ELSEVIER

Urologic Oncology: Seminars and Original Investigations 32 (2014) 43.e23–43.e30

UROLOGIC ONCOLOGY

Original article

Tissue slice grafts of human renal cell carcinoma: An authentic preclinical model with high engraftment rate and metastatic potential

Alan E. Thong, M.D.[a], Hongjuan Zhao, Ph.D.[a], Alexandre Ingels, M.D.[a,b], Maija P. Valta, M.D.,Ph.D.[a,c], Rosalie Nolley, B.A.[a], Jennifer Santos, B.S.[a], Sarah R. Young, B.S.[a], Donna M. Peehl, Ph.D.[a,*]

[a] *Department of Urology, Stanford University School of Medicine, Stanford, CA*
[b] *Department of Urology, Centre Hospitalier Universitaire du Kremlin-Bicêtre, Kremlin-Bicêtre, France*
[c] *Division of Medicine, Turku University Hospital and University of Turku, Turku, Finland*

Received 11 April 2013; received in revised form 14 May 2013; accepted 15 May 2013

Abstract

Objective: Discovery of curative therapies for renal cell carcinoma (RCC) is hampered by lack of authentic preclinical models. Tumorgrafts, generated by direct implantation of patient-derived tissues into mice, have demonstrated superior ability to predict therapeutic response. We evaluated "tissue slice grafts" (TSGs) as an improved tumorgraft model of RCC.

Materials and methods: Cores of fresh RCC were precision-cut at 300 μm and implanted under the renal capsule of RAG2$^{-/-}\gamma C^{-/-}$ mice. Engraftment rate, histology, biomarker expression, genetic fidelity, and metastatic potential were evaluated. Magnetic resonance imaging (MRI) was tested as a noninvasive method to measure tumor volume, and response to a targeted therapy was determined.

Results: All 13 cases of RCC engrafted and displayed characteristic histology and biomarkers. TSG volume quantified noninvasively by MRI highly correlated with graft weights, providing a unique tool for monitoring orthotopic growth. Moreover, in 2 cases, cancer cells from TSGs metastasized to clinically relevant sites, including bone. Microarray analysis and DNA sequencing demonstrated a high degree of correlation of global gene expression and von Hippel-Lindau (VHL) status between TSGs and parental tumors. Treatment of TSGs with sunitinib significantly decreased graft weight and mean vessel density compared with controls.

Conclusion: The TSG model of RCC faithfully recapitulates tumor pathology, gene expression, genetic mutation, and drug response. The high engraftment rate and metastatic potential of this authentic model, in conjunction with the ability to generate large first-generation animal cohorts and to quantitate tumor volume at the orthotopic site by MRI, proffer significant advantages compared with other preclinical platforms.

Keywords: Renal cancer; Tumorgrafts; Metastases

1. Introduction

Realistic preclinical models of renal cell carcinoma (RCC) greatly accelerate the development of new therapeutics and the elucidation of the mechanisms of response and resistance to current therapeutics. Tumorgrafts derived from fresh human tumor tissues, so-called mouse avatars [1], have been shown to possess high predictive power for both patient prognosis and drug response by recapitulating parental tumors both microscopically and molecularly [2–5]. RCC tumorgrafts with genetic and histologic fidelity, metastatic potential, and drug responsiveness have recently been generated successfully by several independent groups [6–8]. However, these investigators all used minced tissues that varied in size, resulting in limited numbers of grafts generated from a single primary tumor specimen with varied growth rates or low engraftment rates or both [6–8]. Moreover, the lack of tracking the exact location in the parental tumor from which each graft is derived makes it impossible to use tumorgrafts derived from adjacent tissues in control and experimental groups to minimize variations

This study is supported by Instrumentarium Science Foundation, Finland, the Finnish Medical Foundation, the Association Française d'Urologie, and funds from the Department of Urology at Stanford University.

* Corresponding author. Tel.: +1-650-725-5531; fax: +1-650-723-4200.
E-mail address: dpeehl@stanford.edu (D.M. Peehl).

1078-1439/$ – see front matter
http://dx.doi.org/10.1016/j.urolonc.2013.05.008

in molecular and cellular composition of the grafts due to the intratumoral heterogeneity of RCC [9].

We previously established prostate cancer tumorgrafts from precision-cut tissue slices of 300 μm thickness and 8 mm diameter as opposed to tissue fragments traditionally used for tumorgrafting [10]. These thin tissue slice grafts (TSGs) offer certain capabilities beyond traditional tumorgrafts. The thinness of the grafts permits exchange of gases and nutrients with the host in the initial days following implantation as the vasculature becomes established, contributing to the high engraftment rate of TSGs. Numerous slices can be generated from small tissue specimens, enabling the establishment of large animal cohorts without serial transplantation of the tumorgrafts. It is easy to track the position of each tissue slice in the parental tumor, making it possible to randomize TSGs derived from adjacent tissue slices into control and experimental groups so that the tumor composition is comparable between the two.

In this study, we extended TSG methodology to human RCC. We examined the engraftment rate, histology, immunophenotype, metastatic potential, and genetic fidelity of TSGs derived from 13 fresh RCC tissues. In addition, we established a noninvasive metric for quantifying orthotopically grafted tumors by magnetic resonance imaging (MRI) and assessed the response of TSGs to a known targeted therapeutic, sunitinib [11].

2. Materials and methods

2.1. Tissue acquisition

Fresh RCC tissues were obtained from patients undergoing nephrectomy between September 2011 and May 2012 at Stanford under an institutional review board–approved protocol with informed consent. Frozen sections were utilized to histologically confirm the diagnosis of RCC. Clinicopathologic features of the cases are summarized in Table 1.

2.2. Precision-cutting and subrenal implantation of tissue slices

Precision-cutting and subrenal implantation of tissue slices were performed as previously described [10]. All animal work was done in accordance with institutional regulations for laboratory animal studies. RAG2$^{-/-}\gamma C^{-/-}$ mice [12] between 6 and 8 weeks of age were engrafted with RCC tissue slices.

2.3. MRI

A Discovery MR901 7.0-Tesla MRI system (Agilent Technologies, Santa Clara, CA) was used at the Stanford University Small Animal Imaging Facility for in vivo imaging. A custom T2-weighted sequence was developed for abdominal imaging of TSGs in situ on the mouse kidney. Three-dimensional volumetric modeling was then performed with OsiriX 4.1 (Pixmeo, Bernex, Switzerland), and calculated volumes were compared with final gross TSG weights.

2.4. Immunohistochemistry

Immunohistochemistry was performed as previously described [10]. Antigen retrieval was achieved by heating in citrate buffer (pH 6.0) for 20 minutes, followed by a 20-minute cooldown. The sources and dilutions of the antibodies used in this study are listed in Table 2.

2.5. Gene expression profiling

Microarray hybridization was performed using Illumina Human HT-12 v4 Beadchips (Illumina Inc., San Diego, CA) according to manufacturer's directions. Raw data were deposited in GEO (GSE44548). Expression data were rank-invariant normalized using BeadStudio software (Illumina Inc.). Average linkage clustering was performed using Cluster software (Eisen Lab, http://rana.lbl.gov/EisenSoftware.htm) and visualized using TreeView (Eisen Lab). Correlation coefficients were calculated using Excel.

Table 1
Pathologic features and engraftment rates of RCC cases in this study

Case	Sex	Age	Pathologic stage	Nodal and metastatic stage	Fuhrman grade	Additional pathologic features	TSG engraftment rate (engrafted/total)
1	F	64	T3a	NxMx	III		90% (25/28) 100% (5/5)[a]
2	F	33	T3b	N1Mx	IV	Sarcomatoid	82% (27/33) 100% (5/5)[a]
3	M	75	T2a	NxMx	III/IV	Rhabdoid, papillary	79% (23/29) 100% (3/3)[a]
4	F	76	T1a	NxMx	II		100% (12/12)
5	F	91	T1a	NxMx	III		100% (5/5)
6	M	62	T3a	NxMx	III		100% (10/10)
7	M	68	T3a	NxMx	Chromophobe		100% (5/5)
8	M	91	T3a	NxM1	III		100% (10/10)
9	M	61	T3a	NxMx	II		100% (3/3)
10	F	83	T3a	NxMx	II	Sarcomatoid	100% (3/3)
11	M	59	T1b	NxMx	II		33% (1/3)
12[b]	F	59	T3a	NxM1	IV	Rhabdoid	66% (19/29)
13	M	42	T3a	NxMx	III		45% (9/20)

[a]Secondary TSGs generated from primary TSGs.
[b]Patient received neoadjuvant therapy of 4 cycles of sunitinib (37.5–50 mg/day, 2 wks on and 1 wk off), which was completed 2 weeks prior to surgery.