Biochemical and Biophysical Research Communications 425 (2012) 775–780

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Neuropilin-1 is expressed by breast cancer stem-like cells and is linked to NF-κB activation and tumor sphere formation

Yelena Glinka [a], Nada Mohammed [a], Venkateswaran Subramaniam [a], Serge Jothy [a,b,c], Gérald J. Prud'homme [a,b,c,*]

[a] Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital, ON, Canada M5B 1W8
[b] Department of Laboratory Medicine, St. Michael's Hospital, ON, Canada M5B 1W8
[c] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 26 July 2012
Available online 2 August 2012

Keywords:
Cancer stem cells
Breast cancer
Mammosphere
Neuropilin
NF-kappaB
Tranilast

ABSTRACT

Cancer stem cells (CSCs) initiate tumors and have a high resistance to conventional cancer therapy. Tranilast is an orally active drug of low toxicity that exerts inhibitory effects on breast CSCs. This appears to depend on its aryl hydrocarbon receptor (AHR) agonistic activity, but this receptor has diverse functions and it is unclear how CSCs are inhibited. CSCs generate tumor spheres in low-adherence cultures, and we employed the mammosphere-forming assay as a functional test for breast CSCs. Because NF-κB has a key role in mammosphere formation and CSC-mediated tumor initiation, we examined that pathway. We also examined the role of neuropilin-1 (Nrp1), which is a growth factor coreceptor linked to the tumorigenicity of some CSCs. We found that tranilast concurrently suppressed mammosphere formation, Nrp1 expression and constitutive NF-κB activation. Flow cytometric analysis revealed that a subpopulation of breast cancer cells bearing breast CSC markers also expressed Nrp1. A blocking anti-Nrp1 antibody suppressed mammosphere formation. We examined whether there was a link between Nrp1 and NF-κB activation. The siRNA knockdown of Nrp1 severely suppressed NF-κB activation and mammosphere formation. The phosphorylation of Akt and ERK1/2 was also reduced, but to a lesser extent. We conclude that Nrp1 plays a key role in mammosphere formation and this activity is linked to NF-κB activation. Thus, Nrp1 might be a target for therapy against breast CSCs, and the anticancer drug tranilast suppresses its expression.

1. Introduction

Cancer stem cells (CSCs) represent a subpopulation of tumour cells with a high capacity to generate new tumors [1–4]. Some important features include a distinctive marker profile, self-renewal, and formation of tumor spheres in low adherence cultures. These cells display marked resistance to anti-cancer drugs, probably through multiple mechanisms including high expression of the ABCG2 drug transporter [3]. This capacity of CSCs to resist chemotherapeutic agents likely contributes to cancer relapse, and the poor prognosis of many cancers. In view of this, there has been a great interest in developing drugs that specifically inhibit CSCs. We recently reported on the anti-CSC properties of tranilast [5], which is an orally active drug of low toxicity developed to treat allergy, but that has anti-proliferative and anti-tumor effects [6]. Our studies revealed that tranilast inhibits tumor sphere (mammosphere) formation in culture and metastasis following xenotransplantation. Furthermore, we found that it is an aryl hydrocarbon receptor (AHR) agonist, and this accounted for its activity against CSCs, at least in vitro [5]. The AHR is the receptor for dioxins and several other toxicants, but it is now clear that it has many physiological functions such as regulation of the cell cycle [7], hematopoietic stem cell survival [8], and interaction with the estrogen receptor (ER) [9] and NF-κB [10]. Because of this diversity of activities, it is unclear which pathway(s) tranilast targets to inhibit CSCs.

Here, we first examined some of the effects of tranilast in the mammosphere-forming assay, as a functional test for breast CSCs [11]. Previous studies have shown that mammosphere culture enriches for cells capable of forming tumors in vivo, i.e., CSC-like cells [11,12]. The formation of spheres by CSC-like cells in non-adherent conditions results from their ability to resist anoikosis, unlike the main population of cells [13]. This assay has the advantage that it does not rely on specific markers to identify CSCs, as markers can vary greatly from one breast cancer or cell line to another [14]. Since NF-κB activation has a key role in the generation of mammospheres and tumorigenicity of CSCs [15], we investigated that pathway. We also examined the potential role of neuropilin-1 (Nrp1), because it appears to play an important in some types

* Corresponding author at: Department of Laboratory Medicine, St. Michael's Hospital, 30 Bond Street, Room 418-LKSKI, Toronto, Ontario, Canada M5B1W8. Fax: +1 416 864 5648.

E-mail addresses: glinkay@smh.ca (Y. Glinka), nada.mohammed@utoronto.ca (N. Mohammed), subramaniamv@smh.ca (V. Subramaniam), jothys@smh.ca (S. Jothy), prudhommeg@smh.ca, prudhommegj@gmail.com (G.J. Prud'homme).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.151

of CSCs [16,17]. Nrp1 is a multifunctional growth factor (GF) coreceptor involved in angiogenesis and cancer [18].

In this report, we demonstrate that tranilast suppresses both Nrp1 expression and NF-κB activation. Furthermore, we establish a link between Nrp1 expression, NF-κB activation, and mammosphere formation, which appears to be interrupted by tranilast. These findings suggest that Nrp1 has a key role in breast CSC function, and might be a good target for anti-CSC therapy.

2. Materials and methods

2.1. Drug

Tranilast (N- [3,4-dimethoxycinnamoyl]anthranilic acid; *MW* = 327.3) was a kind gift of Dr. Richard Gilbert (St. Michael's Hospital, Toronto). It was dissolved in DMSO, and applied as described [19].

2.2. NF-κB assay

We used NK-κB p65 kit by Stressgen (USA), which is an ELISA method to quantify activation of NF-κB, according to the manufacturer's instructions. Extracts obtained from the tumour cells were deposited in 96-well plates coated with a DNA oligonucleotide carrying the NF-κB consensus binding sequence. The attachment of active NF-κB was detected with an antibody against the NF-κB p65 subunit. It was expressed as relative luminescent units per mg of protein in the extract.

2.3. Flow cytometric analysis and cell sorting

Flow cytometry analysis and cell sorting were performed by methods we have previously described [5]. ALDH^{hi} cells were identified by flow cytometric analysis using the ALDEFLUOR^{TM} assay kit (Stem cell technologies, Vancouver, BC) [20,21]. This involves an ALDH-1-dependent enzymatic reaction generating an intracellular fluorescent compound, BODIPY-aminoacetate.

2.4. Mammosphere culture

Mammospheres were grown in serum-free, low adherence cultures, with a CnT-27 medium and growth additives (CellnTEC Advanced cell systems, Bern, Switzerland), as we have previously described [5]. The size and number of mammospheres were quantitated using Image J software (NIH, USA).

2.5. Breast cancer cell lines

The cell lines used in this study were obtained from ATCC (Manassa, VA, USA). We have previously described the properties of these cell lines and growth media [5]. CSC marker expression in these cells has been reported by us and others [5,20–23].

2.6. siRNA knockdown and western blotting

Nrp1 expression was knocked down with siRNA from Santa Cruz Biotechnology and Nrp2 expression was knocked down with Nrp2-targeted SureSilencing siRNA (SA Biosciences/Qiagen), as we have previously described [24]. Cell lysates were prepared and Western blotting performed as described [19].

2.7. Inhibitors and antibodies

NF-κB inhibitors PS1145 dihydrochloride (Sigma) and pyrrolidine dithiocarbamate (PTDC, Tocris) were dissolved in DMSO and used at 0.1–1 μM concentration separately or in combination. PI3 K inhibitor LY294002 in DMSO solution was from Calbiochem. Equivalent concentrations of DMSO were added to the control cells.

Directly-labeled anti-human CD44 and anti-human CD24 antibodies used in flow cytometry were from Biolegend. Monoclonal anti-rat/human Nrp1 antibody (R&D Systems) was labeled with Alexa Fluor 647 (Invitrogen, according to the manufacturer's protocol). Rabbit polyclonal anti-Nrp1 antibody used for blocking was from Santa Cruz Biotechnology.

2.8. Phosphoprotein kinase activity

The phosphorylation of Akt and ERK1/2 was evaluated by cellular ELISA (SA Biosciences/Qiagen), as we have previously described [19]. The relative extent of target protein phosphorylation was calculated by normalizing the values for the phosphoprotein-specific antibodies to the pan-protein-specific antibodies for the same experimental condition.

2.9. Statistical analysis

Statistical analyses were performed with the GraphPad Prism 5.0 program (GraphPad Software Inc., San Diego, CA). In each in vitro experiment, the significance of differences between experimental and control results was determined by either Student's *t* test or analysis of variance (ANOVA). Results are expressed as the mean + SEM, unless stated otherwise.

3. Results and discussion

3.1. Tranilast concomitantly suppresses mammosphere formation, Nrp1 expression and NF-κB activation

We have previously reported that tranilast can inhibit sphere formation by breast cancer cell lines [5]. We examined two well-studied breast cancer cell lines, i.e., MDA-MB-231 (abbreviated MB-231), which is estrogen receptor/progesterone receptor/HER2 (ER/PR/HER2) triple-negative, and BT-474, which is ER/PR/HER2 triple-positive. Here, we show that tranilast inhibits sphere formation and there is concurrent suppression of Nrp1 expression and NF-κB activation (Fig. 1A–C). Tranilast was applied at 200 μM concentration, which is pharmacologically relevant [5,19]. The cell lines differ in that more than 90% of MB-231 cells are Nrp1+ (Fig. 1B), compared to ~55% in BT-474 (Fig. 1C). The suppressive effect was analyzed by flow cytometry, and we observed that tranilast induced $a > 70\%$ reduction in the numbers of Nrp1^{+} cells (Fig. 1B and C).

NF-κB activation was evaluated with an ELISA assay that detects NF-κB binding to its target DNA sequence. This pathway was constitutively activated in both cell lines grown in mammosphere culture, and tranilast-mediated suppression was strong in both cases (Fig. 1A). We also examined the MCF-7 (ER/PR+) breast cancer cell line, with or without selection for resistance to mitoxantrone performed as described [5], and in all cases tranilast suppressed NF-κB (data not shown). Thus, we observed that tranilast suppresses NF-κB activation in a triple-negative (MB-231), triple-positive (BT-474) and ER/PR-positive (MCF-7) cell line.

3.2. Nrp1 expression by breast CSC-like cells

Here, we examined whether cells bearing markers that have been associated with CSC function also express Nrp1. Breast CSC-like cells are found principally in the subpopulation of cells expressing a high level of aldehyde dehydrogenase-1 (ALDH^{hi}), as detected by the ALDEFLUOR assay [20,21]. However, not all these

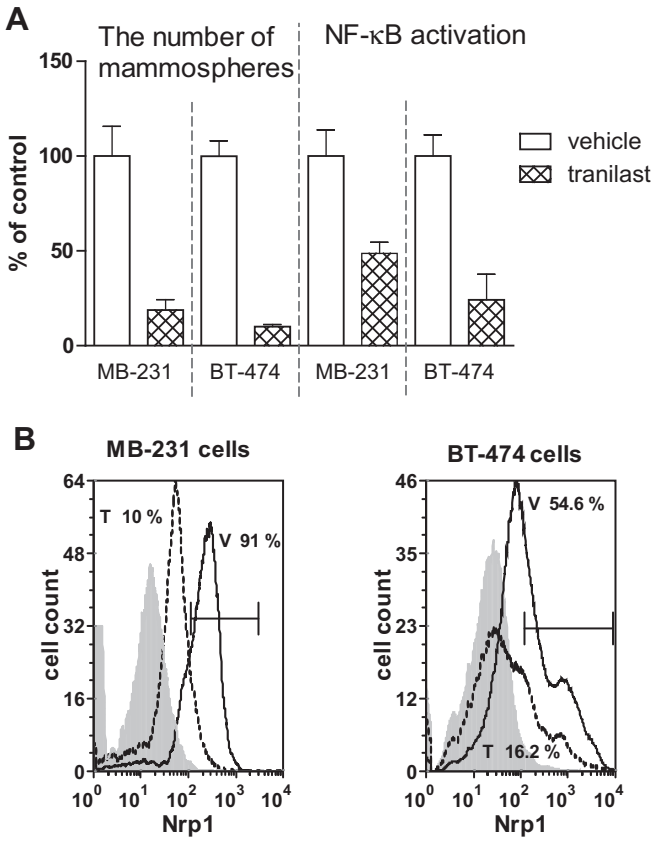

Fig. 1. Tranilast inhibits mammosphere formation, Nrp1 expression and NF-κB activation. (A) MB-231 and BT-474 cells were cultured in the mammosphere assay for 8 days with the partial replacement of the medium every third day. Note that BT-474 forms well-demarcated round spheres, whereas MB-231 forms looser and more irregular spheres, as we have previously described [5]. Tranilast (200 μM) significantly inhibited ($p < 0.01$ vs. control) the number of mammospheres and NF-κB activation in both cell lines. (B) Tranilast markedly inhibited Nrp1 expression by MB-231 cells (flow cytometry). *V* = vehicle; *T* = tranilast. (C) Tranilast markedly inhibited Nrp1 expression by BT-474 cells (flow cytometry). (B) and (C): Gray peak, isotype control; solid-line peak, cells grown in medium with vehicle; dotted-line peak, cells grown with tranilast (200 μM). The numbers of Nrp1 positive cells (as gated from the isotype control) are shown in the histograms. Representative results are shown, and similar results were obtained in three experiments.

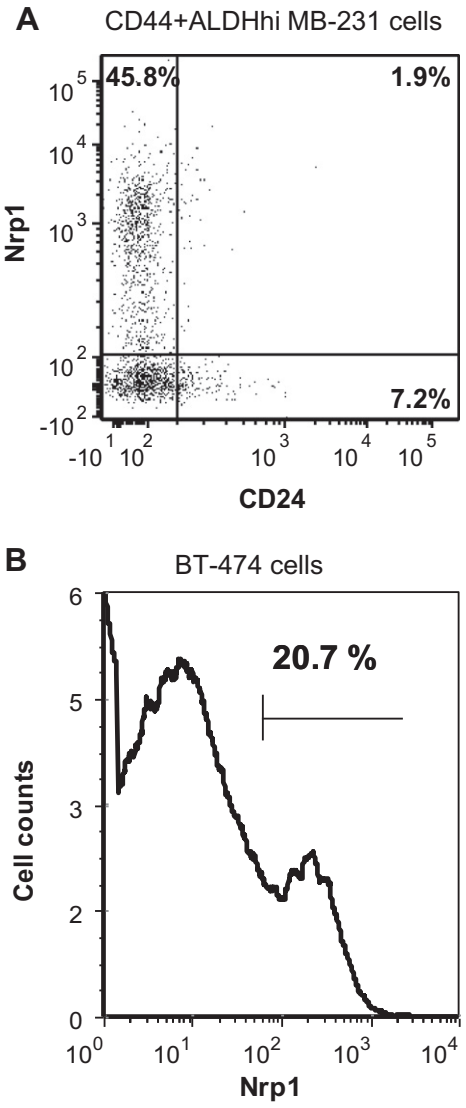

Fig. 2. Nrp1 is expressed by a subpopulation of CSC-like cells. (A) First, MB-231 were sorted by fluorescence-activated cell sorting (FACS) for $ALDH^{hi}/CD44^{+}$ double marker expression. ALDH expression was determined with the ALDEFLUOR reaction, and cell-surface expression of CD44 was detected with an antibody. Second, the sorted cells were stained for cell-surface expression of Nrp1 and CD24 with antibodies. In this cell line, $ALDH^{hi}/CD44^{+}/CD24^{-}$ cells are highly enriched in CSCs, and approximately 45% of these cells were positive for Nrp1 expression (left upper quadrant). Two experiments yielded similar results. (B) BT-474 cells were analyzed for $ALDH^{hi}$ and Nrp1 expression by two-color flow cytometry. In this cell line, $CD44^{+}/CD24^{-}$ are very rare and $ALDH^{hi}$ was used as marker of CSCs. Approximately 20% of $ALDH^{hi}$ cells expressed Nrp1 (appearing as a bright staining peak in the histogram). Two experiments yielded similar results.

cells are CSCs, and the marker profile of CSC-like cells differs considerably from one cell line to another. In the MB-231 cell line, 1–5% of cells are $ALDH^{hi}$ [5,21], and within this population the $ALDH^{hi}/CD44^{+}/CD24^{-/low}$ subpopulation is highly enriched in CSC-like cells [21]. As in previous studies, we observed that $ALDH^{hi}$ MB-231 cells positively selected by fluorescence-activated cell sorting (FACS) could form mammospheres, but the dull ALDH cell population did not (data not shown). To examine Nrp1 expression on MB-231CSC-like cells, we first positively sorted $ALDH^{hi}/CD44^{+}$ cells by FACS, and then stained these cells for CD24 and Nrp1. As seen in Fig. 2A, ~45% of $ALDH^{hi}/CD44+/CD24^{-/low}$ MB-231 cells express Nrp1.

Marker expression is different in the BT-474 cell line, such that very few cells (if any) express the $CD44^{+}/CD24^{-/low}$ phenotype [22,23] and these markers are not useful to identify CSCs. Because the $ALDH^{hi}$ phenotype associates with CSC-like features, we examined these cells. We have previously reported that approximately 5% of BT-474 cells are $ALDH^{hi}$ [5]. Here, we show that ~20% of $ALDH^{hi}$ BT-474 cells expressed Nrp1 (Fig. 2B). Thus, in these two cells lines, a subset of cells expressing markers previously associated with a CSC-like phenotype also expressed Nrp. In both cell lines, the $ALDH^{hi}/Nrp1^{+}$ double-positive subpopulation is a small subset of the total cell population. However, Nrp1 cannot be considered a specific CSC marker, because it is also expressed by main population cells (expressing low levels of ALDH).

3.3. Knockdown or blockade of Nrps prevents mammosphere formation and NF-κB activation

To determine whether Nrps play a role in mammosphere formation, we knocked down Nrp1 and Nrp2 expression with siRNA, with an effective method we have previously described [24]. MB-231 cells express both Nrp1 and the homologous Nrp2 [25]. Because Nrp2 can duplicate many of the properties of Nrp1, in that cell line we knocked down both Nrp1 and Nrp2. BT-474 express only Nrp1, and in that cell line we knocked down only Nrp1. The

downregulation of Nrp expression almost completely abolished the ability of the cells (MB-231 or BT-474) to form mammospheres, whereas sham siRNA knockdown had no effect (Fig. 3A and B). This was not due to cell death, as viability was only slightly reduced (not shown).

We examined NF-κB signalling following siRNA knockdown of Nrps. Remarkably, Nrp knockdown markedly suppressed signaling in this pathway (Fig. 3C). Western blots confirmed effective knockdown of Nrps (Fig. 3D). To determine whether NF-κB blockade was sufficient to prevent mammosphere formation, we added

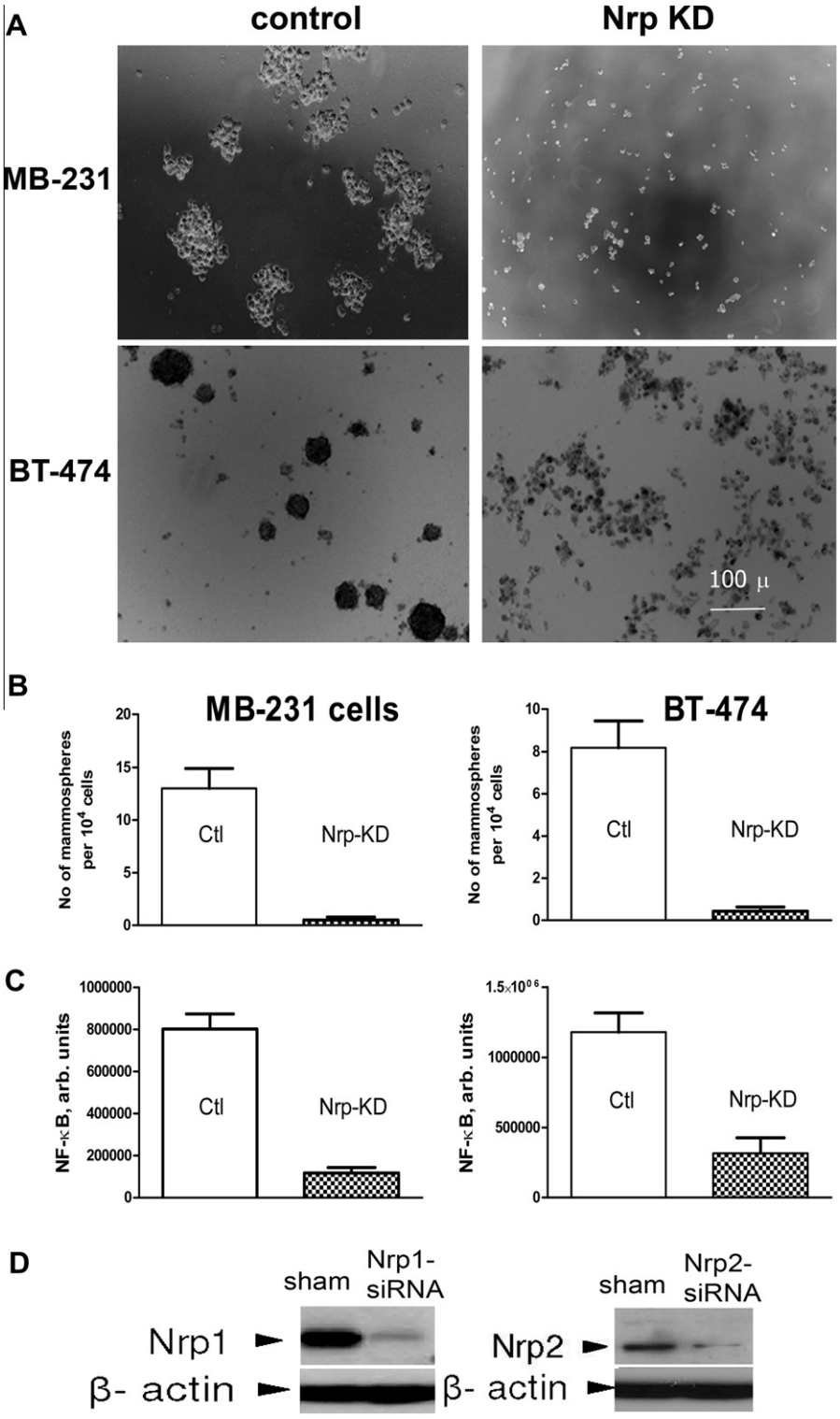

Fig. 3. Knockdown of Nrps with siRNA inhibits mammosphere formation. (A) and (B) MB-231 cells were treated with Nrp1- and Nrp2-targeted siRNA or scrambled siRNA (sham-transfected control). Similarly, BT-474, which expresses only Nrp1, was treated with Nrp1 or scrambled siRNA. The cells were cultured in non-adherent conditions for 8 days, with medium being partially replaced every third day. In both cell lines, knockdown of Nrps almost completely prevented mammosphere formation. (C) In both MB-231 and BT-474 cells lines Nrp knockdown markedly inhibited NF-κB activation ($p < 0.01$ vs. control). (A–C). These results are from a representative experiment, and in each case three experiments yielded similar results. (D) Knockdown of Nrp1 and 2 was confirmed by Western blot. KD, knockdown.

a mixture of two NF-κB inhibitors to cultures, i.e., PS1145 (IKK inhibitor, which prevents activation of NF-κB) and pyrrolidine dithiocarbamate (a potent inhibitor of NF-κB). Indeed, this also prevented mammosphere formation (Fig. 4A). These findings establish a link between Nrps and NF-κB activation in breast sphere-forming cells, and suggest that inhibiting this pathway is sufficient to block mammosphere formation.

NF-κB is a generic name for transcription factors constituted of heterodimers of five subunits, i.e., RelA (p65), RelB, REL (c-Rel), p50, and p52 [26,27]. The NF-κB complex resides in a latent form in the cytoplasm, and is activated by several stimuli including inflammatory mediators and growth factors. Activation depends on the degradation of the inhibitory molecule IκBα by the NF-κB essential modulator (NEMO)/IκB kinase (IKK)γ-containing IKK complex [27]. Thus, once activated, the IKK complex phosphorylates IκBα, which results in its ubiquitination, proteasomal degradation, and release of NF-κB for translocation into the nucleus. In the canonical pathway, the p50/p65 heterodimer enters the nucleus and induces the expression of genes principally regulating cell survival and proliferation, as well as inflammation.

In our experiments, the NF-κB pathway might be activated by several different stimuli [26]. Notably, there is previous evidence that the PI3 K/Akt pathway plays a major role in NF-κB activation in breast cancer cells [28]. Inhibition of Akt phosphorylation interfered with mammosphere formation (Fig. 4A). Furthermore, the Nrps boost responses to several growth factors known to activate PI3 K/Akt [29], and Nrp expression has been linked to Akt phosphorylation in renal carcinoma cells [30]. Interestingly, we found that Nrp knockdown moderately reduced Akt phosphorylation, as well as ERK1/2 phosphorylation, as determined by cellular ELISA (Fig. 4B). These inhibitory effects were not as great as the inhibition of NF-κB. Nevertheless, these findings are consistent with Nrp1 knockdown leading to a lower response of cancer cells to growth factors, and hence a reduction in the activation of all these pathways. However, further investigations are required to confirm this hypothesis.

3.4. Nrp1 blockade with antibodies prevents mammosphere formation

To further establish that this was related to Nrp expression, we treated BT-474 cells with an anti-Nrp1 polyclonal antibody. This treatment also inhibited mammosphere formation (Fig. 4A). Interestingly, this treatment with antibodies induced rapid internalization of Nrp1, as detected by confocal microscopy (data not shown).

In summary, we demonstrate that the Nrps are essential molecules for mammosphere formation in well-studied breast cancer cell lines. We report that mammosphere suppression with tranilast, an AHR agonist drug, was associated with marked suppression of both Nrp1 expression and NF-κB activation. Interestingly, we previously observed that tranilast strongly suppresses Nrp1 expression of tumor cells in vivo, in a 4T1 murine mammary carcinoma model (unpublished observation). Therefore, therapeutic doses of tranilast can suppress Nrp1, but whether this contributes to its anti-cancer effects has not been established. In subsequent experiments we examined the potential role of Nrps.

Other authors have shown that activation of NF-κB is essential for mammosphere formation by the MCF-7 breast cancer cell line [15]. Here, we showed that inhibitors of NF-κB also suppress mammosphere formation by the triple-positive BT-474 cell line. Moreover, it seems likely that tranilast acted at least in part by suppressing this pathway, although it is also inhibits a number of other pathways [19]. Indeed, there have been previous reports of NF-κB suppression by tranilast [31,32], but the target of drug action was not elucidated. Notably, tranilast failed to inhibit key events in the NF-κB signaling pathway [31], and perhaps suppression occurs indirectly, i.e., as a consequence of action in another pathway.

Tranilast inhibits the production of some inflammatory cytokines (e.g., IL-1, TNF-α) and other mediators [19,33,34], which can activate NF-κB. However, these inflammatory mediators might not be present in sufficient amounts in the mammosphere assay. Nonetheless, growth factors added to the sphere-forming assay culture medium (bFGF and EGF), or produced by the tumors cells (e.g., VEGF, HGF and TGF-β) can promote NF-κB activation. Notably, TGF-β is suppressed by tranilast [19]. However, because tranilast reduces the expression of Nrp1 this could mitigate the action of other growth factors. VEGF, HGF, PDGF, TGF-β, and some FGFs are known to interact with the Nrps, which act as coreceptors to enhance the signaling response to these ligands [24,35–38]. It is unclear whether Nrps can signal directly, but it appears more likely that they interact with the conventional signaling receptors of growth factors (e.g., VEGFR2, c-Met, and TGFβRI/RII) to enhance

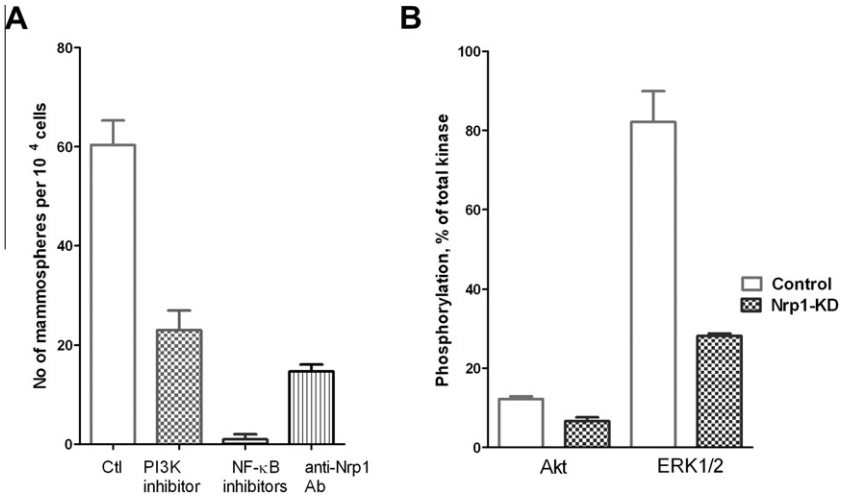

Fig. 4. Blockade or knockdown of Nrp1, and the role of key signaling pathways. (A) Mammosphere formation by BT-474 cells was significantly suppressed ($p < 0.05$ vs. non-treated cells) by an inhibitor of PI3 K, LY294002 (20 μM), or a mixture of NF-κB inhibitors (PS1145 and PDTC at 0.1 and 0.5 μM, respectively), or a blocking anti-Nrp1 antibody (20 μg/ml). A representative experiment is shown, and a repeat experiment yielded similar results. (B) Nrp1-knockdown and sham-transfected cells BT-474 cells, 72 h after the transfection, were cultured in non-adherent plates under mammosphere-forming conditions for 24 h. Phosphorylation of Akt and ERK1/2 was quantified by cell ELISA, and the extent of Akt and ERK1/2 phosphorylation was calculated as described in Methods. Nrp1 knockdown significantly reduced ($p < 0.05$) both Akt and ERK1/2 phosphorylation, and two experiments yielded similar results. KD, knockdown.

signaling. In view of this, we speculate that Nrps stimulate NF-κB activation by boosting the response to growth factors present in the mammosphere assay medium, and this warrants further investigation.

Our studies show that mammosphere formation by CSC-like cells is dependent on Nrp expression, and this is linked to NF-κB activation. The drug tranilast suppresses both Nrp and NF-κB and this might explain at least in part its anti-CSC activity. Therapies blocking the Nrp/NF-κB pathway may be effective against breast CSCs, and of therapeutic benefit in breast cancer.

Acknowledgments

Our studies were supported by Ontario Institute for Cancer Research of the Province of Ontario (Canada), the Canadian Institutes of Health Research, the Canadian Breast Cancer Research Alliance, and the Keenan Research Centre in the Li Ka Shing Knowledge Institute and of St. Michael's Hospital, Toronto, Canada.

References

[1] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (1997) 730–737.
[2] C.A. O'Brien, A. Kreso, J.E. Dick, Cancer stem cells in solid tumors: an overview, Semin. Radiat. Oncol. 19 (2009) 71–77.
[3] G.J. Prud'homme, Cancer stem cells and novel targets for antitumor strategies, Curr. Pharm. Des. 18 (2012) 2838–2849.
[4] J.E. Visvader, G.J. Lindeman, Cancer stem cells: current status and evolving complexities, Cell. Stem. Cell 10 (2012) 717–728.
[5] G.J. Prud'homme, Y. Glinka, A. Toulina, et al., Breast cancer stem-like cells are inhibited by a non-toxic aryl hydrocarbon receptor agonist, PLoS One 5 (2010) e13831.
[6] M. Rogosnitzky, R. Danks, E. Kardash, Therapeutic potential of tranilast, an anti-allergy drug, in proliferative disorders, Anticancer Res. 32 (2012) 2471–2478.
[7] B.A. Barhoover, J.M. Hall, W.F. Greenlee, et al., Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4, Mol. Pharmacol. 77 (2010) 195–201.
[8] F.L. Casado, K.P. Singh, T.A. Gasiewicz, Aryl hydrocarbon receptor activation in hematopoietic stem/progenitor cells alters cell function and pathway-specific gene modulation reflecting changes in cellular trafficking and migration, Mol. Pharmacol. 80 (2011) 673–682.
[9] J. Matthews, J.A. Gustafsson, Estrogen receptor and aryl hydrocarbon receptor signaling pathways, Nucl. Recept. Signal. 4 (2004) e016.
[10] C.E. Ruby, M. Leid, N.I. Kerkvliet, 2,3,7,8-Tetrachlorodibenzo-p-dioxin suppresses tumor necrosis factor-alpha and anti-CD40-induced activation of NF-kappaB/Rel in dendritic cells: p50 homodimer activation is not affected, Mol. Pharmacol. 62 (2002) 722–728.
[11] D. Ponti, A. Costa, N. Zaffaroni, et al., Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties, Cancer Res. 65 (2005) 5506–5511.
[12] M.J. Grimshaw, L. Cooper, K. Papazisis, et al., Mammosphere culture of metastatic breast cancer cells enriches for tumorigenic breast cancer cells, Breast Cancer Res. 10 (2008) R52.
[13] M. Cioce, S. Gherardi, G. Viglietto, et al., Mammosphere-forming cells from breast cancer cell lines as a tool for the identification of CSC-like- and early progenitor-targeting drugs, Cell Cycle 9 (2010) 2878–2887.
[14] S.Y. Park, H.E. Lee, H. Li, et al., Heterogeneity for stem cell-related markers according to tumor subtype and histologic stage in breast cancer, Clin. Cancer Res. 16 (2010) 876–887.
[15] J. Zhou, H. Zhang, P. Gu, et al., NF-kappaB pathway inhibitors preferentially inhibit breast cancer stem-like cells, Breast Cancer Res. Treat. 111 (2008) 419–427.
[16] P. Hamerlik, J.D. Lathia, R. Rasmussen, et al., Autocrine VEGF-VEGFR2-Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth, J. Exp. Med. 209 (2012) 507–520.
[17] B. Beck, G. Driessens, S. Goossens, et al., A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours, Nature 478 (2011) 399–403.
[18] S. Rizzolio, L. Tamagnone, Multifaceted role of neuropilins in cancer, Curr. Med. Chem. 18 (2011) 3563–3575.
[19] R. Chakrabarti, V. Subramaniam, S. Abdalla, et al., Tranilast inhibits the growth and metastasis of mammary carcinoma, Anti-Cancer Drugs 20 (2009) 334–345.
[20] E. Charafe-Jauffret, C. Ginestier, F. Iovino, et al., Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature, Cancer Res. 69 (2009) 1302–1313.
[21] A.K. Croker, D. Goodale, J. Chu, et al., High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability, J. Cell Mol. Med. 13 (2009) 2236–2252.
[22] C. Sheridan, H. Kishimoto, R.K. Fuchs, et al., CD44+/CD24− breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis, Breast Cancer Res. 8 (2006) R59.
[23] S. Ricardo, A.F. Vieira, R. Gerhard, et al., Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype, J. Clin. Pathol. 64 (2011) 937–946.
[24] Y. Glinka, S. Stoilova, N. Mohammed, et al., Neuropilin-1 exerts co-receptor function for TGF-beta-1 on the membrane of cancer cells and enhances responses to both latent and active TGF-beta, Carcinogenesis 32 (2011) 613–621.
[25] A.V. Timoshenko, S. Rastogi, P.K. Lala, Migration-promoting role of VEGF-C and VEGF-C binding receptors in human breast cancer cells, Br. J. Cancer 97 (2007) 1090–1098.
[26] D.S. Bassères, A.S. Baldwin, Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression, Oncogene 25 (2006) 6817–6830.
[27] K. Shostak, A. Chariot, NF-κB, stem cells and breast cancer: the links get stronger, Breast Cancer Res. 13 (2011) 214.
[28] K. Hinohara, S. Kobayashi, H. Kanauchi, et al., ErbB receptor tyrosine kinase/NF-κB signaling controls mammosphere formation in human breast cancer, Proc. Natl. Acad. Sci. USA 109 (2012) 6584–6589.
[29] I.C. Zachary, How neuropilin-1 regulates receptor tyrosine kinase signalling: the knows and known unknowns, Biochem. Soc. Trans. 39 (2011) 1583–1591.
[30] Y. Cao, L. Wang, D. Nandy, et al., Neuropilin-1 upholds dedifferentiation and propagation phenotypes of renal cellcarcinoma cells by activating Akt and sonic hedgehog axes, Cancer Res. 68 (2008) 8667–8672.
[31] M. Spiecker, I. Lorenz, N. Marx, et al., Tranilast inhibits cytokine-induced nuclear factor kappaB activation in vascular endothelial cells, Mol. Pharmacol. 62 (2002) 856–863.
[32] T. Adachi, K. Fukuda, Y. Kondo, et al., Inhibition by tranilast of the cytokine-induced expression of chemokines and the adhesion molecule VCAM-1 in human corneal fibroblasts, Invest. Ophthalmol. Vis. Sci. 51 (2010) 3954–3960.
[33] H. Suzawa, S. Kikuchi, K. Ichikawa, et al., Inhibitory action of tranilast, an anti-allergic drug, on the release of cytokines and PGE2 from human monocytes-macrophages, Jpn. J. Pharmacol. 60 (1992) 85–90.
[34] M. Platten, P.P. Ho, S. Youssef, et al., Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite, Science 310 (2005) 850–855.
[35] Y. Glinka, G.J. Prud'homme, Neuropilin-1 is a receptor for transforming growth factor beta-1, activates its latent form, and promotes regulatory T cell activity, J. Leukoc. Biol. 84 (2008) 302–310.
[36] B. Hu, P. Guo, I. Bar-Joseph, et al., Neuropilin-1 promotes human glioma progression through potentiating the activity of the HGF/SF autocrine pathway, Oncogene 26 (2007) 5577–5586.
[37] C. Pellet-Many, P. Frankel, I.M. Evans, et al., Neuropilin-1 mediates PDGF stimulation of vascular smooth muscle cell migration and signalling via p130Cas, Biochem. J. 435 (2011) 609–618.
[38] D.C. West, C.G. Rees, L. Duchesne, et al., Interactions of multiple heparin binding growth factors with neuropilin-1 and potentiation of the activity of fibroblast growth factor-2, J. Biol. Chem. 280 (2005) 13457–13464.