Upregulated MT1-MMP/TIMP-2 axis in the TSU-Pr1-B1/B2 model of metastatic progression in transitional cell carcinoma of the bladder

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Abstract

Muscle invasive transitional cell carcinoma (TCC) of the bladder is associated with a high frequency of metastasis, resulting in poor prognosis for patients presenting with this disease. Models that capture and demonstrate step-wise enhancement of elements of the human metastatic cascade on a similar genetic background are useful research tools. We have utilized the transitional cell carcinoma cell line TSU-Pr1 to develop an in vivo experimental model of bladder TCC metastasis. TSU-Pr1 cells were inoculated into the left cardiac ventricle of SCID mice and the development of bone metastases was monitored using high resolution X-ray. Tumor tissue from a single bone lesion was excised and cultured in vitro to generate the TSU-Pr1-B1 subline. This cycle was repeated with the TSU-Pr1-B1 cells to generate the successive subline TSU-Pr1-B2. DNA profiling and karyotype analysis confirmed the genetic relationship of these three cell lines. In vitro, the growth rate of these cell lines was not significantly different. However, following intracardiac inoculation TSU-Pr1, TSU-Pr1-B1 and TSU-Pr1-B2 exhibited increasing metastatic potential with a concomitant decrease in time to the onset of radiologically detectable metastatic bone lesions. Significant elevations in the levels of mRNA expression of the matrix metalloproteases (MMPs) membrane type 1-MMP (MT1-MMP), MT2-MMP and MMP-9, and their inhibitor, tissue inhibitor of metalloprotease-2 (TIMP-2), across the progressively metastatic cell lines, were detected by quantitative PCR. Given the role of MT1-MMP and TIMP-2 in MMP-2 activation, and the upregulation of MMP-9, these data suggest an important role for matrix remodeling, particularly basement membrane, in this progression. The TSU-Pr1-B1/B2 model holds promise for further identification of important molecules.

Abbreviations: MMP – matrix metalloprotease; MT1-MMP – membrane type-MMP; STR – short tandem repeat; TCC – transitional cell carcinoma; TIMP – tissue inhibitor of metalloprotease

Introduction

Patients with transitional cell carcinoma (TCC) of the bladder usually present with either superficial (~70% at diagnosis) or muscle-invasive disease [1]. Despite the reasonable prognosis for patients with superficial TCC, resistance to traditional chemotherapy is common [2], eventually leading to the development of muscle invasive disease. Approximately 50% of patients presenting with muscle invasive TCC will develop occult distant metastases [3]. Prognosis for these patients is poor with the 5-year survival rate residing around 7% [4]. Prevention and treatment of metastases is pertinent to the survival of patients suffering from TCC and from many other cancer types. Mechanistic understanding of the metastasis process is a key to this goal.

The anatomical pattern of metastasis in TCC of the bladder identifies the lymph nodes, bone, lung and liver as the most common sites of metastatic lesions [5–7]. Multiple sites are involved in approximately half of patients, with bone being the most common site of metastasis outside the pelvis [5]. Until recently, there has been a scarcity of models in which to study this disease, largely due to the lack of tumorigenicity displayed by TCC cell lines [8–11]. Previous studies in rodent and human metastatic models have relied heavily upon the transfection of oncogenes or chemically induced transformation [8, 12–14] and the paired T24/T24T metastatic model arising from the identification of T24T...
as a variant of T24 [15] to establish metastatic sublines. Lineage-related cell lines are well suited to investigating global genetic changes involved in the spontaneous development of metastatic bladder carcinoma. These include the metastatic M-NBT-II cell line variant of the rat bladder carcinoma cell line NBT-II selected through *in vivo* passaging [16], and two human models – the 253J/253J lung-IV orthotopic metastatic model [9] and the recently developed T24T/FL1-3 model of TCC metastasis to the lung [17].

The metastatic cascade is without doubt a complex process. In order to metastasize, cells must be able to form a primary tumor, extravasate into the circulation and survive there, extravasate into a secondary site and form a secondary tumor in the new environment [18]. A host of molecular pathways have been implicated in this process, however, in many cases their precise roles remain to be defined [19, 20]. Neoplasms consist of biologically heterogeneous cell populations and selection pressures inferred from the microenvironment, as well as commandeered homeostatic pathways, select for subpopulations of cells with different angiogenic, invasive and metastatic abilities [21]. Hence, the development of lineage-related metastatic models is crucial to the understanding of this process.

While the existing bladder TCC metastatic models focus on the lung as a metastatic site, there is yet to be a model that encompasses the propensity for TCC of the bladder to metastasize to the bone. Bone lesions arising from TCC usually manifest as osteoblastic or mixed osteoblastic–osteolytic lesions [22]. This paper describes the development of a novel, *in vivo*-derived model of metastatic transitional cell carcinoma of the bladder. We have utilized the TSU-Pr1 cell line, a derivative of the bladder carcinoma cell line T24 [23], to generate two successive cell lines with increasing metastatic potential to the bone. Our new model provides an excellent platform on which to study multiple aspects of the metastatic cascade, specifically targeting the arrest of cells in the microvasculature, penetration and extravasation into secondary sites, growth of tumor cells in the secondary environment and development of mixed osteoblastic–osteolytic lesions in bone. Initial studies have revealed important changes in the matrix metalloproteases (MMPs) and their inhibitors (tissue inhibitors of MMPs; TIMPs), which reflect an altered capacity for remodeling of the extracellular matrix (ECM).

**Materials and methods**

**Cell culture and reagents**

TSU-Pr1 (‘T24’) human cancer cells (originally from Dr Dan Djakiew, Georgetown University, USA [24]), and derived sublines TSU-Pr1-B1 and TSU-Pr1-B2, were grown in Dulbecco’s modified essential medium (DMEM) (Gibco, Australia) supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Australia).

**Animals**

Mice (3-4-week-old intact male SCID mice) were purchased from the Animal Resource Centre (Perth, Australia), housed in individually ventilated cages under filtered air (Techniplast, Milan, Italy) and acclimatized for 1 week prior to manipulation. Tumor cells were harvested from near confluent conditions, aspirated into single cell suspension, washed twice and resuspended in phosphate-buffered saline (PBS) before inoculation. Anesthesia was achieved by intraperitoneal (ip) injection of ketamine/xylazine (Provet; Australia; 40 μg/g mouse and 16 μg/g mouse, respectively) and tumor cells were inoculated as described below. Mice were allowed to recover from anesthesia before being returned to their cages and monitored daily. All animal studies were conducted with full ethical approval from the St. Vincent’s Hospital Animal Ethics Committee, and in accordance with the Australian National Health and Medical Research Council’s Guidelines for the Care and Use of Laboratory Animals.

**Model derivation**

Mice were anesthetized using ketamine/xylazine (ip) and maintained under isoflurane anesthetic. TSU-Pr1 cells (1 × 10⁶) were inoculated in 0.1 ml PBS into the left ventricle of the heart. From 3 weeks post-inoculation, mice were anesthetized using ketamine/xylazine (ip) and X-rayed weekly to detect bone lesions using high resolution X-ray (Faxitron; Faxitron X-ray Corporation, Wheeling, Illinois). At harvest, tumor tissue was extracted from the tibia into culture media and cultured *in vitro* to form the first TSU-Pr1 subline, TSU-Pr1-B1. In a repeat series, this subline was inoculated into the left ventricle of the heart as described above, tumors were allowed to form, and again tumor was extracted from the bone lesion and cultured *in vitro* to form a subsequent derivative, TSU-Pr1-B2. At harvest, tissues were fixed in 10% neutral-buffered formalin (and bones decalcified in EDTA) before routine processing and embedding in paraffin. Five micron sections were cut, mounted on slides, and stained using hematoxylin and eosin.

**Chromosome preparation and Giemsa banding**

Cells at approximately 70% confluency were treated with Colcemid (0.01 μg/ml) to arrest cells in metaphase, followed by 20 min hypotonic swelling (55 mM KCl; 1% trisodium citrate). Cells were fixed (3:1 methanol:acetic acid) and dropped onto glass slides to visualize metaphases. Slides were aged 1 h at 100 °C, then overnight at room temperature, rinsed with water and G-banded with a 1:9 solution of Giemsa stain (BDH 35086 4x, Selby Anax, Australia).