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Radiogenomics in renal cell carcinoma

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Abstract

Radiogenomics, a field of radiology investigating the association between the imaging features of a disease and its gene expression pattern, has expanded considerably in the last few years. Recent advances in whole-genome sequencing of clear cell renal cell carcinoma (ccRCC) and the identification of mutations with prognostic significance have led to increased interest in the relationship between imaging and genomic data. ccRCC is particularly suitable for radiogenomic analysis as the relative paucity of mutated genes allows for more straightforward genomic-imaging associations. The ultimate aim of radiogenomics of ccRCC is to retrieve additional data for accurate diagnosis, prognostic stratification, and optimization of therapy. In this review article, we will present the *state-of-the-art* of radiogenomics of ccRCC, and after briefly reviewing updates in genomics, we will discuss imaging-genomic associations for diagnosis and staging, prognosis, and for assessment of optimal therapy in ccRCC.

Key words: Radiogenomics—Clear cell renal cell carcinoma—Genomics—CT—MRI

Radiology has traditionally been focused on the relationship between imaging characteristics of a disease and its pathologic correlates. In recent years, the advent of new high-throughput sequencing techniques and the availability of data derived from the Human Genome Project have transformed all fields of biomedical research, due to increased accessibility of genomic information [1, 2]. Radiogenomics represents the imaging complement of this transformation, a field investigating and associating the imaging features of a disease and its gene expression patterns or molecular phenotype [3]. First radiogenomic analyses date back to a decade ago when the association between presence of arterialization on CT and expression of genes related to doxorubicin drug response for hepatocellular carcinoma was identified, and when the association between the contrast to necrosis ratio on MRI and epithelial growth factor receptor overexpression in glioblastoma was found [4, 5]. The goal of radiogenomics has been to non-invasively capture additional data for diagnosis and staging, for prognostication, and ultimately for assessment of optimal therapy based on the imaging features of a disease [6].

Recent advances in genome sequencing of renal cell carcinoma (RCC) have led to the identification of multiple mutations with prognostic significance, which has in turn increased interest in the association of cross-sectional imaging features with these mutations [7–9]. The vast majority of genomic data from Cancer Genome Atlas Program pertain to clear cell RCC (ccRCC), the most common subtype of RCC [7]. For many years, mutations of the von Hippel–Lindau (VHL) tumor suppressor gene have been recognized in ccRCC [10]. VHL is located on the short arm of chromosome 3, and inactivation results in upregulation of hypoxia-inducible factor and triggers the neoangiogenic cascade [10]. More recently, genomic analysis from The Cancer Genome Atlas (TCGA) Research Network has led to the identification of multiple histone-modifying and chromatin-remodeling gene mutations in ccRCC, including polybromo 1 (PBRM1), BRCA1-associated protein 1 (ubiquitin carboxy-terminal hydrolase) (BAP1), SET domain containing 2 (SETD2), and lysine (K)-specific demethylase 5C (KDM5C) [11–15].

The importance of radiogenomics resides in the possibility that imaging contains data not available from genomic testing, since gene mutations and expression are assessed on small samples and may not reflect the heterogeneity of the disease, a common feature of ccRCC [16]. In addition, it has been demonstrated that tumor

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cells with similar genotypes may show different phenotypes, and this discrepancy could be overcome by the analysis of the association between gene expression and imaging phenotypes [17]. In this review article, we will present the current knowledge of radiogenomics of ccRCC; we will introduce recent updates in genomics and discuss imaging-genomic associations with diagnostic, prognostic, and therapeutic implications in ccRCC.

Genomics

Recent comprehensive molecular characterization of ccRCC has revealed several common genetic alterations in ccRCC affecting a relatively small number of genes to date. For example, one study of over 400 ccRCC tumors identified nineteen significantly mutated genes, largely involved in cell metabolism and epigenetic regulation of pathways [7]. Somatic gene mutations commonly involve the short arm of chromosome 3p, which encompasses four of the most frequently mutated genes (VHL, PBRM1, BAP1, and SETD2) [7, 18–20]. Although a relatively small number of genes are commonly mutated in ccRCC, high intratumoral mutation heterogeneity has been observed, underestimating the gene expression profile portrayed from single tumor-biopsy samples, with major challenges to targeted therapy and biomarker development [16]. Thus, radiogenomic analysis is likely particularly valuable in ccRCC, because genomic alterations present in areas not covered by sampling and direct genomic analysis may eventually be inferred from imaging characteristics of the tumor. Table 1 shows the most common mutations occurring in sporadic ccRCC. Deletion involving the short arm of chromosome 3 is observed in 90% of ccRCC [7, 19, 21].

Inactivation of the VHL gene, first observed in ccRCC in 1988, occurs in more than 50% of ccRCC either by mutation or other means of inactivation [10, 20, 21]. The VHL protein forms a complex with elongin C (TCEB1) and other proteins, which has a role in degradation of hypoxia-inducible factor (HIF) [21]. If VHL or TCEB are inactivated, HIF is constitutively activated, promoting neo-angiogenesis and cell growth (Fig. 1) [22, 23]. A recent meta-analysis has shown that VHL gene alteration has no prognostic or predictive value in patients with ccRCC [24].

The second most frequently mutated gene in ccRCC is the tumor suppressor PBRM1, often co-deleted with VHL [7, 19–21]. PBRM1 encodes for BAF180, a nucleosome remodeling complex, limiting DNA accessibility to RNA polymerases and transcription factors [25]. Studies on the impact of PBRM1 on prognosis are not consistent; nonetheless, a recent study has shown that PBRM1 mutational status may influence response to immune checkpoint inhibitors in ccRCC [26–30]. Interestingly, VHL and PBRM1 mutations are thought to occur early in ccRCC tumorigenesis [31].

Table 1. Most commonly mutated genes of clear renal cell carcinoma and their associated imaging features

Gene	Imaging features	Mutation frequencies (%)	Prognostic significance of genomic mutations	Position	References
VHL	Well-defined tumor margins nodular tumor enhancement intratumoral vascularization solid appearance	52.3*	No prognostic value	3p25.3	[7, 8, 20, 21, 23]
PBRM1	Renal vein invasion solid appearance	31–32.9***	No prognostic value	3p21.1	[7, 19–21]
BAP1	Ill-defined tumor margins calcification	10–15***	Decreased survival	3p21.1	[7, 20, 30]
SETD2	NA	10–15***	Non-metastatic ccRCC: unfavorable prognosis metastatic ccRCC: prolonged survival	3p21	[7, 20, 30–32, 34]
KDM5C	Renal vein invasion	6–7***	ccRCC < 4 cm: decreased survival from recurrence or cancer-related death; metastatic ccRCC: prolonged survival	Xp11.22	[7, 20, 32, 33, 35]
MUC4	Exophytic growth	5.66***	Decreased survival in ccRCC who underwent nephrectomy	3q29	[7, 19, 37]
TP53	NA	2.2–6***	Decreased survival	17p	[7, 19, 20, 41]

*According to The Cancer Genome Atlas research network [7]
**According to Forbes et al. [20]
***According to Sato et al. [19]
NA, no association identified; ccRCC, clear cell renal cell carcinoma

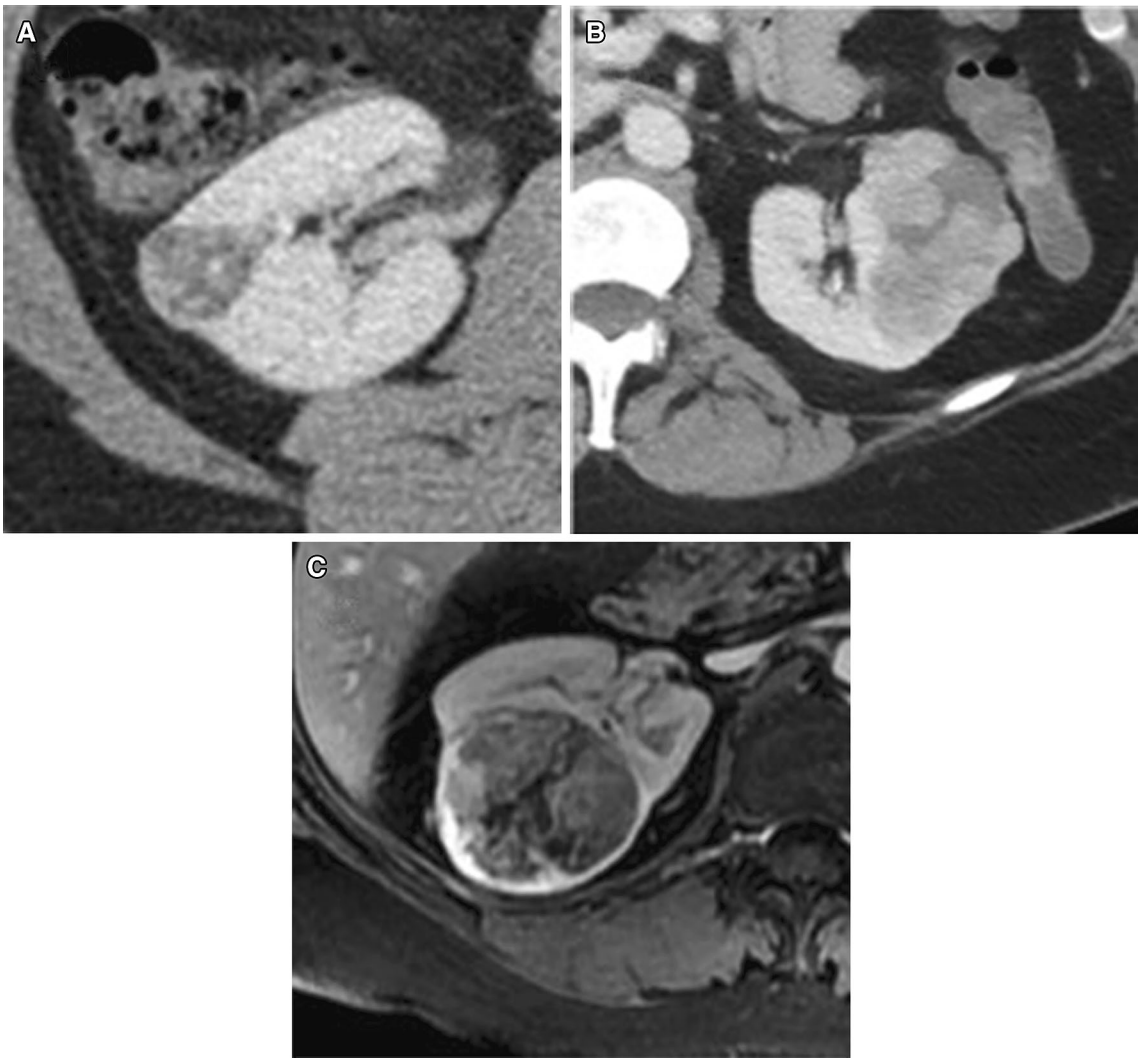

Fig. 1. Representative examples of imaging features associated with von Hippel–Lindau (VHL) mutations in clear cell renal cell carcinoma (ccRCC), including well-defined tumor margins, nodular enhancement, and intratumoral vascularization. Axial contrast-enhanced CT (**A**) of a 56-year-old man with hematuria demonstrates a 2.5-cm ccRCC with documented VHL mutation showing well-defined tumor margins and intratumoral vascularization. Axial contrast-enhanced CT (**B**) of a 61-year-old woman with an incidentally detected ccRCC with VHL mutation showing nodular enhancement within the renal mass. Axial fat-suppressed gadolinium chelate-enhanced T1-weighted image (**C**) of a 63-year-old woman with ccRCC and VHL mutation show well-defined tumor margins and intratumoral vascularization.

The BAP1 tumor suppressor gene is also located on 3p between PBRM1 and VHL [20]. BAP1 is mutated in 10–15% of patients with ccRCC and encodes for a deubiquitinase protein, which regulates protein degradation [20, 21, 32]. Mutation of BAP1 is generally mutually exclusive of PBRM1 and is more commonly associated with the presence of coagulative necrosis, high Fuhrman grade, and mechanistic target of rapamycin complex 1 (mTORC1) activation [14, 33, 34]. Patients with tumors harboring BAP1 mutations show shorter median overall survival when compared with patients harboring wild-type counterparts [35]. Rare ccRCC demonstrating BAP1 and PBRM1 mutations has been shown to be aggressive, associated with shorter survival when compared with BAP1-mutated PBRM1-non-mutated ccRCC [26].

The SETD2 tumor suppressor gene, also located in chromosome 3p, is mutated in 10–15% of ccRCC [7, 20]. SETD2 codes for a histone methyltransferase protein involved in regulation of gene expression [12]. Mutations in SETD2 and low SETD2 expression have been associated with unfavorable prognosis in ccRCC, though in one metastatic ccRCC cohort, SETD2 mutations were associated with prolonged survival [34–38].

KDM5C is a histone demethylase gene involved in transcription regulation and chromatin remodeling,

mutated in 6–7% of ccRCC [7, 20, 39]. In a study of 203 patients with ccRCC smaller than 4 cm, mutation of KDM5C was associated with inferior outcomes related to recurrence or cancer-related death [40]. Interestingly, in patients with metastatic ccRCC, the presence of mutated KDM5C has been associated with prolonged survival [37]. Another recent study showed that "exceptional responses" to vascular endothelial growth factor (VEGF)-targeted therapies or duration of treatment greater than 21 months were more frequent among patients with selected genomic alterations, including KDM5C, which may help to explain the association with prolonged survival in patients with metastatic disease [41].

The mucin-4 (MUC4) gene on chromosome 3q codes for a glycoprotein membrane-bound mucin and whole-exome analysis of ccRCC performed by Sato et al. showed that MUC4 is mutated significantly more often than in normal renal tissue, which may indicate a role in RCC pathogenesis [19]. In general, mucins are involved in protecting cells from injury, differentiation of epithelium, and modulation of cell adhesion and cell signaling [42]. Aberrations in mucin genes and their expression are involved in tumorigenesis and/or tumor growth in multiple cancers, though the prognostic significance of this expression does vary with the type of malignancy [42, 43]. A recent analysis of 198 patients with ccRCC who underwent nephrectomy showed that low expression of MUC4 (as opposed to high expression) is associated with significantly decreased overall survival [44].

The Tumor Protein P53 (TP53) gene on chromosome 17p encodes for a tumor suppressor protein which regulates expression of target genes, ultimately inducing cell cycle arrest and apoptosis [45]. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers related to Li–Fraumeni syndrome [46, 47]. TP53 is mutated in 2–6% of ccRCC [7, 19, 20]. A large pooled analysis of patients with ccRCC showed that mutations in TP53 were associated with decreased survival and resistance to VEGF-targeted agents [27, 36].

Radiogenomics

Various studies on the radiogenomics of ccRCC have yielded promising results in this nascent field [3, 8, 9, 48]. These studies have focused on the identification of imaging-genomic correlates, use of radiogenomics as a tool to retrieve additional data on gene expression profiling, and prognostication in ccRCC. Studies on the role of radiogenomics in defining optimal therapy in patients with ccRCC are currently ongoing [49].

Imaging-genomic correlates and gene expression profiling

Several CT imaging features have been reportedly associated with gene expression in ccRCC. These include the presence of well- or ill-defined tumor margins, nodular tumoral enhancement, intratumoral vascularization, cystic or solid composition, exophytic or endophytic growth pattern, presence of renal vein invasion, and intratumoral calcifications [8, 9]. Association between CT and MRI imaging features and mutated genes is shown in Table 1.

A study on 233 patients with ccRCC investigated the association between CT features of ccRCC and the presence of mutations in several genes (VHL, PBRM1, BAP1, SETD2, and KDM5C) [8]. Well-defined tumor margins, nodular tumor enhancement, and intratumoral vascularization were associated with VHL mutations (Fig. 1), while renal vein invasion was significantly associated with KDM5C and BAP1 mutations (Figs. 2, 3). Mutations of VHL and PBRM1 were more common in solid than in cystic tumors [8]. A study of 103 cases of ccRCC derived from the TCGA and The Cancer Imaging Archive (TCIA) (81 cases were evaluated with CT, 19 cases with MRI and 3 cases with CT and MRI) showed that ill-defined tumor margins and calcifications were associated with BAP1 mutation and exophytic growth was associated with MUC4 mutation (Fig. 4) [9]. In addition, this study highlighted the significant interobserver variability for the various imaging features evaluated when readers from different institutions across the country reviewed the images [9]. Specifically, interobserver agreement was low for margins (well- or ill-defined) ($\alpha = 0.300$), composition (solid or cystic) ($\alpha = 0.558$), presence or absence of necrosis ($\alpha = 0.429$), and growth pattern (endo or exophytic) ($\alpha = 0.640$). Interobserver agreement was relatively higher for the presence or absence of calcifications ($\alpha = 0.722$).

Assessing tumor prognosis

Two types of studies have investigated the role of radiogenomics in assessing ccRCC prognosis: studies inferring prognostic information from the expression of mutated genes and studies investigating the feasibility of a prognostic score to define low- and high-risk ccRCC based on imaging findings.

Known associations between CT and/or MRI findings, mutated genes, and relative prognostic significance are outlined in Table 1. In summary, the presence of ill-defined tumor margins and calcifications are associated with BAP1 mutation, which is commonly seen on ccRCC of high Fuhrman grade, while exophytic growth is associated with MUC4 mutation, which in turn is associated with decreased survival [9]. The presence of renal vein invasion is significantly associated with BAP1 and KDM5C mutation [8]. Mutation of KDM5C in turn is associated with decreased survival in patients with small ccRCC, though paradoxically with prolonged survival in patients with metastatic ccRCC [37, 40].

Regarding studies of prognostic scores, a radiogenomic risk score (RRS) for ccRCC was constructed in

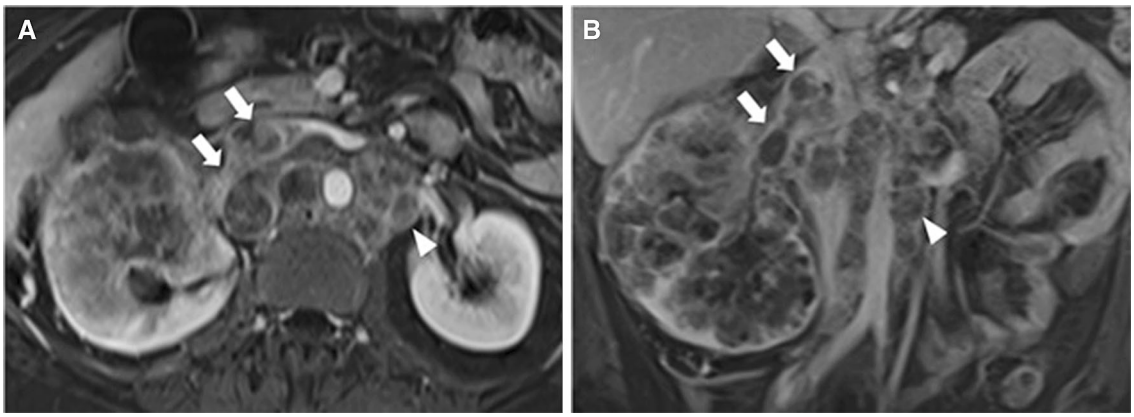

Fig. 2. A 56-year-old woman with ccRCC and BRCA1-associated protein 1 (BAP1) mutation. Axial (**A**) and coronal (**B**) fat-suppressed gadolinium chelate-enhanced T1-weighted images demonstrate a large heterogeneously enhancing ccRCC mass replacing most of the right kidney, with tumor thrombus extension to the renal vein and inferior vena cava (arrows). Radical nephrectomy and thrombectomy were performed, demonstrating a 16-cm ccRCC with sarcomatoid and rhabdoid differentiation (~ 30%) and Fuhrman nuclear grade IV/IV. BAP1 mutations have been associated with high Fuhrman grade, renal vein invasion, and ill-defined tumor margins, each demonstrated in this case. Extensive retroperitoneal adenopathy was also present (arrowheads).

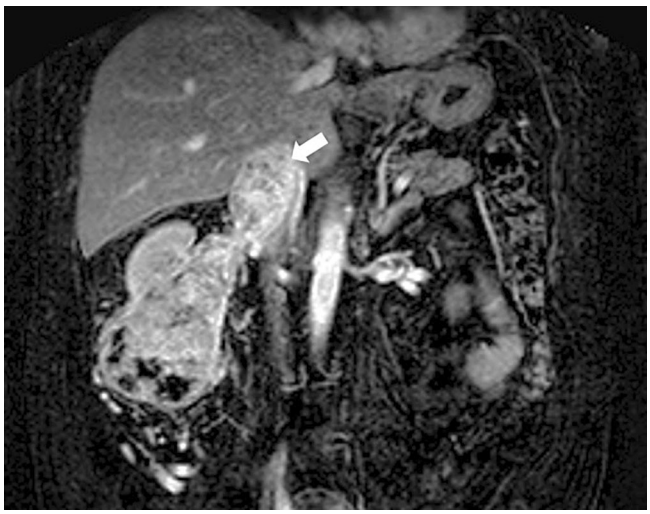

Fig. 3. A 62-year-old man with ccRCC and lysine (K)-specific demethylase 5C (KDM5C) mutation. Coronal fat-suppressed gadolinium chelate-enhanced T1-weighted image at presentation demonstrates a large, heterogeneously hypervascular mass of the lower pole of the right kidney, with retroperitoneal neovascularization and tumor thrombus extending into the inferior vena cava (arrow). KDM5C mutation has been associated with renal vein invasion, inferior outcomes in small renal masses, and "exceptional responses" to vascular endothelial growth factor-targeted agents in metastatic disease. Interestingly, this patient has been on multiple sequential therapies over the last 6 years (sunitinib, pazopanib, everolimus, and others) and remains alive and on treatment today.

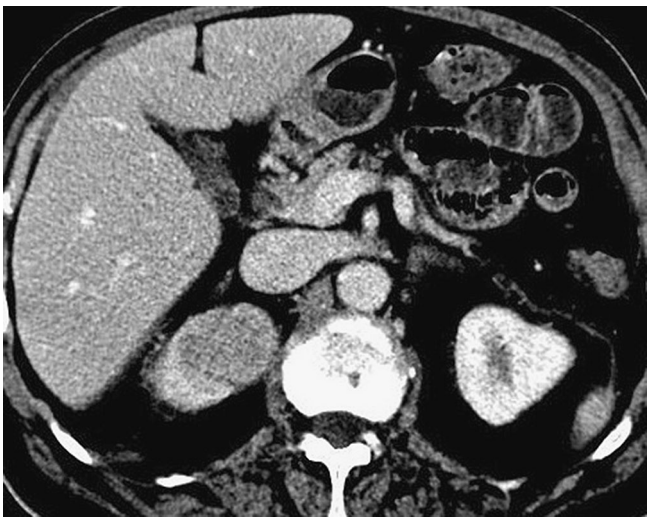

Fig. 4. An 83-year-old man with ccRCC and MUC 4 mutation. Axial contrast-enhanced CT shows a 4-cm mass exophytically arising from the right upper renal pole. MUC4 mutations have been associated with an exophytic growth pattern. Nephrectomy revealed ccRCC with focal sarcomatoid areas, Fuhrman nuclear grade III/IV.

a study of 147 ccRCC [48]. This study combined clinical data, data extracted from genomic analysis, and data from preoperative CT images of ccRCC to build a score predictive of survival with potential applicability in clinical trials [48]. In this study, four imaging features (presence of necrosis, sharp or infiltrating transition zone between tumor and normal renal parenchyma, presence of a discrete enhancing rim or presence of a hypoattenuating rim circumscribing the tumor) were associated with sets of gene transcript (defined as trait-associated gene) to create the RRS. Each imaging trait was given a high or low risk score, and the imaging traits were combined to define a high- or low-risk-score phenotype. Similarly, trait-associated genes were clustered into high- and low-risk gene expression subgroups [44]. Subse-

quently, correlation between the imaging phenotypes and the gene expression subgroups was performed to define a high- and a low-RRS group [48]. Survival analysis confirmed that the high-RRS group had significantly lower disease-specific survival rates than the low RRS, independent of disease stage and grade, in training and validation cohorts.

Assessing optimal therapy

The promising results of the RRS study led to the application of this score for stratifying survival of patients with ccRCC undergoing systemic treatment. A study of 41 metastatic ccRCC patients evaluated the potential of the RRS for stratifying radiological progression-free survival of patients undergoing pre-surgical treatment with bevacizumab, demonstrating a significant difference in progression-free and overall survival between patients with high- and low-RRS values, with the high-risk group having significantly worse outcomes than the low-risk group, independent of grade and stage of disease [49].

Current limitations of radiogenomics

Current ccRCC radiogenomic research has several limitations [50, 51]. The goal of radiogenomics is to associate highly reproducible imaging features with genomic biomarkers that are strongly associated with a clinically meaningful outcome. Nonetheless, most current radiogenomic studies seek association between imaging features and genomic features associated with the disease, some of which do not necessarily relate to a prognostic outcome (Table 1). Furthermore, given the complexity of gene expression mechanisms and intracellular signaling pathways, direct linkage of imaging data to genomic and prognostic features might be biased, and could suggest association between entities not directly associated. In addition, radiogenomics is inherently biased by the difficulties in matching data contained within the imaging studies to large amounts of genomic data. A commonly used approach is to group individual genetic mutations into gene modules or gene traits before performing association analysis with imaging features; however, this may undermine the potential of imaging to predict patient outcomes. Furthermore, there is substantial interobserver variation in the definition and recognition of qualitative imaging features correlated, highlighting the need for quantitative imaging assessments when possible. Moreover, published radiogenomic studies have been performed on small or limited sample sizes, which is likely related to the difficulty in obtaining patients with both appropriate imaging studies and adequate tissue samples for genomic analysis [50, 51]. This limits the generalizability and reproducibility of these studies. Another limitation relates to the discrepancy between the data present on imaging studies and the small tissue sample used for genomic analysis, potentially reflecting the heterogeneity of the tumor within a site or multiple sites [50, 52, 53].

In addition, it should be noted that imaging features of a tumor are not only related to gene expression profile, but also to a variety of factors including patient characteristics, environmental factors, and imaging technique. Associating imaging features only to gene expression, without accounting for other factors, might introduce structural biases in radiogenomic analyses.

Future directions

The field of radiogenomics has the potential to advance insights into cancer biology, elaborate prognostic and predictive markers, and further optimize management in cancer patients with the use of imaging data [51].

Numerous studies in the last 10 years have sought to correlate imaging changes and response kinetics with outcomes in patients with advanced/metastatic disease, without exploring the specific underlying radiogenomic correlates associated with biological behavior. Studies to correlate response patterns and underlying genetics are ongoing. For example, in one cohort, mutations in mechanistic target of rapamycin (MTOR), tuberous sclerosis 1 (TSC1), or tuberous sclerosis 2 (TSC2) were found to be more common in patients who experienced clinical benefit from rapalogs than in those who progressed (Fig. 5) [54]. Given the number of therapies now available in metastatic RCC including targeted therapies and immune checkpoint inhibitors, it may be relevant to characterize radiogenomic features of a patient's disease at diagnosis and over the course of treatment, to capture changes in gene expression patterns and mutational composition to obtain valuable prognostic information and to tailor treatment accordingly.

While published studies have mostly focused on qualitative data, additional information could be obtained to correlate quantitative imaging data and genomic analysis [55]. Conversely, genomic data have been obtained mostly on microarray data. Few studies focused on the prognostic significance of micro-RNAs, non-protein-coding small RNAs which can potentially target hundreds of genes and serve as gene regulators [51, 56–58]. In the field of quantitative imaging, promising results have been obtained with CT and MR-based texture analysis, a histogram-based quantitative technique for assessing heterogeneity in tumor images, which has been successfully used as a non-invasive imaging biomarker of prognosis and treatment response [59–64]. A study of 39 metastatic RCC, 37 of which were ccRCC, showed that entropy, a CT texture analysis quantitative parameter reflecting tumor heterogeneity, is an independent factor associated with time to progression [62]. In another study including 92 ccRCC with unenhanced CT images, multiple quantitative CT

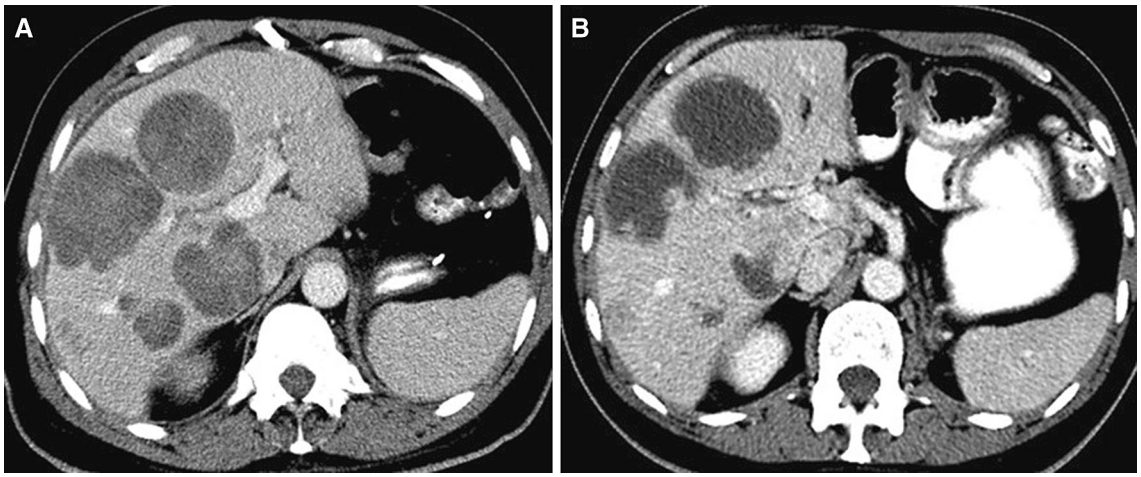

Fig. 5. A 49-year-old man with ccRCC metastatic to liver and tuberous sclerosis 1 (TSC1) mutation. Axial contrast-enhanced CT at baseline (**A**) demonstrates multiple liver metastases. After 8 weeks of mechanistic target of rapamycin (mTOR) inhibitor treatment, liver metastases have mildly decreased in size and decreased in density (**B**). Mutations in MTOR, TSC1, or TSC2 genes have been found to be more common in patients who experience clinical benefit from rapalogs compared to those who progress.

texture parameters were associated with time to disease recurrence and death due to disease [63].

A recent study of 40 patients with ccRCC treated with sunitinib focused on the prognostic value of quantitative parameters of CT-based texture analysis, demonstrating that size-normalized standard deviations of the pixel values measured on pretreatment and follow-up CT were predictors of progression-free survival and overall survival [64].

In recent years, with the expansion of artificial intelligence (AI), radiomics, which builds quantitative tumor phenotypes by using automated high-throughput extraction of quantitative features of medical images, has significantly expanded in every field of cancer imaging, including ccRCC, and has been proposed as a method to accurately predict mutational status in various cancers [65–69]. In addition, radiomic can be used to extract valuable information on pathologic features and prognostic data and to predict tumor response to treatment [70]. Radiomic methods are either based on automated recognition of predefined imaging features or on AI deep-learning methods that can learn imaging feature representation from example data [65, 67, 71]. Nonetheless, studies are often flawed in experimental design or analytic methodology, undermining the acceptance and development of data science in radiology [67].

A laudable approach to advance the quality of analytics in quantitative imaging is to develop publicly available resources containing multidimensional data sets, such as TCIA, or TCGA for genomic data [72, 73]. These resources are particularly useful, given the difficulty in obtaining genomic data, due to need for small fresh tissue samples, and for the great quantity of data that can be extracted from these datasets.

Conclusion

Radiogenomic research in RCC is a promising, developing field. Familiarity with the commonly encountered genetic alterations in this disease and the prognostic significance of these is integral to the identification of clinically meaningful radiogenomic correlates. Limitations to this type of research include difficulties inherent in attempts to match disparate data sets, observer variability in imaging assessment, tumor heterogeneity especially in ccRCC, and the availability of tissue and genetic information. These limitations can be overcome with aims at quantitative methods, increasing publicly available resources, and interdisciplinary collaboration.

Compliance with ethical standards

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Conflict of interest Francesco Alessandrino, MD: no conflict of interest to declare. Atul B. Shinagare, MD: Consultant, Arog Pharmaceuticals; research funding, GTx Inc. Dominick Bossé, MD: no conflict of interest to declare. Toni K. Choueiri, MD: Research funding by AstraZeneca, BMS, Exelixis, Genentech, GSK, Merck, Novartis, Peloton, Pfizer, Roche, Tracon, Eisai. Katherine M. Krajewski, MD: no conflict of interest to declare.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Statement of informed consent is not applicable since the manuscript does not contain any patient data.

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