

An analysis of SARS-CoV-2 cell entry genes identifies the intestine and colorectal cancer as susceptible tissues

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SARS-CoV-2 is the causative agent for the COVID-19 pandemic. COVID-19 has necessitated rapid changes in surgical practice and organisation through both the initial peak and ongoing recovery period ¹. SARS-CoV-2 infects cells by interacting with the host cell surface protein ACE2 and utilises TMPRSS2 in viral spike protein priming to facilitate cell entry (*Fig. 1a*) ². Whilst COVID-19 is predominantly a respiratory disease approximately 15% of patients have concurrent gastrointestinal symptoms ³. SARS-CoV-2 RNA and live virus have been identified in stool from COVID-19 patients and SARS-CoV-2 readily infects intestinal organoids ⁴⁻⁶. Despite these circumstantial data, gastrointestinal transmission has not yet been formally confirmed. Cancers commonly express different genes from the tissue of origin and it is largely unexplored whether tumours can be infected with SARS-CoV-2. We sought to explore the expression of *ACE2* and *TMPRSS2* in large publicly available normal tissue and pan-cancer expression data sets to understand whether levels of these genes identify susceptible tissues.

Analysis of the normal tissue Genotype Tissue Expression project (GTEx) dataset showed high *ACE2* expression in the testis, small intestine, kidney, heart, thyroid and adipose tissue (*Fig. S1a*, supporting information). *TMPRSS2* levels were highest in the prostate, stomach, small intestine, pancreas, lung, salivary gland, kidney, thyroid and liver (*Fig. S1b*, supporting information). Whilst initial analysis suggested only kidney and thyroid co-expressed high levels of *ACE2* and *TMPRSS2* closer inspection of small intestinal and colonic samples revealed heterogeneity with a sub-population (30-40% of patients) also co-expressing high levels that was confirmed by K means clustering (*Fig. 1b,c, Fig. S1c,d*, supporting information). In colonic tissue high levels of both genes were found in younger patients and greater *TMPRSS2* expression in females, although neither factor defined the sub-population observed (*Fig. S2a-j*, supporting information). Biopsy location and tissue compartment from colon samples were explored and higher levels of both genes were found in the mucosa and proximal colon although these factors also failed to fully define the high co-expressing sub-population (*Fig. S2k-m*, supporting information). These normal expression data appear to identify a proportion of individuals

who, in the gastrointestinal tract, express high levels of both genes known to be involved in cell entry of SARS-CoV-2.

The Cancer Genome Atlas program (TCGA) is the largest pan-tumour collection of genomic and transcriptomic sequencing data. Having identified the heterogeneous gene expression within the normal gastrointestinal tract we interrogated TCGA and GTEx gene expression data to identify relative expression of *ACE2* and *TMPRSS2* in tumours compared to their tissue of origin. Generally, there was no correlation between high expression of *ACE2* and *TPMRSS2* in normal tissue and high tumour expression (*Fig. S3a-d , supporting information*). However, we identified colorectal cancer as unique amongst human malignancies by co-expressing higher levels of both *ACE2* and *TMPRSS2* relative to normal (*Fig. 1d,e*). We explored TCGA data to identify if molecular subgroups of colorectal cancers, specific mutations or other commonly collected clinical variables could define tumours with varying expression of *ACE2* and *TMPRSS2*. There was a subtle yet significant trend for higher *ACE2* expression with younger age but no association with sex (*Fig. S4a , supporting information*). Possession of a *BRAF* mutation was found to predict lower levels of tumour *ACE2* however *TMPRSS2* expression was unchanged (*Fig. S4b,c , supporting information*). Strikingly, *ACE2* levels were very low in tumours with microsatellite instability (MSI) (*Fig. 1f*).

Cumulatively these data identify a proportion of healthy individuals as susceptible to putative SARS-CoV-2 intestinal infection and that patients with colorectal cancer may be at even greater risk of infection. Further clinical studies are urgently required to explore this mode of transmission of COVID-19.

Word count - 599

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Fig 1 – ACE2 and TMPRSS2 are expressed heterogeneously in the normal intestine and colorectal cancer

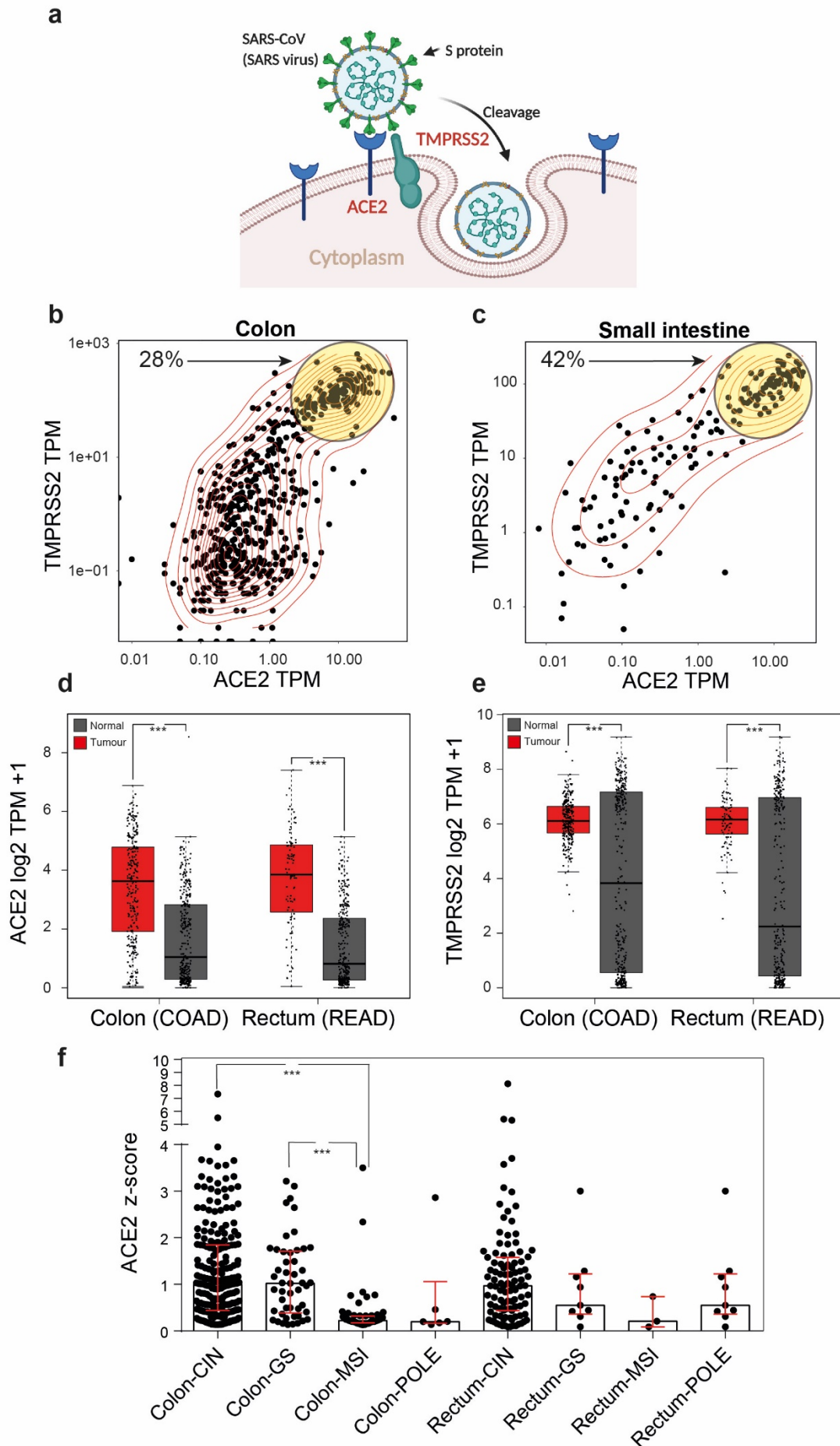


Figure Legends

Figure 1. **(a)** Schematic demonstrating the mode of entry of SARS-CoV-2 into cells via interactions with the cell surface proteins ACE2 and TMPRSS2. **(b)** Scatter plot of the expression of *ACE2* and *TMPRSS2* across all colon GTEx samples. Yellow box highlights high co-expressing samples. **(c)** Scatter plot of the expression of *ACE2* and *TMPRSS2* across all small intestine GTEx samples. Yellow circle highlights high co-expressing samples. **(d)** Box and whisker plot of expression of *ACE2* between normal large intestine (Grey, TCGA and GTEx) and colon and rectal cancer (Red, TCGA) samples. Median +/- IQR. One-way ANOVA. ***, $P < 0.001$. TPM=Transcripts per million. **(e)** Box and whisker plot of expression of *ACE2* between normal large intestine (Grey, TCGA and GTEx) and colon and rectal cancer (Red, TCGA) samples. Median +/- IQR. One-way ANOVA. ***, $P < 0.001$. TPM=Transcripts per million. **(f)** Box and scatter plot of *ACE2* expression levels from TCGA COAD/READ data sets between tumour subtypes CIN=chromosomal instability, MSI=microsatellite instability, GS=genome stable and POLE=DNA polymerase epsilon. Median +/- IQR. Kruskal-Wallis. ***, $p < 0.001$.