NEOPLASIA
www.neoplasia.com

Volume 13 Number 4 April 2011 pp. 327–338 327

Synergy of Nab-paclitaxel and Bevacizumab in Eradicating Large Orthotopic Breast Tumors and Preexisting Metastases[1,2]

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Abstract

INTRODUCTION: Patients with metastatic disease are considered incurable. We previously showed that nab-paclitaxel (nanoparticle albumin-embedded paclitaxel) combined with anti–vascular endothelial growth factor A (VEGF-A) antibody, bevacizumab, eradicates orthotopic small-sized breast tumors and metastasis. Here, we assessed this therapy in two models of advanced (450-600 mm^3) breast tumors and delineated VEGF-A–dependent mechanisms of tumor resistance. *METHODS:* Mice with luciferase-tagged advanced MDA-MB-231 and MDA-MB-435 tumors were treated with saline, nab-paclitaxel (10 or 30 mg/kg), bevacizumab (4 mg/kg), or combined drugs. Lymphatic and lung metastases were measured by luciferase assay. Proinflammatory and survival pathways were measured by ELISA, Western blot and immunohistochemistry. *RESULTS:* Nab-paclitaxel transiently suppressed primary tumors by 70% to 90% but had no effect on metastasis. Coadministration of bevacizumab increased the response rate to 99%, including 71% of complete responses in MDA-MB-231–bearing mice treated concurrently with 30 mg/kg of nab-paclitaxel. This combinatory regimen significantly reduced or eliminated preexisting lymphatic and distant metastases in MDA-MB-231 and MDA-MB-435 models. The mechanism involves paclitaxel-induced NF-κB pathway that upregulates VEGF-A and other tumor prosurvival proteins. *CONCLUSIONS:* Bevacizumab prevents tumor recurrence and metastasis promoted by nab-paclitaxel activation of NF-κB pathway. Combination therapy with high-dosed nab-paclitaxel demonstrates the potential to eradicate advanced primary tumors and preexisting metastases. These findings strongly support translating this regimen into clinics.

Neoplasia (2011) 13, 327–338

Introduction

Chemotherapy is a frontline treatment of breast and other epithelial malignancies, particularly those that are not resectable. Treatment of measurable tumors with chemotherapeutic drugs results in three outcomes: no response occurring in 5% to 10% of breast cancer (BC) patients [1,2], a complete response (CR) occurring in 10% to 20% of patients [1,2], and a partial response (PR) defined as more than 50% of the tumor reduction in response to therapy [1]. PR is the most common outcome with 50% occurrence in patients in the neoadjuvant setting with noninvasive BC [1] and higher frequency in patients with metastatic disease, triple-negative, and therapy-resistant tumors [3]. Incomplete responsiveness to cytotoxic drugs is one of the main reasons for increased mortality due to uncontrolled tumor growth. Delineating the mechanisms underlying PR holds the promise to identify the reasons for tumor resistance to chemotherapy and the potential to improve the efficacy of anticancer drugs.

Abbreviations: ABX, Abraxane, or paclitaxel protein-bound particles for injectable suspension, also abbreviated as nab-paclitaxel; Bev, bevacizumab, humanized anti–human VEGF-A antibody, also known as Avastin; BC, breast cancer; i.p., intraperitoneally; i.v., intravenously; ILN, ipsilateral lymph node; LN, lymph node; MFP, mammary fat pad; MTD, maximal tolerated dose; (p)-, phosphorylated form of protein; PBS, phosphate-buffered saline; PBST, PBS containing Tween-20; RLU, relative light unit; TGD, tumor growth delay; VEGF-A, vascular endothelial growth factor-A

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[1]This work was supported, in part, by a research grant from Abraxis Bioscience, Inc, Los Angeles, CA; the National Institutes of Health (No. 1R01-CA140732-01A1), Illinois William E. McElroy Foundation; and the Department of Defense Breast Cancer Research Program (No. BC086079) awarded to Sophia Ran. Two contributors to this work, Neil Desai and Vuong Trieu, are employed by Abraxis Bioscience, Inc. Lisa Volk, Deena Chihade, and Michael Flister declare no competing interests.

[2]This article refers to supplementary materials, which are designated by Table W1 and Figures W1 to W3 and are available online at www.neoplasia.com.

Received 29 October 2010; Revised 25 January 2011; Accepted 27 January 2011

DOI 10.1593/neo.101490

One of the reasons for tumor chemoresistance is overexpression of P-glycoproteins that pump out cytotoxic drugs, thus preventing intracellular accumulation of the lethal dose [4]. Another mechanism is mediated by vascular endothelial growth factor A (VEGF-A), an angiogenic factor [5,6] that protects tumor cells from apoptosis through autocrine activation of VEGF-A receptors expressed on tumor cells [7]. VEGF-A is upregulated by various chemodrugs including paclitaxel [8], docetaxel [8], carboplatin [9], cisplatin [10], 5-fluorouracil [11], dacarbazine [12], and anthracyclines [13]. Although the mechanism by which these drugs elicit VEGF-A expression is unclear, it might occur through activation of NF-κB [14] and PI3K/AKT pathways [15] that are induced by chemotherapy in both malignant [10,11] and endothelial cells [9]. The crucial role of VEGF-A in chemoresistance was shown in both preclinical [16,17] and clinical studies [18,19] demonstrating superior efficacy of chemodrugs when combined with anti–VEGF-A antibody. In particular, the combination of the anti–VEGF-A antibody, bevacizumab, with 5-fluorouracil, leucovorin, oxaliplatin, or irinotecan, showed an additive or synergistic effect [17]. The E2100 trial also showed that paclitaxel/bevacizumab therapy increased a response rate and significantly prolonged patient survival compared with paclitaxel treatment alone [20,21]. In addition, bevacizumab combined with other taxanes improved the outcome in patients with ovarian tumors, although this benefit was short-lived [18].

Of various taxanes, paclitaxel, a microtubule-stabilizing cytotoxic agent, is widely used against metastatic and refractory tumors [22]. The clinical use of Cremophor-based paclitaxel (Taxol) has been recently improved by formulating it as Cremophor-free, albumin-bound 130-nm nanoparticles coined nab-paclitaxel or Abraxane [23,24]. Nab-paclitaxel demonstrated several advantages over Cremophor-based paclitaxel in clinical [22,25] and experimental [26,27] studies owing to albumin encapsulation of the active component allowing for delivery of a high dose of paclitaxel without the use of solvent [28]. This leads to dose-proportional pharmacokinetics, higher maximal tolerated dose (MTD), and improved efficacy [22,29]. Nab-paclitaxel treatment of metastatic BC patients demonstrated a higher response rate and longer time to progression when compared with Cremophor-based drug [28,29]. The superior efficacy of nab-paclitaxel *versus* conventional paclitaxel was also shown in preclinical xenograft models demonstrating increased incidence of tumor regressions, longer time to recurrence, and extended survival [26]. These advantages are related to the improved delivery of nab-paclitaxel compared with solvent-based paclitaxel, leading to 33% increased intratumoral concentrations and doubling of the MTD [26].

We recently demonstrated that nab-paclitaxel efficacy is further improved by coadministration of anti–VEGF-A antibody [16]. It was shown that combined nab-paclitaxel/bevacizumab therapy eradicated small-sized (150-200 mm^3) orthotopic breast tumors in 40% of the mice and reduced metastatic incidence [16]. Whereas these results were encouraging, the models of small-sized tumors may not adequately reflect the clinical challenges in patients who present with advanced tumor burden and preexisting metastases. In the present study, we allowed luciferase-tagged MDA-MB-231-Luc$^+$ and MDA-MB-435-Luc$^+$ tumors to reach 450 to 600 mm^3 before initiating treatment. In these tumor models, 100% of the animals exhibited preexisting metastases in both lung and lymph nodes (LN). The results showed that bevacizumab combined with nab-paclitaxel at 10- and 30-mg/kg doses eradicated large 231-Luc$^+$ tumors in 33% and 71% of mice, respectively. Although no complete eradications were achieved in the 435-Luc$^+$ model, the combined treatment resulted in 94% tumor inhibition and prolonged time to progression for more than 80 days. Importantly, in both models, the combined therapy substantially decreased the incidence and the burden of preexisting pulmonary and lymphatic metastases. Collectively, these data suggest that the combination of bevacizumab and nab-paclitaxel can be effective in eradicating advanced tumors and preexisting metastases in human cancer patients.

Materials and Methods

Materials

Dulbecco modified Eagle medium and standard additives were obtained from Lonza (Basel, Switzerland). Ketamine and xylazine were from Phoenix Scientific (St Joseph, MO). Endotoxin-free sterile 150 mM NaCl solution (saline) and protease inhibitors were from Sigma (St Louis, MO). Matrigel was from BD Bioscience (Franklin Lakes, NJ).

Antibodies

Primary rabbit anti–Bcl-xL, anti-Akt, anti–p-Akt, anti-p44/42, anti–p-p44/42 anti-p50, anti–p-p50, anti-p65, anti–p p65, anti–VEGF-A, and anti–Bcl-2 antibodies were from Cell Signaling (Danvers, MA) and Thermo Scientific (Waltham, MA). Mouse–anti–β-actin (JLA20) was from Developmental Studies Hybridoma Bank (Iowa City, IA). Antirabbit and antimouse secondary antibodies were from Jackson ImmunoResearch (West Grove, PA).

Study Drugs

Paclitaxel albumin-bound particles for injectable suspension (nab-paclitaxel; Abraxane) was obtained from Abraxis BioScience (Los Angeles, CA). Drugs were reconstituted in saline, prepared fresh daily, and given within 1 hour of preparation. Bevacizumab (Avastin), humanized anti–VEGF-A antibody, manufactured by Genentech (San Francisco, CA), was obtained from a local pharmacy.

Human MDA-MB-231 and MDA-MB-435 Carcinoma Cell Lines and Their Luciferase Derivatives

Luciferase-tagged subline of MDA-MB-231, designated 231-Luc$^+$, has been extensively characterized in previous studies [16,30]. Luciferase-tagged MDA-MB-435 subline, designated 435-Luc$^+$, was a generous gift from Dr Sierra (Universitaria de Bellvitge, Barcelona, Spain) and described elsewhere [16,31]. Cells were cultured in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum and standard additives and were subcultured by using 0.5 mM EDTA diluted in phosphate-buffered saline (PBS).

Mouse Orthotopic Models of Human BC

Orthotopic 231-Luc$^+$ and 435-Luc$^+$ tumor models were previously described [16,30]. Briefly, 4×10^6 cells suspended in 50% Matrigel were implanted into the mammary fat pad (MFP) of 4- to 6-week-old female SCID mice (National Cancer Institute, Frederick, MD). Every 2 to 3 days, perpendicular tumor diameters were measured by digital caliper and used to calculate tumor volume according to the formula: volume = $Dd^2\pi/6$, where D indicates the larger diameter and d indicates the smaller diameter. Animal care was in accordance with institutional guidelines.