studies (columns, n=4,822 samples) are observed in Fig. 5a. The corresponding p values are indicated at the end of each row and in the heatmap obtained, red squares denote large similarity rank (Fig. 5b). We then analyzed these genes across one of the microarray studies (GSE21653) [15], in

Table 2 Immunohistochemical results according to the different subgroups based on the different receptor status

ER	PR	HER2-neu	IDO positive/total (%)
+	+	+	0/7 (0)
+	+	−	22/52 (42)
+	−	−	5/12 (42)
−	+	−	2/4 (50)
−	−	−	8/10 (80)
−	+	+	0/2 (0)
−	−	+	2/4 (50)

which samples are grouped according to breast cancer molecular classification: luminal A, luminal B, erbB2, basal-like, and normal-like. Interestingly, IDO and co-expressed genes predominately are shown in basal and erbB2 subtypes.

In order to identify the molecular pathways that were mainly affected by these 50 genes, we looked for protein/gene interaction networks in the common core of overlapped genes. The protein–protein interaction network was generated using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins, http://string.embl.de/) blue and red spheres (Fig. 5c). Genes from cluster blue are related to regulation of cell proliferation and inflammatory response, while genes from cluster red are mainly associated with apoptosis.

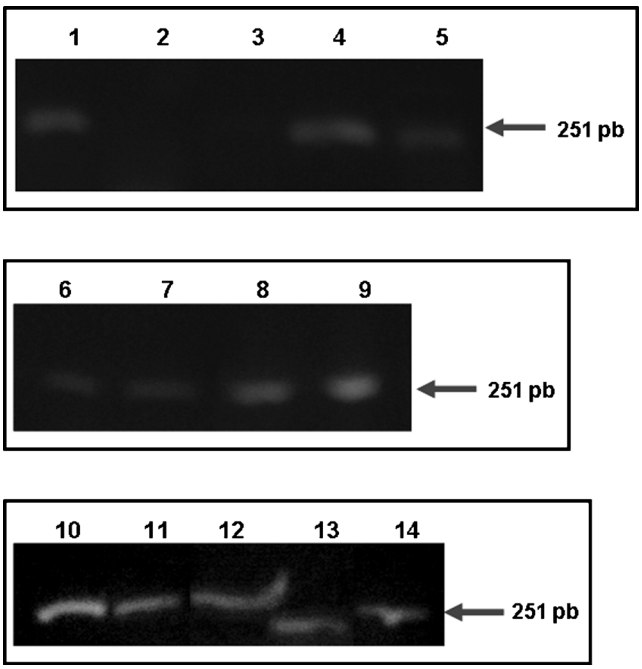

Fig. 3 RT-PCR results. Amplicons of 251 bp are depicted corresponding to IDO bands. *Lanes 1*, *6*, and *10* correspond to MCF7 cell line. *Lanes 7*, *12*, and *14* correspond to MDA-MB-231 cell line, while *lane 11* shows a band from T47D cell line. *Lanes 4*, *5*, *8*, and *9* correspond to primary tumors samples showing bands at 251 bp, while *lanes 2* and *3* show tumors which are negative for IDO. *Lane 13* corresponds to an exosome fraction from MCF7 cell line and shows a band at less than 251 bp

It has been established that triple-negative breast cancer (TNBC) molecular type can be classified into different subtypes: basal-like 1 and 2 (BL1 and 2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) [16]. According to this, we employed the bioinformatics tool TNBCtype [14] to classify those samples labeled as basal in the GSE21653 dataset. We then evaluated IDO 1 expression in the TNBC subgroups, and we found that the IM subtype showed the highest expression of IDO (Fig. 5d).

Discussion

IDO is an important negative regulator of the immune system, and high IDO expression is associated with poor prognosis in a variety of cancer types. Strategies to pharmacologically inhibit IDO may enhance immune-mediated responses following conventional chemotherapy and may be synergistic with other forms of immunotherapy [17, 18].

In this report, we found that IDO expression was detected in 60 % of breast cancer samples, and its expression augmented in relation to increasing disease stage; reaction showed a predominantly cytoplasmic pattern, although some nuclei were also positive. Furthermore, all tumor cell lines here included expressed IDO. Although 43 % of benign breast conditions were positive; reaction was weaker than in cancer samples, while a significant lower expression was detected in normal samples; results were confirmed by RT-PCR.

Considering clinical and histological factors, we found a statistically positive correlation between IDO and tumor pathological stage as well as with tumor nuclear grade, while a negative correlation between IDO and ER expression was obtained. Taken together, these observations account for a more aggressive type of tumor when IDO is expressed.

In previous studies on breast cancer employing IHC and mRNA detection, IDO expression showed variable results. Yu et al. [19] have studied breast cancer, benign breast conditions, and normal adjacent to cancer specimens; they reported an association of IDO expression from normal to carcinoma samples, and with advanced tumor stages and lymph node metastasis; these results are in agreement with our findings.

On the contrary, in a series of 155 breast cancer patients, Jacquemier et al. [20] have found a correlation between IDO expression versus lymph node negative; they did not include normal samples for IHC analysis. Interestingly, considering ER, PR, and HER2-neu expression, they found a positive correlation between high IDO expression and triple-negative breast cancer; regarding the association of IDO expression with negative ER tumors, our results are in coincidence with these authors. Also, and in coincidence with these authors, we found a cytoplasmic and nuclear staining. Finally, in a recent report, Soliman et al. [21] have found that IDO expression was associated with lymph node-negative and estrogen-positive tumors.

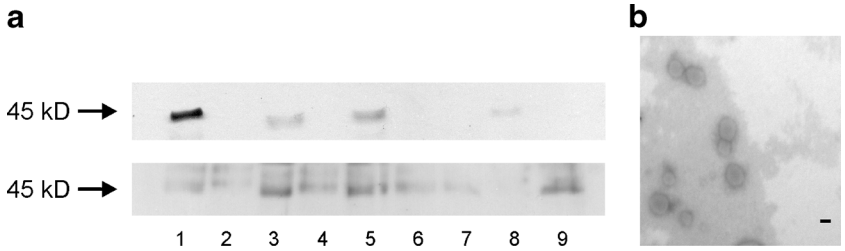

Fig. 4 Results obtained with MV. *Lines 1–9* correspond to MV fractions isolated to breast cancer patients plasma. **a** Western blot results of IDO detection. A single or double band can be observed in the *upper* and *lower lines* in the MV fractions. **b** A representative electron microscope photograph of a MV fraction shows the presence of round microvesicles with accumulation of negative stain (phosphotungstic acid) in the periphery and in the center of the concave exosome-like structures. Size was heterogeneous and ranged from 50 to 200 nm (*scale bar* at the bottom right of the picture represents 50 nm)

Divergences among authors may be related to differences in the composition of the cohorts studied; also, the use of different antibodies may influence the IHC results. Nevertheless, all groups reported a high expression of IDO in breast tumors

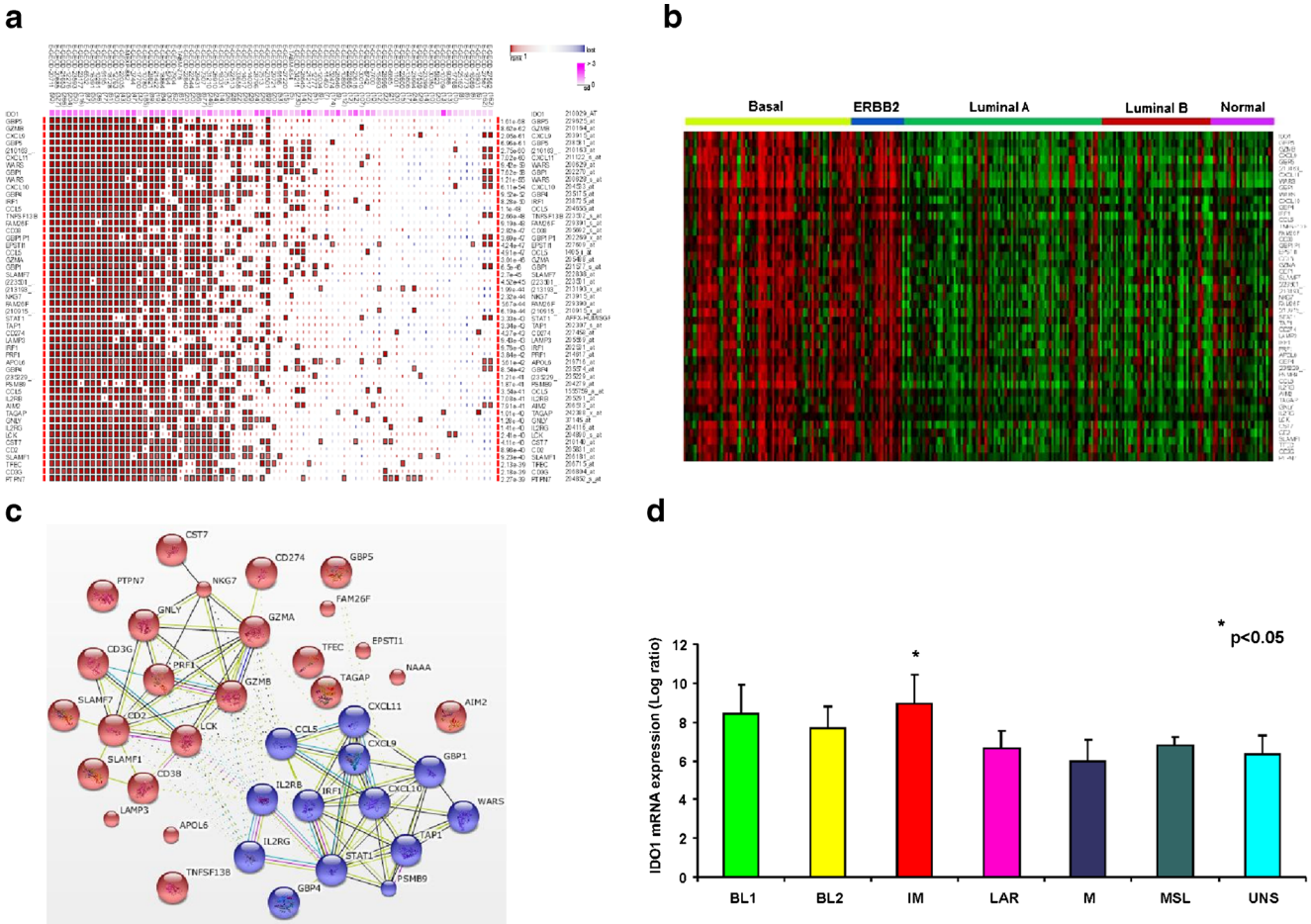

Fig. 5 **a** Analysis of *IDO 1* co-expressed genes through breast cancer platform-specific microarray collections by the web-based bioinformatics tool Multiexperiment Matrix (MEM), http://biit.cs.ut.ee/mem/. The 50 best positive correlated genes, displayed as *rows*, across a total of 76 microarray studies (*columns*, n=4,822 samples) are observed. The corresponding p values are indicated at the end of each row. Heatmap *red squares* denote large similarity rank. **b** Analysis of *IDO 1* and co-expressed genes across one of the microarray studies (GSE21653), in which samples are grouped according to breast cancer molecular classification: *luminal A*, *luminal B*, *erbB2*, *basal-like*, and *normal-like*. *IDO 1* and co-expressed genes predominately are shown in basal and erbB2 subtypes. **c** The protein–protein interaction network was generated using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins, http://string.embl.de/) *blue* and *red spheres*. Genes from cluster blue are related to regulation of cell proliferation and inflammatory response, while genes from cluster red are mainly associated with apoptosis. **d** TNBC (triple-negative breast cancer) molecular subtypes analysis of samples labeled as basal in the GSE21653 dataset employing the bioinformatics tool TNBCtype: IDO is highly expressed in immunomodulatory subtype (IM) (*BL1* basal-like 1, *BL2* basal-like 2, *M* mesenchymal, *MSL* mesenchymal stem-like, *LAR* luminal androgen receptor)

which may be related to the role that IDO would exert in the local immune suppression within the tumor microenviroment.

On the other hand, increased evidence has suggested that microvesicles might act as a vehicle for transmitting signals for immune suppression thus having negative effects on anti-tumor immune responses. In association with microvesicles, different biologically active molecules such as a membrane form of Fas ligand (FasL), APO2/TRAIL, CD44, or major histocompatibility complex (MHC) class I antigens have been found. These findings provide a reasonable explanation for their involvement in immune suppression [11, 22]. Exosomes interfere directly with T cell effectors functions by inducing apoptosis in activated tumor-specific T cells and suppress NK cell tumor cytotoxicity [23].

We found a positive association with circulating microvesicles in 9 out of 25 breast cancer patient. These microvesicles showed a characteristic exosome-like size by electron microscopic analysis, and they express exsome markers CD63 and CD9. The finding of IDO in tumor-derived microvesicles could reinforce the tolerizing role of this enzyme in breast cancer patients not only at local level but also at distant sites as well.

Finally, an in silico analysis of IDO 1 gene expression in breast cancer showed that it is associated with several genes related to immune response and apoptosis. Functions involved in these interactions were, among others, regulation of apoptosis, lymphocyte activation and differentiation, cytokine/chemokine–cytokine/chemokine receptor interactions, Toll-like receptor signaling, and cytolysis.

The "Guilt by association" principle states that gene co-expression might indicate shared regulatory mechanisms and roles in related biological processes. Interestingly, IDO and co-expressed genes are predominately shown in basal and erbB2 subtypes. In order to classify those samples labeled as basal in the GSE21653 dataset, we then evaluated IDO 1 expression in the triple-negative breast cancer subgroups, and we found that the immunomodulatory subtype showed the highest expression of IDO. Lehman et al. [16] established that the immunomodulatory subtype is enriched for gene ontologies in immune cell processes including immune cell and cytokine signaling, antigen processing and presentation, and signaling through core immune signal transduction pathways. The same group also found that it is likely that the IM characteristics are unique to the tumor cells themselves and not a reflection of immune cell infiltrate.

Conclusion

The presence of IDO in breast neoplasms and breast cancer microvesicles supports the hypothesis that this enzyme may contribute to tumoral escape from immunosurveillance and, in consequence, could favor tumor dissemination. In silico analysis reinforces this relationship not only through the high expression of *IDO 1* and co-expressed genes in basal and erbB2 molecular subtypes of breast cancer but also in the immunomodulatory subtype among triple-negative breast cancer. These results might be taken into account to develop new therapeutic strategies.

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Conflicts of interest None

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