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Laboratory-Kidney cancer

Age-related variations in gene expression patterns of renal cell carcinoma

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Abstract

Background: Clear cell renal cell carcinoma (ccRCC) is known to occur across the adult lifetime traversing the spectrum of age-related organismal changes. Little is known as to how the aging process may affect the course of renal cell carcinoma (RCC) and the repertoire of genes involved.

Methods: Using The Cancer Genome Atlas ($n = 436$) and Cancer Genomics of the Kidney ($n = 89$) datasets, we applied regression analysis to examine associations between patient age and gene expression profiles in ccRCC tumors and normal kidney tissues. Pathway enrichment analysis was performed to identify cellular process that is affected by aging in ccRCC. Moreover, connectivity mapping analysis was used to predict age-dependent response to drug treatments.

Results: Our analysis revealed different age-dependent gene expression spectra in ccRCC and normal kidney tissues. These findings were significant and independently reproducible in both datasets examined. Age up-regulated genes, showing higher expression in older patients, were significantly enriched (false discovery rate <0.05) in normal tissues for pathways associated with immune response and extracellular matrix organization, whereas age up-regulated genes in tumors were enriched for metabolism and oxidation pathways. Strikingly, age down-regulated genes in normal cells were also enriched for metabolism and oxidation, while those in tumors were enriched for extracellular matrix organization. Further in silico analysis of potential drug targets predicted preferential efficacy of Phosphoinositide 3-kinase inhibitor or immunotherapy in association with age.

Conclusion: We report on previously unrecognized associations between age and molecular underpinnings of RCC, including age-associated expression of genes implicated in RCC development or treatment.

Keywords: Aging; Cancer genomics; Cancer therapy; Gene expression analysis; Renal cell carcinoma

1. Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer, with 63,340 new cases and 14,970 deaths expected in 2018 in the United States [1]. The most common type is clear cell RCC (ccRCC), accounting for about 85% of cases. ccRCC is molecularly characterized by loss of the von Hippel Lindau gene, an event implicated in de-regulation of processes including hypoxia response and vascular endothelial growth factor (VEGF)-driven angiogenesis. However, the molecular evolution of ccRCC is complex and results in altered expression of genes involved in growth, extracellular matrix (ECM) formation and immunoregulation [2]. Consequently, targeted agents directed at tumor stroma, such as VEGF pathway of angiogenesis and immune checkpoint inhibitors (ICIs), have revolutionized treatment and extended lives of patients with advanced disease [3]. Unfortunately these gains are restricted by the variability

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and transiency of therapeutic responses, the reasons for which remain poorly defined.

Several factors could contribute to the interindividual diversity among cancer patients. Their disease course could be affected not only by cell-intrinsic factors, but also by age-related changes impacting the vasculature, immune system and stroma [4]. Little is known in this regard about ccRCC, a disease which affects adults across a wide age spectrum. Whether and how aging and comorbidities such as atherosclerosis may affect the biology and therapy of ccRCC has scarcely been considered.

Interestingly, earlier studies suggested that blood vessels in ccRCC may exhibit age-related alterations [5]. Moreover, transplantable tumors were shown to grow less and respond favorably to VEGF antagonists in old atherosclerotic mice compared to younger animals [6,7]. These observations suggest that while the core pathways responsible for cellular transformation in ccRCC dictate stromal and vascular responses, their magnitude and effect may be modulated by age-related processes, presently largely unstudied. In order to glean insights as to this relationship, we examined the association between ccRCC patient age and gene expression profiles of tumors and corresponding normal tissues, and investigated the associated translational impacts.

2. Materials and methods

2.1. Patients and datasets

Gene expression and clinical data from 2 independent genomic studies of ccRCC were used; The Cancer Genome Atlas (TCGA) [8] and the Cancer Genomics of the Kidney (CAGEKID) [9] program of the International Cancer Genome Consortium. Information including the number of samples corresponding to sex, tumor grade, and tumor stage for each dataset can be found in Table 1.

2.2. Age-associated gene expression and pathways

To evaluate the association between patient age and gene expression, multiple linear regression was performed independently in R [10] on TCGA and CAGEKID datasets for normal and tumor samples separately, with gene expression as the dependent variable and age, sex, tumor grade, and tumor stage as independent variables. Genes with higher age coefficients were considered to be up-regulated with increased age, while those with lower age coefficients were considered to be down-regulated with age. Correlation tests were performed on the age coefficients for normal and tumor samples to evaluate consistency between results from the TCGA and CAGEKID datasets. Fisher's exact test was then performed to examine significance of overlap for genes with high or low age regression coefficients (within the top or bottom 1,000) in both datasets. The common genesets were subject to pathway analysis using ConsensusPathDB [11].

A heatmap was generated showing the signed logarithm of P value for enrichment of pathways among up-regulated (red) or down-regulated (blue) genes in each patient. Specifically, in each individual, a nonparametric Mann-Whitney U test was used to examine whether genes that belong in each pathway showed a significantly different expression than other genes, and the logarithm of P value was used to visualize the results.

2.3. Association with stage and survival

A Mann-Whitney U test was used to estimate association between patient age and tumor stage. A log-rank test

Table 1
Clinical data breakdown for CAGEKID and TCGA datasets.

Characteristic	Parameter	TCGA	CAGEKID	P value
Type	Tumor	436	89	
	Paired Normal	69 (16%)	43 (48%)	
Age	Range	26–90	35–83	
	Mean	60.9	61.1	
	Median	61	61.7	
Sex	Male	285 (65%)	50 (56%)	0.128
	Female	151 (35%)	39 (44%)	
Grade (Furhman nuclear)	1	8 (2%)	3 (3%)	0.00028
	2	184 (42%)	54 (61%)	
	3	179 (41%)	15 (17%)	
	4	65 (15%)	17 (19%)	
Stage (Tumor, Node, Metastasis)	1	211 (48%)	49 (55%)	0.524
	2	43 (10%)	9 (10%)	
	3	114 (26%)	22 (25%)	
	4	68 (16%)	9 (10%)	

CAGEKID = Cancer Genomics of the Kidney; TCGA = The Cancer Genome Atlas.
The P value represents statistical difference in the proportion between the two datasets for the parameter examined using chi-squared test.

comparing patients below and above median age was performed to determine the association between patient age and overall survival. Cox proportional hazards models using stage as a covariate was additionally used to estimate this association independently of tumor stage.

Further details regarding statistical analyses are provided in Supplementary Information.

3. Results

3.1. Age-associated gene expression patterns in ccRCC

We observed a spectrum of age associations among genes for both normal and cancer tissues (Fig. 1A). Significant correlation was found between results from CAGEKID and TCGA datasets ($R = 0.416$, $P < 2.2 \times 10^{-16}$ and $R = 0.403$, $P < 2.2 \times 10^{-16}$ for tumor and normal samples, respectively, Fig. 1A), demonstrating reproducibility of these associations. Among the top 1,000 age-associated genes in each dataset, 294 and 383 were commonly age up-regulated and age down-regulated, respectively in tumors in both datasets ($P < 2.2 \times 10^{-16}$, Fisher's exact test; Table S1A; Fig. 1B); indicating a significant overlap between genes with the same pattern in independent patient cohorts. Among normal samples, 395 and 402 genes were age up-regulated and age down-regulated, respectively ($P < 2.2 \times 10^{-16}$, Fisher's exact test; Table S1B). Fold-enrichment of overlap compared to chance ranged from 3.51 to 4.58 (Fig. 1C).

3.2. Molecular pathways and processes affected by age-associated gene expression

To gain insight about cellular functions that may be affected by age-related gene expression, we performed pathway analysis of top age-associated genes. Pathways significantly enriched for age-related gene expression (false discovery rate [FDR] <0.05) were different in normal and tumor cells (Table 2, Table S2).

Of particular note was the presence of opposite age-relationship patterns. Whereas ECM and cell adhesion pathways were significantly enriched for genes up-regulated with age in normal kidney, these pathways were down-regulated with age in tumors (Fig. 2A). Opposite relationships were also observed for pathways involved in metabolism and oxidation, which were significantly age down-regulated in normal kidney and age up-regulated in tumors (Table 2). Interestingly, these pathways are known as being de-regulated in ccRCC when compared to normal kidney [9]. Various immune system functions were up-regulated with age in normal cells, with tumors also showing age up-regulation for tumor necrosis factor receptor 2 noncanonical NFκB pathway and toll-like receptor signaling. Similar results were obtained using a more stringent pathway analysis (Table S3). Analysis of enriched Gene Ontology terms was additionally performed revealing down-regulation of angiogenesis with age in tumors (FDR = 1.05×10^{-5}).

3.3. Tumor stage and patient sex as modulators of age-related gene expression in ccRCC

We repeated our analyses using only stage 1 patients, to ensure the results were not being influenced by association between tumor stage and age. The resulting age down-regulated pathways were similar to preceding results, however age up-regulated pathways were more enriched with immune-related pathways compared to when all samples were analyzed.

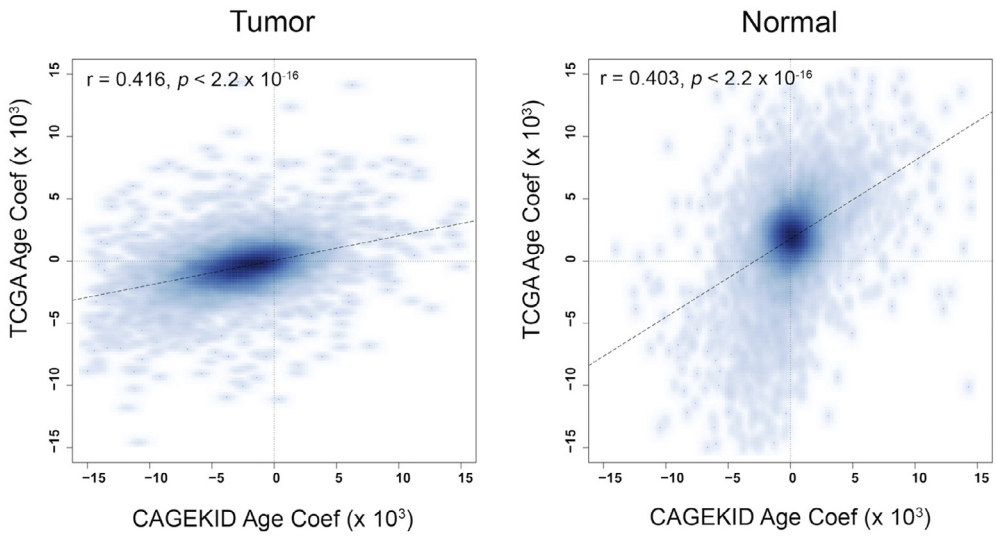
We also separated age-related gene expression changes between male and female patients. Immune system pathways particularly concerning tumor necrosis factor signaling were found more strongly age up-regulated in females ($P = 0.01$ with FDR = 0.069 in males, $P = 0.002$ with FDR = 0.024 in females), and Notch pathways were found preferentially age down-regulated in females ($P = 0.21$ with FDR = 0.38 in males, $P = 0.008$ with FDR = 0.048 in females).

3.4. Age-dependent regulation of stromal genes in ccRCC

Given that ECM and immune system pathways were enriched with age-associated gene expression patterns, and in view of the clinical relevance of stroma and immune cell infiltration in RCC [12,13], we set out to determine to what extent the respective gene groupings were associated with patient age. Yoshihara et al. [14] had previously reported on genes whose expression levels represent the stromal and immune compositions of tumor samples. Using these gene-sets, they assigned unique stromal and immune scores to each of 329 TCGA [8] patient tumors via Gene Set Expression Analysis. Our analysis using those scores revealed a negative association between tumor stromal score and age ($r = -0.186$, $P = 0.00068$), while there was no correlation between immune score and age ($r = 0.001$, $P = 0.98$).

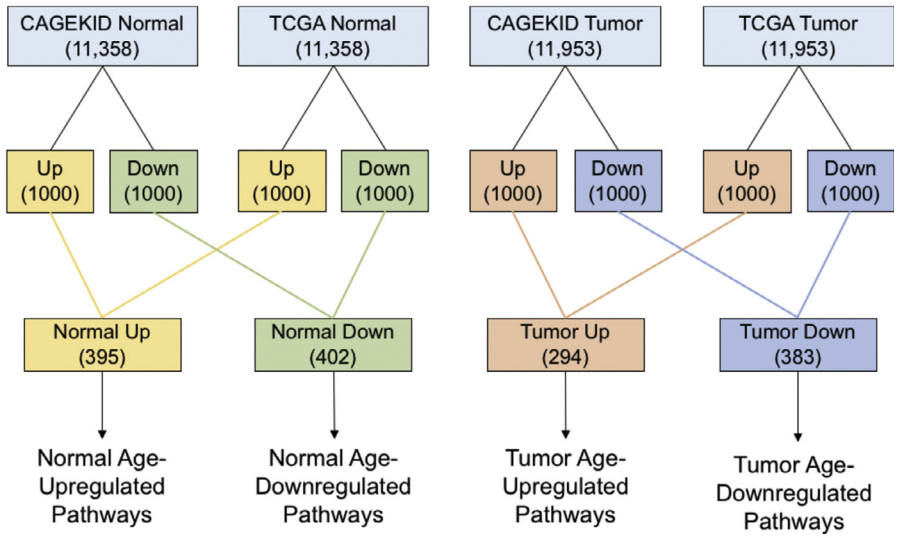
To validate these results, we used Weighted Gene Co-Expression Network Analysis [15] to generate stromal and immune eigengene (E) scores for gene coexpression profiles in tumor samples of 436 TCGA patients and of 89 CAGEKID patients (see Supplementary Information). The stromal score only was indeed significantly correlated with patient age in both TCGA ($r = -0.138$; $P = 0.0038$) and CAGEKID ($r = -0.26$; $P = 0.013$) datasets (Fig. 2B), confirming the association. Yoshihara's stromal signature included several genes with previously reported connection to RCC. Representative genes included ECM members, especially collagens (COL4A1, COL4A2, and COL18A1), and members of the Notch/Jagged signaling pathway (NOTCH3, JAG1, DLL1) (Fig. S1), many of which are involved in tumor stromal interactions and angiogenesis [5,16,17].

A



B

1. Selected genes with 1,000 highest (Up) and lowest (Down) age regression coefficients for each set of results
2. Obtained genes shared between corresponding datasets
3. Performed downstream pathway analysis of genes shared in both datasets



C

Type	Relationship with Increased Age	No. of Genes	Fold-enrichment	*p* value
Normal	Upregulated Downregulated	395 402	4.49 4.57	$< 2.2x10^{-16}$ $< 2.2x10^{-16}$
Tumor	Upregulated Downregulated	294 383	3.51 4.58	$< 2.2x10^{-16}$ $< 2.2x10^{-16}$

Fig. 1. (A) Plot of obtained age beta coefficients from CAGEKID vs. TCGA regression analyses for tumor samples and normal samples. There are significant correlations between the results from both datasets. (B) Flow chart of methods used to obtain age-associated pathways for normal and tumor samples. (C) The overlap between the age-associated genes obtained from CAGEKID and TCGA regression analyses in both normal and tumor samples is significant as compared to chance. CAGEKID = Cancer Genomics of the Kidney; TCGA = The Cancer Genome Atlas.

Table 2
Top age-associated pathways.

Age down-regulated			
Type	Pathway	FDR	Source
Tumor	Extracellular matrix organization	8.24E−28	Reactome
	Protein digestion and absorption	4.85E−16	KEGG
	Collagen formation	7.61E−16	Reactome
	Beta1 integrin cell surface interactions	3.08E−13	PID
	Integrins in angiogenesis	3.24E−09	PID
	Elastic fibre formation	1.40E−08	Reactome
	Integrin cell surface interactions	2.91E−08	Reactome
	Muscle contraction	8.67E−07	Reactome
	Focal adhesion	0.000223	KEGG
	Axon guidance	0.000883	Reactome
	Glycosaminoglycan metabolism	0.00138	Reactome
	Activation of matrix metalloproteinases	0.00139	Reactome
	AGE-RAGE signaling pathway in diabetic complications	0.00153	KEGG
	Developmental biology	0.00222	Reactome
	Signal transduction	0.00245	Reactome
	Regulation of IGF transport and uptake by IGFBPs	0.00298	Reactome
	PI3K-Akt signaling pathway	0.00339	KEGG
	Binding and uptake of ligands by Scavenger receptors	0.00600	Reactome
	Signaling by NOTCH	0.00686	Reactome
Normal	Metabolism	8.45E−08	Reactome
	SLC-mediated transmembrane transport	3.48E−07	Reactome
	Biological oxidations	3.83E−07	Reactome
	Retinol metabolism	8.46E−07	KEGG
	Transport of inorganic cations/anions and amino acids	4.12E−05	Reactome
	Drug metabolism − cytochrome P450	8.28E−05	KEGG
	Transmembrane transport of small molecules	0.000375	Reactome
	Protein digestion and absorption	0.000500	KEGG
	Chemical carcinogenesis	0.000650	KEGG
	FOXA2 and FOXA3 transcription factor networks	0.00166	PID
Age up-regulated			
Type	Pathway	FDR	Source
Tumor	Metabolism	0.000107	Reactome
	Biological oxidations	0.000214	Reactome
	Drug metabolism − cytochrome P450	0.000905	KEGG
	Metabolism of amino acids and derivatives	0.000968	Reactome
	Regulation of TLR by endogenous ligand	0.00596	Reactome
	Drug metabolism − other enzymes	0.00816	KEGG
	Aquaporin-mediated transport	0.0100	Reactome
	TNF signaling pathway	0.0117	KEGG
	GPCR ligand binding	0.0123	Reactome
	Metal sequestration by antimicrobial proteins	0.0196	Reactome
Normal	Immune system	1.21E−08	Reactome
	Hematopoietic cell lineage	5.10E−08	KEGG
	TCR signaling	5.18E−07	Reactome
	PD-1 signaling	3.50E−06	Reactome
	Immunoregulatory interactions between a lymphoid and a nonlymphoid cell	4.92E−06	Reactome
	Cell adhesion molecules (CAMs)	7.23E−06	KEGG
	NF-kappa B signaling pathway	1.92E−05	KEGG
	IL12-mediated signaling events	3.98E−05	PID
	GPCR downstreasm signaling	5.40E−05	Reactome
	Complement cascade	6.18E−05	Reactome
	Innate immune system	0.000102	Reactome
	GPCR ligand binding	0.000131	Reactome
	Extracellular matrix organization	0.000131	Reactome

FDR = false discovery rate.

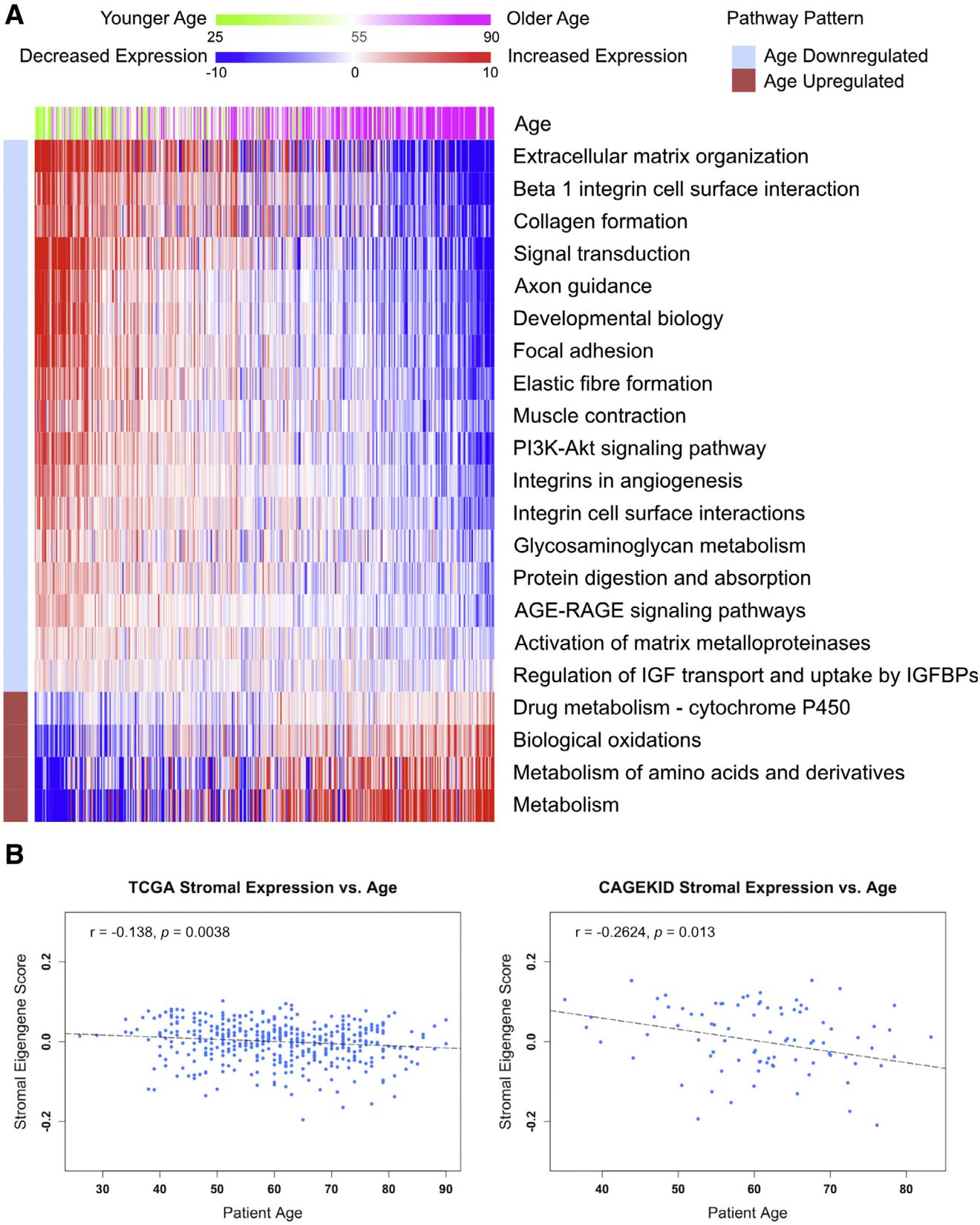


Fig. 2. (A) Heatmap of top age-associated pathways enriched in tumor samples from the TCGA dataset. Pathway expression is represented by the signed log *P* value of a Mann-Whitney *U* test comparing expression level of genes in the pathway and genes not in the pathway. (B) Plots of patient age vs. stromal eigengene scores for CAGEKID and TCGA. There is significant correlation between age and stromal score in both datasets, with increased age being associated with lower stromal activity. CAGEKID = Cancer Genomics of the Kidney ; TCGA = The Cancer Genome Atlas.

3.5. Potential impacts of age-associated gene expression on ccRCC treatments

Of interest was whether our age-associated gene expression patterns could have clinical significance by influencing response to drug treatment [7]. To address this, we used the Broad Institute's Connectivity Map (cmap) [18] datasets, which provide information about changes in gene expression patterns in response to treatment with different molecules. Analysis of age-regulated genes in ccRCC identified 32 agents that could be predicted to possess age-dependent anticancer activity (Table S4), the top-ranked being LY-294002 ($P < 2.2 \times 10^{-16}$, Kolgorov-Smirnov), a known PI3K inhibitor with anti-RCC activity [19]. Although no ccRCC cell line data was available in cmap, we observed that treatment of prostate adenocarcinoma (PC3) cells with LY-294002 resulted in increased expression of genes down-regulated with age, and decreased expression of genes up-regulated with age.

Recent studies have indicated that ICIs may prolong survival in some ccRCC patients [20]. However, what defines responders to ICI therapy remains poorly understood. Given that outcomes depend partly on the extent of immune cells infiltration into the tumor [21], which in turn is influenced by ECM organization, we sought to examine if ccRCC age-associated gene expression show different patterns with regards to potential response to ICI treatment. Although there was no report on gene expression directly related to ICI treatment responses for ccRCC, Hugo et al. [22] reported on genes differentially expressed between melanoma patients who responded or not to anti-programmed cell death protein 1 (PD-1) therapy. Their pathway analysis of the 532 genes overexpressed in nonresponders showed significant enrichment for cell adhesion, ECM organization and angiogenesis, similar to the pathways containing age down-regulated genes in ccRCC (Fig. 3A). Moreover, of the 532 genes associated with PD-1 resistance, 69 were among the 383 age down-regulated genes in RCC tumors; a significant overlap (4.05 fold-enrichment; $P < 2.2 \times 10^{-16}$, Fisher's exact test, Fig. 3B). We also observed that within the melanoma study, mean and median age was higher in PD-1 responders, but no statistical analysis was possible due to small sample size (Fig. S2).

3.6. The impact of age on the clinical course of ccRCC

The aforementioned gene expression studies would be expected to impinge upon age-related clinical characteristics of ccRCC, such as aggressiveness and survival. Indeed, our analysis of the available TCGA treatment-naïve ccRCC dataset showed association between patient age and cancer stage at time of presentation ($P = 0.0051$, Mann-Whitney U test, Fig. 4A). Thus, patients with stage 3 to 4 diseases tended to be older than those with early stage. Moreover, age also correlated with poor survival ($P = 1.88 \times 10^{-5}$, logrank test; Fig. 4B) even after adjusting for tumor stage ($P = 1.4 \times 10^{-7}$, Cox proportional hazards regression), highlighting the role of age in overall outcomes and consistent with previous reports [23,24].

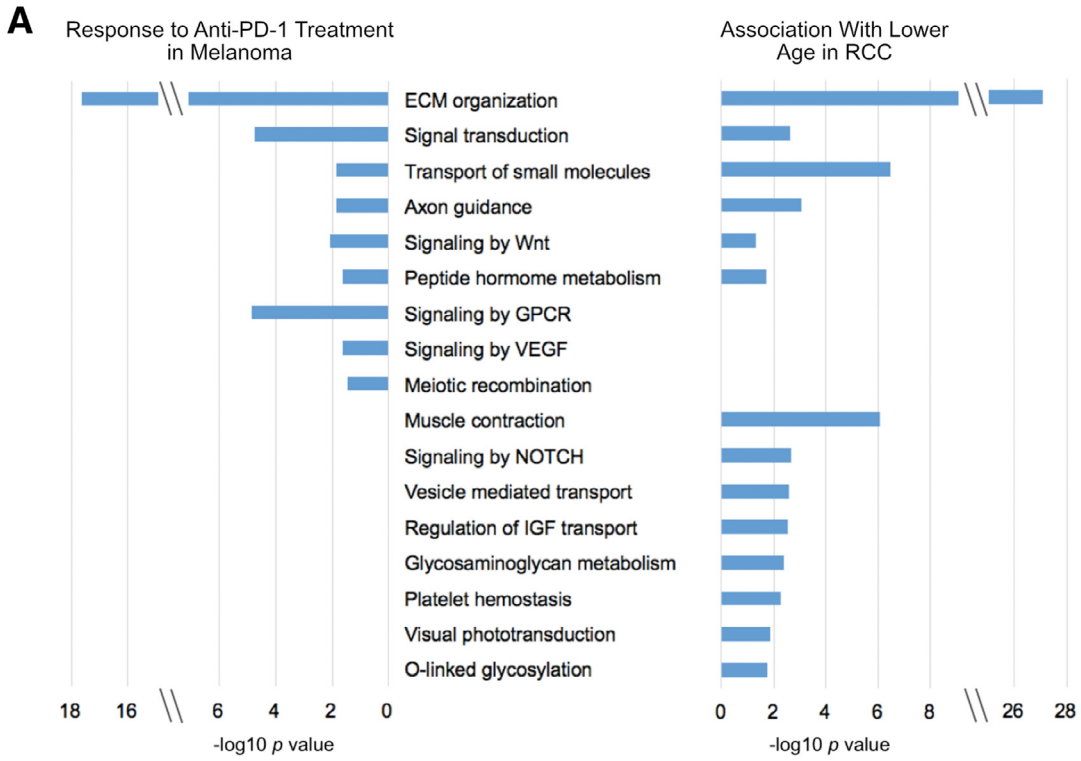
4. Discussion

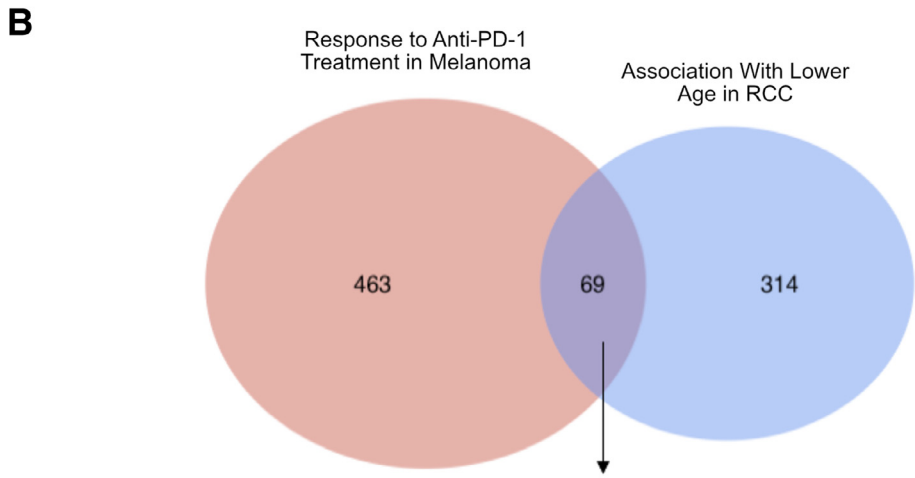
Our study explores the role of aging in shaping the transcriptome of ccRCC. In this regard, we made several novel observations with potential clinical implications. Incidence of many cancers is strongly age-dependent, with specific types often confined to pediatric, adult or elderly populations. CcRCC, however represents an interesting case where histologically and clinically similar disease may occur in patients ranging from 20 to 80 years of age. Moreover, the commonality of von Hippel Lindau mutations and the resulting role of hypoxia and angiogenesis pathways in ccRCC strongly link pathogenesis with vascular and stromal tissue responses. Cellular populations contributing to these responses undergo profound changes during an individual's lifetime, as exemplified by age-related decline in angiogenesis [4]. As these populations are of great interest as targets of current therapies in ccRCC [25] their biological changes due to aging are of considerable relevance.

We identified antithetical age-related de-regulation of genes associated with pathways including metabolism and oxidation – an intriguing discovery meriting further investigation. We particularly noted an age-associated decrease in stromal gene expression signature and genes involved in ECM organization in tumors, as opposed to normal tissue. A large proportion of ECM-related genes encode collagen family of proteins. De-regulation of collagens is involved in angiogenesis and tumor progression [16,26,27], and further exploration of their role in modulating metastasis as a function of age is warranted. Among ECM-related genes, those mapped to the JAG/Notch pathway were also affected. DLL1 expression was previously found to be significantly higher in tumors of younger RCC patients [5], albeit mainly in precapillary endothelial cells.

We observed that genes associated with angiogenesis are age down-regulated in RCC, consistent with previous reports [5]. This suggests that targeted therapies blocking angiogenic pathways may differ in their effects in younger and older patients. Preclinical data suggests that indeed, the effects of sunitinib are greater in old-atherosclerotic mice [7]. Since our study documents age-related changes in targets and modulators of therapeutic antiangiogenesis it is possible that, while active, these agents may exert mechanistically different effects and resistance patterns in patients of different age. Further data are needed to address these questions.

Interestingly, our analysis revealed exclusive age down-regulation of Notch pathways in females. As this signal may reflect vascular or stem cell contributions, it is possible that younger females with ccRCC exhibit different corresponding phenotypes [28] than older patients, with opportunities to develop age/sex-matched therapies. Similarly



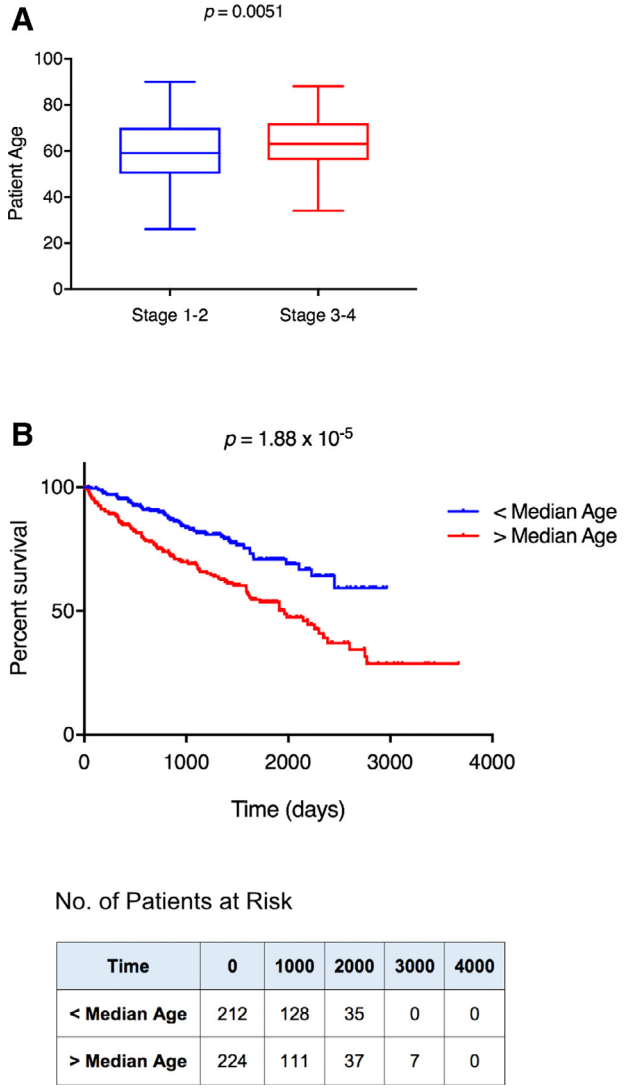


Enriched Pathways	FDR
ECM organization	5.95×10^{-17}
Axon guidance	6.84×10^{-5}
PI3K-Akt signaling pathway	0.00868
Vesicle-mediated transport	0.0108

Fig. 3. (A) Pathways found significantly enriched in genes up-regulated in tumors of melanoma patients who did not respond to anti-PD-1 treatment (Hugo et al.) and in genes down-regulated with age in RCC. Both sets of genes are particularly enriched for ECM organization. (B) Venn diagram of the number of genes significantly up-regulated in melanoma nonresponders (Hugo et al.) and the number of genes down-regulated with age in RCC. Sixty-nine genes are found to overlap and are significantly enriched for ECM organization, axon guidance, PI3K-Akt signaling pathway, and vesicle-mediated transport. ECM = extracellular matrix; RCC = renal cell carcinoma.

intriguing is the increased age up-regulation of immune system pathways in female tumors. In this light, it is of great interest to assess the sex-related responses to ICIs in female patients of different age, as PD-1/PDL-1 inhibitors enter the therapeutic armamentarium in ccRCC [29].

In stage 1 tumors, we found increased enrichment of immune-related pathways among age up-regulated genes, similar to our results from analyzing normal kidney tissue. This is of great interest as an indication of early immune response involvement in development of ccRCC in a manner



No. of Patients at Risk

Time	0	1000	2000	3000	4000
< Median Age	212	128	35	0	0
> Median Age	224	111	37	7	0

Fig. 4. (A) Patient age vs. RCC stage in TCGA dataset. Increased age is significantly associated with higher stage. (B) Kaplan-Meier survival analysis comparing survival between younger patients (<median age) and older patients (>median age) in TCGA dataset. Older age is significantly associated with shorter overall survival. TCGA = The Cancer Genome Atlas.

affected by age. Indeed, immune-related genes were less prominent when tumors of all stages were included in the analysis, suggesting that a therapeutic 'rescue' of this potential should be considered, and indeed, may be the basis of efficacy in the contexts of ICI treatment in metastatic ccRCC.

With regards to anti-PD-1 therapy, the results from Hugo et al.'s data indicating greater effectiveness in older patients would need to be validated by a larger study. Given that ccRCC age-dependent alteration in ECM expression might affect drug efficacy, this is worthy of further investigation. Interestingly a PI3K inhibitor was also the top hit in our cmap analysis. This pathway is suggested as a clinical target [8]. It now appears that PI3K targeting alters age-related gene expression patterns in RCC, which may be key to future effectiveness against RCC as a function of age.

Our study does have several limitations. First, although we used the largest available gene expression datasets, the number of samples was limited and found to differ in distribution of tumor grade (albeit a known subjective factor). Furthermore, it is important to elucidate the relationship between age and ccRCC progression in view of host and tumor cell subsets populating heterogeneous lesions [2]. While this study was not meant to look exclusively at the cancer cell population, we could not confidently discriminate between signals originating from cancer and stromal cells. This can be dissected through single cell transcriptome analysis in the future. It is also important to separate aging as such from age-related diseases, especially those affecting therapeutic ccRCC targets such as the immune system and vasculature. Our study did not have this capacity.

5. Conclusions

Overall our study reveals the impact of age on the molecular repertoire of ccRCC. To the best of our knowledge, such a comprehensive analysis of the interrelationship between aging and RCC has not been described previously. We now have evidence that there are notable differences in tumor-associated pathway regulation between younger and older ccRCC patients, which may be therapeutically actionable. In order to successfully exploit these differences and improve patient outcomes, we must better understand the mechanisms behind those differences. Thus, further efforts are needed to improve our understanding of ccRCC biology and devise a better, more personalized and age-appropriate care for this daunting disease.

Author contributions

Lara Feulner had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Rak, Riazalhosseini, Najafabadi.

Acquisition of data: Feulner.

Analysis and interpretation of data: Feulner, Najafabadi, Tanguay, Rak, Riazalhosseini.

Drafting of the manuscript: Feulner, Riazalhosseini.

Critical revision of the manuscript for important intellectual content: Najafabadi, Tanguay, Rak, Riazalhosseini.

Statistical analysis: Feulner, Najafabadi.

Obtaining funding: Rak, Riazalhosseini.

Administrative, technical, or material support: None.

Supervision: Rak, Riazalhosseini.

Other (specify): None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.urolonc.2018.11.006.

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