Trends in Cancer

CellPress REVIEWS

Opinion

Imaging Tunneling Membrane Tubes Elucidates Cell Communication in Tumors

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Intercellular communication is a vital yet underdeveloped aspect of cancer pathobiology. This Opinion article reviews the importance and challenges of microscopic imaging of tunneling nanotubes (TNTs) in the complex tumor microenvironment. The use of advanced microscopy to characterize TNTs *in vitro* and *ex vivo*, and related extensions called tumor microtubes (TMs) reported in gliomas *in vivo*, has propelled this field forward. This topic is important because the identification of TNTs and TMs fills the gap in our knowledge of how cancer cells communicate at long range *in vivo*, inducing intratumor heterogeneity and resistance to treatment. Here we discuss the concept that TNTs/TMs fill an important niche in the ever-changing microenvironment and the role of advanced microscopic imaging to elucidate that niche.

Intercellular Communication Is Vital to Cancer Pathobiology

Research in cellular oncology has elucidated the importance of intercellular communication networks in cancer and in other diseases. There is untapped potential for targeting modes of communication as a novel strategic approach to treating cancer. Better understanding of how this communication occurs will provide insight to explain at the cellular level why and how tumors are able to evade and evolve to become resistant to many forms of currently used cancer-directed therapies.

Tumors are highly **heterogeneous** (see Glossary) entities [1,2]. This is a well-established fact, but no current modes of treatment are capable of effectively targeting the heterogeneous components of tumors. Effective and efficient intercellular crosstalk in the 3D, complex tumor microenvironment is essential for tumor growth and invasion [3]. Many malignant tumors have a higher proportion of stromatous cells than cancer cells. Because of this tumor–**stroma** heterogeneity, malignant cells are not always in immediate physical contact; furthermore, high stromal content correlates with worse prognosis in a spectrum of cancer types [4–10]. As tumor cells diffusely infiltrate normal tissue, membrane contacts, including gap junction and adhesion molecules implicated in communication, are disrupted. In this context these cells are spatially separated by extracellular matrix components and the formation of **TNTs** could act to maintain long-range contact between diffusely separated cells.

Modes of cancer cell crosstalk are finally being elucidated in greater detail due to advances in microscopy techniques. A major example of effective crosstalk that has merited close attention over the past decade is the field of extracellular vesicles such as exosomes and microvesicles

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Direct cell-to-cell communication between cellular conduits called tunneling nanotubes (TNTs) and tumor microtubes (TMs) is an emerging and novel concept in cancer cell biology.

Over the past decade, the field has moved from studies *in vitro* to the examination of tumors *ex vivo* and, more recently, *in vivo* studies in animal models.

Advanced microscopy techniques, including but not limited to confocal imaging, electron microscopy, stimulated emission depletion microscopy, and *in vivo* laser scanning microscopy, are being harnessed to better characterize the structure of TNTs and TMs at high resolution.

More studies are needed to identify the function and mechanisms of TNTs and to determine the extent to which there is heterogeneity between different cell types.

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[11–18]. A relatively recently discovered form of cellular crosstalk comprises long, membrane-based extracellular extensions called TNTs. Work from our group and others has begun to unravel the structure, function, and mechanism of TNTs, but many questions remain. In this Opinion article, we focus our discussion on the importance of microscopic imaging of TNTs in the natural context of the stromatous tumor microenvironment, share some technical challenges that we have experienced in our own work, and offer a perspective on why this work could be important in addressing relevant questions in clinical oncology.

Knowns and Unknowns about TNTs in Cancer

TNTs are open-ended cellular extensions that connect distant cells. What is known thus far is that they can act as conduits for direct intercellular transfer of important cell cargo such as

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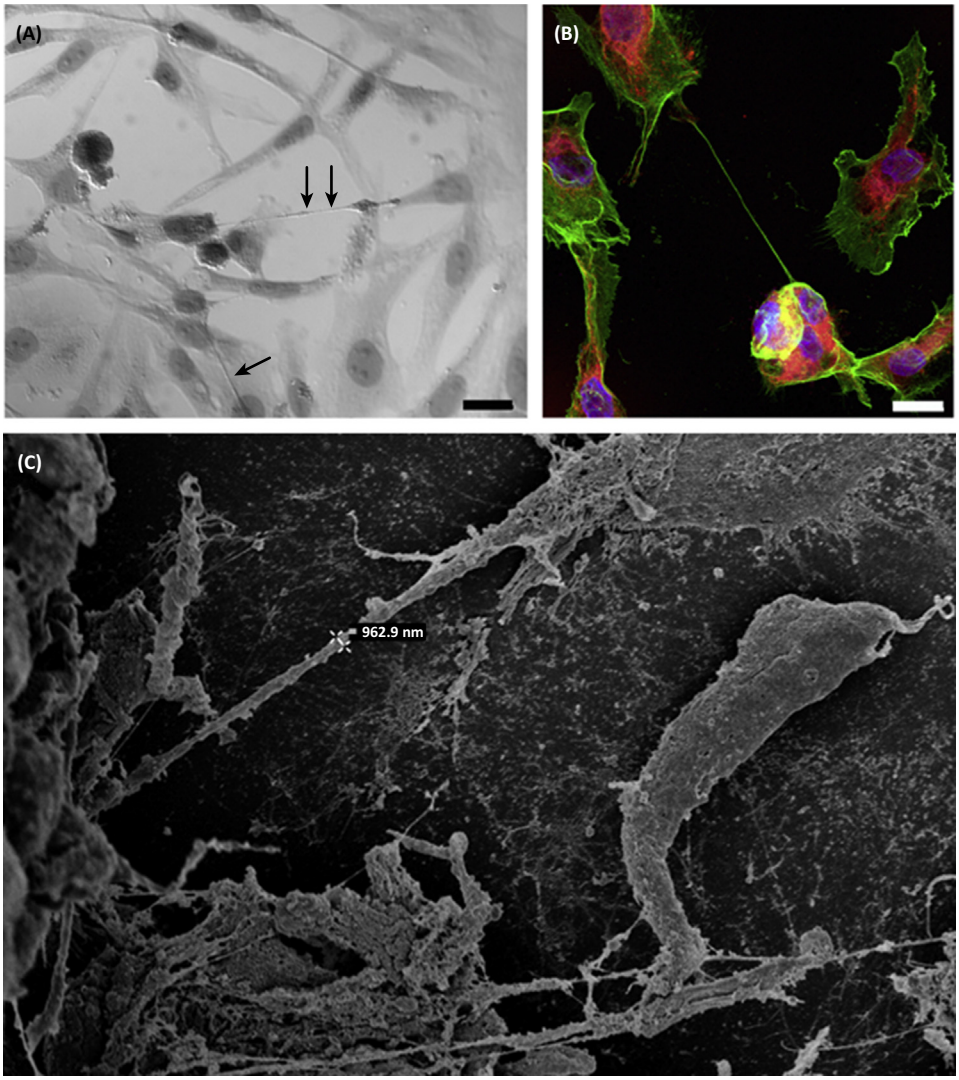

Figure 1. Examples of Tunneling Nanotubes Connecting Mesothelioma Cells *In Vitro*. (A) MSTO-211H (malignant mesothelioma) cells were cultured to semiconfluence in low-serum, hyperglycemic medium. Pap staining was performed. 40× DIC image. Bar, 10 μm. (B) MSTO-211H malignant mesothelioma cells connected by a tunneling nanotube. Green, actin (phalloidin stain); blue, DAPI; red, mitochondria (MitoTracker Red). Images were taken using a Leica TCS SP5 inverted microscope with 40×/1.25 NA oil objectives. Bar, 20 μm. (C) Scanning electronograph demonstrating two MSTO-211H cells connected via a thick tunneling/membranous nanotube.