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Postsurgical Adjuvant Tumor Therapy by Combining Anti-Angiopoietin-2 and Metronomic Chemotherapy Limits Metastatic Growth

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SUMMARY

Antiangiogenic tumor therapy has failed in the adjuvant setting. Here we show that inhibition of the Tie2 ligand angiopoietin-2 (Ang2) effectively blocks metastatic growth in preclinical mouse models of postsurgical adjuvant therapy. Ang2 antibody treatment combines well with low-dose metronomic chemotherapy (LDMC) in settings in which maximum-dose chemotherapy does not prove effective. Mechanistically, Ang2 blockade could be linked to quenching the inflammatory and angiogenic response of endothelial cells (ECs) in the metastatic niche. Reduced EC adhesion molecule and chemokine expression inhibits the recruitment of tumor-promoting $CCR2^{+}Tie2^{-}$ metastasis-associated macrophages. Moreover, LDMC contributes to therapeutic efficacy by inhibiting the recruitment of protumorigenic bone marrow-derived myeloid cells. Collectively, these data provide a rationale for mechanism-guided adjuvant tumor therapies.

INTRODUCTION

Surgery is the standard care of treatment for resectable primary tumors. However, many cancer patients experience fatal metastatic growth despite removal of the primary tumor. For instance, 30% of node-negative and 70% of node-positive breast cancer patients succumb to distant metastases despite surgical intervention (Demicheli et al., 2008), confirming the well-established fact that metastatic seeding has already occurred at the time of diagnosis and subsequent surgery. Hence, better and preferably mechanism-based clinical regimens of postsurgical adjuvant therapy need to be developed to follow surgery, even when no metastases are evident, to lower the risk that the cancer will come back.

Antiangiogenic tumor therapies, targeting the VEGF pathway, have received widespread clinical application for the treatment of advanced primary tumors. Their efficacy is still limited, and antiangiogenic primary tumor therapy has in preclinical models even been proposed to promote metastasis (Ebos et al., 2009; Pàez-Ribes et al., 2009). In the adjuvant setting, anti-VEGF therapy did not prove clinically effective, as shown in colorectal cancer (Allegra et al., 2011; de Gramont et al.,

Significance

There is an unmet need for safe and effective forms of adjuvant tumor therapy following surgical removal of primary tumors, particularly in patients with nonovert, undetectable metastases that would be excluded from more aggressive forms of adjuvant therapy (radiotherapy, chemotherapy). This study has identified the combination of an anti-Ang2 antibody and LDMC as a promising low-adverse effect combination therapy for such setting. Moreover, the study provides mechanistic insights into the endothelial contribution of a proinflammatory microenvironment as an important mediator of tumor progression, which is targeted by the combination therapy of anti-Ang2 antibody and LDMC. These data warrant further preclinical validation of such combination therapy for eventual translation into clinical application.

2012) as well as in triple-negative breast cancer (Cameron et al., 2013).

The Tie2 ligand angiopoietin-2 (Ang2) has recently emerged as a promising target for second-generation antiangiogenic drug development that can be combined with established anti-VEGF/VEGFR therapies (Gerald et al., 2013; Hashizume et al., 2010; Koh et al., 2010). Ang2 is produced by activated endothelial cells (ECs) to facilitate vascular responses to angiogenic and other endotheliotropic cytokines. In fact, Ang2 upregulation may be among the first cellular responses of angiogenic activation to contribute toward the vascular priming associated with the induction of angiogenesis (Holash et al., 1999; Zagzag et al., 1999).

On the basis of its endothelial activation-associated transcriptional regulation, we hypothesized that Ang2 may be an attractive target for postsurgical adjuvant therapy. Conceptually, Ang2-targeting therapies should quench the vascular response and thereby prevent the growth of seeded metastases. To probe this hypothesis, we used two different models of spontaneous metastasis (including an anti-VEGF-refractory model) in which adjuvant therapy was initiated following surgical removal of the primary tumor. Comparatively, we also studied the efficacy of anti-VEGF antibody (VEGF Ab), maximum-tolerated-dose chemotherapy (MTDC), and low-dose metronomic chemotherapy (LDMC) in the same models. The results of these preclinical therapy experiments guided experiments aimed at unraveling the underlying mechanisms to pave the way for mechanism-guided postsurgical adjuvant combination tumor therapies.

RESULTS

Anti-Ang2 Therapy Limits the Growth of Preseeded Micrometastases

To study the effect of Ang2-targeting in the adjuvant setting, we used an orthotopic breast cancer model, which closely mimics lung and bone metastasis as frequently observed during human breast cancer progression (Figure 1A). Ang2 Ab therapy reduced the incidence of bone metastases as well as the growth of lung metastases in the 4T1 breast cancer mouse model (Figures 1B–1G). Exclusion of residual primary tumor growth after surgery (before randomization to treatment groups) (Figure S1A available online) and the decrease in metastatic burden upon therapy was traced by bioluminescence imaging (Figure 1B). Bone metastases were more frequent in the control group (seven of ten versus two of ten) (Figure 1C). Representative images of hematoxylin and eosin (H&E) sections with osteolytic lesions displayed the extent of bone damage inflicted by metastasis (Figure 1D). The lesions caused a corresponding loss of bone density, which was further confirmed by computed tomographic (CT) imaging (Figure 1E). Bioluminescence imaging also revealed reduced lung and lymph node metastasis upon Ang2 blockade (Figures 1B and 1C). H&E sections of lungs identified a significantly decreased incidence of macrometastases (Figures 1F and 1G) and micrometastases (Figures S1B and S1C) in Ang2 Ab-treated mice. Ang2 blockade resulted in a significant decrease of vessel density and increase of intratumor microvessel pericyte coverage compared to the immunoglobulin G (IgG)-treated control group (Figures 1H–1J).

Metronomic Chemotherapy Increases the Antimetastatic Effect of Anti-Ang2 Ab and Promotes Overall Survival

Although Ang2 blockade reduced metastatic growth, residual metastasis was detected upon bioluminescent imaging. This led us to hypothesize that an additional therapeutic regimen may improve the therapeutic benefit conferred by Ang2 blockade. To test this hypothesis, we combined low-dose paclitaxel metronomic chemotherapy [LDMC(PTX)] with Ang2 Ab therapy in the postsurgical adjuvant setting of the 4T1 orthotopic breast cancer model. The combinatorial therapy was significantly more effective than either LDMC (PTX) or MTDC (PTX) given as monotherapy (Figures 2A and 2B). Interestingly, Ang2 targeting alone yielded a significantly better therapeutic response than MTDC (PTX).

Next, we investigated whether the therapeutic benefit conferred by combinations of Ang2 Ab and LDMC (PTX) would also translate to improved overall survival. Combinations of Ang2 Ab and LDMC (PTX) significantly increased overall survival compared with Ang2 Ab alone or control IgG (mean survival of LDMC (PTX) plus Ang2 Ab versus Ang2 Ab alone: 33.5 versus 27 days, $p < 0.04$; mean survival of LDMC (PTX) plus Ang2 Ab versus IgG: 33.5 versus 25 days, $p < 0.0001$; Figure 2C). In contrast to this combination, MTDC (PTX) alone failed to provide any survival benefit (Figure 2C).

LDMC and Anti-Ang2 Ab Combination Therapy Has Fewer Adverse Effects Than High-Dose Chemotherapy

MTDC (PTX) is clinically frequently used as postsurgical adjuvant chemotherapy. We thus analyzed the adverse effects of the different therapeutic regimens used in this study. Histological analysis revealed severe bone marrow suppression in the MTDC (PTX) group (Figure 2D). Reproductive toxicity is a major adverse effect often observed in female breast cancer patients undergoing chemotherapy subsequent to mastectomy. MTDC (PTX) resulted in significantly fewer healthy follicles compared with control or other therapeutic regimens (Figure 2E). Lastly, a significant reduction in body weight was observed in the MTDC (PTX) group, but not in the other treatment groups, including the combination of LDMC (PTX) and Ang2 Ab (Figure 2F).

Anti-Ang2 Ab Therapy Inhibits Metastatic Growth in an Anti-VEGF-Refractory Tumor Model

Following the demonstration of the effect of Ang2 Ab on metastatic growth in the 4T1 orthotopic breast cancer model, we next conducted experiments aimed at examining the effect of Ang2 blockade on the growth of micrometastases in the Lewis lung carcinoma (LLC) model (Figure 3A). Subcutaneously growing LLCs, previously shown to be refractory to anti-VEGF therapy (Shojaei et al., 2007a), were removed at approximately 0.30 g (day 14 following inoculation). No visible metastases were macroscopically detectable at this stage. Intriguingly, primary tumor removal led to the rapid downregulation of circulating VEGF levels (Figure S2).

Postsurgical adjuvant Ang2 Ab therapy inhibited the growth of LLC metastases in the lungs. Macroscopic metastases were detected in 80% of control IgG-treated mice, whereas only 20% of Ang2 Ab-treated mice had macroscopic metastases