RAC1 activity and intracellular ROS modulate the migratory potential of MCF-7 cells through a NADPH oxidase and NFκB-dependent mechanism

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Abstract

In the present study, we demonstrated that changes in Rac1 activity associated with the production of intracellular ROS modulate the migratory properties in MCF-7 and T47D human mammary cell lines. We also described that the NFκB pathway exerts a downstream control on the expression of the ROS-dependent cellular migratory potential. These results emphasize the importance of redox balance in the acquisition of malignancy and support previous data sustaining that an oxidative environment predisposes cells to activate signal-transduction pathways actively involved in cellular oncogenesis. Our data also provides evidence that NADPH oxidase could constitute the main source of intracellular ROS in response to changes in Rac1 activity. We suggest that Rac1 plays a role in cellular migration not only limited to its known function in reorganization of the actin cytoskeleton, but also as part of the intracellular machinery that controls the redox balance.

Keywords: ROS; Rac1; Invasiveness; NFκB

1. Introduction

A causal relationship between inflammation and cancer development arises from data that emphasize the importance of inflammatory chemical mediators in different steps of tumorigenesis [1]. The reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide, are among the most relevant members of this family of mediators [2]. Once released from infiltrating cells in the site of injury, ROS generate an imbalance in the tissular redox status that modifies essential signaling pathways through the reversible oxidation of key intracellular signaling components [3]. In light of this information, a causal link between oxidative stress, induced by a persistent elevation of intracellular ROS, and cancer development has been strongly supported [4].

Extra-mitochondrial generation of ROS in many cellular systems occurs mainly through the activation of the membrane flavoprotein NADPH oxidase (NOX) that, when expressed in phagocytes,

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constitutes the main defense system against microorganisms [5]. The small GTP-binding protein Rac is a positive regulator of NOX, which in its activated form induces the structural assembly of the enzyme [6]. This phenomenon that also is present in a variety of non-phagocytic cells, allows the establishment of a link between the activation of Rac, a pivotal regulator of actin assembly and formation of lamellipodia, with the generation of ROS [7]. It has been clearly demonstrated that both Rac activation and lamellipodia formation constitutes one of the driving forces of cell movement [8]. One of the proposed cellular mechanisms that explains the ROS-dependent enhancement of motile properties is the Rac-induced downregulation of Rho activity and the subsequent formation of lamellipodia. This process has the LMW-PTP phosphatase as the main target of oxidation [9].

Functional association between Rac and intracellular ROS content is not only exerted through the role of the GTPase in NOX assembly. It has been also demonstrated in murine epithelial cells that Rac activation can occur after the stimulus of an enhancement of oxidative intracellular status, as part of an array of other ROS-dependent cellular functional changes that results in the acquisition of invasiveness and motility [10].

One of the proposed functional roles for ROS during tumor progression is the constant activation of transcription factors such as NFκB, which once bound to specific sites in promoter sequences in DNA, induce the activation of genes that further modulate a variety of cellular functions [11]. NFκB cellular function is controlled mainly by the activity of its specific cytoplasmic inhibitor IκB which, under oxidative stress, is phosphorylated and subsequently degraded. Thus, intracellular redox balance modulates NFκB's ability to migrate to the nucleus, bind to DNA and promote gene expression [12]. Sensitivity of NFκB to redox cellular status has provided evidence that this system may represent an effective link between inflammation and cancer [1].

The present study was designed to understand the specific mechanisms by which Rac activity modulates, in a ROS-dependent manner, the expression of cellular migratory properties. Using MCF-7 breast carcinoma cells that over express the active and dominant negative forms of Rac1, we found that Rac activity through the regulation of the intracellular content of ROS by a mechanism that depends on the NADPH oxidase, activates NFκB signalling. This pathway, in turn, is responsible for urokinase production and the expression of an invasive potential.

2. Materials and methods

2.1. Chemicals

tert-Butyl hydroperoxide (H_2O_2), *N*-acetylcysteine (NAC), 2′,7′-dicholoro-dihydrofluorescin diacetate (DCDHF-DA), were from Sigma (St. Louis, MO).

2.2. Cell culture

Mammary MCF-7 and T47D cells were obtained from ATCC (Manassas, VA) and were grown in phenol-red-free DMEM/F12 supplemented with 10% fetal calf serum (FCS).

2.3. Expression vector and reporter construct

cDNA encoding for the dominant negative (N17Rac1) and permanently active (V12Rac1) forms of Rac1 were kindly provided by Dr. Miguel Quintanilla (Instituto de Investigaciones Biomédicas Alberto Sols, CSIC, Madrid, Spain). The luciferase reporter construct (pNFκB-Luc) containing five tandem repeats of the NFκB binding site and IκBα plasmid were a gift from Dr. Luisa Botella, at Centro de Investigaciones Biológicas, Madrid, Spain. Full-length human uPA promoter (−2062 to +27) in luciferase reporter gene plasmid pGL2 basic (p-uPA-Luc) was a generous gift from Dr. Soishi Kojima from the Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki, Japan [13].

2.4. Determination uPA promoter and NFκB activities

For these assays, 40×10^3 cells, cultured on 24-well dishes, were transiently transfected with 500 ng of p-uPA-Luc or pNFκB-Luc and co-transfected with 500 ng N17Rac1 or V12 Rac1 cDNAs or pcDNA3.1 (Empty-Vector) together with 100 ng of β-galactosidase using the Lipofectamine 2000 reagent according to the manufacturer's protocol (Invitrogen CA).

Transfected cells were lysed 48 h later with Luciferase Cell Culture lysis reagent and luciferase assay was performed using the luciferase reporter assay system (Promega, Madison WI). β-Galactosidase activity was measured using the Galacto-Light Plus System (Applied Biosystems, Bedford, MA) to normalize the transfection efficiency. We used the pRL vector (Promega) containing a full-length *Renilla* luciferase under the control of a SV40 constitutive promoter as a positive control. pRL vector without the promoter sequence was used as a negative control. Each transfection was performed in quadruplicate wells [14].

2.5. Cell migration assay

Migration of MCF-7 and T47D cells was studied using a 6.5 mm Transwell chamber with a pore size of 8 μm (Corning NY). The Transwell membranes were coated with 10% fetal bovine serum in culture media for 2 h at 37 °C on only the underside. Cells (50×10^3) were re-suspended in serum-free medium and seeded on the upper compartment of the chamber. The lower chamber of the Transwell was filled with a medium solution enriched with 10% FCS. Migration was allowed to occur for 48 h, in the case of MCF-7 cells, and 72 h for T47D cells, after which cells of the upper membrane surface were removed by a cotton swab and repeatedly washed with PBS. Migration values were determined by counting five (20×) fields per chamber after fixing the membrane in methanol and staining the migratory cells with 0.2% crystal violet [15].

2.6. Determination of ROS

Production of ROS was detected by a fluorescence assay. Briefly, cells were loaded with 10 μM DCDHF-DA in serum free phenol-red-free medium (Gibco Invitrogene, CA) for 30 min at 37 °C. Afterwards, cells were washed and lysed with 0.1 N NaOH. Fluorescence was monitored using a microplate fluorometer (Spectra MAX, Gemini EM, Molecular Devices) using wavelengths of 480 and 530 nm for excitation and emission, respectively [16]. In experiments in which NOX-dependent ROS production was inhibited, cells were subjected to a previous treatment of 12 h with 20 μM DPI previous to ROS determination. In experiments in which the intracellular content of ROS was exogenously modulated, MCF-7 cells were pre-treated 24 h with 10 nM *N*-acetylcysteine (NAC) or 12 h with 50 μM H_2O_2 before fluorometric determination of ROS.

2.7. RT-PCR analysis

Total RNA was isolated with Trizol (GIBCO) from pIκBα or Empty-Vector transfected MCF-7 cells. cDNA were synthesized for 1 h (42 °C) with M-MLV reverse transcriptase (Promega) using oligo dT (Invitrogen), and RT-PCR analyses were carried out as described previously. Primer sequences were as follows: uPA, forward: 5′-GCAGGAACCCAGACAACCG-3′. Reverse: 5′-GACCCAGGTAGACGATGTAG-3′ (amplified a fragment of 357 bp at 26 cycles). GAPDH, forward: 5′-ACCACAGTCCATGCCATCAC-3′. Reverse: 5′-TCCACCACCCTGTTGCTGTA-3′ (amplified a fragment of 452 bp at 23 cycles).

In all cases, the annealing temperature was 58 °C. PCR products were subjected to electrophoresis on a 1.5% agarose gel, and DNA was visualized by ethidium bromide staining.

3. Results

3.1. In MCF-7 cells, Rac1 activity correlates with intracellular ROS level: possible participation of NADPH oxidase

To evaluate whether Rac1 basal activity determines the cellular ROS production, we measured the intracellular ROS content by cell fluorescence after loading with DCDHF-DA, MCF-7 cells over expressing different levels of Rac1 activity. As shown in Fig. 1A, cells that over express the dominant negative version of Rac1 (N17Rac1) display the lowest level of fluorescence, while

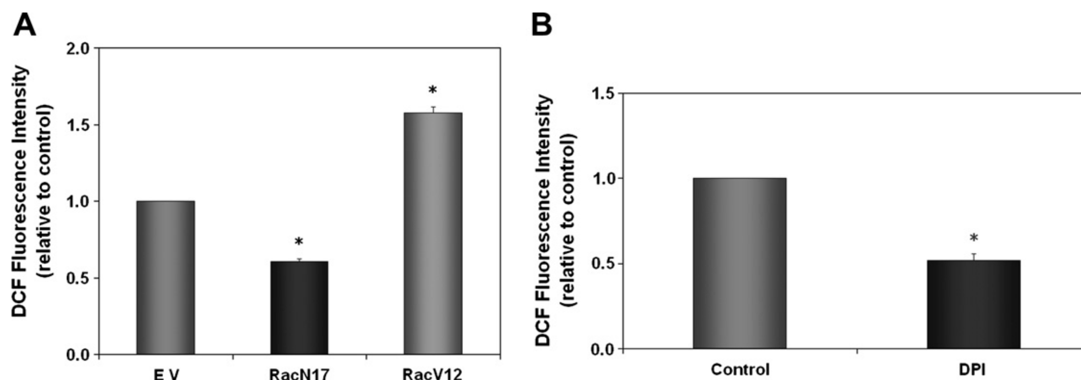

Fig. 1. Rac1 activity correlates with intracellular ROS level in MCF-7 cells. Possible participation of NADPH oxidase. Intracellular ROS was evaluated in MCF-7 cells transiently transfected with the Empty-Vector (EV), the dominant negative (N17) and the permanently active version of Rac1 (V12) using DCDHF-DA as indicated in Section 2 (A). The same methodology was used to measure intracellular ROS in intact and DPI-treated MCF-7 cells as indicated in Section 2 (B). Results are mean values ± SE from three separate experiments. *Differences were calculated against EV (in A) or control (in B) cells and considered significant at $p < 0.01$.

cells that express the permanently active form of this GTPase (V12Rac1) exhibit the highest level relative to cells transfected with a Empty-Vector plasmid (EV). To evaluate the contribution of NADPH oxidase (NOX) to the basal ROS production, we analyzed the ROS content using the same fluorescence procedure in intact MCF-7 cells previously cultured in the presence of the flavoprotein inhibitor diphenyleniodonium (DPI) that inhibits NOX. As shown in Fig. 1B, more than half of the basal level of intracellular ROS in MCF-7 cells was sensitive to DPI and, therefore, presumptively attributable to NOX.

3.2. Rac1 activity is associated with the migratory capacity and uPA expression

We evaluated whether the basal Rac1 activity plays a role in the acquisition of cellular migration and in the basal production of urokinase (uPA), a serine protease that plays a major role in cellular migration. The migratory capacity was assayed in cells that over express different basal Rac1 activity using the Transwell double chamber method. The uPA promoter activity was assessed in these same cells. As shown in Fig. 2A, cells transfected with V12Rac1 exhibit 2.5 times more migration capacity than control EV cells, while N17Rac1 cells display one third of the migration capacity of EV cells. When these cells were used to measure the transcriptional activity and mRNA level of uPA, we obtained a very similar pattern (Fig. 2B and C).

3.3. Intracellular ROS determines migratory activity: possible role of NOX

To evaluate whether intracellular redox balance plays a role determining cellular motility in MCF-7 cells, we assessed the migratory capacity in cells where intracellular ROS was severely modified. In doing so, we cultured cells in the presence of H_2O_2 to enhance the cellular oxidative level, and with *N*-acetylcysteine (NAC) to quench oxidant species. As Fig. 3A shows, migratory capacity of intact MCF-7 cells seems to be directly dependent on the intracellular ROS content modified by the addition of both agents. Furthermore, cells cultured in the presence of H_2O_2 had four times the migratory capacity of control cells. Next, we analyzed whether NOX was in part responsible for the ROS-dependent modulation of cell motility. To this end, we used control (EV) and RacV12 cells treated with the NOX inhibitor DPI. We previously demonstrated that RacV12 cells expressed a higher motility than the control EV transfected cells (Fig. 1). As Fig. 3B shows, NOX inhibition significantly blocked the migratory behaviour in both cases, suggesting that the enzyme exerts a strong influence in the ROS and also Rac-dependent cellular migration.

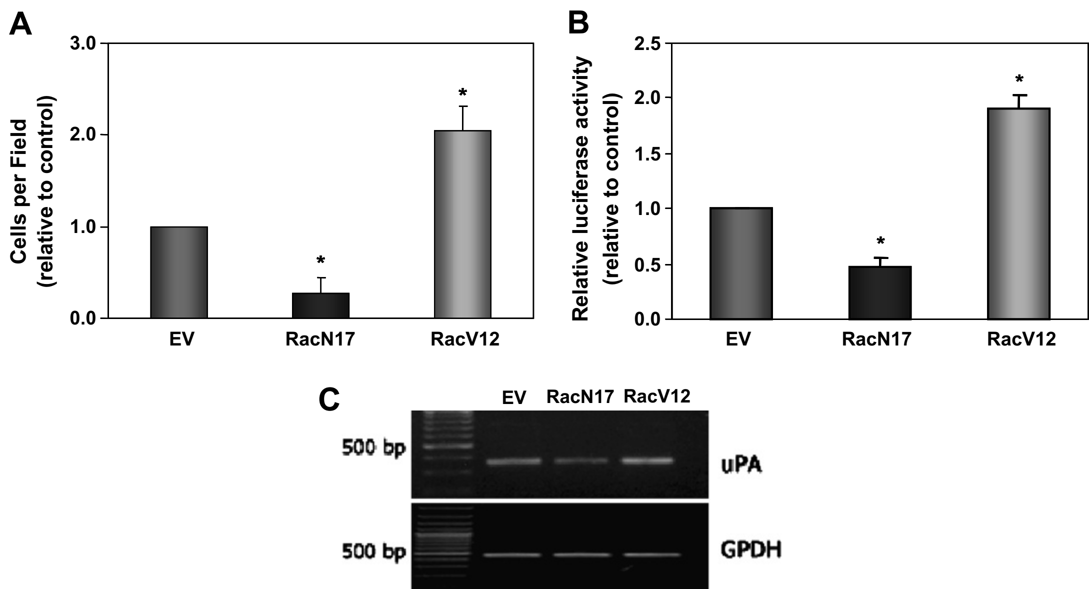

Fig. 2. Rac1 activity correlates with migratory capacity and uPA promoter activity in MCF-7 cells. EV, RacN17 and RacV12 MCF-7 cells were used to determine migratory capacity, evaluated using the Transwell double chamber method as described in Section 2 (A). uPA promoter activity was determined after transient transfection with a −2345 to +32 fragment of the human promoter uPA gene as indicated in Section 2 (B). uPA mRNA expression was evaluated in EV, RacN17 and RacV12 MCF-7 cells by semi-quantitative RT-PCR as indicated in Section 2 (C). Data are mean values ± SE from three separate experiments. *Differences from EV cells were considered significant at $p < 0.01$.

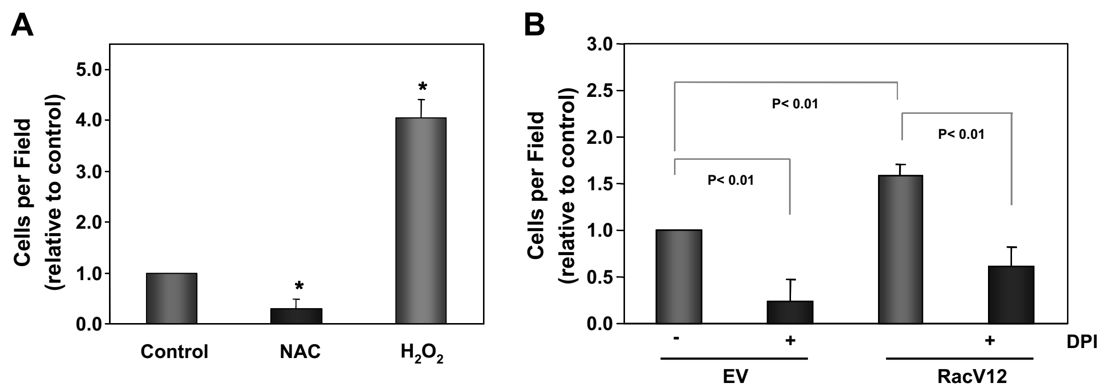

Fig. 3. Intracellular ROS determines migratory capacity in MCF-7 cells. Possible participation of NADPH oxidase. Migratory capacity of MCF-7 cells was evaluated using the Transwell double chamber method in intact cells (control) and cells previously treated with 10 nM *N*-acetylcysteine (NAC) and 50 μM H_2O_2 as indicated in Section 2. Results are mean values ± SE from three separate experiments. *Differences from intact control cells were considered significant at $p < 0.01$ (A). Cells transiently transfected with the Empty-Vector (EV) and the permanently active version of Rac1 (RacV12) treated or untreated with 20 μM DPI were assayed for migratory capacity as in (A). Results are mean values ± SE from three separate experiments. *Differences from EV cells were considered significant at $p < 0.01$ (B).

3.4. T47D mammary cells express a Rac and ROS-dependent migratory capacity

To analyze whether the results obtained in MCF-7 cells implied that Rac activity modified the cellular migratory potential by a mechanism depending of intracellular ROS production and whether this proposed mechanism is also utilized by other cells, we performed a set of similar experiments with T47D cells, a weakly invasive mammary cell line. As Fig. 4A shows, T47D cells over expressing the dominant negative version of Rac1 (N17Rac1) display the lowest level of ROS content as evaluated by fluorescence, while cells that express the permanently active form of this GTPase (V12Rac1) exhibit the highest level, in both cases levels were measured relative to cells transfected with a Empty-Vector plasmid (EV). Consistently with the previous results obtained in MCF-7 cells, T47D cells overexpressing V12Rac1 display the higher migratory capacity evaluated by the Transwell method, and the N17Rac1 cells exhibit the lowest capacity, both relative to the migratory potential of EV cells (Fig. 4B). Finally, in order to verify the contribution of NOX in the ROS-dependent invasiveness in T47D cells, we evaluate cell motility in T47D cells previously treated with DPI, inhibitor of NOX. As Fig. 4C shows, both, the EV and V12Rac1 expressing cells, when treated with DPI, diminished considerably their migratory capacity evaluated by the Transwell method.

3.5. Rac1 activity correlates with NFκB activation level

The transcription factor NFκB has been proposed as one of the most sensitive mechanisms by which cells respond to changes in ROS balance [10]. In fact, we previously found that treatment of intact MCF-7 cells with 10 μM NAC strongly inhibits NFκB activity (data not shown). To determine whether basal Rac1 activity, previously shown to modify ROS intracellular levels (Fig. 1), also modulates NFκB pathway activation, we used cells expressing different levels of basal Rac1 activity to evaluate NFκB activity. The results indicated that in this case, the pattern of NFκB activities (Fig. 5) was highly similar to that previously showed for ROS content and migration assays (Figs. 2 and 3).

3.6. NFκB activity modulates uPA expression and invasiveness in intact MCF-7 cells

To evaluate whether in MCF-7 cells NFκB activity plays a permissive role in cellular migration and to identify a specific target for its activity, we overexpressed pIκBα, the main inhibitor of NFκB nuclear translocation. As Fig. 6A shows, uPA mRNA level, determine by semi-quantitative RT-PCR, decreased in a dose dependent manner when intact MCF-7 cells overexpress pIκBα. On the other and, to confirm that the enhanced migration capacity showed by V12Rac1 cells is the result of a NFκB activation, we use the same experimental approach used in A to analyzed the migration capacity in intact and V12Rac1 transfected cells using the double chamber method. Fig. 6B shows that the blockade of NFκB activation provokes an inhibition of the migration potential both in intact and permanently active Rac1 cells underscoring the importance of NFκB pathway in the Rac-dependent migration of MCF-7 cells.

4. Discussion

In carcinoma cells, particularly in breast cancer cells, GTPases of the Rho family are frequently over

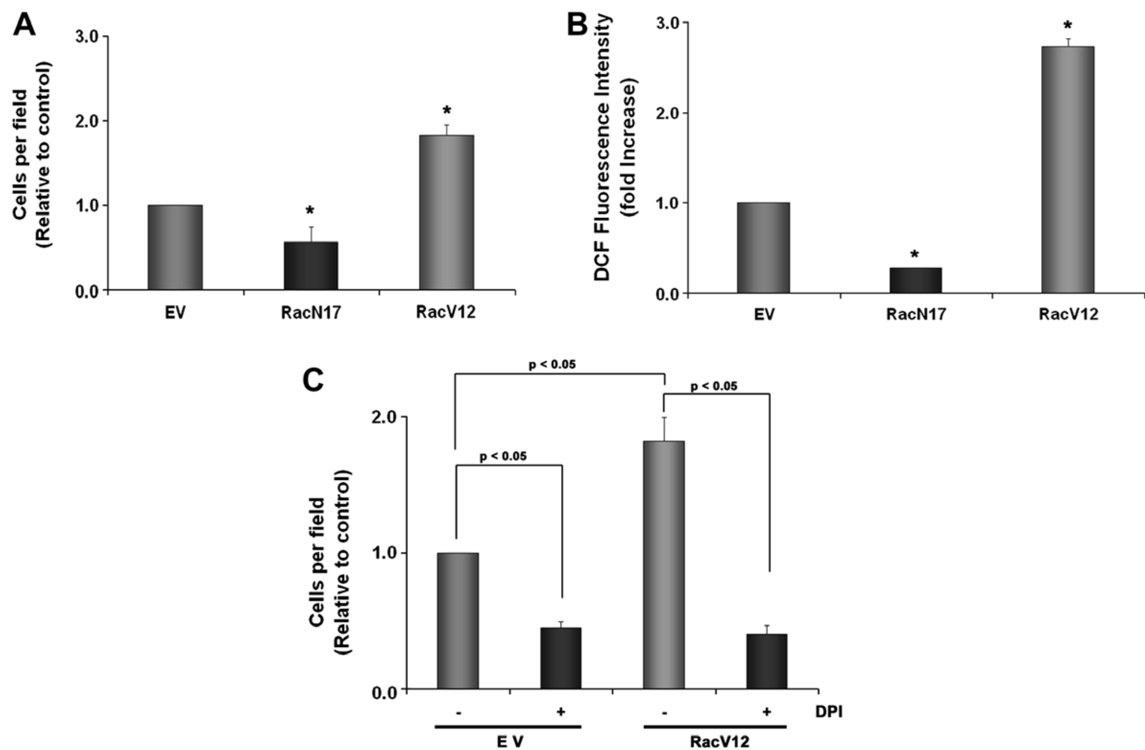

Fig. 4. Intracellular ROS determines migratory capacity in T47D cells. Possible participation of NADPH oxidase. T47D cells transiently transfected with the Empty-Vector (EV), dominant negative (RacN17) and permanently active version of Rac1 (RacV12) were assayed for migration using the Transwell chamber method (A) and intracellular ROS using DCFDA as indicated in Section 2 (B). Results are means ± SE. *Differences were calculated against EV cells and considered significant at $p < 0.05$. Cells transiently transfected with Empty-Vector (EV) and permanently active version of Rac1 (RacV12) treated or not with 20 μM DPI were assayed for migratory capacity as in A. Results are mean values from three separate experiments ± SE and considered significant at $^{*}p < 0.05$ (C).

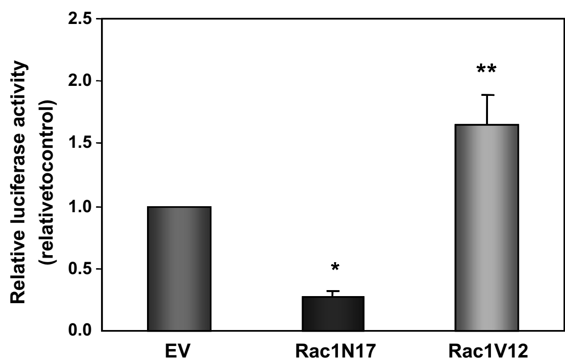

Fig. 5. Rac1 activity correlates with NFκ-B activity. MCF-7 cells were transiently transfected with 1 mg of 5xNFκB/Luc plus and co-transfected with the Empty-Vector (EV), dominant negative (N17) and permanently active version (V12) of Rac1. NFκB activity was evaluated as indicated in Section 2. Significant differences from EV (Student's *t* test), $^{*}p < 0.01$, $^{**}p < 0.05$.

expressed, an event that usually correlates with a poor prognosis [17]. This association is done not only by the acquisition of motile properties resulting from a cytoskeleton arrangement to more favorable cell movement, but also by the generation of a misbalance in redox equilibrium that activates signaling routes that favor invasiveness [18].

Invasiveness in epithelial cells is the main result of a complex process collectively known as epithelial–mesenchymal transition (EMT), by which cells lose epithelial traits and acquire fibroblastic characteristics [19]. EMT has emerged as a central process during cancer progression because its development induces cancer cells to acquire motile properties that allow them to change position within the tissues and to disseminate and grow on distant organs [20]. Using different cellular models, it has been proposed that ROS can play a central role in different aspects of EMT. For example, in the TPA-dependent EMT in human hepatoma cells, ROS triggers sustained PKC-ERK signaling that promotes cell scattering and migration [21]. In mouse mammary epithelial cells, the MMP-3-induced EMT that is mediated by the expression of a Rac1b, an alternative spliced

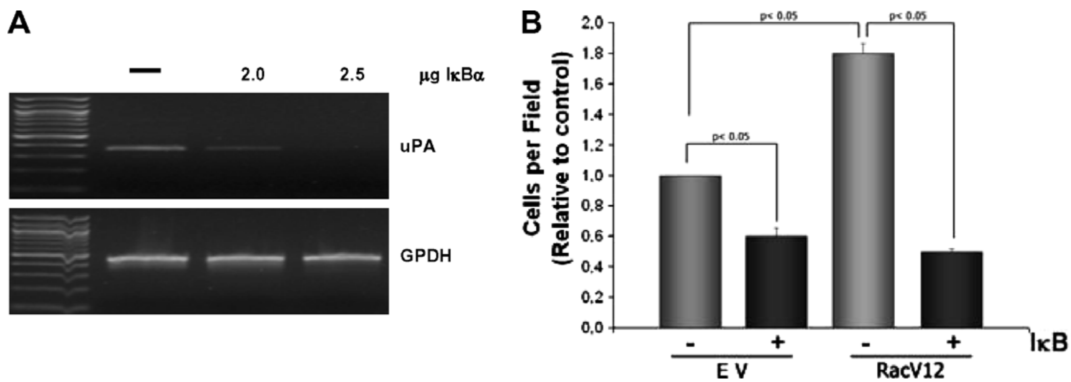

Fig. 6. NFκB activity is necessary for basal mRNA uPA expression and migratory capacity. MCF-7 cells transiently transfected with an Empty-Vector or a plasmid encoding for IκBα were used to determine uPA mRNA level by semi-quantitative RT-PCR as indicated in Section 2 (A). To evaluate migratory capacity these cells were co-transfected with Empty-Vector (EV) or permanently active version of Rac1 (RacV12) and a plasmid codifying to IκBα. Migration were measured using the Transwell chamber method. Data are mean of values ± SE obtained from three separate experiments. *Differences among groups were calculated against EV cells and considered significant at $p < 0.05$ (B).

isoform of Rac1, and the enhancement of the expression of the transcription factor Snail, could be blocked by treatment with the ROS-quenching agent NAC [7]. Collectively, these data suggest that oxidative conditions cause a loss of epithelial properties, switching to a more invasive phenotype.

Rac1 GTPase constitutes an upstream regulator of actin reorganization and adhesive properties associated with cellular shape and motility [22]. It has been proposed that the main targets for Rac and Cdc42 that mediate actin polymerization for driving membrane protrusion are the WASP/WAVE family of Arp2/3 complex activators. In fact, Rac stimulates lamellipodial extension by activating the WAVE family [23]. However, the fact that Rac1 also constitutes part of the structure of NOX and, in this manner, participates in the control of the intracellular ROS machinery, provides an alternative approach to analyze Rac1 function. Results in Fig. 3B show that near a 60% of the migratory capacity resulting from the expression of the permanently active RacV12 version is sensitive to NOX inhibitor DPI and thereby, results of the intracellular ROS generation. These data underscore the role of Rac-dependent intracellular ROS in cell motility.

It has been demonstrated that intracellular ROS activates the NFκB route, but the redox-sensitive pathways triggering this activation are quite different depending on the cell type considered [24]. Recently, Li et al. demonstrated in MCF-7 cells that IL-1β stimulation induces the endocytosis of the loaded receptor IL-1βR1, a mechanism that is required for redox-dependent NFκB activation. In this phenomenon, NOX2 is recruited to the endosomal compartment in a Rac1-dependent fashion [25]. Our data in Figs. 1, 5, and 6 showing that Rac1 activity mirrored both the ROS intracellular content and the NFκB activity along with the fact that pIκBα exerts an inhibitory effect on uPA expression and invasiveness, strongly confirm that in MCF-7 cells, the modification of ROS balance induced by Rac activity is one of the main components of the downstream modulation of NFκB system activity. A functional link between NFκB activation, uPA production and motility has been proposed in the invasive MDA MB-231, a cell line that produces a large amount of uPA [26]. Our results, linking NFκB activation to Rac activity and to NOX-dependent intracellular ROS production, constitute a new contribution to understand the role of intracellular redox balance on cellular invasiveness.

Data presented here open a new scenario to analyze the acquisition of metastatic phenotype, expanding our view of the role of the oxidative balance in cellular functions, and providing a potential therapeutic role for natural and synthetic antioxidants.

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References

[1] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867.

[2] M. Benhar, D.E. Engeberg, A. Levitzki, ROS, stress-activated kinases and stress signaling in cancer, EMBO Rep. 3 (2002) 420–425.
[3] W. Dröge, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (2002) 47–96.
[4] S. Toyokuni, Novel aspects of oxidative stress-associated carcinogenesis, Antioxid. Redox Signal. 8 (2006) 1373–1377.
[5] B.M. Babior, NADPH oxidase: an update, Blood 93 (1999) 1464–1476.
[6] G.M. Bokoch, B.A. Diebold, Current molecular models for NADPH oxidase regulation by Rac GTPase, Blood 100 (2002) 2692–2696.
[7] D.C. Radisky, D.D. Levy, L.E. Littlepage, H. Liu, C.M. Nelson, J.E. Fata, D. Leake, E.L. Godden, D.G. Albertson, M.A. Nieto, Z. Werb, M.J. Bissell, Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability, Nature 436 (2005) 123–127.
[8] V. Patel, H.M. Rosenfeldt, R. Lyons, J.-M. Servitja, X.R. Bustelo, M. Siroff, J.S. Gutkind, Persistent activation of Rac1 in squamous carcinomas of the head and neck: evidence for an EGFR/Vav2 signaling axis involved in cell invasion, Carcinogenesis 28 (2007) 1145–1152.
[9] A.S. Nimnual, L.J. Taylor, D. Bar-Sagi, Redox-dependent downregulation of Rho by Rac, Nat. Cell Biol. 5 (2003) 236–241.
[10] K. Mori, M. Shibanuma, K. Nose, Invasive potential induced under long-term oxidative stress in mammary epithelial cells, Cancer Res. 64 (2004) 7464–7472.
[11] N. Li, M. Karin, Is NFκB the sensor of oxidative stress?, FASEB J 13 (1999) 1137–1143.
[12] M. Karin, Nuclear factor-κB in cancer development and progression, Nature 441 (2006) 431–436.
[13] Y. Suzuki, J. Shimada, K. Shudo, M. Matsumura, M.P. Crippa, S. Kojima, Physical interaction between retinoic acid receptor and Sp1: mechanism for induction of urokinase by retinoic acid, Blood 93 (1999) 4264–4276.
[14] J.F. Santibañez, JNK mediates TGF-β1-induced epithelial mesenchymal transdifferentiation of mouse transformed keratinocytes, FEBS Lett. 580 (2006) 5385–5391.
[15] M. Cáceres, J. Guerrero, J. Martínez, Overexpression of RhoA-GTP induces activation of the epidermal growth factor receptor, dephosphorylation of focal adhesion kinase and increased motility in breast cancer cells, Exp. Cell Res. 309 (2005) 229–238.
[16] M. Benhar, I. Dalyot, D. Engelberg, A. Levitzki, Enhanced ROS production in oncogenically transformed cells potentiates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase activation and sensitization to genotoxic stress, Mol. Cell. Biol. 20 (2001) 6913–6926.
[17] G. Fritz, I. Just, B. Kaina, Rho GTPases are over-expressed in human tumors, Int. J. Cancer 81 (1999) 682–687.
[18] D. Ferraro, S. Corso, E. Fasano, E. Panieri, R. Santangelo, S. Borrelo, S. Giordano, G. Pani, T. Galeotti, Pro-metastatic signaling by c-Met through RAC-1 and reactive oxygen species (ROS), Oncogene 25 (2006) 3689–3698.
[19] J.P. Thiery, Epithelial–mesenchymal transitions: twist in development and pathologies, Curr. Opin. Cell Biol. 15 (2003) 740–746.
[20] P. Friedl, K. Wolf, Tumor-cell invasion and migration: diversity and scape mechanisms, Nat. Rev. Cancer 3 (2003) 362–374.
[21] W.S. Wu, R.K. Tsai, C.H. Chang, S. Wang, J.R. Wu, Y.X. Chang, Reactive oxygen species mediated sustained activation of protein kinase C alpha and extracellular signal-regulated kinase for migration of human hepatoma cell Hepg2, Mol. Cancer Res. 4 (10) (2006) 747–758.
[22] A. Hall, GTPases and the actin cytoskeleton, Science 279 (1998) 509–514.
[23] A.J. Ridley, M.A. Schwartz, K. Burridge, R.A. Firtel, M.H. Ginsberg, G. Borisy, J.T. Parsons, A.R. Horwitz, Cell migration: integrating signals from front to back, Science 302 (2003) 1704–1709.
[24] G. Gloire, S. Legrand-Poels, J. Piette, NF-kappaB activation by reactive oxygen species: fifteen years later, Biochem. Pharmacol. 72 (2006) 1493–1505.
[25] Q. Li, M.M. Harraz, W. Zhou, L.N. Zhang, W. Ding, Y. Zhang, T. Eggleston, Ch. Yeaman, B. Banfi, J.F. Engelhardt, Nox2 and Rac1 regulate H_2O_2-dependent recruitment of TRAF6 to endosomal Interleukin-1 receptor complexes, Mol. Cell. Biol. 26 (2006) 140–154.
[26] D. Sliva, M.T. Rizzo, D. Denis English, Phosphatidylinositol 3-kinase and NFκB regulate motility of invasive MDA-MB-231 human breast cancer cells by the secretion of urokinase-type plasminogen activator, J. Biol. Chem. 277 (2002) 3150–3157.