Identification of Stem-like Metastatic Bone Tumor-Initiating Cells (TICs) Utilizing Human Patient Derived Xenograft (PDX) Cancer Models

Vinagolu K. Rajasekhar, PhD, Nagavarakishore Pillarsetty, PhD, Daniel Thorek, PhD, Jackie Bromberg, MD, PhD, John H. Healey, MD.
Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Disclosures:

Introduction: Goal: Identify, isolate, and characterize stem-like human bone metastatic TICs through PDX models for human (prostate, breast, lung, etc.) epithelial cancers for developing precise therapeutic strategies.

Hypothesis: Although TICs represent a rare cell population within bulk tumor tissue, we expect that metastatic tumor tissues would form an enriched and proven source of functional TICs. Because of their extreme minority in the bulk tumors and the lack of known markers specifically associated with them, purification of these TICs becomes a big challenge which otherwise could offer a valuable resource for developing faithful PDX models for cancer metastasis. Such investigations will facilitate identification of novel markers for early clinical cancer diagnosis, and also for developing functional screens to identify precise therapeutic targets.

Background: Most cancer patients die as a result of metastatic tumors rather than primary tumors. Conventional therapies like surgery and chemotherapy do not provide durable responses in patients with cancer. Surgery is not an option for all types of solid tumors, and chemotherapies are often associated with burdensome adverse effects. The use of targeted therapies against the overexpression of a particular oncogene or the loss of a tumor suppressor is often hampered by the emergence of therapy resistant disease. It is unknown whether therapy resistance is a consequence of the elimination of predominant tumor-initiating clones and subsequent growth and emergence of new tumor-initiating clones, or is due to new mutations that bypass the intended targeted signaling in a given cancer-associated pathway, or results from a combination of these two possibilities. Therefore, it is vitally important to identify and target the functionally relevant metastatic TICs to obtain a durable clinical response.

Bulk tumor tissue is highly heterogeneous, comprised of tumor cells, stroma cells, endothelial cells, and infiltrated immune cells. In addition, there are undifferentiated TICs, which have stem cell characteristics such as long term self-renewal and multipotent differentiation, characterized as spheres in vitro and tumor in vivo, respectively. But this cell population comprises only a minute fraction of total bulk tumor tissue. We have established conditions to enrich stem-like self-renewing tumor cells by in vitro sphere-forming assays from a variety of tumor specimens, and we have confirmed the utility of these methods with the human prostate cancer patient derived primary tumor tissues (Fig. 1A)\(^1\). Many putative therapeutic targets may actually have been derived from differentiated tumor cells with short-term renewal ability and have been identified only because of their abundant representation in total bulk tumor tissue. Despite successfully reducing solid tumor burden, targeted therapies often fail to prevent the development of therapy resistant disease. Thus, it is important to identify the specific markers that will enable the purification of TICs from solid tumors, similar to what has been accomplished with hematologic (liquid) tumors\(^3,4\). For example, our in-depth studies demonstrated that the human prostate TICs do not express the otherwise established markers of well differentiated human prostate tumor cells such as androgen receptor (AR) and the prostate specific antigen (PSA) (Fig. 1B); this theory has been well substantiated independently\(^2,5,6\). Simultaneous targeting of stem-like TICs and differentiated tumor cells is therefore expected to inhibit tumor initiation and tumor burden, respectively, forming an effective treatment strategy for human cancers.

Methods: As the overall metabolism of any given tumor is dependent on its microenvironment and its niche factor (cytokines etc.)-specific signaling\(^7\), we performed only the orthotopic transplantation of TICs that are also transduced with a fluorescent reporter-luciferase-expressing lentiviral vectors. Fresh surgical specimens obtained under pre-approved IRB protocol were labeled as above and implanted within a few hours in immunocompromised nude, NOD/SCID, and NSG mice (JAX labs). While the growth time varies with specimens, we restrict our studies to early passages (up to 5) to generate the metastatic TICs.

Results: We isolated self-renewing stem-like TICs from human PDX-derived prostate, breast, and lung tumors and re-transplanted orthotopically (intra-prostatically for prostate, in the mammary fat pad for breast, and in lungs for lung TICs). We then imaged tumor growth through mouse PET imaging for luciferase activity. About 4-6 weeks post-transplantation at the orthotopic sites (Fig. 1C); we carried out necropsy studies to detect bone metastases (Fig. 1D). However, during this period, metastasis was observed only with TICs and not with total tumor cells transplanted at orthotopic sites. Furthermore, no detectable metastases were observed following subcutaneous transplantations of the same TICs.

Discussion: Our data suggest that TICs are the selectively enriched cell population for metastasis ability into bone and that the original parent tumor microenvironment may provide necessary and specific signals for stem-like TICs to appropriately
metastasize and survive. Tumorigenicity of prostate TICs was restricted to male mice only. We are also utilizing similar approaches to validate and characterize these phenomena with various other metastatic cancer types.

**Significance:** These studies provide direct evidence for stem-like TICs-derived distant metastasis of some epithelial cancers, open avenues for discovery of novel functional bio-markers in these TICs, and present new and unique approaches for prospectively purifying metastatic TICs. These PDX-model systems are, therefore, very appropriate for delineating the molecular mechanisms associated with cancer metastasis and are comparable to those metastases that occur naturally in patients. As such, these models offer novel screening strategies for therapeutics against the lethal metastatic spread of human cancers.

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**References:**
7. Joyce JA. Therapeutic targeting of the tumor microenvironment. Cancer Cell 7, 513-520

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**Figure 1.**
A: Confocal microscopy of prostate sphere cells (GFP-green; DAPI-blue) and the Bright field image mammary spheres (10X);
B: Androgen receptor (AR) and prostate specific antigen (PSA) expression in parent prostate tumor, spheres, and the sphere (-derived) tumor (Rajasekhar et al, 2011);
C: Orthotopic and subcutaneous prostate tumors;
D: X-ray and small animal PET image with[18F] of bone metastasis of prostate and lung tumors.

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