Letter to the Editor

High CT attenuation of clear cell renal cell carcinoma as a possible radiogenomic sign of *GPX8* gene mutation

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Background: Glutathione peroxidase 8 (GPX8) has an active role on *de novo* lipid synthesis in clear cell renal cell carcinoma (ccRCC). GPX8 knockout or downregulation, in fact reduces lipid droplet levels, *de novo* fatty acid synthesis and triglyceride esterification in vitro, regardless of lipid uptake. The objective of this study is to investigate the presence of *GPX8* gene mutation in ccRCC using radiogenomic approach on computed tomography (CT) images.

Methods: In this retrospective study, we enrolled 13 ccRCC patients divided into two groups: 1 ccRCC patient with *GPX8* gene mutation and 12 ccRCC patients without *GPX8* gene mutation. Hounsfield Unit (HU) values on unenhanced CT images were acquired in solid non-hemorrhagic tumor tissue to estimate the levels of intracellular lipid droplets.

Results: The tumor of patient with *GPX8* gene mutation showed sharply higher attenuation value (i.e., HU 51) than the mean HU values of the 12 ccRCC patients without *GPX8* gene mutation (i.e., mean HU 40.9). The high HU value detected in ccRCC with *GPX8* gene mutation may be related to the reduction of intracellular lipid droplets that characterizes the loss of normal activity of GPX8 on *de novo* fatty acid synthesis.

Conclusions: The results of this study lead to hypothesis that high intratumor attenuation values detected on unenhanced CT images may be linked to expression of *GPX8* gene mutation in ccRCC. Future studies, on larger series, are needed to confirm this hypothesis.

Keywords: Clear cell renal cell carcinoma (ccRCC); computed tomography (CT); gene; GPX8; radiogenomics

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Introduction

The glutathione peroxidase (GPX) family is composed by GPX1-4 and GPX6, with a selenocysteine at the active site and catalyzes reactive oxygen species (ROS) detoxification. Moreover, GPX5 and GPX7-8 show a cysteine which can perform additional functions (1). GPX7 and GPX8 have a remarkable similarity and are localized in the endoplasmic reticulum. GPX8 differs from GPX7 due to the presence of an additional sequence that binds the membrane and serine instead of otherwise conserved glutamine at the catalytic tetrad. Peroxides formed during oxidative protein folding in the endoplasmic reticulum by protein disulfide isomerase and endoplasmic reticulum oxidoreductase 1 alpha (ERO1A) are removed by GPX7 and GPX8. These common functions

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are due to their very similar molecular structure (2). GPX8 holds several functions including the regulation of calcium homeostasis in endoplasmic reticulum, protection against chemical-induced colitis and microsomal lipid composition regulation (3-5).

The cytoplasm of clear cell renal cell carcinoma (ccRCC) can contain abundant lipids and glycogen, with the characteristic clear appearance of ccRCC (6). It is known that the high lipid content has been implicated in tumor progression and pathophysiology of ccRCC (6), suggesting a connection between ccRCC and metabolic status.

Nguyen *et al.* reported the role of GPX8 on *de novo* lipid synthesis in ccRCC, demonstrating how, independently from lipid uptake, GPX8 knockout or downregulation decreased lipid droplet levels, fatty acid *de novo* synthesis and triglyceride esterification *in vitro* (7). Indeed, the link between lipid metabolism, genomics and pathogenesis of ccRCC is well known (8-10).

Knowledge of metabolic profiles is critically important to understand ccRCC pathogenesis. For example, ferroptosis is a type of cell death dependent on iron and linked to oxidative stress (11). This process is based on the accumulation of cytoplasmic lipid reactive oxygen species and the disintegration of mitochondria (12). The analysis of the expression of Acyl-CoA synthetase long-chain family member 4 (ACSL4) and the evaluation of ferroptosis indicators, such as lipid reactive oxygen species, lead to the hypothesis that a lower level of ferroptosis is linked to the pathogenesis and development of ccRCC (13). ACSL4, in addition to being an indicator and modulator of ferroptosis, determines the degree to which ferroptosis can modify the cellular lipid content (14). ACSL4, in fact, is able to catalyze the synthesis of fatty acids (14).

Radiogenomics is a research field targeting the association between molecular processes, genomic of diseases, and imaging phenotypes or macroscopic appearance visible as imaging features (15,16).

Unenhanced computed tomography (CT) Hounsfield Unit (HU) values of adipose tissue range from -100 to -50, while unenhanced CT HU values of renal cell carcinoma (RCC) without calcified regions range from 20 to 70 (17).

Starting from these concepts we hypothesized that *GPX8* gene mutation in ccRCC could be suspected at unenhanced CT images.

Methods

A total of 13 caucasian male patients with ccRCC from TCGA-KIRC (kidney renal clear cell carcinoma) was selected: patients without *GPX8* gene mutation (n=12; mean age: 56.5 years, range, 39–79 years) and 1 patient with *GPX8* gene mutation (age: 46 years). Cancer staging was T2N0M0 in 7 patients and T2NxM0 in 6 patients (18,19).

To measure HU CT-based attenuation, a region of interest (ROI) was placed for each patient at the level of solid tissue without calcification that appeared more hyperdense to the naked eye at unenhanced CT. Calcifications were skipped to avoid an increase of density not related to the solid tumoral portion.

Results

The tumor of patient with *GPX8* gene mutation showed sharply higher attenuation value (i.e., HU 51) than the mean HU values of the 12 ccRCC patients without *GPX8* gene mutation (i.e., mean HU 40.9) (*Figure 1*). The high attenuation found in ccRCC with *GPX8* gene mutation was clearly distinguished from a possible hyperdense blood component due to the homogeneous contrast enhancement in the postcontrast images during nephrographic phase. This feature confirmed that the ROI was placed on the solid non-hemorrhagic tumor tissue (*Figure 2*).

Discussion

The high HU value detected in ccRCC with *GPX8* gene mutation can be interpreted as loss of the normal activity of GPX8 which, with reduction or abolishment of fatty acid *de novo* synthesis and consequent reduction of intracellular lipid droplets.

Kidney cancer with “clear cell” with high lipid content is the most common subtype of kidney cancer, while in tumors of other organs such as lung, breast, liver, and brain it is very rare (7). This has sparked great interest on how this high lipid content is deposited in ccRCC (7).

The study of GPX8 in lipid metabolism showed clear differences between the wild type and GPX8 knockout (GPX8-KO) cell lines, as well as a reduction in intermediates for glycerophospholipid metabolism in the GPX8-KO cells (7). Differences were also found in RNA-seq transcriptomic analysis, in particular GPX8-KO cells showed 484 downregulates and 662 upregulates genes compared to wild type cells (7). Functional analysis showed a downregulation of glycosphingolipid biosynthesis, a downregulation of lysophospholipid pathways, and an upregulation of lipid breakdown (7). Specific biochemical

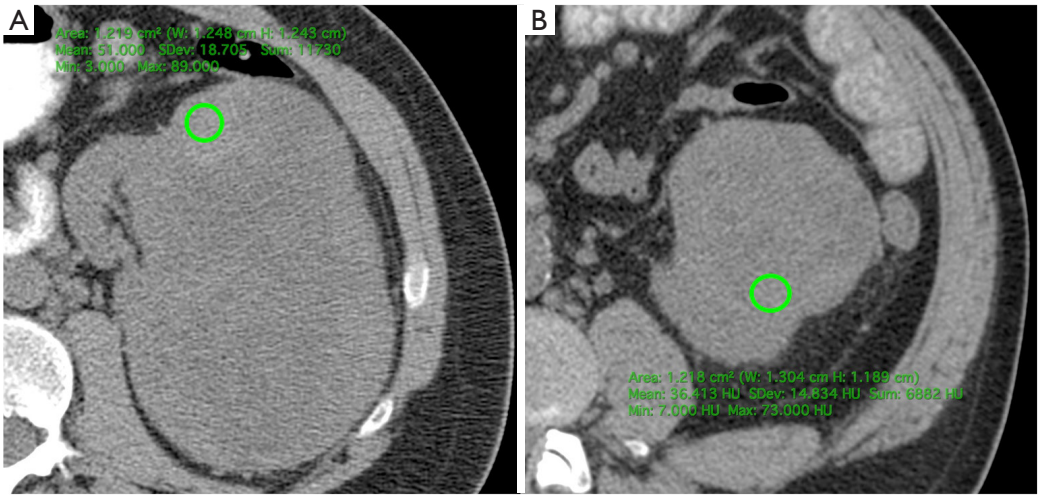

Figure 1 Unenhanced axial CT images of male ccRCC patients with *GPX8* gene mutation (A) and without *GPX8* gene mutation (B) show green ROIs with different mean attenuation values of ccRCC solid tissues without calcified regions (HU 51 and 36.4 respectively). CT, computed tomography; ccRCC, clear cell renal cell carcinoma; ROI, region of interest.

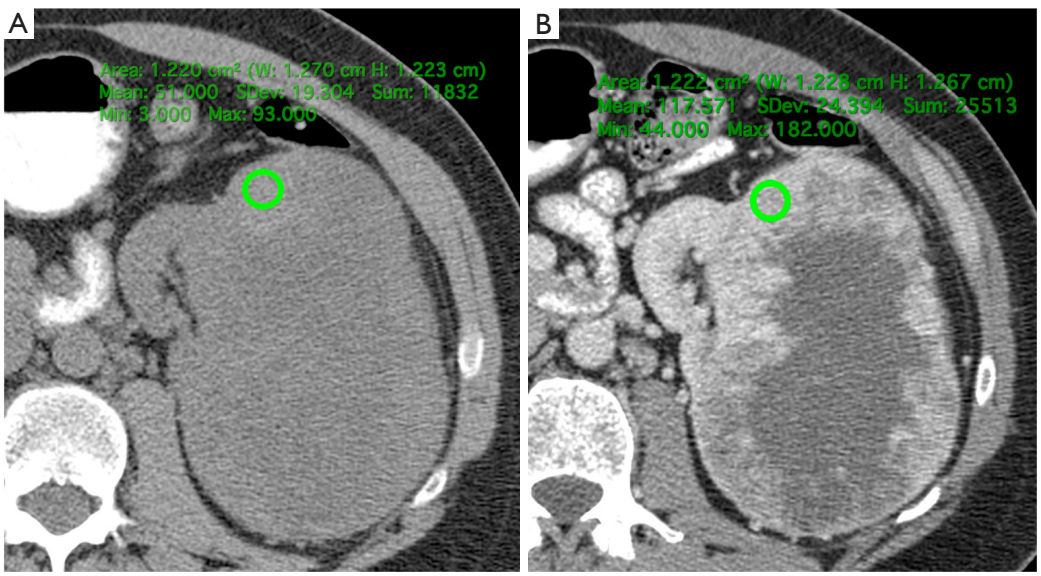

Figure 2 Unenhanced and postcontrast axial CT images of a male ccRCC patient with *GPX8* gene mutation (A and B respectively) show green ROIs positioned on solid tissue in the unenhanced image (A) and the related homogeneous contrast enhancement during nephrographic phase (B). CT, computed tomography; ccRCC, clear cell renal cell carcinoma; ROI, region of interest.

tests also further evaluated lipid metabolism by detecting fewer lipid droplets in GPX8-KO cells (7). In GPX8-KO cell lines grown in the lipid-depleted medium, the lipid droplets almost disappeared compared to wild-type cells which still retained slightly fewer lipid droplets, further reinforcing the roles of GPX8 in *de novo* lipogenesis (7).

De novo lipogenic processes of GPX8 in ccRCC were also evaluated focusing attention on 5' adenosine monophosphate-activated protein kinase (AMPK) as it has a central role in metabolism and is involved in the tumorigenesis/progression of ccRCC (20). AMPK expression and its phosphorylated form showed a negative

correlation with GPX8 and a positive association with overall survival (7). This negative regulation of AMPK with GPX8 is coherent with the reduced *de novo* lipogenesis in GPX8-KO cells, as AMPK inhibits acetyl-coenzyme A carboxylases (ACC), an important enzyme in *de novo* lipogenesis (7). Pharmacologically, the use of compound C, a widely used AMPK inhibitor (21), increased both fatty acids and triglyceride synthesis (7). The inhibition of AMPK indicates how GPX8 acts in ccRCC through inhibition of AMPK, which is involved in ccRCC growth and prognosis (7).

It has been demonstrated that the tumor microenvironment plays a fundamental role in tumor progression and could significantly influence clinical outcomes (22). Tumor-infiltrating immune cells, a considerable part of the tumor microenvironment, are associated with the growth, invasion, and metastasis of almost all cancers (23,24). For example, it has been shown that the cancer-associated fibroblasts have an active role in the generation of the interstitial matrix contributing desmoplastic stroma of advanced carcinomas. Furthermore, cancer-associated fibroblasts have been found to correlate with poor prognosis in numerous types of cancer and may play a role in tumor aggressiveness and immune evasion by determining an environment of cytokines and extracellular matrix (25-28). Immune cells recruited in the tumor microenvironment could determine the secretion of cytokines and chemokines, activating an inflammatory process that would contribute to pathological angiogenesis, tumor growth, invasion, and metastasis (29-31). A correlation has been demonstrated between GPX8 expression and tumor microenvironment characterized by the presence of cancer-associated fibroblasts and immune infiltration in ccRCC (32).

The limitations of this study are: the low number of samples which does not allow to perform a statistical analysis but only to formulate a hypothesis, the retrospective nature which could limit the generalization of results, and the age difference between the single ccRCC patient with *GPX8* gene mutation and ccRCC patients without *GPX8* gene mutation.

Conclusions

This present report is likely to stimulate future studies, on larger series, aimed to evaluate whether high CT attenuation of solid portion in ccRCC can be considered as a radiogenomic clue to *GPX8* gene mutation. A proper statistical analysis on this hypothesis will potentially allow to non-invasively draw genetic status information of ccRCC. Moreover, further studies could investigate the possible role of GPX8 in ccRCC pathogenesis. This would be of fundamental importance in the field of precision medicine, so that patient-tailored therapies could be developed and tested in ccRCC according to the specific genomic status.

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Footnote

Provenance and Peer Review: This article was a standard submission to the journal. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tro.amegroups.com/article/view/10.21037/tro-23-16/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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