Oncologic trogocytosis with Hospicells induces the expression of N-cadherin by breast cancer cells

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Abstract. In breast cancers, the appearance of metastasis is synonymous with poor prognosis. The metastatic process is usually associated with epithelial-mesenchymal transition (EMT) which is often induced by several soluble factors produced either by the tumour cells themselves or by cells constituting the tumour microenvironment. The aim of the present study was to determine whether the mesenchymal properties given by some molecules such as N-cadherin, for instance, could be acquired by cancer cells via the trogocytosis process with cells of the tumour microenvironment. Hospicells are stromal cells which were first isolated from cancer cell aggregates of patients with ovarian cancer. We recently showed that these cells are immunosuppressive for T lymphocyte functions and confer chemoresistance to cancer cells by the transfer of the MDR protein via trogocytosis. In this study, we showed that a mammary cancer cell line (MDA-MB-231) acquires patches of membrane via oncologic trogocytosis with Hospicells. This unidirectional and active process depends on actin polymerization and can be increased via inhibition of the Src family and decreased via inhibition of PI3K. Trogocytosis between Hospicells and MDA-MB-231 does not lead to the direct acquisition of N-cadherin but rather it leads to the production of soluble factor(s) which induce *de novo* expression of N-cadherin by the cancer cells. The novelty here is that this factor is produced only if cancer cells interact and undergo trogocytosis with Hospicells. This new expression could confer a more invasive phenotype to the cancer cells and thus can explain the correlation of the presence of Hospicells with the number of invaded lymph nodes in patients with mammary adenocarcinoma.

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Introduction

Breast cancer is the most common malignant disease in Western women. In these patients, metastasis to distant sites and not the primary tumour are the cause of death. Therefore, the appearance of metastasis is synonymous with a poor prognosis (1).

The metastatic process is the ability of tumour cells to migrate through their surrounding host tissue, enter the circulatory blood stream, arrest in capillary beds of distant organs and invade the host tissue and proliferate (1-3). This series of steps is associated with the process of epithelial-mesenchymal transition (EMT), in which epithelial cells, such as cancer cells, lose their characteristic polarity, disassemble cell-cell junctions and become more migratory (2,4-8). For this, cancer cells progressively redistribute and down-regulate their apical and basolateral epithelial-specific tight and adherent junction proteins (such as E-cadherin) and re-express mesenchymal molecules (such as vimentin and N-cadherin).

Epithelial-mesenchymal transition (EMT) is induced by several growth factors (TGF-ß, HGF, EGF, IGFs, FGFs, MMPs), which are produced either by the tumour cells themselves or by different types of cells constituting the tumour microenvironment (2,3,6). Among these cells, the mesenchymal stem cells (MSCs), which are pluripotent bone marrow-derived cells, have been recently described to localize to breast carcinoma (9). After recruitment in significant numbers to the primary tumour sites, MSCs accelerate the growth of tumour cells (2,9). Moreover, these cells, which serve as local sources of dormant stem cells, are also able to produce metastatic factors to home the cancer cells to tumour-specific pre-metastatic sites and form cellular clusters before the arrival of tumour cells (10).

Even though many studies have shown that the soluble factors produced by the cellular components of the tumour microenvironment are implicated in the metastatic process, interactions between tumour cells and stromal cells have not been studied. In fact, cellular interactions leading to an exchange of membrane patches, called trogocytosis, can have several biological implications in some cases. This phenomenon is largely described in immune systems with specific interactions between immune cells, called effectors, and cells recognized as targets, leading to different immune responses, as reviewed by Davis (11) and more recently by Ahmed and colleagues (12). Through trogocytosis with target cells, the effector cells can acquire certain proteins, such as the CMH-associated antigen in the case of dendritic cells recognized by specific T lymphocytes (13), the viral receptor by natural killer (NK) cells (14), the inhibitory receptors of NK cells by cancer cells (15) and the H-ras oncogene by NK cells from transfected lymphoblastic cell lines (16). Despite the transient state at the membrane of these acquired proteins, they can be active for recipient cells such as NK cells which are activated for their proliferation or for the production of cytokines after acquiring the H-ras protein (16), or they are inhibited after acquiring HLA-G1 (17). Furthermore, trogocytosis is also present in non-immunological systems. For example, tetraspanin can be transferred from oocytes to the fertilizing spermatozoa present in the perivitelline space leading to membrane reorganization of the spermatozoa required for the fusion with the oocyte (18). In an oncological context, we first showed the homotypic trogocytosis with B lymphoma (19). Secondly, we recently showed the acquisition by ovarian cancer cells of a membrane molecule conferring chemoresistance (P-gp protein) from an original type of stromal cells, the Hospicells (20). The intercellular transfer of the P-gp protein was also described between drug-resistant and drug-sensitive human neuroblastoma cell lines, leading to a protection of the sensitive cells and to the formation of permanently resistant cells with a *de novo* expression of P-gp (21).

Hospicells were first isolated from cancer cell aggregates contained within peritoneal samples of patients with ovarian cancer, and then they were also found inside ovarian tumours. We recently described this new subset of stromal cells which do not express any of the common markers for other cell types but instead co-express the cell-surface markers CD9, CD10, CD29, CD146, CD166 and HLA-I (20). This co-localization of Hospicells and ovarian cancer cells induces, or is the result of, specific strong interactions between these two types of cells giving an advantage to cancer cells via the acquisition of the P-gp protein through oncologic trogocytosis.

Some of the mammary cancers are metastatic and can acquire the capacity to disseminate either by EMT (22) or not, as demonstrated by a recent work showing that cancer cells are able to spread even if they have not undergone EMT (23). If EMT is a necessary step for the metastatic process, the acquisition of mesenchymal properties could be induced by soluble factors but could also be made via oncologic trogocytosis between cancer cells and cells within the tumour microenvironment. As in ovarian tumours, it was thought that Hospicells would be found in mammary tumours and that the interactions between cancer cells and Hospicells would lead to an acquisition by the cancer cells of metastatic characteristics through, for example, the *de novo* expression of N-cadherin.

We show here that the breast carcinoma cell line MDA-MB-231 can undergo trogocytosis with Hospicells and that this phenomenon is an active process which depends on adhesion molecules and on certain cytoskeleton elements. This oncologic trogocytosis does not lead to the direct acquisition of N-cadherin but rather it leads to the production of soluble factor(s) which induce N-cadherin expression by the cancer cells. This process could be a step in EMT.

Table I. Immunoreactive score (IRS) calculated using the formula: IRS = intensity of stain x percentage of labelled cells.

Stained cells no.		Stain intensity		IRS	Conclusion
<1%	0	0	0	0-3	Negative
1-10%	1	+	1	4-6	1+
11-50%	2	++	2	7-9	2+
51-80%	3	+++	3	10-12	3+
>80%	4				

Materials and methods

Tissue micro-array construction and immunohistochemistry. Immunohistochemistry was performed on 5-μm thick routinely processed paraffin sections. Using a tissue micro-array instrument (Beecher Instruments, Alphelys™), we removed representative areas of the tumour from paraffin-embedded tissue blocks.

The antibodies were incubated for 30 or 60 min and then revealed by a system of polymers coupled to the peroxidase (EnVision™ kit, Dako Cytomation, Glostrup, Denmark). The interpretation of the immunostaining was made with the Herceptest™ score (Dako). The semi-quantitative analysis was chosen for the immunomarquages of the oestrogen receptor, progesterone receptor, CXCR4, CD10, CD31, BNH9, KBA62 and LYVE-1 given the percentage of labelled cells and the intensity of the stain (weak, moderate, strong). The IRS (immunoreactive score) was calculated using the following formula (IRS = intensity of stain x percentage of labelled cells) (Table I).

Cell culture. The mammary cancer cell line MDA-MB-231 was purchased from the ATCC. The Hospicells used in this study were isolated from ovarian carcinoma patients as described previously (20) and immortalized with SW40.

Immortalization of Hospicells. The immortalization of ovarian carcinoma-derived Hospicells with the SV40 large T antigen was performed in the laboratory at the Thérapie Génique et d'Amplification de Vecteurs, Inserm U649, Hôtel Dieu, Nantes. Briefly, primary Hospicell cultures isolated from five different ovarian carcinoma patients were infected

with an LT-expressing retroviral vector containing supernatants in the presence of polybrene. Then, SV40 large T antigen-expressing Hospicells were selected in medium containing 500 μg/ml neomycin. Immortalized Hospicell lines were grown at 37˚C in 5% CO_2 in RPMI medium supplemented with 5% FCS (Invitrogen, San Diego, CA), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Cambrex Bioscience, Verviers, Belgium) until they were used. Intraperitoneal and subcutaneous injections of immortalized Hospicells in SCID/beige mice did not generate tumours, demonstrating that these cells had not been transformed by retroviral transfection. The immortalized Hospicells retained their ability to specifically interact with ovarian tumour cells. The phenotype of the immortalized Hospicells was analysed by flow cytometry.

Cells culture conditions. The cells were cultured at 37˚C in a humidified incubator with 5% CO_2 in air in RPMI-1640 culture medium supplemented with penicillin, streptomycin (Cambrex Bio Science), sodium pyruvate and 10% heat-inactivated FCS (Invitrogen Corporation, Paisley, UK).

Reagents and cellular treatments. Cytochalasin D, SU6656, quadripeptid RGDS and pronase (Sigma-Aldrich, St. Louis, MO) were used at 10 μM, 1 μM, 2 mM and 1 mg/ml, respectively. Latrunculin B (Merck Biosciences, Darmstadt, Germany) was used at 25 μM and wortmannin and Ly-294002 (Sigma-Aldrich) at 1 μM. The mammary tumoural cell line was treated with these previously mentioned molecules for 1 h then washed twice with PBS before use.

Trogocytosis. Hospicells or MDA-MB-231 cells were stained with the lipophilic green-emitting dye PKH67 (Sigma-Aldrich) according to the manufacturer's instructions. Then, PKH67-positive cells were co-incubated for 3 h with PKH67-negative cells in 96-well U-bottomed culture plates at a cell ratio of 2:1 and with a total of $6x10^5$ cells in 120 μl of complete RPMI-1640 culture medium. The cells were pelleted by gentle centrifugation (110 x g for 1 min) and left in co-incubation for 3 min, 3 h, 5 h or 3 days at 37˚C (or 4˚C, as indicated) and then washed with 0.5 mM PBS/EDTA. Trogocytosis was measured as the acquisition of PKH67 fluorescence, which was characterized via the increase in mean fluorescence intensity (mfi) of PKH67 by a flow cytometry analysis. Live cells were gated on the basis of FSC/SSC parameters (P1 in Fig. 2) and 10^4 events were acquired in each experiment using the FL1 channel (log scale) for PKH67 on an LSR-II cytometer using Diva software (both from BD Biosciences, San Jose, CA, USA).

When specified, the tumour cells were treated by different molecules, as detailed in the reagents section, for 1 h before contact with the Hospicells. For the confocal microscopy, the Hospicells were stained with PKH67 and with the cytoplasmic Cell Tracker™ Orange-CMTMR [5-(and-6)-(((4-chloromethyl) benzoyl) amino)tetramethylrhodamine), Molecular Probes, Oregon, USA] according to manufacturer's instructions. After contact (see above), the cells were then gently suspended and plated onto poly-L-lysine (Sigma-Aldrich)-coated slides for 5 min at 37˚C. After fixation with PBS containing 4% *p*-formaldehyde, the cells were washed and directly mounted in PBS containing 90% glycerol and 2% 1-4-diazabicyclo (2.2.2) octane (DABCO, Sigma-Aldrich). The samples were examined using a Carl Zeiss LSM 410 confocal microscope (Carl Zeiss, Jena, Germany).

Adhesion test. The Hospicells were seeded in 96-well flat-bottomed culture plates at a cell concentration of $5x10^6$ cells/ml with a final volume of 80 μl and kept for 4 h at 37˚C in a humidified incubator with 5% CO_2 in the air. The tumour cells stained with 5 μM of CFSE [5(6)-carboxyfluorescein di-acetate N-succinimidyl ester, Sigma-Aldrich] were deposed on the layer of Hospicells at a concentration of $5x10^6$ cells/ml in a volume of 40 μl. The co-culture was kept for 1 h at 37˚C in a humidified incubator with 5% CO_2 in the air. Then, the non-adherent tumour cells were eliminated by two washings with PBS and the adherent tumour cells were quantified by measuring the mean CFSE fluorescence with a Mithras fluorometer.

Western blot analysis. Western blotting was performed using standard protocols.

Protein extraction. Cells ($5x10^6$) were diluted in 100 μl of lysis buffer: Tris 50 mM pH 8.0, NaCl 150 mM, Triton X-100 1%, sodium desoxycholate 1% (NP40), SDS 0.1%, EDTA 5 mM, DTT 1 mM, $NaVO_3$ 1 mM, ß glycerophosphate 10 mM, NaF 50 mM, leupeptin 2 μg/ml, pepstatin 2 μg/ml, aprotinin 10 mg/ml, PMSF 0.1 mM for 30 min at 4˚C. After centrifugation at 10000 x g for 15 min at 4˚C, the supernatant was kept at -80˚C. The proteins were quantified using the Bradford process.

Electrophoresis and revelation. The migration of proteins was performed at 120 V in 25 mM Tris, 192 mM glycin and 0.1% SDS. The proteins were then transferred onto a nitrocellulose membrane before incubation with 0.1 mg/ml of mAb against human N-cadherin (Sigma-Aldrich) for 12 h at 4˚C. After washing, the membrane was incubated for 30 min with purified goat anti-human IgG horseradish peroxidase conjugate (1/5000) (Bio-Rad Laboratories, CA, USA). The anticorps-protein complexes were revealed by chemiluminescence with an ECL kit (Amersham, Biosciences AB, Uppsala, Sweden).

Measurement of N-cadherin expression by flow cytometry. Staining for flow cytometry was performed using the mAb against human N-cadherin (Sigma-Aldrich) or the appropriate IgG isotype mAb as the control, where $0.6x10^6$ cells were diluted in 60 μl of PBS containing 5% FCS and 74 μg/ml of mAb and were then incubated for 45 min at 4˚C. The cells were then stained with phycoerythrin-cyanin 5-conjugated goat anti-mouse IgG (Beckman-Coulter-Immunotech, Marseille, France). The cells were washed, resuspended in PBS, and 10^4 cells were analysed on a LSR-II cytometer using Diva software (both from BD Biosciences). Live cells were gated on the basis of FSC/SSC parameters.

Statistical analysis and graphical representation. The data of Figs. 1B and C, 4A and B were analysed using two-tailed Student's t-tests. The results shown are representative of at least three separate experiments and the values are expressed as the mean ± SEM with $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.005$.

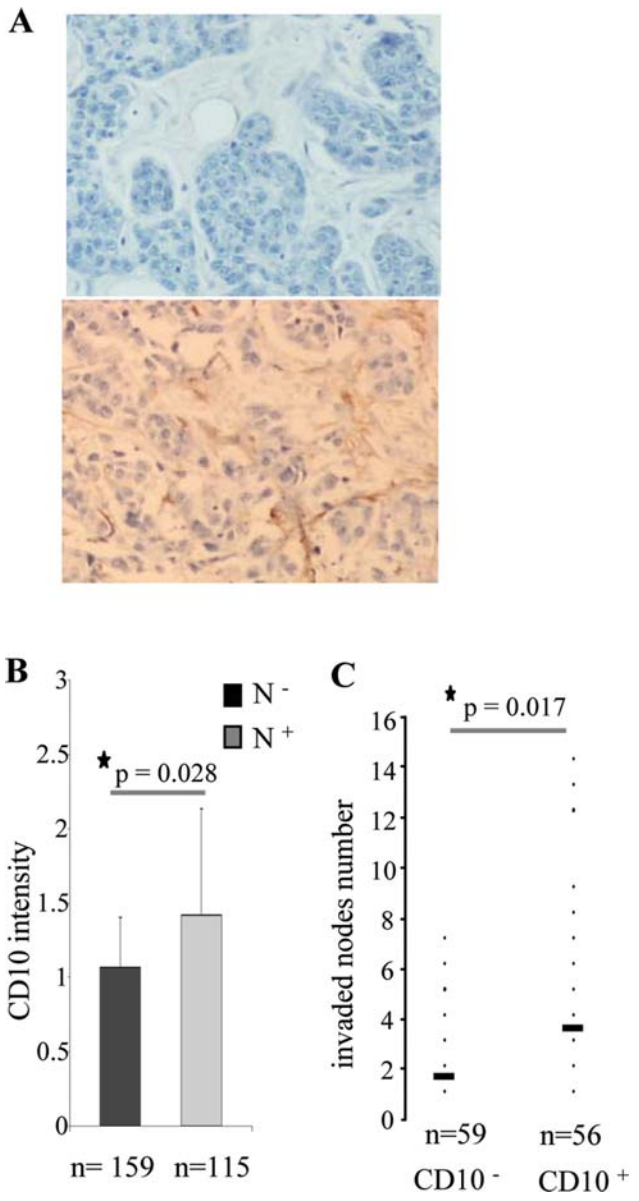

Figure 1. Aggressiveness of the tumour correlates with the presence of Hospicells in the stroma. (A) Tissue micro-arrays of mammary adenocarcinoma with the positive immunohistochemical expression of CD10 in brown for the lower panel and negative for the upper panel. (B) CD10 intensity for the group of patients with a high risk of metastatic development (N^+) and for the group with a low risk (N^-). (C) Number of invaded nodes for the CD10-negative patients and for the CD10-positive patients in the N^+ group.

Results

Detection of Hospicells in mammary adenocarcinoma. To investigate whether or not breast tumours, like ovarian tumours, contain Hospicells, we studied a series of 274 mammary adenocarcinomas for the immunohistochemical expression of CD10. The tissue micro-array analysis in Fig. 1A shows the expression of CD10, which was characteristic for the presence of Hospicells in some mammary tumours (lower panel) whereas other mammary tumours did not contain Hospicells (upper panel). Moreover, the presence of Hospicells in the tumour stroma correlated with the presence of axillary lymph node metastasis. Patients with tumour-positive axillary lymph nodes and a high risk of metastasis development were classified N^+ as opposed to the N^- patients. The CD10 intensity was statistically higher for the series of the 115 N^+ patients compared to the series of the 159 N^- patients (Fig. 1B). Furthermore, the number of invaded lymph nodes constituted a prognostic marker of the locoregional relapse and morbidity. We could distinguish two groups of patients: one group with an intermediate prognosis, having one to three invaded nodes, and a group with a very poor prognosis, comprised of patients having more than three invaded nodes. Among the 115 N^+ patients, we showed that the presence of Hospicells correlated with a higher number of invaded lymph nodes; the mean number was 3.36 for the $CD10^+$ group compared to 1.86 for the $CD10^-$ group (Fig. 1C).

Oncologic trogocytosis between Hospicells and breast cancer cells. The presence of Hospicells in breast adenocarcinoma led us to analyse the *in vitro* interactions between these stromal cells and the breast cancer cell line MDA-MB-231 (24).

First, once seeded onto the Matrigel pre-coated plates containing Hospicells, the eGFP-MDA-MB-231 cells mainly grew on the network previously formed by the stromal cells (Fig. 2A). Then, the electronic microscopy analysis showed membrane fusion domains in the contact zone when the MDA-MB-231 cell line was co-cultured with Hospicells (Fig. 2B).

Thus, we tested the capability of breast cancer cells to undergo oncologic trogocytosis with the Hospicells. After 3 h of contact, the cancer cells acquired fluorescence from the stromal cells which were preliminarily stained with the lipophilic membrane fluorochrome PKH67. Since this green fluorochrome was stable in the membrane, the acquisition of fluorescence by the MDA-MB-231 cells meant that these cells acquired membrane patches containing the green molecule from the Hospicells. This acquisition of PKH67 fluorescence after 3 h of contact was visualized by confocal microscopy (Fig. 2C, D) and measured by flow cytometry (Fig. 2E). To distinguish the two cell types in microscopy, the Hospicells were also stained with a red cytoplasmic fluorochrome, CMTMR. Interestingly, when the cancer cells were stained with PKH67, the Hospicells did not acquire a significant amount of PKH67 fluorescence in the 3 h of contact (Fig. 2F).

We then verified that the acquisition of membrane patches by the cancer cells was really a trogocytosis contact-dependent process and not a fusion with exosomes produced by the Hospicells. We showed that this acquisition after 3 h was inhibited when a 0.4-μm porous membrane (Transwell™ system) was used to separate the Hospicells from the cancer cells (Fig. 3A, bold line), compared to the experiment when the two cells were in contact (Fig. 3A, grey line). Moreover, trogocytosis was totally inhibited when the Hospicells were preliminarily treated with the *Streptomyces griseus* proteinase, which digests the extracellular parts of membrane proteins (Fig. 3B, bold line). Finally, the total inhibition of trogocytosis between Hospicells and MDA-MB-231 cells at 4˚C showed that this process depends on an intracellular signalling process which is abolished at low temperatures (Fig. 3C, bold line).

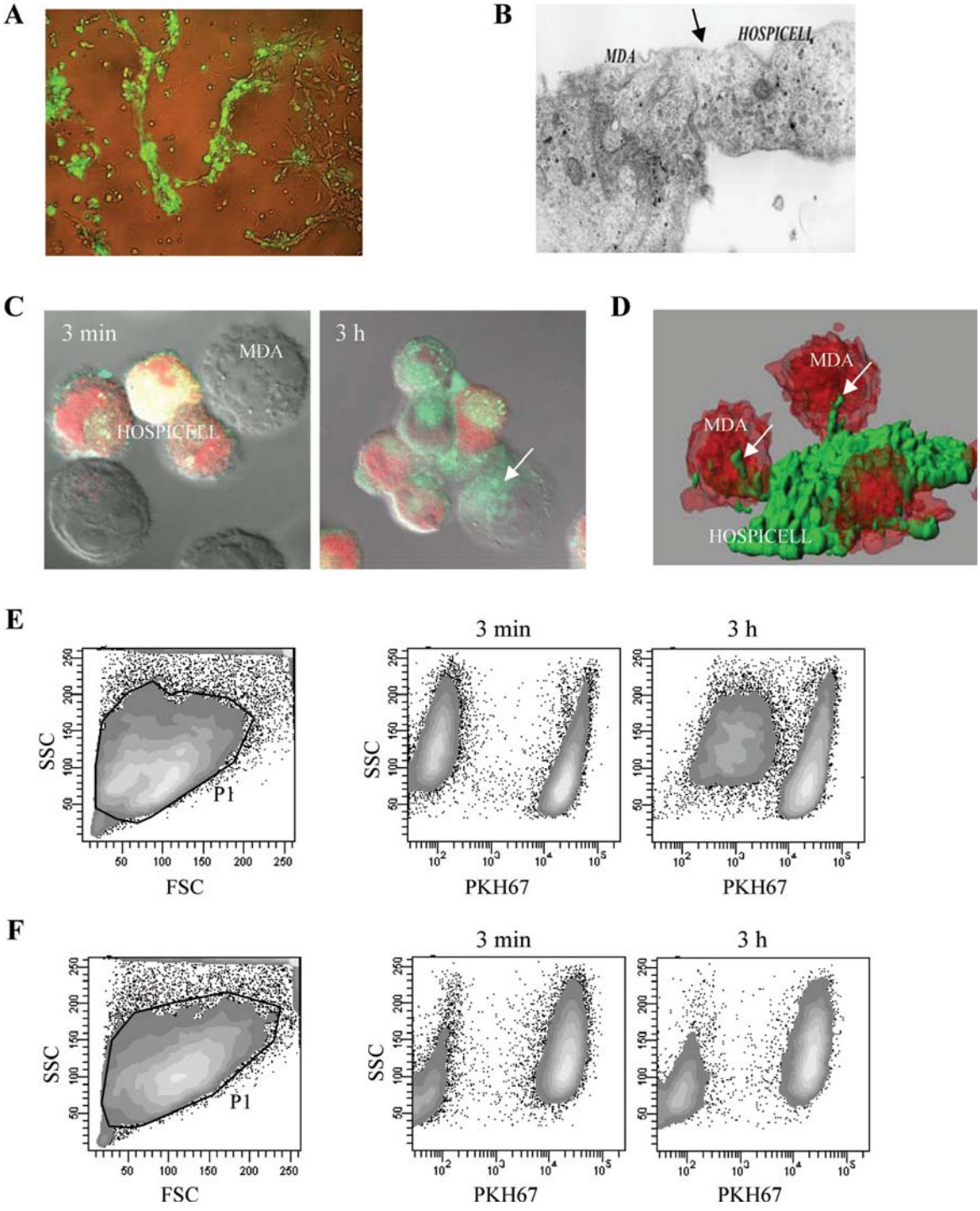

Figure 2. Oncologic trogocytosis between Hospicells and the mammary tumour cell line. (A) Culture in Matrigel of MDA-MB-231 eGFP and Hospicells. (B) Electron microscopy of the contact between MDA-MB-231 cells and Hospicells. (C) Confocal microscopy of conjugates formed after 3 h co-incubation of PKH67-positive and CMTMR-positive Hospicells and the MDA-MB-231 cell line. Arrows: large green patches transferred from PKH67-positive cells on unstained tumour cells. (D) Flow cytometry contour plots of PKH67 versus SSC of the co-culture of PKH67-positive Hospicells and unstained MDA-MB-231 cells at 3 min and 3 h. The live cells were selected by P1 and were detected by their PKH67 fluorescence. (E) Flow cytometry contour plots of PKH67 versus SSC of the co-culture of PKH67-positive MDA-MB-231 cells and unstained Hospicells at 3 min and 3 h. The live cells were selected by P1 and were detected by their PKH67 fluorescence.

The Hospicell/MDA-MB-231 trogocytosis is dependent on actin polarization and on an Src/PI3K-dependent signal. To depict the signalling pathway, which is involved in the oncologic trogocytosis between Hospicells and mammary cancer cells, we pre-treated MDA-MB-231 with different families of inhibitory molecules. We showed that cytochalasin D and latrunculin B, which inhibit actin polymerization, induced a 50% reduction of trogocytosis. In contrast, a microtubule polymerization inhibitor, taxol, did not affect trogocytosis (Fig. 4A).

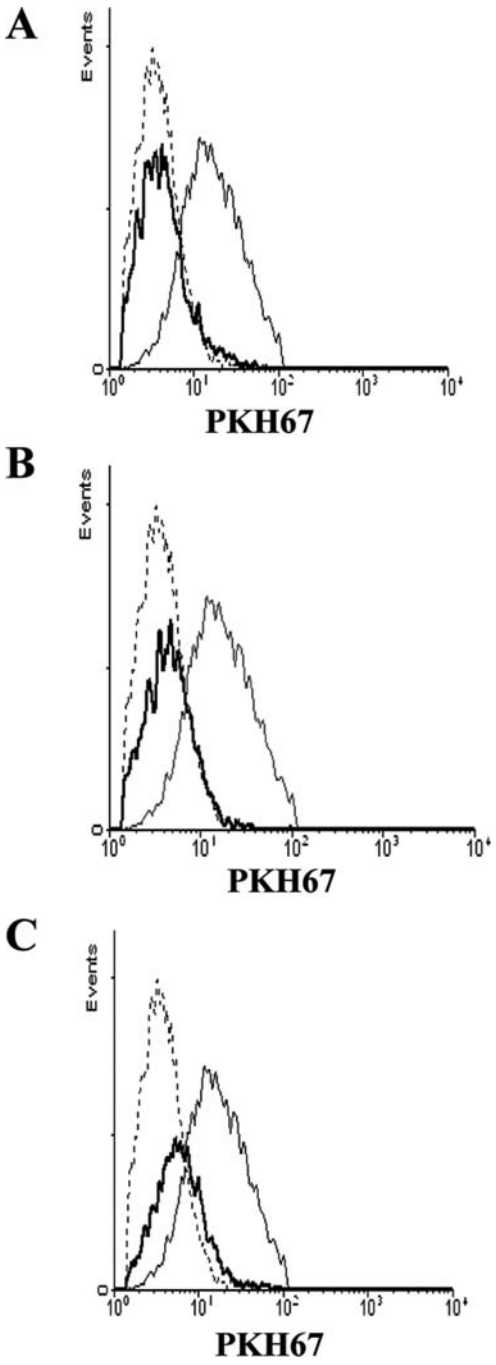

Figure 3. Oncologic trogocytosis is a contact- and signalling-dependent process. (A) The 3-h trogocytosis between PKH67-positive Hospicells and unstained MDA-MB-231 cells is inhibited by using the Transwell system for the co-culture (grey line) compared to 3 h trogocytosis without the Transwell system (black line), the reference being trogocytosis at 3 min (dotted line). (B) The 3-h trogocytosis between PKH67-positive Hospicells and unstained MDA-MB-231 cells is largely reduced by the preliminary action of a proteinase on the tumour cell (grey line). (C) The 3-h trogocytosis between PKH67-positive Hospicells and unstained MDA-MB-231 cells is largely reduced when processing the co-culture at 4˚C (grey line) compared to 3 h trogocytosis at 37˚C (black line).

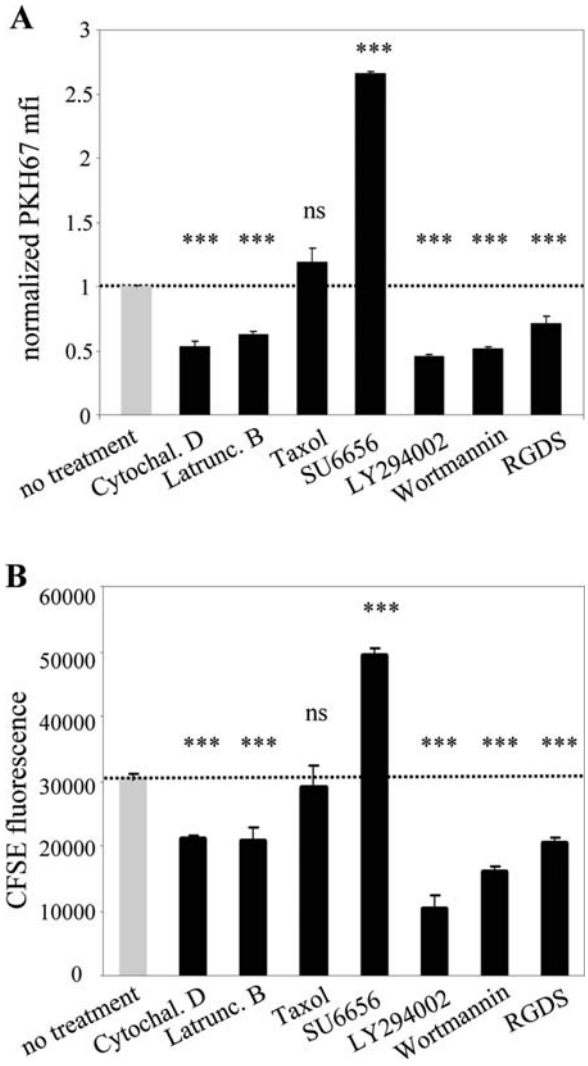

Figure 4. Oncologic trogocytosis is actin dependent and Src/PI3K-dependent. (A) The PKH67 mfi from 3 h trogocytosis between PKH67-positive Hospicells and MDA-MB-231 cells pre-treated for 1 h with different inhibitory molecules. The results are represented by the normalized PKH67 mfi from the experiment with no treatment. (B) The CFSE fluorescence measured from the adhesion of CFSE-positive MDA-MB-231 cells to the adherent Hospicells after a 1 h pre-treatment of the former with different inhibitory molecules. For (A) and (B), the error bars show standard deviations from four independent experiments.

Moreover, we tested inhibitors of the Src family (SU6656) and of phosphatidylinositol-3-kinase (PI3K) (LY294002 and wortmannin), which target transduction modules essential for cell-to-cell adhesion. Inhibition of the Src kinases led to an increase of trogocytosis while the inhibition of PI3K decreased it. Finally, integrin saturation with the RGDS peptide also reduced the Hospicell/MDA-MB-231 trogocytosis (Fig. 4A).

The intensity of immunological trogocytosis is determined by the affinity of the T-cell receptor (TCR) for its antigen: the higher the affinity the more important the trogocytosis between the T lymphocyte and its target (25). In our system, we tried to test the strength of the interactions between Hospicells and the breast cancer cell line using adhesion tests. Using spectrofluorometry, we determined the intensity of the interactions by the measuring the fluorescence intensity after washing a 1-h co-culture of Hospicells and MDA-MB-231 preliminarily stained with CFSE. As with trogocytosis, actin polymerization inhibition reduced adhesion of the stromal cells to the breast cancer cells whilst inhibition of microtubule polymerization had no effect. Furthermore, this

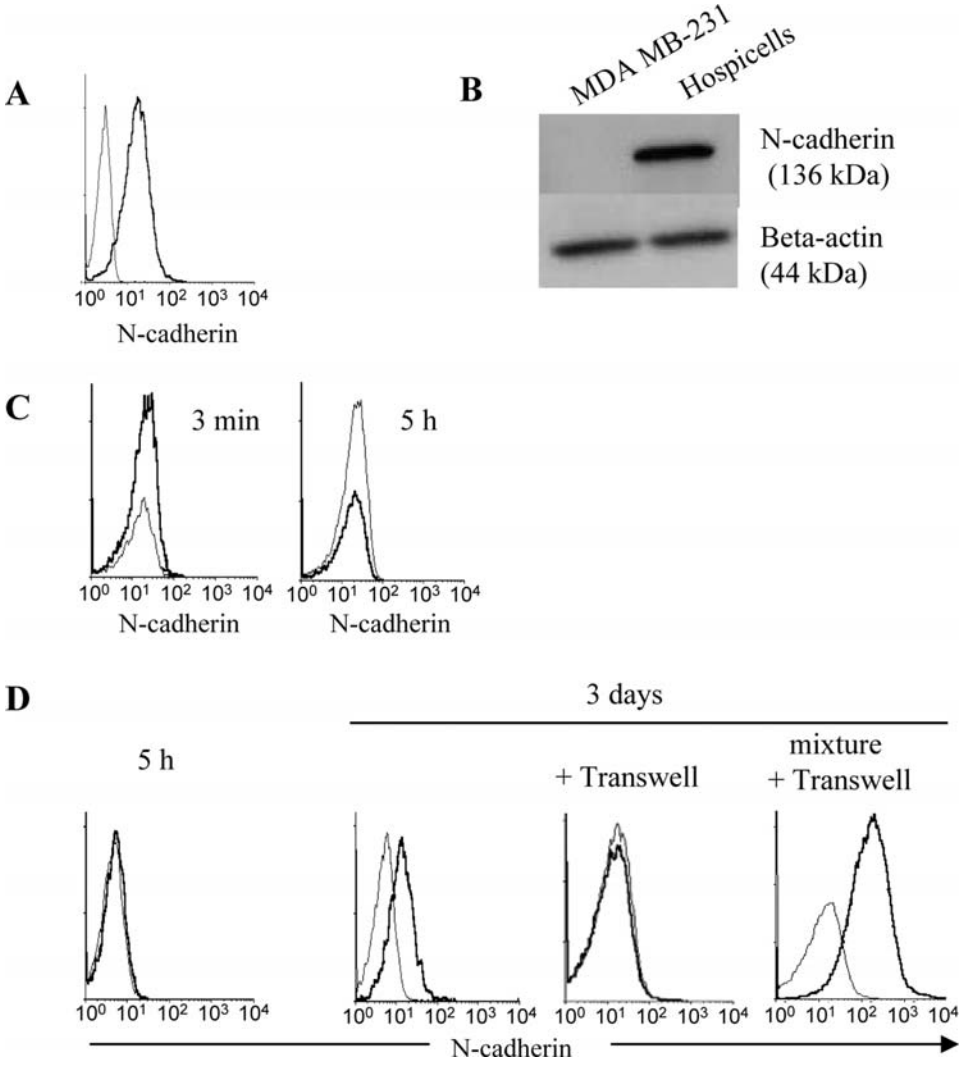

Figure 5. Expression of N-cadherin induced by trogocytosis. (A) N-cadherin expression on Hospicells as measured by flow cytometry. (B) N-cadherin expression on MDA-MB-231 cells and Hospicells measured by Western blot with ß-actin as a positive control. (C) N-cadherin expression by MDA-MB-231 cells after co-culture with Hospicells for 3 min and 5 h. (D) N-cadherin expression by MDA-MB-231 cells after co-culture with Hospicells for 5 h or 3 days with or without the Transwell system and after co-culture with a mixture of MDA-MB-231 cells and Hospicells separated by a Transwell system. For each histogram: black line, N-cadherin; grey line, isotype control.

adhesion was also decreased by the inhibition of PI3K and use of the RGDS peptide, and increased by the inhibition of the Src kinases (Fig. 4B).

Trogocytosis with Hospicells induces expression of N-cadherin by breast cancer cells. Expression of N-cadherin during EMT is a crucial step in the dissemination of mammary adenocarcinomas (22). Since trogocytosis allows the transfer of membrane proteins from stromal cells to ovarian cancer cell lines (20), we decided to explore whether the breast cancer cell line MDA-MB-231 could acquire N-cadherin through trogocytosis with Hospicells. First, by using flow cytometry (Fig. 5A) and Western blot (Fig. 5B), we showed the expression of N-cadherin by Hospicells. Then, according to the literature (26), we verified the non-expression of N-cadherin by MDA-MB-231 cells (Fig. 5B).

The acquisition of N-cadherin by the cancer cells was not observed when a specific mAb in a 5 h co-culture of Hospicells and cancer cells was used (Fig. 5C). However, a *de novo* expression of N-cadherin by the MDA-MB-231 cell line after 3 days of co-culture with the Hospicells was observed (Fig. 5D). This induction was found to be contact dependent when the use of a Transwell system for the 3 days co-culture, which separated the cancer cells from the Hospicells, abolished the *de novo* expression of N-cadherin. Interestingly, MDA-MB-231 showed *de novo* expression of N-cadherin when cultivated for 3 days in a mixture of Hospicells and MDA-MB-231 cells when separated from the mixture by a Transwell system (Fig. 5D).

Discussion

We recently showed that a new type of stromal cells, called Hospicells, isolated from the ascitis of patients with ovarian carcinoma, is able to confer chemoresistance to an ovarian cancer cell line through the transfer of the multi drug resistance protein by trogocytosis (20).

In the current study, we showed that the breast cancer cell line MDA-MB-231 shows *de novo* expression of the membrane N-cadherin protein following trogocytosis between

the cancer cells and the Hospicells. First, we demonstrated that MDA-MB-231 acquires a high amount of membrane patches from Hospicells through trogocytosis, and that this transfer was not reciprocated.

This trogocytosis is an active phenomenon which depends on actin polymerization and can be increased with inhibition of the Src family and decreased with inhibition of PI3K. The induction of cell clustering, as a result of stabilizing transient cell-cell contacts, could explain the increase in trogocytosis when Src catalytic activity was inhibited (27,28). Since the signalling of PI3K is directly linked to cell-cell adhesion, its inhibition correlates with a disruption of cell-cell contacts in intestinal and mammary epithelial cells via the inhibition of F-actin at the site of cell-cell contact (29,30). Inhibition of PI3K signalling destabilizes cell-cell contacts and thus decreases trogocytosis.

This membrane acquisition leads to a new expression of N-cadherin by MDA-MB-231 cells after 3 days of close contact between Hospicells and cancer cells, whilst the use of a Transwell system, which separated the two cell types, prevented this expression. Moreover, the culture of MDA-MB-231 cells for 3 days with a mixture of this cancer cell line and the Hospicells, but separated from this mixture by a Transwell system, led to the expression of N-cadherin at the surface of MDA-MB-231 cells. Thus, trogocytosis between this cancer cell line and the Hospicells did not lead to a direct transfer of N-cadherin from the Hospicell to the cancer cell, but instead it induced the production of a soluble factor which stimulated the *de novo* expression of N-cadherin by MDA-MB-231 cells. This soluble molecule could be produced by the Hospicells or through an autocrine process by the cancer cell line. Collagen I could be a good candidate because of its capability for inducing the up-regulation of N-cadherin in mouse mammary epithelial cells (31) and in human pancreatic cancer cells (32). The growth factors EGF and HGF are also implicated in the promotion of N-cadherin expression in ovarian carcinoma (33). The tumour suppressor TGF-ß which inhibits cell proliferation (34), can also promote metastasis in ovarian cancer by the elevation of matrix metalloproteinease secretion or by an increase in the expression of N-cadherin (35). Besides this, human lung cancer cells respond to collagen I by increasing production of TGF-ß, which then promotes EMT changes which include, amongst others, the *de novo* expression of N-cadherin (36). Finally, TGF-ß is also able to promote the expression of N-cadherin in normal and malignant oral keratinocytes, which is correlated with an increase in the motility of these cells (37). A further reason why TGF-ß could also be a good candidate is that recent data showed that ARNm for TGF-ß is expressed by Hospicells (38); however, this work did not analyse the expression of EGF or collagen I in these cells. The follow-up to our study will be to determine which soluble factor is produced by the co-culture of Hospicells and the MDA-MB-231 cancer cell line which leads to the expression of N-cadherin by the MDA-MB-231 cells.

N-cadherin is an important molecule in the acquisition of a metastatic phenotype for cancer cells by promoting motility and invasion in carcinoma cells and specifically in breast carcinoma (26,39). Effectively, this molecule contributes directly to the migratory and invasive phenotype through stable and labile cellular interactions which facilitate dynamic processes which lead to the metastatic effect (40). Moreover, N-cadherin cooperates intimately with the FGF receptor by increasing the sensitivity of cancer cells to FGF-2, leading to an increase in the expression of MMP-9 (matrix metalloproteinase-9) which is an extracellular matrix-degrading enzyme associated with tumour angiogenesis, invasion and metastasis (41). Concerning the Hospicell/MDA-MB-231 interactions, the *de novo* expression of N-cadherin by the MDA-MB-231 cell line increased sensitivity to FGF-2 produced by the Hospicells (38). Possibly cross-talk between these two cells is necessary for inducing the capability of the cancer cell to response to the messages of the stromal cell, leading to an increase of its metastatic properties.

Thus, the *de novo* expression of N-cadherin in a carcinoma following trogocytosis with stromal cells can promote a signalling cascade which is implicated in the metastatic process. During this process, cancer cells could change their phenotype to acquire more motility and invasive capabilities by the up-regulation or the acquisition of N-cadherin following tight interactions with stromal cells present in the tumour microenvironment. The MDA-MB-231 cell line used in this study is constitutively E-cadherin and N-cadherin negative but cadherin-11 positive, which confers an invasive phenotype (26). However, cadherin-11-expressing cells are not as invasive or motile as N-cadherin-expressing cells (26). Thus, the *de novo* expression of N-cadherin by this type of cell could enhance its invasive phenotype.

The correlation of the presence of Hospicells with the number of invaded lymph nodes in mammary tumours (Fig. 1) could be explained on one hand by the more invasive phenotype of the cancer cells acquired through interactions with the Hospicells. On the other hand, we recently showed that the Hospicells are immunosuppressive for the proliferation and cytokine production of T lymphocytes (42). Therefore, the Hospicells provide favourable support to tumour development in two ways.

Several works have shown the importance of the microenvironment in the development or the repression of a tumour. Moreover, in a non-pathological system, such as in the bone marrow niche, intercellular transfers between hematopoietic stem-progenitor cells (HSPC) and osteoblasts induce a down-regulation of Smad signalling, increasing the production of stromal-derived factor-1 (SDF-1), a chemokine responsible for HSPC homing to bone marrow (43).

Thus, intercellular transfers are as important in pathological conditions as in physiological systems. Here, we reinforced this idea by showing the possible acquisition of metastatic characteristics by the cancer cell through interactions with the cells in its environment. The novelty here is that interactions between stromal cells and cancer cells induce the production of a soluble factor which leads to new expression of a metastatic phenotype for the cancer cell.

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