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Urologic Oncology: Seminars and Original Investigations 25 (2007) 401–406

UROLOGIC ONCOLOGY

Seminar article

RhoGDI2: A new metastasis suppressor gene: Discovery and clinical translation☆

Michael A. Harding, Ph.D.[a], Dan Theodorescu, M.D., Ph.D.[a,b,*]

[a] *Department of Urology, University of Virginia, Charlottesville, VA 22908, USA*
[b] *Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA 22908, USA*

Abstract

The greatest risk for morbidity and mortality caused by bladder cancer is due to metastasis. For this reason, we have developed a paradigm for discovering the molecular mechanisms underlying bladder cancer progression to an invasive and metastatic phenotype. Results of microarray gene expression analysis of a cell culture model were parsed by identifying overlapping genes that correlate with increasing stage and grade of human tumors. One gene identified by this method, RhoGDI2, was tested in various in vitro and in vivo model systems and confirmed to be a metastasis suppressor gene. Using a similar strategy of gene identification by concordance of microarray gene expression results from cells expressing RhoGDI2 and human bladder cancers, two molecular effectors of RhoGDI2 signaling were identified. These targets, endothelin-1 and Neuromedin U are excellent potential targets for therapeutic intervention in the metastatic cascade.

Keywords: GTPases; Bladder cancer; Biomarker; Metastasis

Introduction

Bladder cancer is a serious health care problem and a significant contributor to cancer-caused deaths. There are approximately 50,000 newly diagnosed cases of bladder cancer each year in the United States [1,2]. Approximately 5% of these patients present with metastatic disease at diagnosis. However, on average, an additional 50% of patients who are originally diagnosed with locally advanced disease later develop metastases, commonly to the lung, resulting in significant mortality among patients with bladder cancer [1,2].

Metastasis is a complex, multi-step process, which requires the acquisition of several novel phenotypes by cancer cells of epithelial origin. For a superficial transitional cell carcinoma (TCC) of the bladder, the cells must first acquire the ability to disrupt the basement membrane, thus exiting the mucosal layer, and to then invade the surrounding muscle. Concomitant to invasion, it is believed that the tumor must induce angiogenesis in order for its growth to not out-strip its nutrient and oxygen supply. Development of a neovasculature also provides a conduit for cancer cells to extravasate into the bloodstream, travel to distant sites and intravasate into the parenchyma of a new host organ. In order to accomplish this feat, cancer cells must be able to degrade intervening matrix, move around and through surrounding tissue, survive without adhesion to a substrate, tolerate the shear forces found in the circulation, and thrive in a foreign microenvironment. Indeed, it has been shown that very few cells within a primary tumor have the requisite phenotypes to accomplish metastasis [3].

In recent years it has been discovered that one way cancer cells acquire metastatic competency is through the loss of metastasis suppressor gene expression (reviewed in [4]). This loss of gene expression may be due to somatic mutation or to epigenetic mechanisms. Metastasis suppressor genes have been functionally defined as genes whose re-expression in metastatically competent cells inhibits the formation and growth of metastases. It is hypothesized that metastasis suppressor proteins function by restoring normal homeostatic signaling mechanisms, which inhibit the aforementioned phenotypes necessary for metastasis. In the most stringent definition, metastasis suppressors differ from tumor suppressor genes in that they do not inhibit growth of the primary tumor. However, this distinction is not absolute

☆ This work was supported by NIH grant CA075115 to D.T.

* Corresponding author. Tel.: +1-434-924-0042; fax: +1-434-982-3652.

E-mail address: dt9d@virginia.edu (D. Theodorescu).

1078-1439/07/$ – see front matter
doi:10.1016/j.urolonc.2007.05.006

and may be tissue- and model-specific. There are examples of metastasis suppressor genes that act as tumor suppressors in different contexts [4].

Over the past several years, our laboratory has endeavored to understand mechanisms involved in bladder cancer progression. Our focus has been the transition of superficial cancers to invasive and metastatic disease, because this transition seems to be a promising target for meaningful therapeutic intervention. Since detailed, mechanistic studies are not possible in patients, much of our efforts have centered on the development of in vitro and in vivo models to study metastasis. We have been particularly interested in developing a paradigm for identifying and validating candidate genes that suppress bladder cancer metastasis to lung, a common site in patients. The following discussion outlines the methodology and experimental results, which led to our discovery that the RhoGDI2 gene (rho GDP dissociation inhibitor 2) is a metastasis suppressor. The hope is that this approach will allow us to elucidate the signaling pathways involved, and to identify and test promising therapeutic targets.

The T24/T24T model system

Our investigations into RhoGDI2 function began with the characterization of a paired cell line model for bladder cancer progression [5]. The T24 cell line was established from a transitional cell carcinoma of the bladder in a female patient in 1973. Reports in the literature indicated a wide range of tumorigenicity for T24 when injected subcutaneously in immunocompromised murine hosts. Initial studies from our laboratory using two variations of T24 cells, obtained from different sources, exhibited quite disparate subcutaneous tumorigenicity. Microsatellite repeat analysis confirmed that our two variant T24 cell lines were from the same patient [Harding and Theodorescu, unpublished data]. For simplicity, we retained the T24 designation for the less tumorigenic cell line and labeled the more tumorigenic variant "T24T."

In vitro investigations were conducted to more clearly define the phenotypic differences in the T24/T24T system [5]. Growth in monolayer cultures on plastic tissue culture dishes at subconfluent cell densities revealed no significant difference in the growth rates of T24 and T24T. However, when cells reached confluence, proliferation of T24 cultures dramatically slowed but T24T cells continued to grow by piling up on each other. In a similar fashion, differences in T24 and T24T motility only became evident at high densities. Blind well chemotaxis assays were performed using Boyden chambers and serum as a chemoattractant. At low cell plating numbers, T24 cells transited the pores of the filters just as well as T24T. However, at high cell densities, the motility of T24T was nearly twice that of T24. Growth in soft agar is often used to measure contact inhibition of proliferation and ability to grow in suspension. T24T cells readily formed colonies in soft agar, whereas T24 formed virtually no colonies. The conclusion drawn from these experiments is that T24 cells do not have an inherent defect in proliferation or motive ability, but have retained homeostatic mechanisms, like contact inhibition, which limit their growth and motility under certain conditions. T24T cells have apparently lost these homeostatic mechanisms.

In vivo experiments demonstrated that T24T was clearly more invasive than T24 in an orthotopic environment, and that T24T had an increased capacity to metastasize to lung. Upon inoculation into the bladder lumen of nude mice, T24 cells did grow and form flat "sheet-like" tumors, but they were confined to the mucosal layer, whereas T24T formed muscle invasive tumors [Theodorescu et al., unpublished observations]. To measure each cell line's ability to metastasize to lung, we injected T24 and T24T cells into the venous circulation via the tail vein of immunocompromised mice. Mice were autopsied 8 weeks after injection and the number of nodular lung metastases was counted. All of the T24T injected mice developed lung metastases but only about a third of T24 injected mice developed lung metastases. The number of metastases in the lungs was also quite different. T24 produced only 1 to 2 nodules per mouse and T24T had an average of 28 nodules per mouse [6]. These observations indicated that T24 and T24T cells might be an ideal model system in the search for metastasis suppressor genes.

The search for genetic differences in T24 and T24T began with detailed studies of the T24 and T24T genomes. These included karyotyping [5], spectral karyotyping (SKY), comparative genomic hybridization (CGH), and positional expression profiling [7]. Several chromosome structural rearrangements that differed in the two cell lines were revealed. These chromosomal rearrangements were intriguing because they suggested a mechanism by which metastasis suppressor genes might be silenced, thus explaining the cells' phenotypic differences. Of particular interest for the discussion presented here, the number of copies of the short arm of chromosome 12 (12p) was less in T24T than in T24, and a corresponding decrease in expression from genes located on 12p in T24T was noted. The RhoGDI2 gene, which will be discussed below, is located on chromosome 12p.

Identification and screening of candidate metastasis suppressor genes

Hybridization of RNA to microarrays allows the interrogation of expression for tens of thousands of genes simultaneously. We harnessed the power of microarray technology and our novel, well characterized T24/T24T cell model system to search for genes that inhibit bladder cancer cell metastasis to lung [8]. RNA extracted from each cell line was hybridized to Affymetrix oligonucleotide arrays and the