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Core Needle Biopsy of Breast Cancer Tumors Increases Distant Metastases in a Mouse Model[1,2]

Edward Gitau Mathenge[*,†], Cheryl Ann Dean[†,‡], Derek Clements[†], Ahmad Vaghar-Kashani[†], Steffany Photopoulos[‡], Krysta Mila Coyle[†], Michael Giacomantonio[§], Benjamin Malueth[‡], Anna Nunokawa[‡], Julie Jordan[†], John D. Lewis[¶], Shashi Ashok Gujar[‡,#], Paola Marcato[†,‡], Patrick W.K. Lee[†,‡] and Carman Anthony Giacomantonio[*,†]

*Department of Surgery, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; †Department of Pathology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; ‡Department of Microbiology and Immunology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; §Department of Biology, Saint Francis Xavier University, Antigonish, Nova Scotia, Canada; ¶Department of Oncology, University of Alberta, Edmonton, Alberta, Canada; #Strategy and Organizational Performance, IWK Health Center, Halifax, Nova Scotia, Canada

Abstract

INTRODUCTION: Incisional biopsies, including the diagnostic core needle biopsy (CNB), routinely performed before surgical excision of breast cancer tumors are hypothesized to increase the risk of metastatic disease. In this study, we experimentally determined whether CNB of breast cancer tumors results in increased distant metastases and examine important resultant changes in the primary tumor and tumor microenvironment associated with this outcome. *METHOD:* To evaluate the effect of CNB on metastasis development, we implanted murine mammary 4T1 tumor cells in BALB/c mice and performed CNB on palpable tumors in half the mice. Subsequently, emulating the human scenario, all mice underwent complete tumor excision and were allowed to recover, with attendant metastasis development. Tumor growth, lung metastasis, circulating tumor cell (CTC) levels, variation in gene expression, composition of the tumor microenvironment, and changes in immunologic markers were compared in biopsied and non-biopsied mice. *RESULTS:* Mice with biopsied tumors developed significantly more lung metastases compared to non-biopsied mice. Tumors from biopsied mice contained a higher frequency of myeloid-derived suppressor cells (MDSCs) accompanied by reduced CD4+ T cells, CD8+ T cells, and macrophages, suggesting biopsy-mediated development of an increasingly immunosuppressive tumor microenvironment. We also observed a CNB-dependent up-regulation in the expression of *SOX4*, *Ezh2*, and other key epithelial-mesenchymal transition (EMT) genes, as well as increased CTC levels among the biopsy group. *CONCLUSION:* CNB creates an immunosuppressive tumor microenvironment, increases EMT, and facilitates release of CTCs, all of which likely contribute to the observed increase in development of distant metastases.

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Abbreviations: CCAC, Canadian Council on Animal Care; CNB, core needle biopsy; CTCs, circulating tumor cells; EMT, epithelial-mesenchymal transition; H&E, hematoxylin and eosin; MDSCs, myeloid-derived suppressor cells; PGE2, prostaglandin E2

Address all correspondence to: Carman Anthony Giacomantonio, MD, MSc, Departments of Surgery and Pathology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada.
E-mail: edward.mathenge@gmail.com

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Introduction

Breast cancer is the most common cancer affecting women and accounts for the second highest incidence of cancer-related death, after lung cancer [1]. There are numerous factors known to influence the metastatic potential of any given breast cancer (tumor size, receptor status, lymph node involvement at the time of diagnosis, age of the patient, menopausal status, and family history) [2–5]. However, the most consistent predictors of metastasis continue to be tumor size and lymph node involvement at the time of diagnosis [6,7]. Because of this, breast cancer screening and early detection are critically important in improving outcomes for women with breast cancer.

Suspicious lesions detected on screening mammograms are generally biopsied to confirm or rule out a diagnosis of cancer. The most common form of biopsy administered today is a core needle biopsy (CNB) [8–14]. A CNB is a form of incisional biopsy whereby a portion of a tumor is removed for histologic evaluation leaving the remainder *in vivo* to be removed at a later date following a definitive diagnosis. Typically with breast cancer, tissue samples are collected by administering three to nine passes with a 14G biopsy needle [15,16]. Biopsy needle sizes can range from 9G to 18G depending on the particular form of image guidance and system of sample acquisition used for the core biopsy [17–19].

Other breast cancer biopsy techniques include complete excisional biopsy, open incisional biopsy, and fine needle aspiration. CNB and fine needle aspiration are favored over open incisional or excisional biopsies because they are less invasive, produce a smaller cosmetic post-operative footprint, and result in faster patient recovery. This is particularly appealing given that the majority of biopsied breast lesions are ultimately ruled benign [20,21]. Furthermore, in addition to distinguishing invasive from non-invasive cancer, tissue obtained from CNB can be used to perform nucleic acid analysis, immunohistochemistry, or analysis of prognostic biomarkers [22–24]. As a consequence, stereotactic or ultrasonographically guided CNB is currently the predominant biopsy method employed in breast cancer management [16].

Incisional surgical procedures, including incisional biopsies, on cancers have historically been associated with higher local recurrence rates and elevated incidence of lymph node metastasis [25–29]. There is also a growing body of evidence suggesting that surgical trauma in the presence of an established neoplasm can potentiate its growth and metastatic proliferation [30–33].

Current literature also increasingly notes the occurrence of post-surgical immunosuppressive changes and their relevance to metastatic spread and disease recurrence [33]. These observations suggest that surgically instigated changes in the tumor and subsequent host interaction with residual disease can influence the tumor's metastatic potential.

The clinical impact and potential risk associated with performing a CNB has long been debated. There is compelling evidence that CNB increases the risk of needle track seeding and local tumor recurrence in patients with breast cancer [28,34,35]. There is also little question that cancer cells from both invasive and non-invasive breast cancers enter lymphatic channels and migrate to lymph nodes following a biopsy procedure [27,36]. However, whether or not cancer cells displaced into lymphatic and vascular channels are capable of effectively establishing distant metastases remains unproven [34,37–39].

To test the hypothesis that surgically initiated changes in the tumor microenvironment due to CNB results in increased metastatic spread, we used the 4T1-BALB/c mouse model, a well-established, immune-competent, cancer animal model considered to closely mimic metastatic breast cancer in humans. Murine mammary 4T1 tumor cells were orthotopically implanted in BALB/c mice and tumors large enough to biopsy developed within 2 to 3 weeks. Biopsies were then performed in a manner designed to experimentally replicate the human clinical experience of using CNB for the diagnostic workup of breast cancer as well as study the impact of the CNB on metastatic outcomes. In this model, tumors spontaneously metastasize from the mammary fat pad to lymph nodes, lung, and bone in a similar pattern to that observed in human breast cancers [40]. This immunologically intact model also enabled study of the immunologic changes associated with CNB within the local tumor microenvironment, in distant organs, and peripheral circulation. These changes were assessed to detect those events that might be associated with tumor progression and metastasis [41]. Gene expression profiles were evaluated to detect changes in known key epithelial-mesenchymal transition (EMT) genes [42]. Recent studies have drawn associations between surgical procedures and increased levels of tumor cells in circulation [43]. In consideration of this finding, and the documented link between circulating tumor cell (CTC) levels and metastasis [44,45], the impact of CNB on CTC levels was also measured.

Methods

Cells

Metastatic murine breast cancer cell line 4T1 (ATCC CRL-2539) cells were cultured in RPMI-1640, supplemented with 10% FBS and antibiotic-antimycotic, at 37°C at 90% humidity and 5% CO_2. All media, sera, and supplements used for the murine metastasis model were purchased from Invitrogen, Life Technologies (Burlington, Ontario).

Animals

Female BALB/c mice were obtained from Charles River Laboratory (St-Constant, Canada) and housed in the Carleton Animal Care Facility at Dalhousie University. After a 1 week acclimatization period, the mice were used in the study. All protocols (No. 09-044 and No. 13-088) were approved by the Dalhousie University Committee on Laboratory Animals in keeping with guidelines established by the Canadian Council on Animal Care (CCAC).

Metastasis Model

Mice received a subcutaneous orthotopic injection of 7×10^3 4T1 tumor cells into the third thoracic mammary fat pad. Primary tumors were palpable in 10 to 16 days and reached the biopsy size of 6 to 8 mm by 15 to 20 days after implantation. Mice with slow-growing tumors that failed to achieve the required size for experimentation were excluded from the study (this was typically no more than 3 mice from a starting set of 23 mice). Tumor growth was determined by measuring two axes of the tumor and determining the mean tumor diameter. At the CNB time point, mice were alternately assigned to biopsy and non-biopsy groups to eliminate bias. Mice assigned to the biopsy group were fully anesthetized using inhaled isoflurane (2% body weight) and their biopsy sites were shaved and subjected to aseptic surgical preparation; they then received CNB with an 18-gauge needle attached to a 5-ml syringe. Six to eight cutting passes were administered in a palmate-radiating pattern through a single cutaneous insertion point so as to reduce injury to the skin. On occasion, when a tumor was multilobed or irregularly shaped, an