Plasma glycosaminoglycan scores in early stage renal cell carcinoma

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Abstract

Background: Previous studies have found an outstanding role in the regulation of metabolism of clear cell renal cell carcinoma (ccRCC; Gatto et al., 2014; Creighton et al., 2013). We discovered that glycosaminoglycan (GAG) biosynthesis was prominently regulated in ccRCC, and measurements of circulating GAGs could be condensed into scores that distinguished metastatic ccRCC with accuracy ranging 92.7% to 100% (Gatto et al., 2016). However, it is still unknown if GAG scores could detect cancer at earlier stages and across other histologies.
Methods and Results: We measured plasma GAGs in pre-operative samples from a retrospective consecutive series of 218 patients with a radiographic finding of renal mass. A control group was formed with 19 healthy volunteers and 25 historical healthy samples. In clustering analyses, plasma GAGs distinguished the 179 RCC samples as a separate group in an unbiased fashion. The previous GAG score was updated and achieved an area-under-the-curve (AUC) equal to 0.994 (95% CI: 0.985 - 1) in the validation set with a sensitivity of 95.7%. The GAG score was not significantly associated with age or gender nor with any histopathologic features.
Conclusions: Plasma GAG scores are specifically altered in RCC patients and can detect the disease irrespective of stage and histology with elevated accuracy.

Methods

We measured GAG profiles in pre-operative plasma samples versus healthy controls with the aim to characterize GAG scores for the detection of early stage RCC.

The study was retrospective and double-blinded. Inclusion criteria were: patients with radiographic finding of a renal mass; healthy volunteers without any history of malignancy. Exclusion criteria were: no records on date of surgery; a pre-operative sample was obtained 50 days or earlier with respect to the date of surgery; absence of pre-operative samples following filtering out outliers or laboratory assay failures. Participants were enrolled at Memorial Sloan Kettering Cancer Center, New York City, United States between May 2011 and February 2014. Eligible participants formed a consecutive series.

Laboratory measurements of the GAG profile quantified 19 properties using capillary electrophoresis with laser induced fluorescence, as previously described (Volpi and Lindhardt, 2010).

Study population

In total, 237 subjects were retrospectively enrolled in this study, 218 patients and 19 healthy volunteers as controls. Following eligibility, 194 patients with pre-op samples were included in this study.

The median age at diagnosis was 60 years (IQR: 52-67, Table 1). The most common pathologic diagnosis was RCC (N=162 patients, 90%), followed by oncocytoma (N=7, 4%) and angiomyolipoma (N=6, 3%). The median age at diagnosis in the healthy cohort was 55 years (IQR: 50-60), and included 6 males and 13 females.

Limited to the sub-cohort of 162 RCC cases, the demographic characteristics were similar to the patient cohort (Table 2). The most common histological subtype was ccRCC (N=113, 70%). Of the remaining 49 non-ccRCC, the most common histological subtype was papillary RCC (pRCC, N =25), followed by chromophobe RCC (chRCC, N =17). Most RCC cases were localized (pT1, N =86 [53%]), with the vast majority below 4 cm in size (pT1a, N = 66). The remaining RCC cases were predominantly locally-advanced (Stage II or III, N = 66). Finally, there were 12 cases of advanced disease (pM1, N =11 and pT4, N =1).

Table 1

Factors	Patients	Healthy
	N = 179	*N* = 19
Age	60 [52-67]	55 [50-60]
Gender		
Female	56	13
Male	123	6
Race		
White American	161	0
African American	9	0
Asian American	3	0
Other/Not Available	6	19
Diagnosis		
Renal cell carcinoma	162	
Oncocytoma	7	
Angiomyolipoma	6	
Urothelial cell carcinoma	2	
Other benign	2	

Table 2

Factors	RCC
	N = 162
Age [years]	59.5 [52-67]
Gender	
Female	45
Male	117
Histological subtype	
Clear cell	113
Non-clear cell	49
Chromophobe	17
Mucinous tubular and spindle cell	2
Papillary Type I	18
Papillary Type II	3
Papillary (unspecified)	4
Unclassified	5
Tumor size [cm]	4.5 [2.6 – 7]
AJCC stage	
Stage I	86
Stage II	6
Stage III	57
Stage IV	12
Not Available	1
Grade	
Not Available	35
Fuhrman nuclear grade	111
2	31
3	59
4	21
Other grading system	17
High	7
Low	10

Clustering analysis reveals plasma GAG alterations across RCC stages and histologies

We performed a principal component analysis (PCA) to ascertain in an unbiased fashion how similar the overall GAG profiles were across pre-operative RCC and healthy volunteers' samples (Figure 1A). The PCA plot evidenced that samples obtained from RCC patients pre-operatively tended to cluster as a separate group with limited overlap with those obtained from healthy volunteers.

We used unsupervised hierarchical clustering based on the between-sample GAG profile correlation to validate the PCA results and to highlight the GAGs that contributed most to the separation of RCC from healthy subjects (Figure 1B). Five properties were noticeably altered in RCC va. healthy both in this cohort and in historical cohorts (Figure 2).

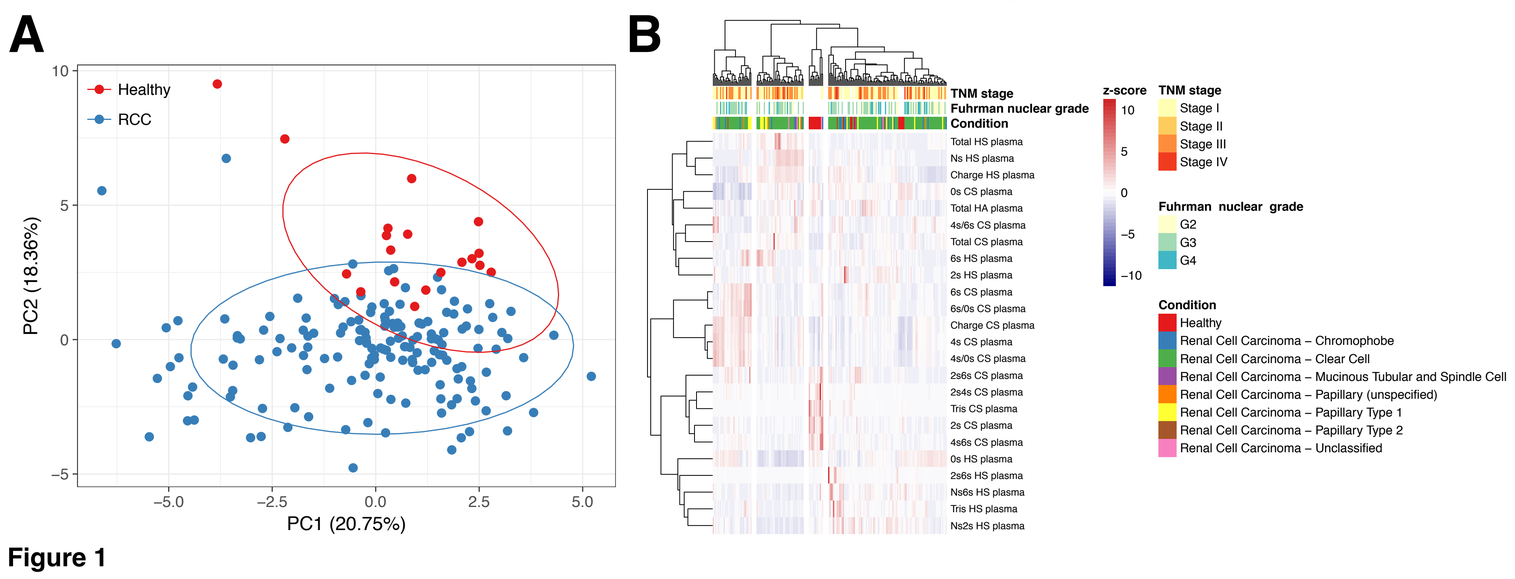

Figure 1

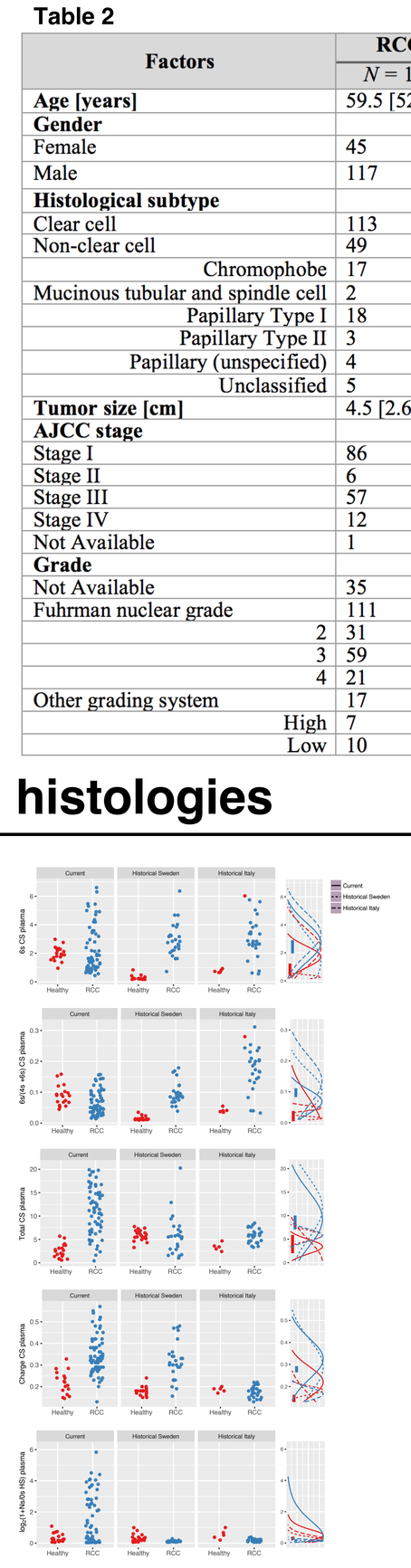

Figure 2

GAG scores detected any stage RCC with 95.7% sensitivity

We sought to redefine the GAG scores in order to incorporate GAG property alterations observed in the current cohort as well as those observed in historical cohorts.

The new plasma GAG score achieved an AUC equal to 0.999, with 98.8% accuracy, 94.7% specificity and 100% sensitivity at an optimal cut-off equal to 0.87 (Figure 3). The new GAG score was elevated in all RCC samples, irrespective of stage and histology. The new GAG score performed similarly in our historical cohorts, with AUC = 1 in the Italian cohort (N=28) and 0.988 in the Swedish cohort (N=46, Figure 4).

The new GAG score achieved an AUC equal to 0.994 (95% CI: 0.985 - 1) in the validation set. At the pre-specified cut-off, the validated sensitivity was 95.7% (Figure 3).

The new GAG score did not correlate with age, gender, nor histopathologic features. However, two of its constituent GAG properties showed a weak correlation with tumor stage, size and/or histology (Figure 5).

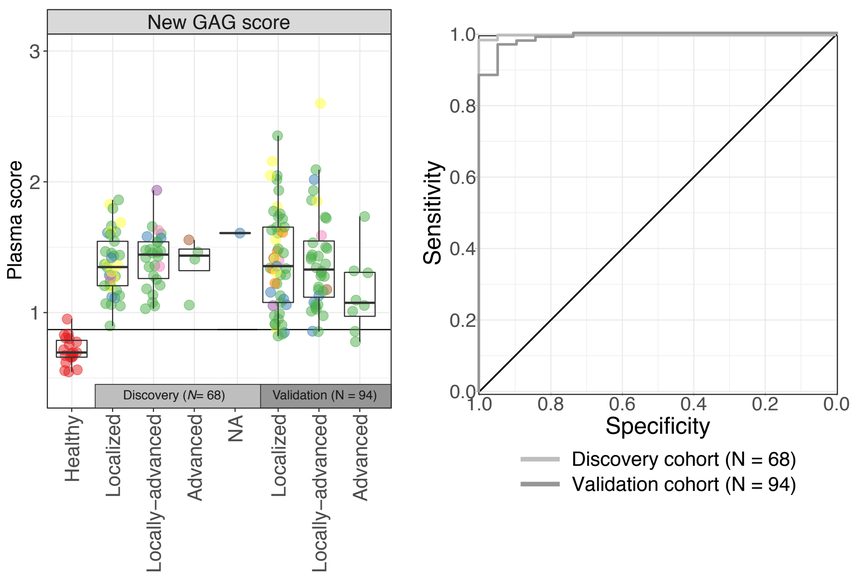

Figure 3

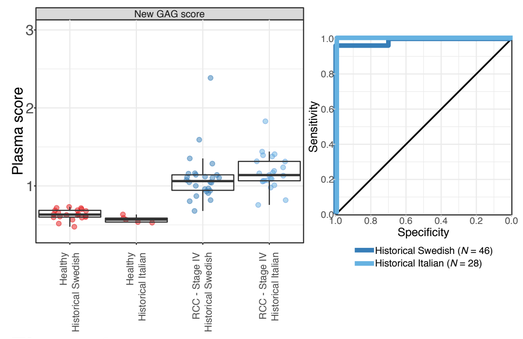

Figure 4

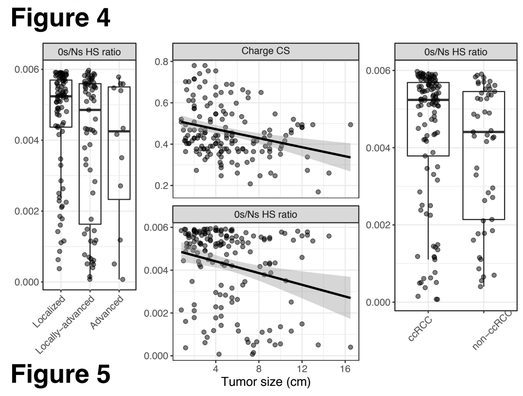

Figure 5

Conclusions

Plasma GAGs defined a specific signature in RCC patients vs. healthy subjects (N=179). By updating the definition of GAG scores, it was possible to detect RCC irrespective of its stage or histology with 95.7% sensitivity in an independent validation set. The lack of correlation with pathologic features suggests that the process beyond the GAGs' alterations is triggered concomitantly with tumor formation but independent of its progression.
This large scale retrospective study warrants prospective trials to validate the clinical utility of multiple applications for GAG scores as diagnostic biomarkers in RCC.

References
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