FEATURED NEW INVESTIGATOR

Tumor-stromal cross talk: direct cell-to-cell transfer of oncogenic microRNAs via tunneling nanotubes

VENUGOPAL THAYANITHY, ELIZABETH L. DICKSON, CLIFFORD STEER, SUBBAYA SUBRAMANIAN, and EMIL LOU

MINNEAPOLIS, MINN

Tunneling nanotubes (TnTs) represent a novel mechanism by which intercellular components such as proteins, Golgi vesicles, and mitochondria can be transferred from cell to cell in the complex tumor microenvironment. Here, we report data showing that microRNAs (miRNAs) are transferred through TnTs in osteosarcoma (OS) and ovarian cancer as in vitro model systems. miRNA array analysis demonstrated significant upregulation of miR-19a in OS tumors resected from human patients, and differential expression of miR-199a in ovarian cancer cell lines resistant or sensitive to platinum chemotherapy. K7M2 murine OS cells were transfected with miR-19a and cultured with nontransfected K7M2 cells in low-serum, hyperglycemic medium for up to 72 hours to induce TnT formation. miRNA transfer via TnTs was detected by time-lapse microscopic imaging. miR-19a was also transported via TnTs connecting transfected K7M2 cells and nontransfected stromal MC3T3 murine osteoblast cells. Similar findings were observed in studies of TnT-mediated transport of miR-199a among SKOV3 ovarian cancer cells and nonmalignant immortalized ovarian epithelial cells. To quantify TnT-mediated transport of miRNAs, we used modified Boyden chambers to separate miR-19a–transfected K7M2 cells (top chamber) and DiI-labeled MC3T3 cells (bottom chamber) compared with open culture of these cells. Fluorescence-activated cell sorting analysis of cells collected after 48 hours of culture indicated that miR-19a–positive MC3T3 cells were 3-fold higher in open culture; this finding suggests that miR-19a transfer occurred via TnTs, exclusive of other forms of cell-cell communication. These studies demonstrate that TnTs mediate direct transfer of genetic material between tumor and stromal cells. (Translational Research 2014;164:359–365)

Abbreviations: FACS = fluorescence-activated cell sorting; FBS = fetal bovine serum; miRNAs = microRNAs; nM = nanomolars; nm = nanometers; ng = nanograms; OB = osteoblasts; OS = osteosarcoma; TnTs = Tunneling nanotubes

Emil Lou, MD, PhD, *is an Assistant Professor in the Division of Hematology, Oncology, and Transplantation at the University of Minnesota. Dr. Lou's laboratory focuses on investigation of the role of tunneling nanotubes in invasion, recurrence, and chemoresistance of solid tumor malignancies.*

From the Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota, Minneapolis, Minn; Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Minnesota, Minneapolis, Minn; Division of Gastroenterology, Department of Medicine, University of Minnesota, Minneapolis, Minn; Department of Surgery, University of Minnesota, Minneapolis, Minn.

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Reprint requests: Emil Lou, Division of Hematology, Oncology and Transplantation, Department of Medicine, MMC 480, University of Minnesota, 420 Delaware Street SE, Minneapolis, MN 55455; e-mail: emil-lou@umn.edu.

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AT A GLANCE COMMENTARY

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Background

Tunneling nanotubes (TnTs) represent a novel mechanism by which intercellular components such as proteins, Golgi vesicles, and mitochondria can be transferred from cell to cell in the complex tumor microenvironment.

Translational Significance

Here, we report that microRNAs (miRNAs) are transferred through TnTs in osteosarcoma and ovarian cancer as in vitro model systems. This finding has potential relevance to the emerging paradigm that tumor-stroma interactions are important properties of tumor formation, progression, and recurrence and that miRNAs play an important role in regulating these processes. TnTs represent a potential therapeutic target for cancers through disruption of intercellular transfer of miRNAs and other vital stimulants of malignant progression and proliferation.

INTRODUCTION

Intercellular communication among distant and proximal cells in the heterogeneous tumor microenvironment has emerged as an important paradigm for understanding tumor growth and invasion. Gap junctions, chemical messengers (eg, cytokines), and exosomes are well-established forms of intercellular communication[1,2]; however, tunneling nanotubes (TnTs) represent a novel mechanism by which intercellular components can be transferred from cell to cell in the complex tumor microenvironment.[3,4] TnTs are long, thin, actin-based, cytoplasmic extensions that form de novo and can serve as conduits for intercellular shuttling of cargo such as proteins, Golgi vesicles, and mitochondria. We identified TnTs in solid tumors resected from patients with mesothelioma and lung adenocarcinomas[4]; this observation provided the first evidence of the potential in vivo relevance of TnTs in human solid tumor malignancies.

Intercellular exchange of genetic materials has the potential for inducing malignant transformation and affecting gene regulation in recipient cells. Small noncoding RNAs, in particular, have gained strong interest for their critical role in tumor cell regulation, specifically by post-transcriptional modification of key regulator gene products. We hypothesized that TnTs were capable of transporting microRNAs (miRNAs) as a novel mechanism of tumor-tumor and tumor-stromal cross talk among neighboring and distant cells. Using osteosarcoma (OS) and ovarian cancer as model systems, we examined whether miRNAs are transferred via TnTs in invasive cancers of high metastatic potential.

MATERIALS AND METHODS

For additional pertinent details, please also see Supplemental Information section. This paper conforms to the relevant ethical guidelines for human and animal research.

Culture conditions for TnT formation. TnT formation was assessed in standard and hyperglycemic culture conditions. Standard conditions consisted of 10% fetal bovine serum (FBS) and 25 mM glucose RPMI-1640. Cells were also cultured in a low-serum, high-glucose environment to assess the growth of TnTs as previously described.[4] Specifically, culture conditions we used to stimulate TnT formation consisted of an RPMI-1640 medium with 2.5% FBS, 50 mM glucose, 1% penicillin-streptomycin, 2% L-glutamine, and 10 nM ammonium lactate, at pH 6.6.

Transfection and coculture of cells and time-lapse imaging. For OS studies, K7M2 cells were reverse-transfected with Alexa-488–labeled miR-19a (250 ng of RNA per 1×10^6 cells) using Lipofectamine RNAimax following protocol of the manufacturer (Life technologies, Carlsbad, CA). Transfection efficiency was assessed by visual inspection by fluorescent microscopy (Olympus IX70; Olympus America Inc, Center Valley, PA) 15 hours after transfection. Cells were collected by trypsinization 16 hours after transfection and subjected to coculture experiments as needed. DiI and DiO are commercially available lipophilic dyes that fluoresce in the red and green channels, respectively (Life Technologies, Carlsbad, CA), and the use of these for TnT studies has been described previously.[4] Equal proportions of miR-19a–transfected K7M2 cells were cocultured with DiI-stained MC3T3 cells in a 1:1 mixture of 10% FBS and antibiotic-containing alpha minimum essential media (MEM) and Dulbecco's Modified Eagle Medium (DMEM) under standard conditions. The cells were allowed to adhere at standard cell culture conditions for 16 hours before imaging.

For ovarian cancer studies, SKOV3 cells were reverse-transfected with Alexa-488–labeled miR-199a. Equal proportion of miR-199a–transfected SKOV3 cells was cocultured with DiI-stained immortalized ovarian epithelial cells (IOSE) cells in a 1:1 mixture of 10% FBS and antibiotic-containing alpha MEM and DMEM under standard conditions.