Renal cancer

https://doi.org/10.1038/s41585-023-00749-8

Uncovering a link between COVID-19 and renal cell carcinoma

Samuel A. Gold & Vitaly Margulis

The cellular action of SARS-CoV-2, the virus responsible for the COVID-19 global pandemic, is dependent on a specific combination of receptors and cofactors. These proteins are now known to be expressed in normal kidney tubule tissue and renal cell carcinoma cells, representing possible targets for budding therapeutic modalities.

REFERS TO Choong, O. K. et al. SARS-CoV-2 replicates and displays oncolytic properties in clear cell and papillary renal cell carcinoma. *PLoS ONE* **18**, e0279578 (2023).

SARS-CoV-2 enters host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, a transmembrane protein[1]. Cleavage and priming of the viral spike proteins by enzymes such as FURIN or transmembrane protein serine 2 (TMPRSS2), as well as the co-receptor activity of neurolipin 1 (NRP1), facilitate cell entry[2,3]. Although ACE2 is expressed in multiple organs, the cofactors necessary for SARS-CoV-2 uptake are not always expressed alongside this receptor. One location in which ACE2, TMPRSS2 and NRP1 are expressed is in the proximal tubules of normal kidney tissue, which leads to viral uptake and is thought to be a mechanism for the direct kidney injury seen in patients with COVID-19 (refs. [2–4]). The proximal tubules are also the cellular origin for most renal cell carcinomas (RCCs), including clear cell RCC (ccRCC) and papillary RCC (pRCC).

Choong and colleagues[2] sought to determine whether malignant transformation of renal cells altered expression of SARS-CoV-2 entry factors, with the hypothesis that RCC cells are susceptible to SARS-CoV-2 infection. The authors focused on ccRCC and pRCC, as these cancers originate at the proximal tubules and comprise 90% of RCC cases. Chromophobe RCC (chRCC), which is the third most common RCC and originates at the cortical collecting ducts, was also included. Nephrectomy specimens were obtained from treatment-naive patients and included both benign and malignant renal tubule tissue. Bioinformatics, histology, quantification of protein and mRNA expression, and cell culture with viral exposure were all used to test the authors' hypothesis. Further analyses were conducted on tissue obtained from a patient with serologically confirmed COVID-19 before nephrectomy for RCC.

Transcriptional levels of *ACE2*, *TMPRSS2* and *NRP1* mRNA in RCC were investigated using The Cancer Genome Atlas (TCGA). In ccRCC and pRCC, *ACE2* and *NRP1* were highly expressed, and *TMPRSS2* expression was also elevated but to a lower extent. By contrast, *ACE2*, *NRP1* and *TMPRSS2* expression was reduced in chRCC. When compared against data from TCGA for 32 different malignancies, ccRCC and pRCC maintained the first and second highest levels of *ACE2* and *NRP1* mRNA expression. Western blot analysis confirmed protein expression of ACE2, TMPRSS2 and NRP1 in normal kidney, ccRCC and pRCC. Tissue microarray staining localized ACE2, TMPRSS2 and NRP1 to the proximal tubules of normal kidney tissue as well as in the majority of ccRCC (76%, 81% and 75%, respectively) and pRCC (93%, 56% and 66%, respectively) specimens. However, chRCC specimens did not display positivity for ACE2 or NRP1.

Benign and malignant cells were then exposed to SARS-CoV-2. Infection and propagation were quantified by PCR and visually assessed for viral cytopathogenic effect. Of three cultures each for normal kidney, ccRCC and pRCC, two normal kidney cultures, one ccRCC culture and one pRCC culture showed minimal viral mRNA and no cytopathogenic effect. For comparison, RCC4 (a *vhl* mutant cell line) was also studied. The investigators did not find evidence of staining for ACE2, TMPRSS2 or NRP1 at the RCC4 cell membrane, and viral replication of SARS-CoV-2 was not evident in RCC4 cells.

In tissue from the patient who had a history of COVID-19 infection and nephrectomy for RCC, the authors noted distinctly irregular features of the ccRCC cells, including diffuse necrosis, cancer cell syncytia and negative PCR for SARS-CoV-2. To expand on these findings, ccRCC cell cultures were exposed to the Delta variant of SARS-CoV-2 in vitro. Again, ccRCC cell syncytia were evident.

> "Choong et al.[2] demonstrate that the receptors and cofactors necessary for SARS-CoV-2 viral uptake are maintained by ccRCC and pRCC cells"

Based on publicly available data from TCGA and experimental analyses on various RCC cell lines, Choong et al.[2] demonstrate that the receptors and cofactors necessary for SARS-CoV-2 viral uptake are maintained by ccRCC and pRCC cells. When these cell lines were exposed to SARS-CoV-2, evidence of intracellular viral replication and distinct cytopathogenic effect was observed. Lack of viral activity in RCC4 cell cultures further supported the importance of ACE2, TMPRSS2 and NRP1 in SARS-CoV-2 infection. This study contributes to the growing body of work seeking to better elucidate the cellular mechanisms of SARS-CoV-2 infection and introduces a potential link between two seemingly unrelated pathologies — RCC and COVID-19. In a separate analysis utilizing TCGA, Huang et al.[5] surveyed over 300 SARS-CoV-2 infection-related genes found in patients with ccRCC to generate a validated prognostic risk model based on expression of five differentially expressed genes between malignant and normal renal

tissue. Huang et al. describe a clear association between a five-gene prognostic scoring model and overall survival, noting that patients in the high-risk category displayed higher immune checkpoint expression levels and more severe immunosuppressive tumour microenvironments than patients in the low-risk category. These findings suggest a potential role for immune checkpoint inhibitors in patients with greater expression of these SARS-CoV-2 infection-related genes, but data are lacking to measure this effect.

> **"Whether SARS-CoV-2 infection directly kills cancer cells or generates enhanced immunogenicity is a question worth investigating"**

Choong et al.[2] hint at the potential clinical utility of their study during discussion of the findings from a patient with prior COVID-19 infection followed by nephrectomy for ccRCC, and of their in vitro examination of ccRCC cells exposed to the SARS-CoV-2 Delta variant. These findings could be evidence of a virally mediated oncolytic effect on the cancer cells, or perhaps viral infection of cancer cells promotes antigenic expression and enhanced immune response.

Ottaiano and colleagues[6] reported on three cases of patients with metastatic colorectal cancer who demonstrated disease reduction after developing COVID-19. In each case, patients had already received multiple rounds of chemotherapy and demonstrated metastatic progression or stabilization before infection. Immunohistochemistry staining for ACE2 was positive in the tumour cells, and natural killer cell analysis showed augmented immunogenic activity (that is, degranulation) towards cells with high expression of ACE2 and NRP1. Notably, seven patients with similar clinical courses and COVID-19 infections did not demonstrate metastatic regression.

Whether SARS-CoV-2 infection directly kills cancer cells or generates enhanced immunogenicity is a question worth investigating. A study on the use of recombinant poliovirus injected into human melanoma, breast cancer and prostate cancer cells showed increased immune activity in the tumour microenvironment[7]. Furthermore, attenuated herpesvirus oncolytic treatment (talimogene laherparepvec) injected directly into melanoma serves as a model of viral instigation of immune response against tumour cells[8]. Although far from a certainty, these examples might provide a framework for future initiatives building on the findings of Choong et al.[2].

Drawing too many conclusions regarding potential RCC treatments from this study would be premature. The data rely heavily on in vitro experiments and, although evidence of expression of SARS-CoV-2 receptors and cofactors in ccRCC and pRCC is made clear, much remains to be uncovered regarding the potential involvement of these proteins in tumorgenicity, prognosis and treatment. These findings should prompt continued investigation into patients with histories of COVID-19 infections and RCC, which could further elucidate the link between these pathologies and stimulate developments in therapeutic modalities.

Samuel A. Gold & Vitaly Margulis ✉
Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA.
✉e-mail: vitaly.margulis@utsouthwestern.edu

Published online: 13 March 2023

References

1. Hoffmann, M. et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181**, 271–280.e8 (2020).
2. Choong, O. K. et al. SARS-CoV-2 replicates and displays oncolytic properties in clear cell and papillary renal cell carcinoma. *PLoS ONE* **18**, e0279578 (2023).
3. Hossain, M. G., Akter, S. & Uddin, M. J. Emerging role of neuropilin-1 and angiotensin-converting enzyme-2 in renal carcinoma-associated COVID-19 pathogenesis. *Infect. Dis. Rep.* **13**, 902–909 (2021).
4. Chen, Q.-L. et al. Localization of cell receptor-related genes of SARS-CoV-2 in the kidney through single-cell transcriptome analysis. *Kidney Dis.* **6**, 258–270 (2020).
5. Huang, Y. et al. A novel prognostic signature for survival prediction and immune implication based on SARS-CoV-2-related genes in kidney renal clear cell carcinoma. *Front. Bioeng. Biotechnol.* **9**, 744659 (2022).
6. Ottaiano, A. et al. Unexpected tumor reduction in metastatic colorectal cancer patients during SARS-Cov-2 infection. *Ther. Adv. Med. Oncol.* **13**, 17588359211011455 (2021).
7. Brown, M. C. et al. Cancer immunotherapy with recombinant poliovirus induces IFN-dominant activation of dendritic cells and tumor antigen-specific CTLs. *Sci. Transl Med.* **9**, eaan4220 (2017).
8. Andtbacka, R. H. I. et al. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. *J. Immunother. Cancer* **7**, 145 (2019).

Competing interests

The authors declare no competing interests.