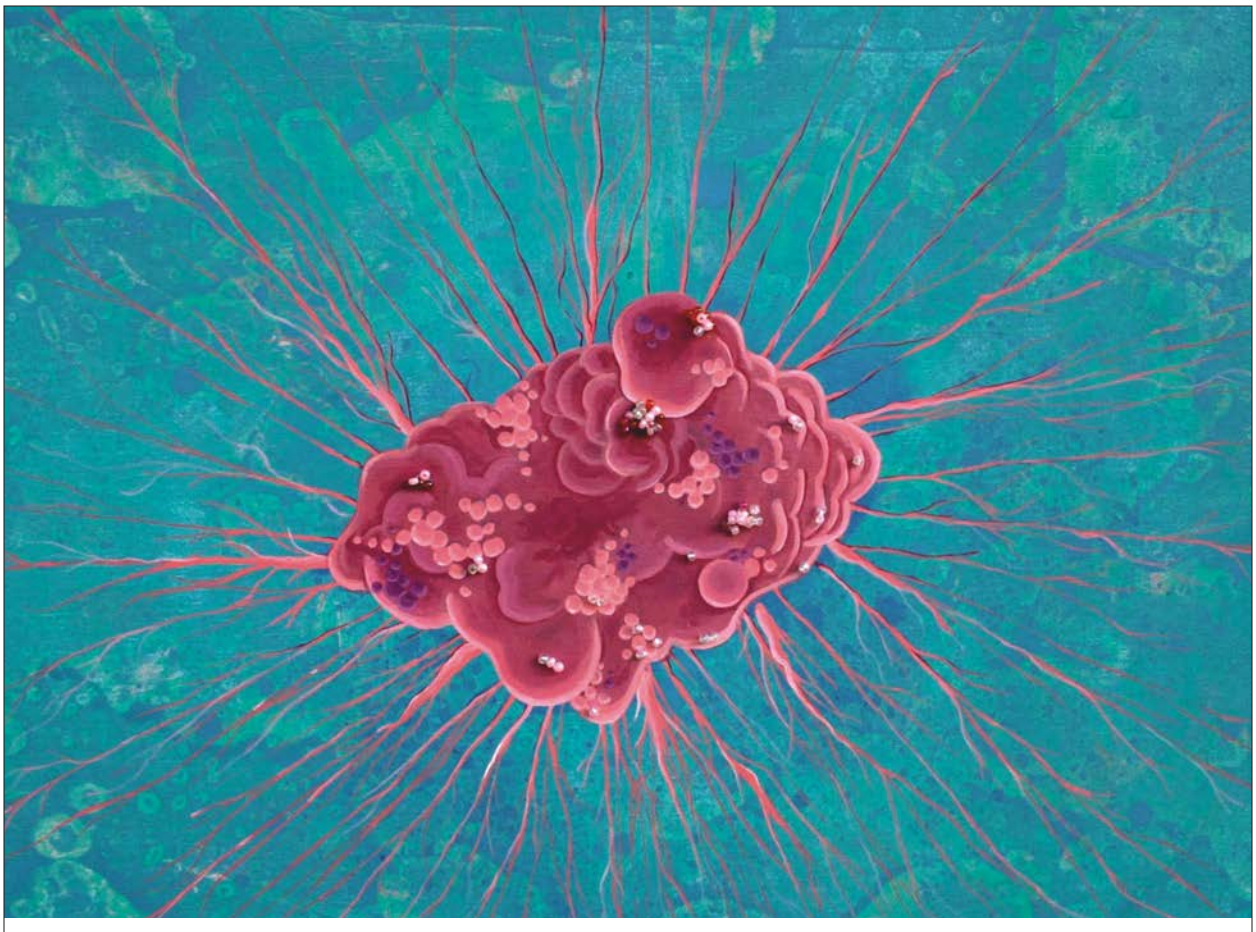

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Research Article

CD74 a Potential Therapeutic Target for Breast Cancer Therapy: Interferon Gamma Up-regulates its Expression in CAMA-1 and MDA-MB-231 Cancer Cells

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Abstract

Background and Objective: Cluster of differentiation (CD) 74 is well known for its diverse proinflammatory and immunological functions. This study was undertaken to investigate the role of interferon (IFN)-γ on CD74 expression in breast cancer cells. **Materials and Methods:** The CAMA-1 and MDA-MB-231 cancer cells were stimulated with IFN-γ and expression of CD74 was determined by flow cytometry and western immunoblotting using Raji cells as a positive control. The MTT assays were used to determine the effect of IFN-γ on the proliferation and migration of cells. Whereas, cell surface expression of HLA-ABC and HLA-DR were acquired by flow cytometry. **Results:** Treatment of CAMA-1 and MDA-MB-231cells with IFN-γ significantly increased the expression of CD74. Not only have these, IFN-γ also stimulates the expression of HLA-ABC and HLA-DR in a dose-dependent manner in CAMA-1 and MDA-MB-231cells. Moreover, IFN-γ treatment also inhibited the proliferation and migration of CAMA-1 and MDA-MB-231cells. **Conclusion:** The IFN-γ up-regulates CD74 expression in human breast cancer cells. The results concluded that IFN-γ played a key role in modulating the expression of CD74, as well as in the proliferation and the migration of CAMA-1 and MDA-MB-231 cancer cells. Data suggest that CD74 plays a key role in tumor immunogenicity as well as in breast cancer immunoediting in humans.

Key words: CD74, IFN-γ, proinflammatory response, tumorigenesis, breast cancer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cluster of differentiation (CD) 74 is also known as major histocompatibility complex (MHC) class II-associated invariant chain, is classified as a type II transmembrane glycoprotein that has diverse immunological functions[1]. In human, CD74 is post-transnationally glycosylated and exists in four isoforms, P33, P35, P41 and P43. Among all reported isoforms of CD74, P33 is the most common[2]. The CD74 is restrictedly expressed by antigen presenting cells and the most well-known function of CD74 is its ability to associate with MHC class II α and β chains, directing the transport of the αβIi complex to the endosome and lysosome[3,4]. Importantly, CD74 has begun to emerge as a more versatile molecule beyond its well-known function of regulating class II MHC trafficking as CD74 is highly expressed in various inflammatory disorders and also in several types of tumors[5]. Moreover, it has also been classified as a new prognostic factor for patients with malignant pleural mesothelioma[6] and was reported to have a high potential in treating thyroid carcinoma[7]. Furthermore, it is also reported that CD74 may function as a cytokine and a receptor for bacterial entry[8]. As it been identified as the high-affinity receptor for the cytokine macrophage migration inhibitory factor (MIF) and binding through this stimulates several proinflammatory cell signaling events such as ERK and PI3K/AKT signaling[8,9]. Moreover, CD74 involves in the regulation of several pro-tumorigenic molecules, such as interleukin (IL)-8 and interferon-gamma (IFN-γ) and anti-apoptotic factor BCL-2[10,11]. Although studies reported CD74 expression in breast cancer[12] but its role in breast cancer remains unclear.

Interferon-gamma (IFN-γ) is a potent proinflammatory cytokine, known to perform numerous critical roles such as promoting immune responses, immunopathological processes, cell maturation, differentiation, activation and apoptosis[13]. The IFN-γ is a member of a family of proteins originally identified by their capacity to non-specifically protect cells from viral infections. It is known that IFN-γ plays a critical role in cancer progression by controlling apoptosis, cell proliferation, angiogenesis and the expression of MHC class I and II as well as CD74[13,14]. The main aim of the present study was to investigate the role of CD74, the cell-surface form of the invariant chain, in respect to immunological molecules in breast cancer cell lines. This was achieved by studying the effect of IFN-γ on the expression of CD74 on CAMA-1 and MDA-MB-231 cells and by quantifying the cell-surface expressed CD74 receptors as well as proliferation and cell migration. To evaluate the response of the cells to stimulation, the cell- surface expression of HLA-A, B, C and HLA-DR was investigated after IFN-γ treatment. To achieve the aim of this study, flow cytometry and Western blotting were employed.

MATERIALS AND METHODS

This study was conducted in School of Biological Sciences, University of Essex, Colchester, United Kingdom between May, 2016-January, 2018. Data interpretation and manuscript drafting were performed in the College of Medicine, Qassim University, Buraidah, Saudi Arabia.

Cell lines and cell culture: The human mammary gland cell lines, CAMA-1 and MDA-MB-231 were purchased from American Type Culture Collection (ATCC, Breast Cancer Cell Panel # ATCC® 30-4500K™, Middlesex, UK). The CAMA-1 cell lines were maintained in RPMI 1640 medium (LONZA-Belgium), supplemented with 10% (v/v) fetal calf serum (FCS, Imperial Laboratories, UK). The MDA-MB-231 cell line was maintained in D-MEM (high glucose), supplemented with 10% FCS. Whereas, Raji cells (human negroid Burkitt's lymphoma) were cultured in RPMI 1640 containing 10% FCS. All cell lines were cultured in a humidified atmosphere of 5% CO_2 at 37°C. All media used for this study were purchased from PAA Laboratories (GmbH, Pasching, Austria).

Reagents: The monoclonal primary antibodies mouse anti-human CD74 (clone: By2) and mouse anti-human α-tubulin (clone: TU-02) were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology Inc., CA, USA). Mouse anti-human HLA-A, B, C (clone: W6/32) was purchased from Cambridge-Biosciences (Cambridge, UK). Mouse anti-human HLA-DR (clone: LN3) was purchased from Bio-legand (Bio-legand Inc., UK). Recombinant Human Interferon Gamma (IFN-γ) was purchased from Immuno Tools (GmbH, Friesoythe, Germany). The secondary antibody used for flow cytometry was a goat anti-mouse antibody conjugated with the fluorophore FITC (clone: poly4053) and was purchased from Bio-legend (Bio-legand Inc., UK). The secondary antibody used for Western blotting was goat anti-mouse (IRDye 800CW) and was purchased from LI-COR Biosciences, USA.

Cell stimulations: Synthetic IFN-γ is a single, non-glycosylated, polypeptide chain containing 144 amino acids, in white lyophilized (freeze dried) powder. IFN-γ was dissolved in 1 mL of sterile H_2O (stock 1), 50 µL from stock 1 was added to 450 µL of sterile H_2O (stock 2) and was stored at -18°C in accordance with the manufacturer's instructions. The CAMA-1 and MDA-MB-231 cells were grown in RPMI-1640 and D-MEM

respectively, containing 10% FCS overnight before stimulation. Cells were then stimulated with IFN-γ at a concentration of 100, 500 and 1000 IU mL^{-1} for 72 h. Cells were counted using a hemocytometer, checked for viability and harvested for sample preparation.

Flow cytometry analysis: Cell lines were lifted with Accutase (Sigma-Aldrich, Dorset, UK) and 10×10^6 cells were used per sample. Monoclonal antibodies By2 (anti-CD74), (anti-HLA-A, B, C) and LN3 (anti-HLA-DR) were employed in indirect immunofluorescence staining. Cells were preincubated with saturating concentrations of primary antibody, followed by washing and labelling with FITC-conjugated goat anti-mouse IgG (Bio-legend, UK). For cell-surface staining, cells were fixed with 4% formaldehyde solution and washed with 1X PBS. The cells were then blocked with blocking buffer (PBS/0.1% BSA) and washed in PBS. Primary and secondary antibodies were diluted with 0.1% BSA in PBS. Cells were sorted on a BD FACSAria and analysed by FlowJo 8.8.6.

Immunostaining: Cell monolayer culture and double staining: CAMA-1, MDA-MB-231 and Raji cell lines were separately cultured in LabTek 8 well chambers (Thermo Fisher Scientific) at a density of 86 10 Å~ 103 cell per well for 2 days following seeding. For the staining procedure, all the steps were carried out at room temperature. The cells were fixed with 4% PFA (Sigma, UK) for 20 min and blocked with 2% (w/v) BSA (Bovine serum albumin) prepared in 1X PBS for 1 hr at room temperature. The cells were incubated with anti-CD74 monoclonal antibody for 1 h and washed three times with PBS. For the secondary antibody, anti-mouse IgG conjugated with Alexa Fluor® 488 (Invitrogen, Carlsbad, CA, USA) was used for 1 h. Isotype controls were stained with only secondary antibody. The slides were then examined under a Nikon confocal microscope using an ×60 oil immersion objective (numerical aperture 1.4) and FITC filter for Alexa Fluor® 488. The images obtained were then analysed using the Fiji software and NIS elements.

MTT assay: Breast cancer cells CAMA-1 and MDA-MB-231 (1×10^4 cells/well) cells were cultured in 96 well flat bottom plates at 37°C. After 24 h incubation that allowed cells to adhere, cells were exposed to varying concentrations of IFN-γ or no treatment (10% FCS medium only). Then cells were incubated for 72 h after treatments. The MTT assay was then used to assess cell proliferation. Briefly, 20 µL of MTT solution (5 mg mL^{-1} in PBS) was added per well and cells were incubated at 37°C with 5% CO2 in a humidified chamber for 4 h for color development. The resultant formazan crystals were dissolved in dimethyl sulfoxide (100 µL) and the absorbance intensity measured at 595 nm using a micro plate reader (Versamax, USA). The percentage of cell proliferation was calculated relative to the rate of proliferation in untreated cells.

Migration assay: Both CAMA-1 and MDA-MB-231 cells were detached, counted and seeded in serum free medium in the upper chamber of a 6-well cell culture plate placed with 24 mm polyester membrane trans well insert (pore size: 4.0 µm) from Corning Incorporated Life Sciences, UK. The cells were then allowed to attach with medium alone that was added to the upper chamber of the plate. Either medium containing 10% FCS was added or serum free medium was added to the lower chamber and the cells were allowed to migrate and invade for 24 h. Migrated cells were washed twice in PBS and fixed with 4% paraformaldehyde (PFA) for 20 min at room temperature. The cells were then permeabilized using 100% methanol for 20 min at room temperature followed by washing in PBS. The cells were then stained using Giemsa stain for 15 min and then washed three times in PBS. Non-migrated cells were then carefully scraped off with cotton swabs. Migrated cells were mounted and the slides were visualized under low magnification on a BX41 microscope.

Western blotting and immunodetection: Total cell lysate from treated and non-treated cells was prepared as described previously[15,16]. The total cell lysate was electrophoresed on 10% SDS-PAGE gel with 2.5% stacking and proteins were detected by immunoblotting as described previously[17,18]. Briefly, a total of 30 µg protein was loaded per well then after electrophoresis, protein was transferred to a polyvinylidene fluoride (PVDF) membrane (Immobilon-FL, Merck Millipore, Germany). Membranes were blocked with 5% skimmed milk in PBS-Tween-20 (Sigma, UK) for 1 h at room temperature and incubated in anti-CD74 (clone: By2) at a concentration of 1:200. As a control, an alpha subunit-specific tubulin mouse monoclonal antibody was used to probe the cell extracts at a concentration of 1:200, followed by washing in PBS-T for 30 min. The membranes were then incubated with IRDye 800CW donkey anti-mouse IgG (Li-Cor Bioscience, USA) at a concentration of 1:1000 for 1 h followed by washing in PBS-T for 30 min. Signals were detected using the ODYSSEY Infrared Imaging System (Li-Cor Biosciences, USA). Fermentas PageRuler™ plus Prestained Protein Ladder (Thermo Fisher Scientific, USA) was used in order to estimate the molecular weight of the respective protein bands.

RESULTS

Intracellular expression of CD74 in CAMA-1 and MDA-MB-231 cells: Intracellular expression of CD74 was detected in CAMA-1 and MDA-MB-231 cells by confocal microscopy and the results are shown in Fig. 1. Both CAMA-1 and MDA-MB-231 cells expressed CD74 in their intracellular compartments. Raji cells also showed expression of CD74 and were used a positive controls.

Role of IFN-γ on the proliferation migration of CAMA-1 and MDA-MB-231 cells: The effect of varying concentrations of IFN-γ (100, 500 and 1000 IU mL^{-1}) for 72 h on proliferation of CAMA-1 and MDA-MB-231 cells was examined. Cells without IFN-γ treatment served as the control group. The IFN-γ could inhibit the proliferation of CAMA-1 and MDA-MB-231 cells (Fig. 2a). Both cell lines showed a similar pattern of decreasing the proliferation. The ability of non-invasive CAMA-1 and highly invasive MDA-MB- 231 cells to migrate was measured in the presence and absence of 10% FCS, as controls, using a trans well insert from Corning Life Science (Fig. 2b). The result showed that both cell lines had a tendency to migrate towards the serum. However, MDA-MB-231 cells migrated more than CAMA-1 cells. The migration of both CAMA-1 and MDA-MB-231 cells cultured with IFN-γ for 24 h was also evaluated. In the absence of 10% FCS, 1000 IU mL^{-1} of IFN-γ were added in the upper chambers of the Trans well insert. The results confirmed that both cell lines migrate against IFN-γ even in the absence of FCS, which functions as an effective chemoattractant for cells in the culture. This suggests that IFN-γ a clear effect in the migration of both cell lines.

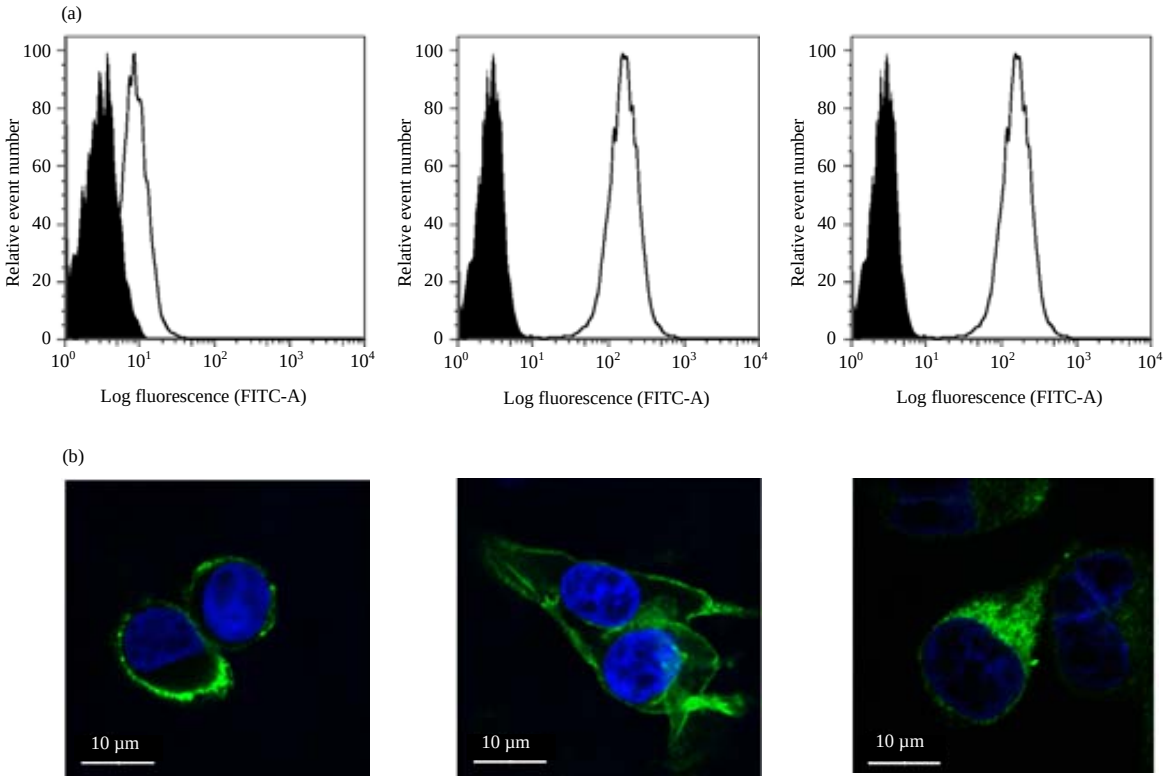

Fig. 1(a-b): Cell surface expression of CD74 on CAMA-1, MDA-MB-231 and Raji cells. (a) Cells were cultured in the appropriate media and were acquired by flow cytometry. Empty histograms represent the aforementioned cell lines labeled with anti-CD74 antibody. Black-filled histograms show the isotypes serving as negative controls. Data are representative of three independent experiments. (b) Cells were stained either with CD74 labelled with Alexa Fluor® 488 (green). Cell nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI) that is shown in blue. Fluorochromes were acquired separately to evaluate the expression of CD74, CD44 and MIF using the Fiji software. Photomicrographs are representative of three independent experiments. Scale bar 10 µm

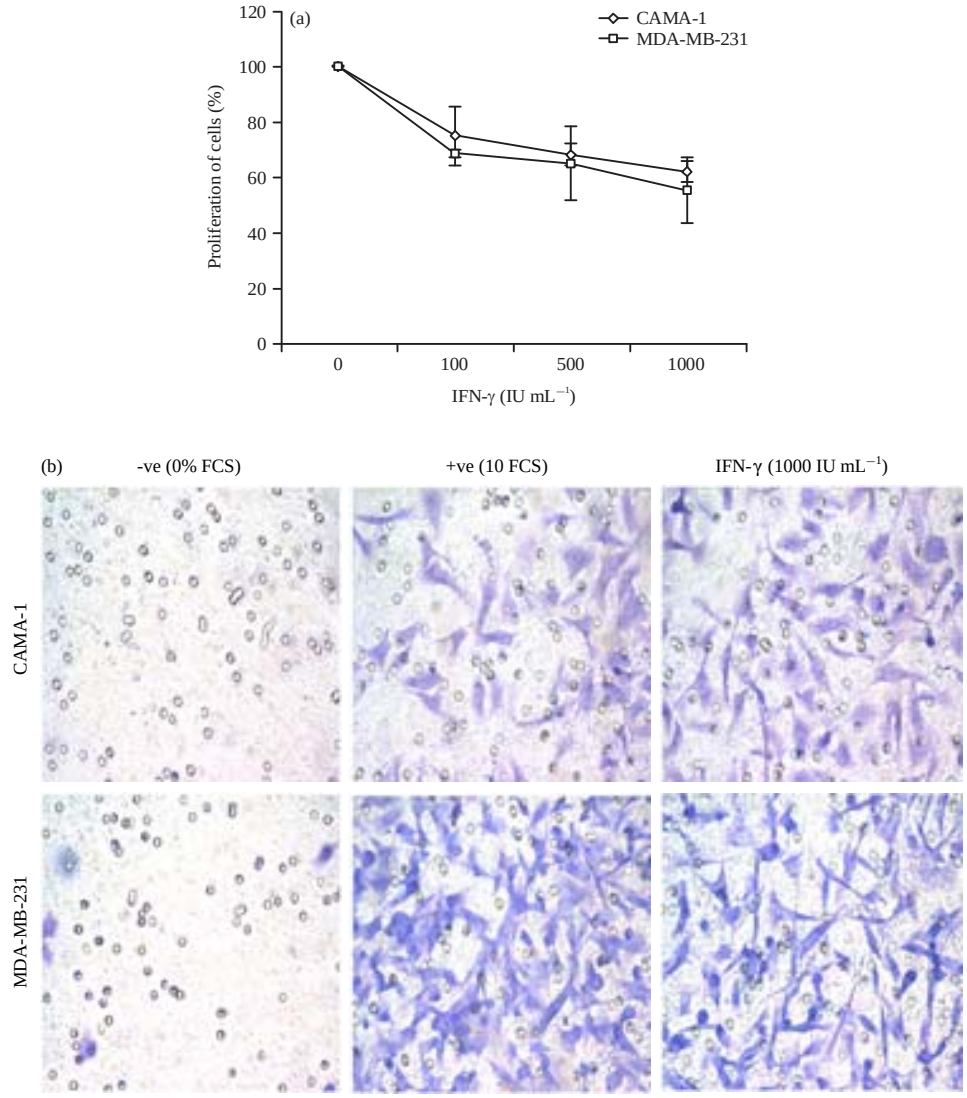

Fig. 2(a-b): Effect of IFN-γ on the proliferation and Migration of CAMA-1 and MDA-MB-231 cells by MTT assay. (a) CAMA-1 and MDA-MB- 231 cells were seeded in 96 well-plates and treated with indicated concentrations of IFN-γ for 72 h. Each data point represents a mean of at least three independent experiments with triplicate wells and error bars represent SEM from three separate experiments. (b) CAMA-1 and MDA-MB-231 cells were cultured with an appropriate medium in the presence or absence of 10% FCS and were allowed to migrate overnight through the trans well membrane, 24 mm diameter inserts and a pore size of 0.4 μm

Effect of IFN-γ on the expression of HLA-A, B, C, HLA-DR and CD74: Weak expression of HLA-DR was detected on the cell-surface membrane of untreated CAMA-1 and MDA-MB-231 cells. In contrast to CAMA-1 cells, which showed a negative expression of HLA-A, B, C molecules, MDA-MB-231 cells showed high expression of the same molecules. It was observed that the expression of HLA-A, B, C and HLA-DR increased when CAMA-1 and MDA-MB-231 cells were incubated with (100, 500 and 1000 IU mL^{-1}) IFN-γ for 72 h (Fig. 3). However, maximum HLA-A, B, C and HLA-DR expression was detected in CAMA-1 cells treated with 1000 IU mL^{-1} of IFN- γ. In the same manner, MDA-MB-231 cells treated with 1000 IU mL^{-1} of IFN-γ showed the maximum expression of HLA-A, B, C and HLA-DR molecules. The CD74 was found to be moderately expressed on the cell-surface in untreated CAMA-1 and MDA-MB-231 cells. The expression of CD74 increased when CAMA-1 and MDA-MB-231 cells were incubated with IFN-γ (100, 500 and 1000 IU mL^{-1}) for 72 h (Fig. 4a, b). However, maximum CD74 expression was detected in CAMA-1 and MDA-MB-231 cells treated with either

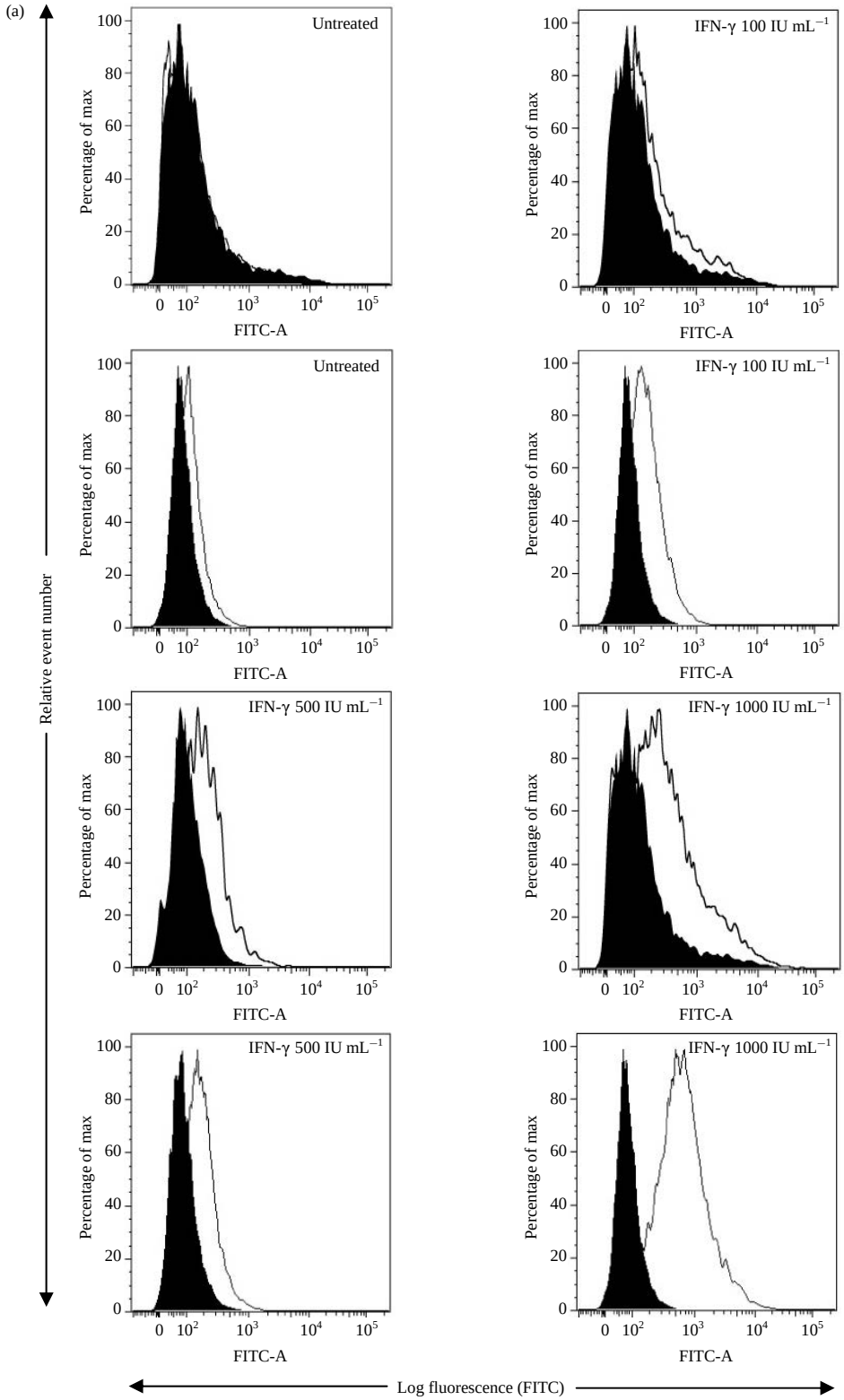

Fig. 3(a-b): Continue

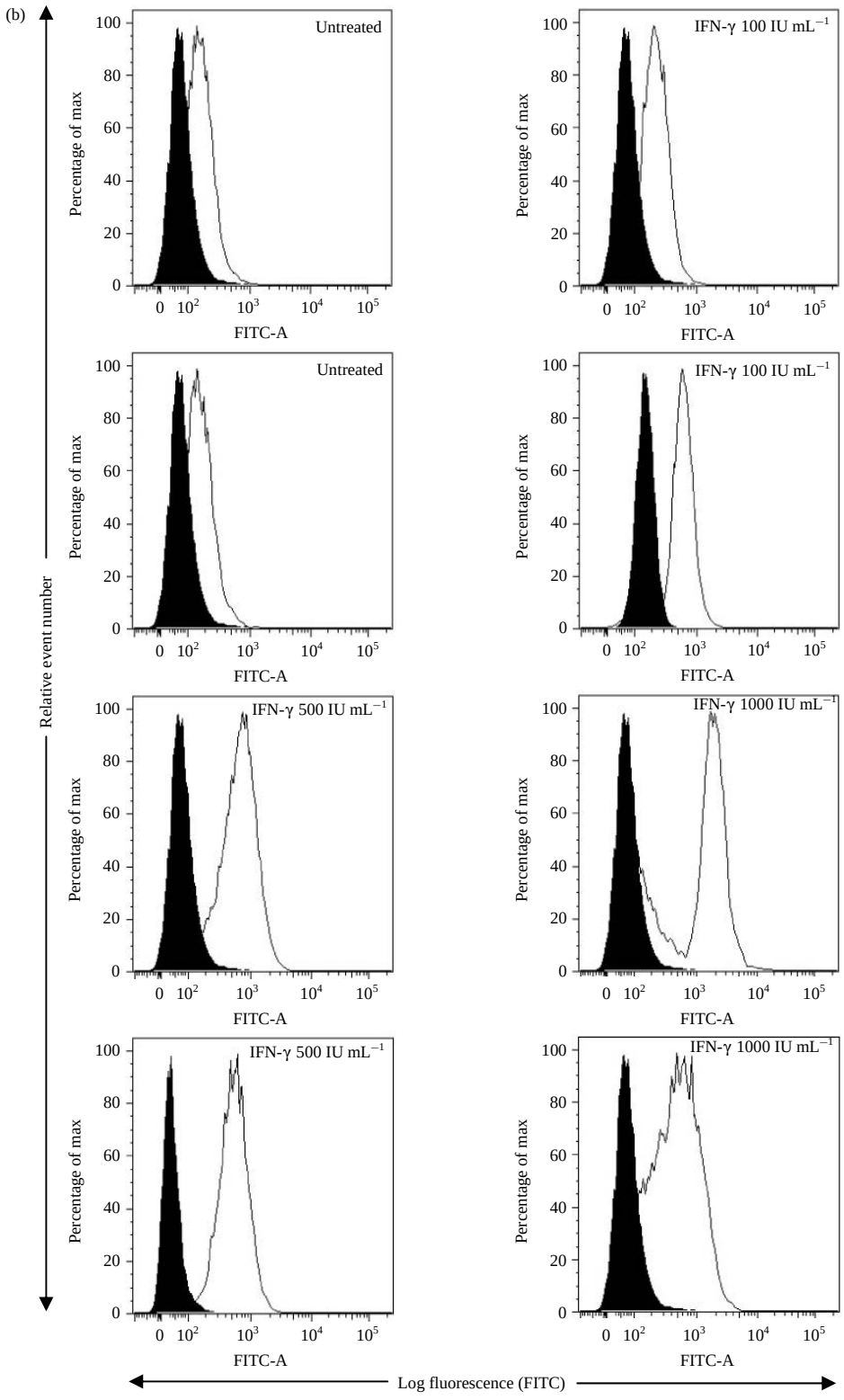

Fig. 3(a-b): Cell-surface expression of HLA-A, B, C and HLA-DR on IFN-γ treated CAMA-1 and MDA-MB-231 cells. The CAMA-1 and MDA-MB-231 cells were cultured in the presence of the indicated concentrations of IFN-γ (100-1000 IU mL^{-1}) for 72 h and were acquired by flow cytometry using w6/32 (anti-HLA-A, B, C) and L243 (anti-HLA-DR). Empty histograms represent the cells with indicated concentrations of IFN-γ and antibody. Black filled histograms show negative controls. Cells were labelled with FITC-labelled secondary anti-mouse antibody

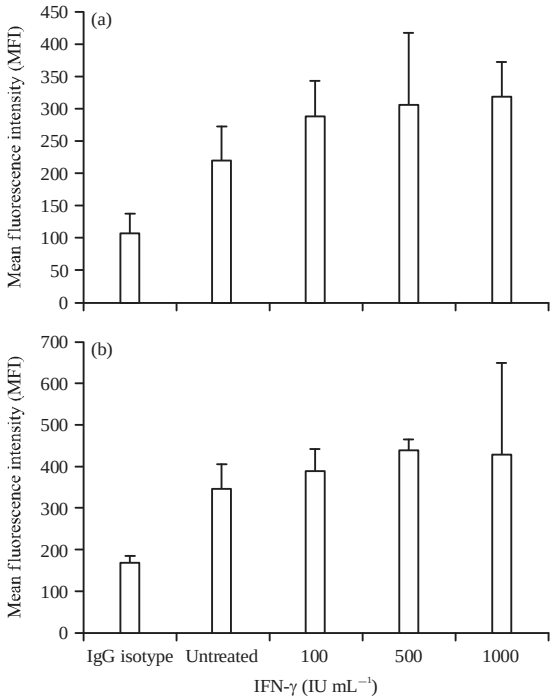

Fig. 4(a-b): Graphical representation of CD74 surface protein expression in (a) Untreated CAMA-1 cells and (b) MDA-MB-231 cells (B) treated with IFN-γ treated CAMA-1 MDA-MB-231cells. Bar graphs represent level of each protein in values Mean±SD in each cell line. It was noted that level of CD74 expression increased after IFN-γ treatment. Data are representative of three independent experiments

500 or 1000 IU mL^{-1} of IFN-γ. Figure 4a and b showed cell-surface expression of CD74 depicted by graphical representation of mean fluorescence intensity (MFI) in IFN-γ treated CAMA-1 and MDA-MB-231.

Western blot analysis: Total cell lysate was extracted and loaded on a gel from untreated and IFN-γ treated CAMA-1 and MDA-MB-231 cells, cultured in the presence of the indicated concentrations of IFN- γ (100, 500 and 1000 IU mL^{-1}) for 72 h (Fig. 5a, c). The presence of bands corresponding to the expected molecular weight of CD74 confirmed its expression. The differences in band intensity between untreated and treated CAMA-1 or MDA-MB-231 cells confirmed that CD74 was upregulated in cells incubated with IFN-γ (fold change). CD74 isoforms were detected at expected molecular weights, 33 and 41 kDa. Maximum CD74 isoform expression was detected in CAMA-1 cells treated with 500 IU mL^{-1} of IFN-γ. Density of the bands was normalized with internal α-tubulin loading control (Fig. 5a, d).

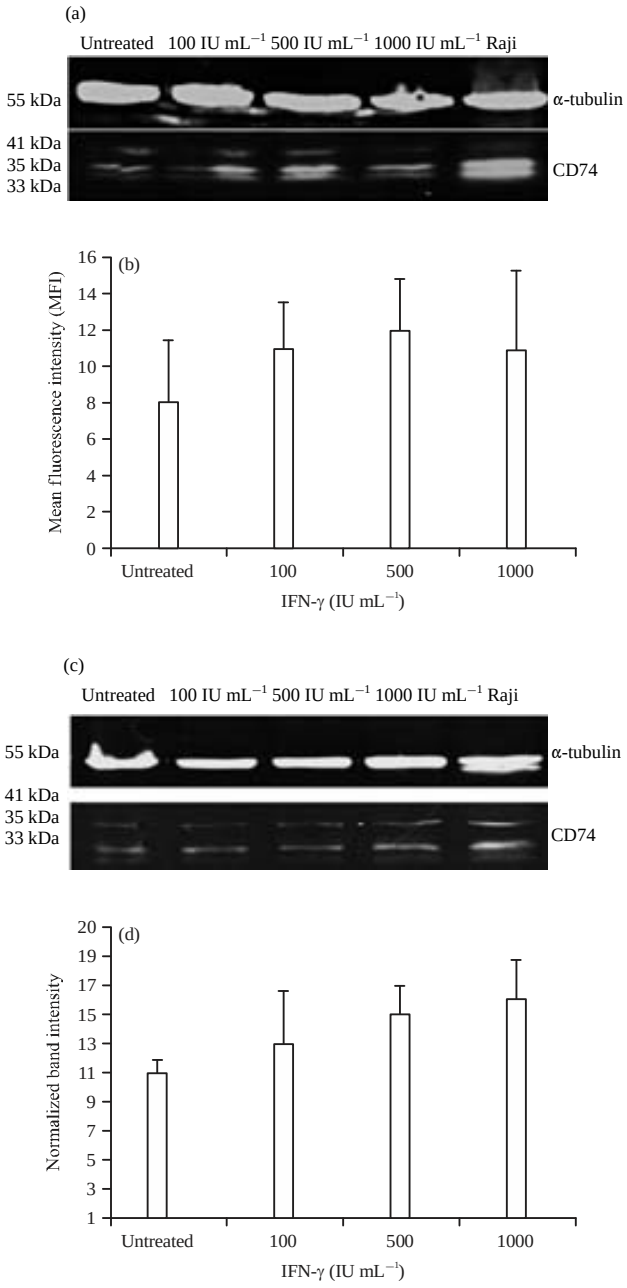

Fig. 5(a-d): CD74 and α-Tubulin expression according to their molecular weight in IFN-γ treated CAMA-1 cells (A,B) and MDA-MB-231 cells (C,D). A representative immunoblot of three independent experiments

DISCUSSION

In this study, it was determined that the MIF-receptor CD74 as an independent prognostic factor in CAMA-1 and MDA-MB-231 breast cancer cells. Growing interest focuses on the role of inflammatory signals in the initiation and development of breast cancer diagnosis and prognosis.

Therefore, following profiling of CD74 expression, the effect of proinflammatory cytokine IFN-γ on CD74 expression in CAMA-1 and MDA-MB-231 cancer cells was investigated in the present study. It was found that CD74 positivity is significantly enhanced when CAMA-1 and MDA-MB-231 cancer cells were treated with IFN-γ. The results indicated that IFN-γ plays an important role in up-regulated expression of CD74 in the breast cancer cells CAMA-1 and MDA-MB-231. Moreover, in previous report similar findings were also proved through flow cytometry using anti-CD74 (clone: By2)[19].

Not only have these, results also showed IFN-γ played a key in the proliferation and the migration of these breast cancer cells. These findings clearly suggested that CD74 might play a role in tumor immunogenicity as well as in cancer immunoediting of CAMA-1 and MDA-MB-231 cells. The characterization of the determinants of tumor immunogenicity may potentially help to inform the development of cell-based vaccines, therefore the present study sought to map of immunological and related molecules in breast cancer cells to investigate the involvement of CD74 in proinflammatory and inflammatory pathways. As it was explained above, CD74 is a receptor for the proinflammatory cytokine, MIF. This ligand/receptor complex initiates survival pathways and cell proliferation and it triggers the synthesis and secretion of major proinflammatory factors and cell adhesion molecules[20,21]. For this reason the influence of IFN-γ on the behavior of the breast cancer derived cell lines, CAMA-1 and MDA-MB-231 was studied. It is suggested that over expression of CD74 by tumor cells might enhance their escape from immunoediting during the cancer immunoediting process[22]. Moreover, studies also have shown evidences to link CD74 to tumor survival, suggesting that systemic inflammation and postoperative infections lead to cancer recurrence and survival[23]. Therefore, investigation of the role of IFN-γ in the expression of CD74 in breast cancer cell lines has become critical. Present study, investigated the role of IFN-γ in proliferation and migration of CAMA-1 and MDA-MB-231 cells. Treatment of CAM-1 and MDA-MB231 cells with IFN-γ ranging from 100-1000 IU mL^{-1} showed declined in the cells number, indicating the increasing concentration of IFN-γ produces toxic effect on the cell viability. The results from migration assays indicated that MDA-MB-231 cells migrated towards FCS at a higher level than CAMA-1 cells. This was carried out as an evaluation of the ability of cells to display migratory properties in an *in vitro* assay. The effect of the optimal concentration 1000 IU mL^{-1} of IFN-γ on cell migration and invasion was also investigated. In this study, it was observed that 1000 IU mL^{-1} of IFN-γ was the optimal concentration. In this context, Zhao *et al.*[24] have shown that AGS, HGC-27 and GES-1 gastric cancer cells that were exposed to IFN-γ had significantly reduced colony formation, cell proliferation and migration ability[24]. However, flow cytometry showed no effect of IFN-γ on apoptosis of the cells and no effect on cell ageing as assessed by beta-galactosidase (β- gal) staining[24]. In contrast, results obtained from flow cytometry revealed that IFN-γ arrested the cells in the transition stage (G1/S) phase. It has also been shown that IFN-γ induces apoptosis in ovarian cancer cells *in vivo* and *in vitro*[25]. Tate, Jr. *et al.*[26] have shown that IFN-γ inhibits the proliferation of murine renal cell carcinoma cells[26]. In this respect, Zhang *et al.* stated that, in breast epithelial cells, the anti-proliferative effects of IFN-γ occur by up-regulation of apoptotic members of the bcl-2 family[27]. However, it has been reported that IFN-γ has an apoptotic effects which promoted by stimulation of p21 and inhibits G1 and S phase of cell cycle in prostate cancer cells[26,27]. In the same manner, García-Tuñón *et al.* showed that in breast cancer cell lines, IFN-γ treatment produces an increase in p21wich suggested IFN-γ could be non-functional and unable to activate p21 to stop the cell cycle[28].

The expression of CD74 can be induced in tumor cells upon their exposure to proinflammatory cytokines such as MIF and IFN-γ[29,30]. Several researches have shown that IFN-γ reduces tumourgenicity *in vivo* by upregulating the expression of MHC and related molecules such as CD74. The IFN-γ in cancer cells can be modified by epigenetic changes in the promoter region of class II, major histocompatibility complex, transactivator[31]. This may potentially disrupt the IFN-γ signaling pathway and impair T-cell activation, thus representing a possible tumor escape mechanism of cancer cells from immunosurveillance[32]. In the same manner, it was suggested that systemic inflammation and postoperative infections lead to cancer recurrence[33]. It has been shown that CD74 expression is increased by chronic inflammatory conditions and *Helicobacter pylori* infection[27]. Thus, to investigate tumor cell responsiveness to treatment with different reagents, the expression of HLA-A, B, C and HLA-DR molecules on the surface of CAMA-1 and MDA-MB-231 cancer cells, following their exposure to IFN-γ, was determined in the present study. This data confirmed that the viability of the cells in response to external reagents such as IFN-γ. The data also pointed out that CD74 expression increased upon treatment with IFN-γ in both the CAMA-1 and MDA-MB-231 cell lines. There were several potential explanations for increasing CD74 expression after IFN-γ treatment. In provision of these, it has been suggested that IFN-γ may directly induce CD74 intramembrane cleavage and release to the nucleus or alternative indirect mechanism via cathepsin S upregulation

or by an as yet unknown mechanism[34]. The CD74 is classified as MIF receptor, which suggests that its expression might be induced directly after cytokine treatment[5,35]. As an example, MIF induces the expression of CD74 via BCL-2 and BCL-XL (proteins family involved in the regulation of apoptosis) in tumor cells such as bladder cancer, prostate cancer, gastric cancer and breast cancer[36,37]. Verjans *et al.*[38] confirmed that the expression of CD74 and MIF is somehow associated, since the cell lines that express high levels of MIF show higher expression of CD74 compared to those that have low expression of MIF[38]. This might indicated that IFN-γ induces CD74 expression directly via the JAK-STAT1 pathway, even though the exact mechanism is not well known yet. In support of these studies, the data from the present study showed the MIF-receptor CD74 as an independent prognostic factor in CAMA-1 and MDA-MB-231 breast cancer cell lines. The data clearly pointing out the CD74 positivity is significantly increased after incubation with IFN-γ. The results suggested that IFN-γ can play a key role in modulating the expression of CD74 in CAMA-1 and MDA-MB-231cancer cells as well as on the proliferation and the migration of both cancer cell lines. This clearly indicated that CD74 might play a role in tumor immunogenicity as well as in cancer immunoediting of breast cancer cells. However, further research into CD74 and its effect on cellular processes, including the complex interactions between CD74 and its binding partners, such as MIF and CD44, will undoubtedly translate into clinical benefit for patients. In short, the present data support a role for CD74 in the inflammatory cascade during tumourigenesis. Its biological functions and its association with surrogate markers, such as invasion and migration, could implicate CD74 as a potential therapeutic target in breast cancer therapy.

CONCLUSION

This study concluded that treatment of breast cancer cells CAMA-1 and MDA-MB-231 with potent proinflammatory cytokine, IFN-γ up-regulates CD74 expression. The findings indicated that IFN-γ played a key role in modulating the expression of CD74, as well as in the proliferation and the migration of CAMA-1 and MDA-MB-231 cancer cells. Current findings suggested that CD74 plays a key role in tumor immunogenicity as well as in breast cancer immunoediting in humans.

SIGNIFICANCE STATEMENT

This study investigates the key role of CD74 expression in tumor immunogenicity as well as in breast cancer immunoediting in human. Tumor immunology is a growing field of research that aims to discover innovative cancer immunotherapies to treat the disease and prevent its progression. This study assists the researchers to uncover the critical areas of breast cancer prevention that many researchers were not able to explore. There are still key questions that could be further investigated in future research. A major challenge in the future will be to understand CD74 interactions and modifications with different molecules, CXCR1 and CXCR2 in breast cancer. It may then become possible to target specific pathways and provide new therapeutic interventions.

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