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Quantitative tumour necrosis is an independent predictor of overall survival in clear cell renal cell carcinoma

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Summary

Previous studies have reached conflicting results regarding whether tumour necrosis is a predictor of survival in clear cell renal cell carcinoma. In addition, studies quantifying the extent of necrosis are limited. The aim of this study was to determine if quantifying tumour necrosis could improve its predictive value for survival in clear cell renal cell carcinoma. We reviewed the clinical pathological information contained in The Cancer Genome Atlas for clear cell renal cell carcinoma and correlated it with overall survival using a Cox proportional hazard model. Necrosis was quantified on a single frozen section slide taken at the time of tissue harvesting for molecular studies. For all tumours, the presence of tumour necrosis was a significant predictor of overall survival ($p < 0.001$) on univariate analysis. When quantitated, >10% necrosis was associated with survival, but ≤10% necrosis was not. On multivariate analysis, age ($p = 0.004$), T3b stage ($p = 0.02$), M1 stage ($p < 0.001$), necrosis >30% ($p < 0.001$), and elevated serum calcium ($p = 0.003$) remained significant. For clinical stage 1–2 (T1-T2N0M0) tumours, necrosis >20% was significant on univariate analysis ($p \leq 0.005$), and remained so on multivariate analysis ($p < 0.001$). We conclude that quantitating the extent of tumour necrosis adds prognostic information in clear cell renal cell carcinomas, including organ confined tumours.

Key words: Clear cell, necrosis, prognosis, quantitative, renal cell carcinoma, survival.

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INTRODUCTION

Previous studies have shown conflicting results regarding whether coagulative tumour necrosis is a predictor of survival in renal cell carcinoma. Most studies have agreed that the presence of tumour necrosis is associated with worse cause specific survival[1–6] and overall survival[7–11] in renal cell carcinomas of clear cell renal cell and chromophobe type,[1] including in organ confined (T1-T2N0M0) clear cell tumours.[4,8] Nevertheless, some studies have failed to show that tumour necrosis is an independent predictor of prognosis when considered in the context of other prognostic features.[12] In addition, previous studies examining the extent of necrosis are limited. To our knowledge only two previous studies attempted to stratify the extent of necrosis.[6,8] One study suggested that tumour necrosis of greater than 50% of the entire tumour was associated with worse disease specific survival but not overall survival, and the other suggested that quantification was not of value in predicting overall survival.

However, there were significant limitations in the way in which the extent of necrosis was quantified in these studies. In particular, the extent of necrosis was estimated for the entire tumour and the extent of necrosis was stratified into a few very large groups. Stratification into smaller groups, especially in those tumours with lesser degrees of necrosis, was not assessed.

We recently noticed that in a previously published study addressing the molecular characteristics of clear cell renal cell carcinoma from the Tumour Cancer Genome Atlas (TCGA),[13] tumour necrosis was assessed as part of a quality assessment measure for molecular studies. In this study, tumour necrosis was estimated from examination of a single frozen section slide of tissue that was taken for molecular studies. We wondered if this assessment might shed light on the significance of quantifying necrosis in clear cell renal cell carcinoma.

METHODS

All data were taken from the supplementary tables of the Cancer Genome Atlas Research Network publication[13] (Data File S2, clinical dataset) which also contain additional methodological information. In brief and as described in the methods of that paper and paraphrased here, tumours were flash-frozen and shipped to a centralised processing centre (Biospecimen Core Resource, BCR) for additional pathological review and nucleic acids extraction. Biospecimens were collected from newly diagnosed patients with renal clear cell carcinoma undergoing surgical resection who had received no prior treatment for their disease, including chemotherapy or radiotherapy. All cases were collected, regardless of surgical stage or histological grade. Cases were staged according to the American Joint Committee on Cancer (AJCC) staging system. Cases were graded using the Fuhrman grading system, which was the standard at the time the study was performed. Each frozen tumour specimen submitted to the BCR weighed at least 30 mg and was typically under 200 mg. Specimens were shipped overnight from one of 13 tissue source sites using a cryoport that maintained an average temperature of less than −180°C. Tumour necrosis was assessed microscopically from a single frozen section of this biorepository specimen.

Complete clinical data elements were compiled for all specimens included and reflected current data as of 13 April 2012. Clinical/demographic data included: sample code, primary site (kidney for all specimens), gender, age at diagnosis, race, ethnicity, and year of tumour collection. Samples of questionable authenticity as clear cell RCC tumours due to unexpected molecular analysis results were evaluated by secondary pathological review.

Tumour information recorded complete pathological information regarding the tumour. All specimens included in this analysis were coded as kidney clear cell renal carcinoma. The table records: laterality (right/left), Fuhrman nuclear grade, maximum tumour dimension (cm), T stage, lymph node involvement (based on pathological staging), and M stage (intended to be indicative of a review of clinical evidence for metastatic disease, but was often provided from available pathological information only, so should be interpreted with caution).

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A compiled tumour stage using standard AJCC staging criteria using the Tumour Node Metastasis universal schema was reported. Pathological data came from board certified pathologists in the originating institution. Additional laboratory data including lactate dehydrogenase, calcium, haemoglobin, white blood cell count, and platelet count were also included.

Clinical status of patients at the point of enrolment, and as available at last follow-up was recorded. Sites were asked to indicate if patients following surgical resection were tumour free, or with tumour. We also recorded vital status (living/deceased) at the time of enrolment. Follow-up data were requested for subjects out to a minimum of 2 years from the time of sample collection. The patient tumour status (tumour free/with tumour) was again recorded, along with vital status (living/deceased) from the most recent follow-up data form completion at the time of data collection. Time to recurrence was recorded as the number of days to a new tumour event. We also recorded the days to last contact at the point of enrolment or most recent follow up. Finally, the days from diagnosis (sample collection) to death were recorded at both enrolment and in the most recent follow-up forms. These data provided the information to explore survival-based outcomes and median follow-up for patients included in this study. Cause of death, from cancer or other causes, was not recorded.

Survival analysis was performed using a Cox proportional hazard ratio (HR). A threshold of $p < 0.05$ was considered significant.

RESULTS

A total of 446 cases were included and there were 145 deaths; 257 organ confined (T1-T2N0M0) cases were included, and included 45 deaths. Tumour necrosis was present in 181 (40.6%) tumours, and 79 (30.7%) organ confined tumours, and ranged from 1–30%. Necrosis of greater than 5% was present in 34% of tumours.

For all cases, age, type of surgery, size, T stage, M stage, presence of necrosis, elevated calcium, elevated WBC, low haemoglobin, and elevated platelet count were all significantly associated with overall survival on univariate analysis (Table 1). When quantitated, necrosis >10% was associated with survival, but necrosis ≤10% was not (Table 2). On multivariate analysis, age ($p = 0.004$), T3b stage ($p = 0.02$), M1 stage ($p < 0.001$), necrosis >10% ($p \leq 0.04$), necrosis >30% ($p < 0.001$), and elevated serum calcium ($p = 0.003$) remained significant (Table 3). Kaplan–Meier curves for tumours with >10% necrosis versus ≤10% necrosis are shown in Fig. 1.

For clinical stage 1–2 (T1-T2N0M0) tumours, necrosis >20% was significant on univariate analysis ($p \leq 0.005$), and remained so on multivariate analysis ($p < 0.001$) (Tables 4 and 5). Kaplan–Meier curves for tumours with >20% necrosis versus ≤20% necrosis are shown in Fig. 2.

DISCUSSION

In this paper we have shown that tumour necrosis is a significant independent prognostic factor for clear cell renal cell carcinoma both in general and for organ confined disease as well as when evaluated with both traditional pathological factors and additional clinical laboratory data. In addition, we have shown that stratifying necrosis into different quantities creates subgroups with significantly different overall survival.

Overall, the cases included in this series are similar to other studies, as has been discussed in prior publications.[13] One notable difference is that in this series Fuhrman grading was not a significant prognostic factor. At the time of this study, Fuhrman grading was the standard for grading renal cell carcinoma. While there are several possible reasons for this, one factor may be that the grades were assigned by multiple board certified pathologists rather than a single pathologist. It has previously been shown that Fuhrman grading is not completely reproducible,[2,4,14] and the results in this study may reflect that fact. In addition, the cases in this study may represent a selection bias for larger higher stage tumours (which may be more easily harvested for molecular studies).

As others have also shown, in this series an elevated serum calcium was a significant negative prognostic factor for renal cell carcinoma in general.[15–19] However, its significance in organ confined disease is less clear. Other authors in much larger series have shown that it is a significant prognostic factor in this setting as well, and for this factor our current series may be underpowered. Similar issues may also apply to the other laboratory values reviewed in this study.[20–26]

Although several laboratory values including serum calcium have been examined in series that assessed necrosis, a direct comparison between the two has not been previously made. Specifically, in one study the significance of laboratory values after stratifying for the SSIGN score (Stage, Size, Grade, and Necrosis), which includes tumour necrosis, was performed.[15] To our knowledge the current study is the first study to directly compare the relative importance of these laboratory values against tumour necrosis as an independent value.

Table 1 Univariate analysis for all renal cell carcinomas for overall survival ($n = 446$)

Factor	p value	95% CI
Age	<0.001	1.02–1.05
Sex	0.43	0.62–1.22
Partial nephrectomy (vs total nephrectomy)	<0.001	0.18–0.62
Grade	0.99	NA
Tumour size	<0.001	1.09–1.19
T2 stage (vs T1)	0.06	0.98–3.15
T3a	<0.001	2.32–5.24
T3b	<0.001	2.24–6.16
T3c	0.004	1.92–33.49
T4	<0.001	3.95–25.64
N1 stage (vs N0)	<0.001	1.81–7.94
M1 stage (vs M0)	<0.001	3.15–6.20
Necrosis present	<0.001	1.06–1.08
Elevated LDH	<0.001	3.56–3.99
Elevated calcium (vs normal)	<0.001	1.98–8.87
Low calcium (vs normal)	0.01	0.42–0.89
Elevated WBC (vs normal)	0.004	0.38–0.83
Low WBC (vs normal)	0.09	0.87–4.59
Elevated Hgb (vs normal)	0.003	1.79–19.00
Low Hgb (vs normal)	<0.001	1.31–2.83
Elevated platelets (vs normal)	<0.001	2.10–5.16
Low platelets (vs normal)	0.01	1.16–3.11

CI, confidence interval; Hgb, haemoglobin; LDH, lactate dehydrogenase; NA, not applicable; WBC, white blood cell count.

Table 2 Effect of tumour necrosis quantitation on overall survival for all renal cell carcinomas compared with no necrosis ($n = 446$)

Extent of necrosis	p value	95% CI
1–5%	0.33	0.75–2.36
6–10%	0.09	0.90–4.07
11–15%	0.01	1.21–5.45
16–20%	0.03	1.14–11.95
21–25%	0.04	1.09–18.66
26–30%	<0.001	4.43–9.65

CI, confidence interval.