Mithramycin A sensitizes therapy-resistant breast cancer stem cells toward genotoxic drug doxorubicin

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Chemotherapy resistance is a major clinical challenge for the management of locally advanced breast cancer. Accumulating evidence suggests a major role of cancer stem cells (CSCs) in chemoresistance evoking the requirement of drugs that selectively target CSCs in combination with chemotherapy. Here, we report that mithramycin A, a known specificity protein (Sp)1 inhibitor, sensitizes breast CSCs (bCSCs) by perturbing the expression of drug efflux transporters, ATP-binding cassette sub-family G, member 2 (ABCG2) and ATP-binding cassette sub-family C, member 1 (ABCC1), survival factors, B-cell lymphoma 2 (Bcl-2) and X-linked inhibitor of apoptosis (XIAP), and, stemness regulators, octamer-binding transcription factor 4 (Oct4) and Nanog, which are inherently upregulated in these cells compared with the rest of the tumor population. In-depth analysis revealed that aberrant overexpression of Sp1 in bCSCs transcriptionally upregulates (1) resistance-promoting genes to protect these cells from genotoxic therapy, and (2) stemness regulators to sustain self-renewal potential of these cells. However, mithramycin A causes transcriptional suppression of these chemoresistant and self-renewal genes by inhibiting Sp1 recruitment to their promoters. Under such antisurvival microenvironment, chemotherapeutic agent doxorubicin induces apoptosis in bCSCs via DNA damage–induced reactive oxygen species generation. Cumulatively, our findings raise the possibility that mithramycin A might emerge as a promising drug in combinatorial therapy with the existing chemotherapeutic agents that fail to eliminate CSCs. This will consequently lead to the improvement of therapeutic outcome for the treatment-resistant breast carcinomas. (Translational Research 2015;165:558–577)

Abbreviations: bCSCs = breast cancer stem cells; ChIP = chromatin immunoprecipitation; CR = chemotherapy resistant; CS = chemotherapy sensitive; DCF-DA = dichlorofluorescein diacetate; FAC = 5-fluorouracil, adriamycin, and cyclophosphamide; FITC = fluorescein isothiocyanate; H_2O_2 = hydrogen peroxide; LABC = locally advanced breast cancer; NAC = N-acetyl cysteine; NACT = neoadjuvant chemotherapy; PBS = phosphate-buffered saline; PBMC = peripheral blood mononuclear cells; pH2AX = phospho-H2A histone family, member X; PE = phycoerythrin; PUMA = p53 upregulated modulator of apoptosis; ROS = reactive oxygen species; XIAP = X-linked inhibitor of apoptosis

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AT A GLANCE COMMENTARY

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Background

Accumulating evidence suggests a major role of cancer stem cells (CSCs) in chemoresistance evoking the requirement of drugs that selectively target CSCs in combination with chemotherapy.

Translational Significance

Here, we have demonstrated the potential of mithramycin A to overcome the intrinsic chemoresistance and self-renewal potential of breast CSCs. On the basis of this study, we suggest that mithramycin A might emerge as a promising chemosensitizer in combinatorial therapy with chemotherapeutic agent doxorubicin that alone fails to eliminate CSCs within physiologically feasible dose range. This combination strategy, therefore, may open a new avenue for more effective therapies for treatment-resistant breast carcinomas.

INTRODUCTION

Breast cancer is the leading cause of cancer death in women with more than a million newly diagnosed cases annually worldwide.[1] Despite significant advances in screening for early detection of breast cancer, locally advanced breast cancer (LABC), which has poor prognosis, accounts for 30%–60% of newly diagnosed cases in developing countries.[2] LABC includes a heterogeneous group of diseases characterized by the presence of advanced primary tumors, advanced nodal disease, and inflammatory carcinomas.[3] Neoadjuvant systemic therapy, that is, preoperative chemotherapy, is an integral part of the multidisciplinary management of LABC.[4,5] It provides the opportunity to assess the efficacy of systemic therapy *in vivo* and makes breast-conserving surgery a possibility for these patients. However, the high percentage of nonresponders and failures after initial responses to chemotherapy manifests chemotherapy resistance as a major clinical challenge for the management of patients with LABC.[6-8] Understanding the mechanisms by which chemoresistance can occur is important for developing novel therapeutic approaches to treat cancer.

Accumulating evidence indicates that rare self-renewing stem cells, termed cancer stem cells (CSCs), are resistant to chemotherapy and radiation therapy.[9-13] This population retains the capacity to self-renew and regenerate the total bulk of a heterogeneous tumor comprising mostly non–stem cancer cells. Furthermore, clinical studies demonstrating the enrichment of breast CSCs (bCSCs) assessed by $CD44^{+}/CD24^{-/low}$, mammosphere assays,[14,15] or by aldehyde dehydrogenase expression[16] in human patients with breast cancer after neoadjuvant chemotherapy (NACT) have provided clear evidence for the therapeutic resistance of bCSCs. Moreover, reports have shown that increased percent of bCSCs significantly associates with shorter cumulative disease-free survival and overall survival of patients.[17,18] Taken together, these studies suggest that CSCs may be a critical factor in determining the therapeutic response of patients with LABC and raises the question of whether conventional anticancer therapies target the correct cells because the actual agents appear to be evasive of current treatment modalities. These studies suggest that a significant improvement in patient outcome will require drugs that selectively target CSCs, particularly in combination with chemotherapy.

A range of mechanisms is believed to be involved in the resistance phenomena; however, antitumor drug efflux caused by adenosine triphosphate (ATP)-driven pumps, in particular by multi-drug resistance (MDR)-associated protein1 (ABCC1) and breast cancer resistance protein (ABCG2), is considered as the primary reason for chemoresistance of bCSCs.[19] Investigators have designed numerous methods to evade, neutralize, or even exploit drug efflux pumps to overcome drug resistance. Notably, several functional inhibitors, for example, verapamil, cyclosporine A, tamoxifen, dexverapamil, valspodar, and biricodar, that can interact with ATP-binding cassette (ABC) transporters to inhibit multidrug resistance have been tested. However, thus far none are clinically successful because of the dose-limiting toxic effect of these modulators.[20,21] An alternative strategy targeting ABC transporters, therefore, involves regulating the expression levels of these transporters.

Recent data suggest that specificity protein (Sp) transcription factor family member, Sp1, plays a pivotal role in transcriptionally regulating the expression of several ATP-driven drug efflux pumps.[22-24] It also plays a critical role in various cellular processes associated with tumorigenesis-like cell growth, invasiveness and metastasis, angiogenesis, and cell apoptosis.[25-27] Moreover, an increased expression of Sp1 in several cancers including breast is associated with poor prognosis of disease.[28] Very recently, few reports have shown that use of the Food and Drug Administration (FDA)-approved Sp1 inhibitor, mithramycin A, can suppress the growth of colon CSCs[29] and can also circumvent chemoresistance potential of lung CSCs.[30] But, there is hardly any report describing either the contribution of Sp1 to bCSC chemoresistance or the