Norepinephrine Promotes the β1-Integrin–Mediated Adhesion of MDA-MB-231 Cells to Vascular Endothelium by the Induction of a GROα Release

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Abstract

The migratory activity of tumor cells and their ability to extravasate from the blood stream through the vascular endothelium are important steps within the metastasis cascade. We have shown previously that norepinephrine is a potent inducer of the migration of MDA-MB-468 human breast carcinoma cells and therefore investigated herein, whether the interaction of these cells as well as MDA-MB-231 and MDA-MB-435S human breast carcinoma cells with the vascular endothelium is affected by this neurotransmitter as well. By means of a flow-through assay under physiologic flow conditions, we show that norepinephrine induces an increase of the adhesion of the MDA-MB-231 cells, but not of MDA-MB-468 and MDA-MB-435S cells to human pulmonary microvascular endothelial cells (HMVEC). The adhesion of MDA-MB-231 cells was based on a norepinephrine-mediated release of GROα from HMVECs. GROα caused a β1-integrin–mediated increase of the adhesion of MDA-MB-231 cells. Most interestingly, this effect of norepinephrine, similar to the aforementioned induction of migration in MDA-MB-468 cells, was mediated by β-adrenergic receptors and therefore abrogated by β-blockers. In conclusion, norepinephrine has cell line–specific effects with regard to certain steps of the metastasis cascade, which are conjointly inhibited by clinically established β-blockers. Therefore, these results may deliver a molecular explanation for our recently published retrospective data analysis of patients with breast cancer which shows that β-blockers significantly reduce the development of metastases. Mol Cancer Res; 10(2); 197–207. ©2011 AACR.

Introduction

In his article on the war against cancer, Sporn pointed out already in 1996 that "it is local invasion and distant metastasis that kill rather than excessive cell proliferation per se." (1). Accordingly, today more than 90% of breast cancer–related deaths are because of the development of metastases. Thus, future therapeutic strategies for cancer treatment must include the inhibition of metastasis formation, and therefore the understanding of the molecular mechanisms of this process is an essential prerequisite. The metastasis cascade is a multistep process of highly regulated events including tumor cell migration as well as the intra- and extravasation into and out of the blood stream. Each step of the metastasis cascade comprises the interaction of the tumor cells with their environment in cell–cell and cell–matrix contacts and the susceptibility of these interactions to the regulation by soluble signal substances. We have reported previously that the stress hormone norepinephrine is the most potent physiologic inducer of the migration of breast carcinoma cells (2), as well as of colon (3), and prostate carcinoma cells (4) in vitro. The promigratory effect is mediated by β-adrenergic receptors because it could be blocked significantly by clinically established β-blockers (i.e., propranolol; refs. 2–4). These findings were confirmed in a xenograft mouse model with PC-3 prostate carcinoma cells. In this model, parallel application of propranolol decreased lumbar lymph node metastases formation induced by norepinephrine (5). Furthermore, other groups have shown that norepinephrine increases the migration and invasion of pancreatic (6) and ovarian (7) cancer cell lines. Besides studies using the direct application of norepinephrine, there are increasing numbers of studies showing that social stress, for example, by isolation, is associated with an increased tumor growth (8) and metastasis (9) in mouse models.

Recently, 3 independent epidemiologic studies provided evidence of the clinical relevance of these experimental findings: retrospective data analyses of patients with breast cancer showed that those women who received β-blockers because of hypertension developed metastases significantly...
later than untreated patients or patients treated with other anti-hypertensive drugs and had consequently an increased cancer-specific survival time (10–12). Immunohistochemical analysis of tissue microarrays from patients with breast cancer showed that α- and β-adrenergic receptors are differentially overexpressed in distinct subtypes of breast cancer. Especially, the α1b- and α2c-adrenergic receptors were associated with a poor prognosis in basal-like tumors (13).

However, not only direct effects of the adrenergic signaling on the tumor cells but also indirect effects on cells of the tumor environment might promote metastasis formation, as was recently shown by Sloan and colleagues (14): they provide evidence that the sympathetic (noradrenergic) activation of macrophages promotes metastasis formation in an orthotopic mouse model. This puts forward the hypothesis of parallels between a tumor and inflammation (15, 16). Proinflammatory chemokines, as delivered by macrophages, regulate the site at which leukocytes leave the blood stream by the activation of integrin-mediated adhesion (17, 18), and a similar role has been attributed to chemokines for the localization of metastases in several types of cancer (19). CXC chemokines, such as CXCL8/IL-8 and CXCL1/GROα, bind to the G-protein–coupled receptors, CXCR1 and CXCR2, which are described to play an important role with regard to disease progression and metastasis formation (20). In colorectal cancers, GROα expression was shown to be positively associated with tumor size, stage, invasion, lymph node metastasis, and patient survival (21).

In the present study, we now question whether norepinephrine does not only directly affect the migratory activity of breast carcinoma cells but also other parts of the metastasis cascade in a direct or indirect manner. Therefore, we investigated the ability of tumor cells to adhere to vascular endothelium, a further important step of the hematogenous tumor metastasis cascade during extravasation. The rationale for these experiments is based on the observation that the microvasculature of organ sites, especially of lymphoid organs, is surrounded by nerve fibers that release catecholamines at high local doses thereby affecting the endothelium itself as well as circulating leukocytes (22). With regard to the involved receptors in the adhesion of the tumor cells to the endothelium, we especially focused on the role of integrins, as (i) they are known to be key receptors in the extravasation process (23) and (ii) their affinity to the ligand is regulated by the so-called “inside-out-signaling” which is mediated by chemokines (24). In addition, we investigated the involvement of chemokines in the interaction of breast cancer cells with endothelial cells and the regulation of their expression by the transcription factor NF-κB.

Materials and Methods

Cell lines and cell culture

The human breast carcinoma cell lines MDA-MB-468, MDA-MB-435S, and MDA-MB-231 (all from the American Type Culture Collection) were cultured in Dulbecco’s Modified Eagle’s Media (DMEM; PAA) containing 10% fetal calf serum (PAA) at 37°C humidified atmosphere with 5% CO2. Confirmatory experiments were carried out with PC-3 human prostate carcinoma cells (DSMZ). These cells were cultured in HAM’s F-12 medium and RPMI-1640 medium (1:1; both PAA) containing 10% heat-inactivated fetal calf serum (PAA) under the same conditions as the breast carcinoma cells. All cell lines were authenticated by the supplier and used no longer than 6 months after resuscitation. For experiments, cells were collected by short accutase (PAA) treatment at a subconfluent (75% confluency) stage of growth.

Human pulmonary microvascular endothelial cells (HMVEC; Lonza) were cultured in EBM-2MV media with supplements (Lonza) in a humidified incubator with 5% CO2. Endothelial cells were only used within 6 passages and routinely screened for Mycoplasma infection (MycoAlert; Lonza).

Migration experiments

For the investigation of the migratory activity, migration experiments were carried out as described in detail previously (2). In brief, a cell suspension of 8 × 103 tumor cells was mixed with a carbonate-buffered collagen solution (1.63 mg/mL collagen; PureCol, Inamed) containing minimal essential medium. In test samples, norepinephrine (Sigma-Aldrich) was added to a final concentration of 10 μmol/L. The suspension was filled into self-constructed migration chambers consisting of a microscopic slide, wax walls on 3 sides and a coverslip on top, and polymerized within 30 minutes at 37°C humidified atmosphere with 5% CO2. After polymerization, the residual chamber volume was filled with PBS with or without norepinephrine, and the fourth open side of the chamber was sealed with wax. The migratory activity of the cells within the collagen matrix was monitored for 15 hours by time lapse video microscopy. After recording, the paths of 30 randomly selected cells were digitized by computer-assisted cell tracking. The migratory activity was calculated in 15-minute intervals as the portion of cells (in percentage), which was locomotory active, for example, when 15 of 30 cells moved, the migratory activity at this certain time point was 50%.

Flow-through adhesion assay

The flow-through adhesion assay was conducted as described previously (25). HMVECs were seeded on collagen IV–coated flow chambers (μ-slides I, IBIDI) and grown to confluence for 2 days. Tumor cells (1.5 × 105/mL in DMEM with 2% fetal calf serum) were drawn over the endothelial monolayer by a perfusion pump (Perfusor IV, B. Braun Melsungen AG) at a flow rate of 12.2 mL/h, which results in a shear stress of 0.25 dyne/cm2 and represents physiologic blood flow conditions in small vessels. The suspension flow was recorded by video microscopy for 20 minutes using a 2.5-fold objective. To investigate the impact of norepinephrine and/or the β-blocker propranolol (both Sigma-Aldrich), the endothelial monolayer was preincubated with norepinephrine...
and/or propranolol for 2 hours at the indicated concentrations. Furthermore, the substances were also added to the tumor cell suspension drawn over the endothelium. The NF-κB activation inhibitor (Merck Biosciences) was used in the same manner at a concentration of 10 nmol/L. This concentration was used because of previous studies on cell migration (unpublished results) and corresponds to the highest concentration that showed no effect on the spontaneous migratory activity of tumor cells.

For blocking experiments, MDA-MB231 were preincubated for 10 minutes with monoclonal mouse anti-CXCR1 and anti-CXCR2 (each 5 μg/mL; R&D Systems), mouse IgG2a (10 μg/mL; R&D Systems), mouse anti-β1-integrin clone 4B4 (5 μg/mL; Beckman Coulter), or mouse IgG1 (5 μg/mL; Beckman Coulter). The highest possible concentration of IgG that showed no effect on the adhesion of the tumor cells (data not shown) was chosen as final concentration for blocking antibodies.

For chemokine-dependent adhesion experiments, the HMVEC monolayer was overlaid for 3 minutes at room temperature with GROα and/or interleukin (IL)-8 each at 1 μg/mL (both R&D Systems). Denaturated GROα and IL-8 (10 minutes at 95°C) served as negative control. The HMVEC monolayer was washed 3 times with PBS and used directly in the flow-through assay.

MDA-MB-231 cells were found to adhere to the endothelium mostly in clusters. Cluster formation was analyzed using ImageJ Software (NIH, Bethesda, MD). Thus, the area covered by MDA-MB-231 clusters was calculated in pixels (Fig. 1), which used to quantify the intensity of adhesion. At least 2 different areas were analyzed per recorded video.

RNA isolation and cDNA synthesis
Total RNA from $5 \times 10^5$ HMVECs or $3 \times 10^6$ tumor cells was isolated using the Total RNA Isolation Kit from Macherey Nagel according to the manufacturer’s instructions. The cDNA was synthesized using the First Strand cDNA Synthesis Kit with RevertAid H Minus M-MuLV as reverse transcriptase and random hexamer primers (Fermentas).

Quantitative real-time PCR
Real-time PCR was carried out on an ABI StepOnePlus (Applied Biosystems) system using “MESA Blue qPCR Master Mix Plus” (Eurogentec) to determine target amplification. The expression level of β-actin was used as reference for normalization, and fold expression changes were calculated using the standard ΔΔCT method. The following program was used: 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds; this was followed by melting curve analysis to verify single product amplification. The primers were:

- β-actin: fwd-GGA CTT CGA AGA GAT GG and rev-AGG AAG GAA GCC TGG AAG AG; GROα: fwd-ATT CAT TGG CGC ATG GAA AGA and rev-TGG TGC CAC GAT GAG AAG; CXCR1: fwd-TTT GTT TGT CTT GGC TGC TG and rev-GCC AAG AAC TCC TTG CTT AC (annealing temperature, 57°C; ref. 27); CXCR2: fwd-CAG TTA CAG CTC TAC CCT GCC and rev-CCAGGAGCAAGACACGCC (annealing temperature, 57°C; ref. 28); β1-adrenergic receptor: fwd-TCA TCC GAG CAA AAG AGA AA and rev-TCA CAT CCC TCC CCA AAC TT (annealing temperature, 58°C); and β2-adrenergic receptor: fwd-TCA CCT TTC AAG TAC CAG AGC CT and rev-ACA CAA TCC ACA CCA TCA GAA TG (annealing temperature, 58°C).
Amplification programs were as follows: β-actin: 94°C for 5 minutes, 30 cycles of 94°C for 45 seconds, 65°C for 30 seconds, 72°C for 90 seconds, finally followed by 72°C for 5 minutes; CXCRL1: 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 60 seconds, finally followed by 72°C for 5 minutes; CXCRL2: 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 20 seconds, finally followed by 72°C for 5 minutes; and β1- and β2-adrenergic receptors: 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, finally followed by 72°C for 5 minutes.

Primers for chemokine receptors and β-actin were chosen to span the template for at least one intron so that any genomic DNA contamination would result in a larger product band. Because primers for the β-adrenergic receptors were not exon–exon junction spanning and not separated by at least one intron, we included in these analyses, a "no amplification control" to ensure that the product is not due to genomic DNA contamination. PCR products were visualized on a 1.0% (1.5% for β-actin) ethidium bromide agarose gel.

Enzyme-linked immunosassay
HMVECs were incubated for 2 hours with 10 μmol/L norepinephrine and propranolol alone or in combination; all samples were prepared as doublets. Supernatants were collected, and the concentrations of IL-8 and GRoα were measured by an enzyme-linked immunosassay using the "Quantakine kit" (R&D Systems) according to the manufacturer’s protocol.

Flow cytometry
Samples of 5 × 10^5 endothelial cells or tumor cells were fixed with 2% paraformaldehyde for 10 minutes, permeabilized with 0.5% Triton X-100/PBS for 10 minutes, and then stained in 2% goat-serum/PBS with 10 μg/mL of the primary antibody (V-19 for the β1-adrenoceptor and H-20 for the β2-adrenoceptor; both from Santa Cruz Biotechnology) or with a rabbit isotypic control antibody (Santa Cruz Biotechnology) for 15 minutes. After washing, the cells were incubated with 10 μg/mL of a secondary anti-rabbit fluorescein isothiocyanate (FITC)-conjugated antibody (Santa Cruz Biotechnology) in 2% goat-serum/PBS for further 10 minutes. All incubation steps were conducted at room temperature. The mean fluorescence intensity was measured using a FACSCalibur flow cytometer (Becton Dickinson).

Western blot analysis
The expression of the β1- and β2-adrenergic receptors was confirmed by immunoblotting using the same primary antibodies that were used for flow cytometry. Cells (3 × 10^5) were lysed in Laemmli sample buffer and incubated at 95°C for 10 minutes. Proteins were separated using an 8% PAGE (28) and then transferred to an Immobilon-P transfer membrane, with a pore size of 0.45 μm (Millipore Corporation). After transfer, the membranes were blocked for 1 hour at room temperature in TBS containing 5% non-fat milk powder, followed by 3 washing steps. The membranes were incubated overnight at 4°C with the primary antibodies and a rabbit anti-eIF4E-antibody (Cell Signalling) as control for the applied protein amounts (all antibodies were used in a 1:1,000 dilution). Subsequently, the membranes were washed vigorously with TBS containing 0.1% Tween 20 (AppliChem) and then incubated at 1:1,000 dilution with a horseradish peroxidase (HRP)-linked secondary goat anti-rabbit IgG antibody (Cell Signalling) at room temperature for 2 hours. The luminescence signals were detected using a Hamamatsu C 4742-98 system (Hamamatsu).

Statistics
Statistical analyses were conducted using Student t test (two-tailed, unpaired) if not stated differently. A P value less than 0.05 was considered statistically significant.

Results
As introduced above, we have reported previously that several carcinoma cells lines from different tissue origin significantly increase their locomotory activity in response to norepinephrine (2–4). This neurotransmitter or so-called stress hormone is to our knowledge the strongest physiologic inducer of the migratory activity of MDA-MB-468 breast carcinoma cells (2). Likewise, MDA-MB-231 and MDA-MB-435S both present the relevant β1- and β2-adrenergic receptors as was shown by flow cytometry and immunoblotting using the same antibodies for both techniques (Fig. 2A and B). However, as the Western blot analysis showed only weak bands, we additionally confirmed the receptor expression by PCR (Fig. 2C). In addition to the tumor cells lines, HMVECs were tested for β-adrenergic receptor expression. A human embryonic stem cell line served as positive control (29). Human pulmonary smooth muscle cells were used as positive control for the β2-adrenergic receptor and negative control for the β1-adrenergic receptor. Human peripheral blood mononuclear cells (i.e., the T lymphocytes of this fraction) and human prostate carcinoma cells of the cell line PC-3 were investigated for β-adrenergic receptor expression by flow cytometry previously and served as further positive controls (4, 30). However, although the relevant receptors were expressed on MDA-MB-231 and MDA-MB-435S cells, norepinephrine did in contrast to MDA-MB-468 cells not increase the migratory activity of these cells (MDA-MB-231, Fig. 3A: control 52.4% ± 7.0% vs. norepinephrine 40.9% ± 10.8% locomoting cells; MDA-MB-435S, Fig. 3B: control 43.7% ± 5.0% vs. norepinephrine 38.5% ± 5.9% locomoting cells; MDA-MB-468, Fig. 3C: control 50.4% ± 11.1% vs. norepinephrine 62.5% ± 8.7% locomoting cells).

However, the effect of norepinephrine on the adhesion of the breast carcinoma cells to endothelium under flow conditions revealed a completely different picture (Fig. 4). The adhesion of MDA-MB-468 cells and MDA-MB-435S cells was not influenced by norepinephrine (MDA-MB-468; Fig. 4A: control 7,646 ± 2,418 vs. norepinephrine...
8,428 ± 1,824 pixels, and MDA-MB-435S; Fig. 4B: control 8,651 ± 3,598 vs. norepinephrine 8,595 ± 2,854 pixels), but in contrast, norepinephrine treatment led to a significant and dose-dependent increase of the adhesion of MDA-MB-231 cells with 10 μmol/L being most effective (10,111 ± 12,825 to 39,906 ± 19,927 pixels; \( P = 0.019 \); Fig. 4C). This increase of adhesion was mediated by \( \beta \)-adrenergic receptors, as the \( \beta \)-blocker propranolol abolished the effect of norepinephrine (control 10,822 ± 2,979 to norepinephrine 30,121 ± 10,501 pixels; \( P = 0.008 \); Fig. 4D).

As already shown by PCR (Fig. 2C), not only the tumor cells but also the HMVECs express both the \( \beta \)-1- and \( \beta \)-2-adrenergic receptor. This was confirmed by flow cytometry to show that the receptors are present on the surface of the HMVECs (Fig. 5A). In response to norepinephrine, the HMVECs significantly increased the release of the chemokines GROα (50.2 ± 19.3 to 137.5 ± 44.3 pg/h/100,000 cells; \( P = 0.011 \)) and IL-8 (53.3 ± 11.7 to 139.2 ± 9.9 pg/h/
100,000 cells; $P = 0.016$; Fig. 5B, left). Accordingly, the relative expression of the relevant genes was more than 4-fold increased ($GRO\alpha$: 4.67 and IL-8: 4.41), as was assessed by quantitative real-time PCR (Fig. 5B, right). The release of both chemokines was mediated by $\beta$-adrenergic receptors, as a blockade of these receptors using propranolol strongly reduced the release ($GRO\alpha$: 72.1 $\pm$ 2.4 pg/h/100,000 cells and IL-8: 100.5 $\pm$ 1.1; $P = 0.032$). With regard to GRO$\alpha$, this reduction was not significant due to the high SD of the norepinephrine sample.

GRO$\alpha$ and IL-8 bind to the same chemokine receptors, which are CXCR1 and CXCR2. PCR analysis revealed that the RNA for both receptors is expressed in high amounts in MDA-MB-231 cells, whereas the MDA-MB-435S and MDA-MB-468 cells showed considerable less amounts (Fig. 6A). Confirmatory experiments were carried out with PC-3 human prostate carcinoma cells, which showed overall less adhesion to the endothelium, but a similar increase in response to norepinephrine from 5,004 $\pm$ 1,785 to 8,663 $\pm$ 2,363 pixels (Fig. 6B, left). Furthermore, these cells express high amounts of both chemokine receptors CXCR1 and CXCR2 too (Fig. 6B right). We thus concluded that the MDA-MB-231 cells should be susceptible to the chemokines' signaling and hypothesized that the proadhesive effect of norepinephrine might be transmitted by these chemokines. Therefore, we inhibited the function of these receptors on the MDA-MB-231 cells by preincubation with blocking antibodies and subsequently subjected the cells.
The chemokine receptor blockade (12,123 ± 3,589). Accordingly, the combination of both chemokines was of similar efficacy as GROα alone (19,866 ± 2,266; P = 0.011; Fig. 6D).

Subsequently, we investigated the molecular basis of this interaction between MDA-MB-231 cells and HMVECs. It is well known that integrins are important adhesion molecules for the extravasation of tumor cells (23). Pretreatment of the MDA-MB-231 cells with the β1-integrin–blocking antibody 4B4 significantly (P = 0.011) inhibited the norepinephrine-induced adhesion from 27,043 ± 5,917 to 18,383 ± 3,299 pixels, but not the spontaneous adhesion of the control [15,028 ± 5,033 (IgG1 isotype control) vs. 16,110 ± 5,320 pixels (4B4); Fig. 7A].

![Image](image_url)

**Figure 4.** Adhesion of the tumor cell lines to endothelium under flow conditions. The field that was covered by adhesive tumor cells was calculated in pixels as explained in the Materials and Methods section. Each experiment was carried out independently 3 times; mean values and SDs are shown. *, statistically significant changes (P < 0.05); A, B, D, norepinephrine (Nor) was used at 10 μmol/L. D, propranolol (Prop) was used at 10 μmol/L.

To the flow-through adhesion assay (Fig. 6C). Such a receptor blockade abrogated the norepinephrine effect. In the control, there was no difference between the cells treated with either an isotypic control antibody (IgG2a; 16,390 ± 3,243 pixels) or anti-CXCR1 and anti-CXCR2 antibodies (αCXCR1/2; 13,311 ± 3,136 pixels). In contrast, norepinephrine treatment led to a significant increase of adhesion in IgG2a-treated cells (25,966 ± 3,505 pixels; P < 0.001), which was significantly reduced down to the control level by the chemokine receptor blockade (12,614 ± 2,039 pixels; P < 0.001; Fig. 6C). In turn, overlay of the endothelium with GROα significantly (P < 0.001) induced adhesion (from 10,158 ± 3,914 to 21,829 ± 4,173 pixels; Fig. 6D), whereas IL-8 had only a very weak and nonsignificant effect (12,123 ± 3,589). Accordingly, the combination of both chemokines was of similar efficacy as GROα alone (19,866 ± 2,266; P = 0.011; Fig. 6D).

![Image](image_url)

**Figure 5.** Expression of β-adrenergic receptors (A) and influence of norepinephrine on the chemokine release (B) of HMVECs. A, the expression of the β1- and β2-adrenergic receptor (AR) in HMVECs was analyzed by flow cytometry. The shown graphs are representative for 3 independent measurements. B, the release of chemokines GROα and IL-8 was investigated by an enzyme-linked immunoassay. Norepinephrine (Nor) and propranolol (Prop) were used at 10 μmol/L. In addition, the expression level of the relevant genes was assessed by quantitative real-time PCR. The results of the immunoassay are shown as mean values and SDs of 3 independent measurements. The PCR analysis is shown as box plot of a single experiment. *, statistically significant changes (P < 0.05). ISO, isotypic.
Furthermore, inhibition of endothelial NF-κB led to a slight, however not statistically significant reduction of the adhesion of the control cells from 16,872 to 13,080 pixels, but significantly (P = 0.002) reduced the norepinephrine-mediated adhesion from 28,285 to 16,779 pixels (Fig. 7B).

Discussion

Proinflammatory chemokines are supposed to play an important role in tumor progression and metastasis formation. Several animal studies gave evidence that blocking CXCR1 and especially CXCR2 signaling, either by use of neutralizing antibodies or small-molecule inhibitors, inhibits colon cancer metastasis to liver (31, 32). One earlier study showed similar results for a CXCR1/CXCR2 blockade on lung metastasis development in a renal cell carcinoma model (33). But in this study, the anti-metastatic effect is explained by inhibition of tumor-associated angiogenesis. Direct effects of the CXCR1/CXCR2 blocking the extravasation of tumor cells were not investigated in this context. With our in vitro flow-through adhesion assay, we show that GROα, and to a lesser extent, IL-8, can promote the adhesion of CXCR1/CXCR2-positive tumor cells to endothelium. Chemokines are released by endothelial cells in response to certain stimuli and then become bound on the apical side of the endothelial cell layer by glycosaminoglycans (34, 35). By this action, they are presented to rolling cells leading to the rapid activation of integrins (17, 36). Accordingly, our own results show that overlay of the endothelium with the chemokines GROα and, to a much lesser extent, IL-8 lead to a β1-integrin–mediated increase in the adhesion of MDA-MB-231. Therefore, we provide in vitro arguments for a possible involvement of GROα in regulating integrin-mediated adhesion of MDA-MB-231 cells, which might have parallels to the known role of proinflammatory chemokines in the extravasation of leukocytes. Our work complements recent results by Yao and colleagues showing that the inhibition of the α5β1-integrin prevents the extravasation of MDA-MB-231 cells in the lungs of athymic nude mice (37). Similar effects of α5β1-integrin blocking on lung metastasis were shown in other studies for a metastatic rat prostate cancer cell line (38) and a metastatic murine melanoma cell line (39). These and our data point toward an important role of β1-integrin in lung metastasis.

The following question is why or in response to which stimuli these chemokines are released. It is well known that the generation of inflammatory conditions comprises
the release and action of chemokines and that such inflammatory conditions contribute to the progression of cancer (15, 16). However, in these conditions, the chemokines are largely provided by leukocytes. It is well documented that the vasculature is, at least in part, innervated by noradrenergic sympathetic nerves (40–45), and as shown herein, norepinephrine induces the release of GROα and IL-8 from the vascular endothelium. This induction by norepinephrine is mediated via β-adrenergic receptors, as the blockade of these receptors by the clinically established β-blockers (48) and thereby regulates the expression of proinflammatory chemokines and cytokines (49, 50). In accordance with these findings, the addition of a β-blocker significantly reduced the norepinephrine-induced adhesion of MDA-MB231 breast carcinoma cells to lung endothelium in our flow-through adhesion assay. Therefore, our study adds further evidence of a direct effect of norepinephrine on endothelial cells, in addition to the recent observation that β2-adrenoceptor−deficient mice show dysfunctional angiogenesis (51), which possibly explains why β-blockers are effective in the treatment of hemangiomas (52). In summary, our study shows 3 possible points to inhibit the norepinephrine-induced adhesion of MDA-MB231 breast carcinoma cells to lung endothelium: (i) inhibition of norepinephrine signaling by β-blockers, (ii) blocking of β1-integrins, and (iii) inhibition of chemokine signaling/release. Among these possibilities, β-blockers are clinically well established in long-term hypertension therapy, and epidemiologic studies confirm that they have no effect in causing or promoting cancer (53). Furthermore, we and others have reported previously by mice studies (5, 14), and retrospective patient data analysis (10–12), that the use of β-blockers inhibits metastasis formation. One molecular explanation for these observations was our in vitro migration studies, in which the migratory activity of carcinoma cells from breast (2), prostate (4), and colonic (3) tissue origin increased by treatment with norepinephrine; this effect was inhibited by β-blockers as well. In addition, other groups have shown evidence that propranolol inhibits the norepinephrine-induced migration and invasion of pancreatic (6) and ovarian (7) cancer cells. However, metastasis formation is a multistep process, which requires not only the induction of migratory activity (54) but also the ability of tumor cells to enter the blood stream and extravasate at the site of metastasis development; at least in hematogenic dissemination (55). Here, we provide a further molecular explanation for how β-blockers can inhibit metastasis formation by the attenuation of the β1-integrin−mediated adhesion of tumor cells to the vascular endothelium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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