Engraftment of tumorgrafts predicts for development of metastasis in patients with localized renal cell carcinoma

Felix Thomas, Xiaoyue Li, Andrea Pavia-Jimenez, Vanina Toffessi, Shannon Cohn, Alana Christie, James Brugarolas, MD, PhD.

University of Texas Southwestern Medical Center

ABSTRACT

Purpose: This retrospective study compares tumorgraft engraftment with development of metastatic renal cell carcinoma (RCC) in patients after the resection of localized tumor in order to determine the potential clinical applications of tumorgraft models.

Materials and Methods: We analyzed tumorgraft lines derived from primary tumor samples of 180 patients. Odds ratios and Kaplan-Meier analyses were used to determine the correlation between tumor engraftment and patient outcome.

Results: There were primary tumor samples from a total of 22 patients who had metastatic disease at the time of surgery. These tumors engrafted at a higher frequency than those of patients who did not have metastatic disease at the time of surgery (OR=3.39, p=0.0099). Of the 158 patients who had localized RCC at the time of surgery, patients whose tumors engrafted developed metastasis at a higher frequency (OR=3.53, p=0.01174) than those whose tumors did not engraft. Patients with engrafted tumors also had a marked decrease in progression-free survival and RCC-specific progression-free survival, but not overall survival.

Conclusions: Selecting tissue from patients with metastatic RCC at the time of surgery can be used to increase the efficiency of engraftment in RCC tumorgraft models. Engraftment of tumors in mice may be an independent predictor of patient outcome and thus has the potential to become a powerful clinical tool.

INTRODUCTION

The employment of tumorgrafts in animal models for the evaluation of cancer mechanics has been established for over 40 years[1-3], but became especially important after it was suggested that traditional cell line models were not as representative as previously thought; in particular, drug effect correlations between patients and matching derived cell lines were poor[1, 4, 5]. Tumorgrafts serve as representative models of cancer because of their ability to retain tumor-specific genotypic[6-10] and phenotypic[6, 7, 9-11] qualities. However, the tumorgraft process has not been very efficient, with engraftment rates of 27-28%[8, 10]. In an effort to increase the efficiency of tumorgrafts, studies were conducted to determine independent factors of successful engraftment of tumor tissue.

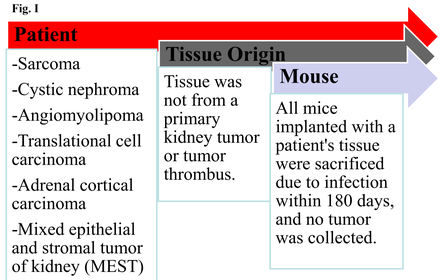
We recently reported a validated tumorgraft mouse model of renal cell carcinoma (RCC) which suggested that metastatic RCC can be used as a predictor of engraftment [9, 13]. Our goal was to determine if the reverse was also true: whether or not successful engraftment of primary tumor tissue could be used to predict for future development of metastatic disease in patients. RCC tumorgrafts have been used for accurate genomic analysis, effective drug trials, and for tumor cell characterization[9], but their clinical significance has yet to be analyzed in depth. In other cancer types, successful engraftment is correlated with poor prognosis, decreased survival rates, increased recurrence and metastasis development[10-12]. In addition to the known predictors of metastasis, which include TNM staging[14] and tumor histology[15], our goal was to determine if tumorgraft engraftment could also predict for future development of metastasis in RCC.

MATERIALS AND METHODS

Patient and Tissue Selection

- Patient tissue information was collected from an established database in the lab.
- Tumor implantation was done based on prior protocol [9].
- Mice were sacrificed at various times and any tissue resembling RCC was processed to study histology
- A tumor graft (TG) line was considered "engrafted" if at least one mouse in the line grew a tumor within 180 days that was no less than 4 mm in largest dimension and histology was consistent with RCC .
- Certain patients were excluded based on the exclusion criteria presented in Figure I. Overall, 180 patients were included.

Fig. I



Patient Follow-up

- Patient follow-up information was collected by accessing electronic patient records and patient date of death using the social security death index (SSDI).
- A standardized database with patient surgical information and corresponding TG lines was generated.
- Patients were categorized as:
  - free of metastatic RCC
  - metastasis at the time of surgery
  - metastasis development following surgery

Analysis

- Odds ratio analyses with 95% confidence interval (CI) were used to study overall survival (OS).
- The Fisher's exact test was used for categorical variables and the Student t-test for continuous variables to determine any association with future development of metastasis.
- Kaplan-Meier curves along with the log-rank test were used to evaluate the RCC-specific progression-free survival (RPFS), OS, and Progression free survival (PFS).
- Hazard ratios were calculated using Cox regression analyses.
- All p-values are two-tailed and were calculated at the 0.05 significance level without adjustment. All statistical analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Table 1: Predictors of Metastatic Development

	Future Metastasis/Total	P-value
Engraftment	10/36 (27.8%)	0.012
No engraftment	12/122 (9.8%)	
RCC histology		0.11
Clear cell	16/112 (14.3%)	
Papillary	1/19 (5.3%)	
Chromophobe	3/14 (21.4%)	
Oncocytoma	0/8 (0%)	
Tubulocystic	0/1 (0%)	
Unclassified	2/3 (66.7%)	
Sarcomatoid differentiation	7/14 (50.0%)	0.00095
No sarcomatoid differentiation	16/145 (11.0%)	
Focality		0.74
Unifocal	19/127 (15.0%)	
Multifocal	2/21 (9.5%)	
Fuhrman nuclear grade		0.0068
1	0/4 (0%)	
2	2/54 (3.7%)	
3	11/66 (16.7%)	
4	7/22 (31.8%)	
Size (cm)		0.0037
≤4	2/35 (5.7%)	
>4-7	3/51 (5.9%)	
>7-10	5/38 (13.2%)	
>10	11/34 (32.4%)	
Pathologic tumor grade		0.00013
T1	1/59 (1.7%)	
T2	1/28 (3.6%)	
T3	17/59 (28.8%)	
T4	2/5 (40.0%)	
Pathologic lymph node stage		0.11
N0	9/42 (21.4%)	
N1	5/10 (50.0%)	

RESULTS

A

	Metastasis	No metastasis
Engraftment	11	36
No engraftment	11	122

B

	Engraftment	No Engraftment
Metastasis	10	12
No Metastasis	26	110

C

	Engraftment	No Engraftment
Deceased	6	11
Not Deceased	30	111

Fig. II. Contingency tables comparing (**A**) engraftment between patients with and without metastasis at diagnosis, (**B**) future development of metastatic disease between patients with and without engraftment, and (**C**) death among patients with and without engraftment.

RESULTS

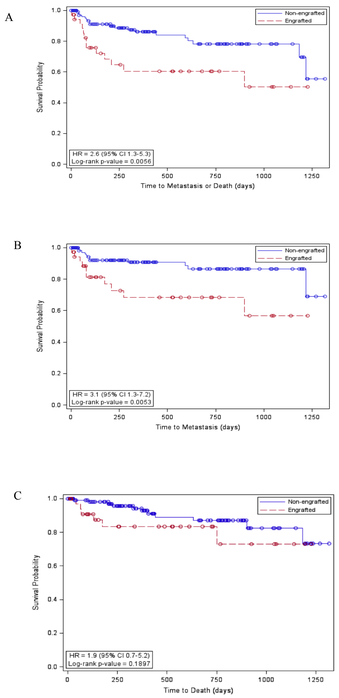


Fig. III. Evaluation of patient outcomes as a function of engraftment of primary tumors using Kaplan-Meier curves. (**A**) Progression-free survival for groups with and without engraftment. (B) Metastasis-free survival for groups with and without engraftment. (**C**) Overall survival for groups with and without engraftment.

Results/Discussion

Eleven patients who had metastatic RCC at diagnosis had tumors that did not engraft successfully in mice (Fig. IIA).

Tumor heterogeneity - Mutations that favor the ability to metastasize may not be ubiquitous and thus may only be present in a small subset of the tumor cell population. Thus, only samples of tissues containing metastasis-favoring mutations would show an increased likelihood of successful engraftment, and it is possible that the tissues that did not engraft were collected from areas of the tumor that did not contain these mutations.

Specificity of mutations - There is also the possibility that metastasis-inducing mutations only confer the ability to grow in specific new environments. In the patients who developed metastasis but did not have engrafted tissue, tumor cells may have acquired mutations that favored growth in only a subset of locations rather than any generalized new location (mouse kidney).

Twenty-six patients had tumors which engrafted, but the patients did not develop metastasis (Fig. IIB).

Inconclusive follow-up - Patients in this category may still develop metastasis in the future; however, this can only be verified with further follow-up.

Complete surgical resection - Some tumors with metastatic potential are still treatable by surgery alone.

CONCLUSION

Metastasis at the time of surgery is a predictor of engraftment.

The condition of metastatic disease at the time of diagnosis correlated with successful tumor engraftment.

There is a significant correlation between tumorgraft engraftment and future development of metastatic disease in patients.

The odds of developing metastatic RCC were higher for patients with engrafted tumors in mice compared to patients whose tumors did not engraft.

The Kaplan-Meier curves revealed a significant difference in PFS. The difference in RPFS was also significant.

REFERENCES

1. Garber, K., *From human to mouse and back: 'tumorgraft' models surge in popularity.* J Natl Cancer Inst, 2009. **101**(1): p. 6-8.
2. Cobb, L.M., *The behaviour of carcinoma of the large bowel in man following transplantation into immune deprived mice.* Br J Cancer, 1973. **28**(5): p. 400-11.
3. Steel, G.G. and M.J. Peckham, *Human tumour xenografts: a critical appraisal.* Br J Cancer Suppl, 1980. **4**: p. 133-41.
4. Voskoglou-Nomikos, T., J.L. Pater, and L. Seymour, *Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models.* Clin Cancer Res, 2003. **9**(11): p. 4227-39.
5. Sausville, E.A. and A.M. Burger, *Contributions of human tumor xenografts to anticancer drug development.* Cancer Res, 2006. **66**(7): p. 3351-4, discussion 3354.
6. Grisanzio, C., et al., *Orthotopic xenografts of RCC retain histological, immunophenotypic and genetic features of tumours in patients.* J Pathol, 2011. **225**(2): p. 212-21.
7. Mischek, D., et al., *Molecularly characterised xenograft tumour mouse models: valuable tools for evaluation of new therapeutic strategies for secondary liver cancers.* J Biomed Biotechnol, 2009. **2009**: p. 437284.
8. Monsma, D.J., et al., *Genomic characterization of explant tumorgraft models derived from fresh patient tumor tissue.* J Transl Med, 2012. **10**: p. 125.
9. Sivanand, S., et al., *A validated tumorgraft model reveals activity of dovitinib against renal cell carcinoma.* Sci Transl Med, 2012. **4**(137): p. 137ra75.
10. Nemati, F., et al., *Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors.* Clin Cancer Res, 2010. **16**(8): p. 2352-62.
11. DeRose, Y.S., et al., *Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes.* Nat Med, 2011. **17**(11): p. 1514-20.
12. John, T., et al., *The ability to form primary tumor xenografts is predictive of increased risk of disease recurrence in early-stage non-small cell lung cancer.* Clin Cancer Res, 2011. **17**(1): p. 134-41.
13. Angevin, E., et al., *Human renal cell carcinoma xenografts in SCID mice: tumorigenicity correlates with a poor clinical prognosis.* Lab Invest, 1999. **79**(7): p. 879-88.
14. Belsante, M., et al., *Lymphovascular invasion in clear cell renal cell carcinoma-Association with disease-free and cancer-specific survival.* Urol Oncol, 2013.
15. Leibovich, B.C., et al., *Histological subtype is an independent predictor of outcome for patients with renal cell carcinoma.* J Urol, 2010. **183**(4): p. 1309-15.
16. Gerlinger, M., et al., *Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.* N Engl J Med, 2012. **366**(10): p. 883-92.