Tissue-specific ramp sequences correspond with increased gene expression in humans and SARS-CoV-2

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Article

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Abstract

Translational ramp sequences are essential regulatory elements that have yet to be characterized in specific tissues. Ramp sequences increase gene expression by evenly spacing ribosomes and slowing initial translation. Therefore, the relative codon adaptiveness within different tissues changes the existence of a ramp sequence without altering the underlying genetic code. Here, we present the first comprehensive analysis of tissue and cell type-specific ramp sequences, and report 3,108 genes with ramp sequences that change between tissues and cell types. The Ramp Atlas (<https://ramps.byu.edu/>) is an accompanying web portal that allows researchers to query ramp sequences in 18,388 genes across 62 tissues and 66 cell types. We also identified seven SARS-CoV-2 genes and seven human SARS-CoV-2 entry factor genes with tissue-specific ramp sequences that may help explain viral proliferation within those tissues. We anticipate that The Ramp Atlas will facilitate future tissue-specific ramp sequence analyses to develop targeted therapeutics for human disease.

Introduction

Tissue-specific regulation and resource availability directly affect gene transcription and translation, resulting in widespread tissue-specific differential gene expression^{1–5}. The Human Protein Atlas⁶, The Genotype-Tissue Expression (GTEx) Project⁷, and The Functional Annotation of Mammalian Genomes (FANTOM5)^{8,9} report gene expression in different tissues and cell types, which can be leveraged to identify disease biomarkers^{6,10–20} for a variety of diseases such as cancers^{1–5}, drug resistance¹⁸, cystic fibrosis¹⁴, cardiovascular disease¹⁶, and Alzheimer's disease²⁰.

Since codon usage biases correlate with local tRNA pools and tissue-specific RNA binding proteins^{21,22}, codon usage biases often differ across different tissues^{21,23}. Variations in tRNA abundance affect gene expression by altering tRNA competition and codon optimality, which changes the translational efficiency of various codons^{24,25}. Therefore, although the mRNA transcript remains unchanged between tissues, the efficiency at which those codons can be translated differs between tissues and cell types^{22,26–30}. While genome-wide codon usage biases are highly correlated with genome-wide guanine-cytosine (GC) content³¹, translational selection may play an important role in maintaining gene-specific codon usage biases^{32–34} because codon usage biases alter translational efficiency^{32,35,36}, gene expression^{35,37,38}, mRNA secondary structure^{34,39}, and protein structure and function³⁶. However, selection for tissue-specific codon adaptation in humans has been highly controversial: Doherty and McInerney⁴⁰ and Chamary, et al.³² argue that tRNA gene families undergo precise regulation to generate anticodon pools that directly correlate with available mRNA, while Pouyet, et al.⁴¹ concludes that global GC-content biases affect meiotic recombination and preclude tRNA optimization. Translational selection can also significantly shape codon usage biases across vertebrates, even when high GC-content biases are present⁴². Regardless of selection or GC-content biases shaping the underlying tRNA pool within each tissue, gene expression can be significantly impacted by codon choice.

Often, tissue-specific tRNA levels correlate with the codon usage biases of highly expressed genes, suggesting that tRNA abundances play a role in tissue-specific gene and protein expression²¹. The tRNA pools within tissues and cell types can differ up to tenfold^{21,43–50}, and unique tRNA pools cluster into subsets of proliferating and differentiating cells when characterized by cell type³⁰. These cell types maintain tRNA levels that match the codon usage biases and increase the expression of genes that promote proliferation or differentiation³⁰. Similarly, cellular tRNA levels may dynamically change to increase the expression of certain genes in response to stress. For example, heterologous protein overexpression, diminished growth rate, and translational imbalances trigger tRNA pool readjustments to increase expression of needed genes^{51–55}. Additionally, cells significantly increase tRNA levels in the G2 phase of the cell cycle to temporarily increase the translational efficiency and expression of cycle-regulated genes with non-optimal codon usage bias⁵⁶.

Various diseases also trigger tRNA pool alterations. For example, the tRNA pool in breast cancer cells is altered to increase expression of genes that promote metastasis⁵⁷. The interplay between tissue and cell type-specific codon usage biases, tissue and cell type-specific tRNA abundances, and synonymous mutations can have a significant effect on various human diseases^{30,43–45,57–63}. During the later stages of infection, HIV-1 changes cellular tRNA levels to increase expression of viral genes with codon usage biases that are poorly adapted to the original host tRNA pool⁶⁴. Similarly, viral genes in SARS-CoV-2 alter tRNA levels and deprive tRNA from normally highly-expressed host genes by monopolizing the tRNA supply, thereby decreasing host gene expression and inducing additional damage⁶⁵.

Viruses that infect humans often use similar codon usage biases as highly expressed genes within the tissues that they infect to facilitate co-opting available cellular machinery^{65–67}. For example, genes in SARS-CoV-2 utilize codon usage biases that match very highly expressed genes in lung tissue, likely contributing to increased viral gene expression, decreased host gene expression, and infection in the lungs^{65,68}. In SARS-CoV-2, codon usage similarities between viral genes and highly-expressed host genes likely improve viral translation and replication, which may help predict which human genes will be downregulated during an infection⁶⁹. Similarly, codon usage biases in *Papillomavirus* genes promote gene expression in differentiated epithelial cells to more effectively spread infection⁷⁰.

Although many tissue-specific codon usage biases have previously been reported, the tissue-specific effects of ramp sequences have yet to be characterized. Ramp sequences increase overall translational efficiency by utilizing slowly-translated codons at the beginning of genes to evenly space ribosomes and reduce downstream ribosomal collisions. Ramp sequences occur when 20–40 suboptimal codons concentrate at the beginning (5' end) of highly-expressed gene sequences and occur in approximately 10% of genes⁷¹. Ramp sequences generally increase gene expression by counterintuitively slowing initial translation so that ribosomes evenly distribute^{21,49,71–74} and the remaining transcript can be more efficiently translated without ribosomal traffic jams^{75,76}. Increased translational efficiency increases mRNA stability and gene and protein expression, especially in genes that have higher ribosome density,

higher mRNA levels, and a strong correlation between mRNA and protein expression^{75,77}. However, the mechanisms by which ramp sequences affect gene expression have yet to be fully characterized, and ramp sequences may also impact transcriptional efficiency by requiring fewer dsDNA bonds to be broken⁷⁸. While variations exist within different human populations⁷⁹, orthologous ramp sequences are evolutionarily conserved across all domains of life^{80–82}. However, the extent to which ramp sequences differ between tissues and cell types has never been studied, despite the direct effect that ramp sequences have on gene expression.

Here, we present the Ramp Atlas, which is the first web portal to analyze tissue and cell type-specific ramp sequences. We identified 3,108 genes with ramp sequences that were alternatively present, or absent, depending on the relative translational efficiencies in tissues and tissue-stratified cell types calculated from gene expression in the Human Protein Atlas^{83–85}, GTEx Project⁷, and FANTOM5 datasets^{8,9}. We allow researchers to query ramp sequences in 18,388 genes spanning 62 tissues and 66 cell types. Embedded Tableau charts allow user-specific querying to facilitate targeted ramp sequence analyses. Additionally, we identify ramp sequences in seven SARS-CoV-2 genes and seven human SARS-CoV-2 entry factor genes, both present in a variety of tissues. The SARS-CoV-2 analyses indicate that virus-specific ramp sequences may play an integral role in viral proliferation. We anticipate that the Ramp Atlas will facilitate future ramp sequence analyses on tissue or cell type-specific gene expression impacted by ramp sequences, genetic variant effects on tissue-specific gene expression, viral adaptations to specific tissues or cell types, and therapeutic developments that aim to modulate tissue-specific gene expression.

Results

The Ramp Atlas (<https://ramps.byu.edu>) is a comprehensive online repository of tissue and cell type-specific ramp sequences spanning 18,388 human genes in 62 tissues and 66 cell types. Additional interactive comparisons of SARS-CoV-2 ramp sequences and human entry factors are also included in this database. This resource provides a template for conducting ramp sequence analyses on viruses and identifying ramp sequences that are correlated with tissue or cell type-specific differential gene expression. Interactive graphics enable comprehensive user-specific analyses that can be integrated into a variety of downstream genetic analyses.

The Ramp Atlas contains the first comprehensive analysis of tissue and cell type-specific ramp sequences and their correlation with protein levels. All results can be queried online at The Ramp Atlas. Table 1 shows that the presence of a ramp sequence significantly correlates with higher gene expression in each dataset: FANTOM5 (odds=1.1152; p-value=3.00x10⁻⁹⁹), GTEx (odds=1.1578; p-value=9.48x10⁻¹⁵⁵), Human Protein Atlas (odds=1.1947; p-value=1.27x10⁻³⁰⁶), and consensus (odds=1.1477; p-value=1.00x10⁻²⁵⁴). Genes with tissue-specific ramp sequences (i.e., ramp sequences that were present in only some tissues) were also significantly more likely to increase gene expression in the consensus dataset (odds: 1.1937; p-value=5.56x10⁻⁵), FANTOM5 (odds: 1.1314; p-value=0.00589), and the Human

Protein Atlas (odds: 1.1897; p-value= 2.74×10^{-5}), although the difference was not significant in the GTEx dataset (odds: 0.9242; p-value=0.15236). Notably, GTEx has fewer tissue samples than all other datasets, with only 34 tissues compared to 43 in the Human Protein Atlas, 45 in FANTOM5, and 62 in the consensus dataset.

A chi-squared test shows that ramp sequence prevalence differs significantly across all tissue types in every dataset (FANTOM5 p-value $<2.23 \times 10^{-308}$, GTEx p-value= 8.52×10^{-81} , Human Protein Atlas p-value $<2.23 \times 10^{-308}$, consensus p-value $<2.23 \times 10^{-308}$, cell type p-value= 6.06×10^{-20}). Pairwise two-proportion z-tests that correct for multiple testing in each dataset showed that the proportion of genes with ramp sequences also differed significantly in many pairwise tissue comparisons: 43.99% of tissue comparisons in the consensus dataset, 50.51% of comparisons in FANTOM5, 35.65% of comparisons in GTEx, and 62.57% of comparisons in the Human Protein Atlas. Similarly, pairwise two-proportion z-tests in the Human Protein Atlas cell type dataset show that proportions of ramp sequences differed significantly in 4.34% of tissue-stratified cell type comparisons after correcting for multiple tests. The proportion of unique ramp sequences in the thymus, rectum, and total peripheral blood mononuclear cells (PBMC) were the highest compared to all other tissues (see Figure 1). We found that all 66 tissue-stratified cell types had at least one significant z-test result. Ramp sequence presence is most likely to differ in the seminiferous ducts in testis, cells in the white pulp in the spleen, and glandular cells in the parathyroid gland. Cells in the white pulp in the spleen use fewer ramp sequences than average, while the other two tissue-stratified cell types have more ramp sequences than average (see Figure 2). Figure 3 shows the percentage of ramp sequences shared between each tissue in the consensus dataset. The average percentage of ramp sequences shared between the urinary bladder and all other tissues is the highest (87.46%), while the rectum has the fewest overlapping ramp sequences with other tissues (57.26%). Figure 4 shows ramp sequence overlap between different cell types from the Human Protein Atlas tissue-stratified cell types. Trophoblast cells in the placenta have the highest percentage of shared ramp sequences with other tissue-stratified cell types (94.96%), while cells in the seminiferous cells in the testes have the lowest (76.89%).

The "Search Database" page on The Ramp Atlas contains several interactive Tableau charts that allow users to query 18,388 genes for ramp sequences and explore differences between each dataset. For convenience, users can view ramp sequence results for multiple genes and datasets side-by-side. These interactive Tableau charts also display normalized gene expression levels across tissues, cell types, and datasets. Users can quickly view the extent to which ramp sequences differ between different tissues and visually determine if ramp sequences potentially affect gene expression. Interactive versions of Figures 1-4 (introduced above) are also available on the "Search Database" page, which enable users to view tissue and cell type differences in ramp sequence presence in more detail. These ramp sequence results are also available for bulk download on the "Downloads" page (<https://ramps.byu.edu/Downloads>).

SARS-CoV-2 Ramp Sequence Analysis

We identified ramp sequences in seven SARS-CoV-2 genes: the 2'-O-RNA methyltransferase, the leader protein (non-structural protein 1), non-structural protein 6, the nucleocapsid phosphoprotein, the ORF3a protein, the ORF7a Nanoluciferase protein, and the surface glycoprotein. Ramp sequences in all but two of these genes (non-structural protein 6 and the surface glycoprotein) are tissue-specific (see Figure 5). Similarly, seven of the 26 analyzed human genes that encode SARS-CoV-2 entry factors⁸⁶ show tissue-specificity: Transmembrane protease, serine 2 (*TMPRSS2*); Membrane alanyl aminopeptidase (*ANPEP*); Charged Multivesicular Body Protein 2A (*CHMP2A*); Cathepsin L (*CTSL*); Furin, Paired Basic Amino Acid Cleaving Enzyme (*FURIN*); RAB1A, Member RAS Oncogene Family (*RAB1A*); and Transmembrane Anterior Posterior Transformation 1 (*TAPT1*) (see Figure 6). By combining these two analyses, we ranked tissues by ramp sequence prevalence in both SARS-CoV-2 and human entry factor genes (see Figure 7). A one-tailed t-test using these data shows that tissues with high SARS-CoV-2 proliferation have significantly more SARS-CoV-2 and human entry factor genes with ramp sequences than tissues that show less SARS-CoV-2 proliferation (p-value=0.009918). Both the rectum and duodenum (tissues with high SARS-CoV-2 proliferation⁸⁷) showed the most unique ramp sequence usage (see Figure 8).

Users can interactively query Tableau charts on the SARS-CoV-2 page of The Ramp Atlas to thoroughly explore ramp sequences within the SARS-CoV-2 genome as well as in seven human entry factors for SARS-CoV-2. An interactive heat map at the top of the page shows the percentage of SARS-CoV-2 ramp sequences that are shared between different tissues in the consensus dataset. Below, another interactive chart presents data on the presence of ramp sequences in different SARS-CoV-2 genes and across the 62 tissues in the consensus dataset. Under the "Ramp Sequences in Human Entry Factors" section of the webpage, an interactive Tableau chart presents data for the presence of ramp sequences in SARS-CoV-2 human entry factors across all tissues in the consensus dataset. These interactive charts allow users to thoroughly explore ramp sequences in SARS-CoV-2.

Discussion

Tissue-Specific Ramp Sequence Regulation

We show that ramp sequences change based on the relative codon adaptiveness within a specific tissue or cell type. Those changes often result in gene-specific ramp sequences that are unique to a tissue or cell type. Tissue and cell type-specific ramp sequences are highly associated with increased gene expression when a ramp sequence is present. This study is the first comprehensive analysis of variable ramp sequences and presents a framework for conducting future tissue and cell type-specific ramp sequence analyses online. We propose that future ramp sequence calculations should consider ramp sequence

variability that may occur within an organism based on tissue-specific codon optimality. We also propose that variable ramp sequences might be an additional mechanism for regulating tissue and cell type-specific differential gene expression that warrants further exploration.

SARS-CoV-2 Ramp Sequence Analysis

Ramp sequences may play a significant role in determining tissue-specific SARS-CoV-2 infection and proliferation because tissues that experience higher rates of SARS-CoV-2 infection have significantly more ramp sequences in SARS-CoV-2 and associated human entry factor genes. These tissue-specific ramp sequences are predicted to increase expression of SARS-CoV-2 genes as well as the human genes that facilitate SARS-CoV-2 entry into host cells, resulting in tissue-specific increased rates of infection and proliferation. Specifically, tissues and tissue-stratified cell types with increased expression of human entry factors *ACE2*, *TMPRSS2*, and *CTSL* show increased SARS-CoV-2 infection^{87–91}. While both *TMPRSS2* and *CTSL* contain ramp sequences, only ramp sequences in *TMPRSS2* are tissue-specific and therefore may influence which tissues are most infected by SARS-CoV-2. Additionally, tissue-specific ramp sequences in the SARS-CoV-2 leader protein, which inhibits host gene expression^{92,93} and helps the virus escape type-1 interferon cellular responses^{92–94}, may also influence tissue-specific infection and proliferation.

In addition to potentially contributing to SARS-CoV-2 tissue-specific infection and proliferation, ramp sequences may also influence other fatal complications commonly found in SARS-CoV-2 patients. SARS-CoV-2 genes *ORF3* and non-structural protein 1 (*NSP1*) respectively significantly upregulate and downregulate *APOB* expression⁹⁵. Overexpression of *APOB* is associated with atherosclerosis⁹⁶, a condition that contributes to the severity of SARS-CoV-2 infection^{95,97}. Both *ORF3* and *NSP1* have ramp sequences specifically in the liver and small intestine, where *APOB* expression is highest^{83,98}. These ramp sequences may significantly increase *ORF3* and *NSP1* expression within the liver and small intestine, likely affecting *APOB* expression. While *ORF3* and *NSP1* have opposite effects on *APOB* expression, ramp sequences may play an important role in determining the magnitude of those effects, and consequently, the complications of atherosclerosis associated with increased *APOB* expression. The Ramp Atlas provides a framework to conduct similar analyses on novel viral strains and viral variants, which may help prioritize tissues and cell types likely to be affected by a virus.

Conclusion

The Ramp Atlas is the first comprehensive analysis of tissue and cell type-specific ramp sequences. We identified ramp sequences in SARS-CoV-2 that likely contribute to its widespread proliferation, and we show that those ramp sequences are more likely to occur in tissues known to be affected by SARS-CoV-2. Additionally, we provide a template for conducting similar ramp sequence analyses using a web resource to facilitate the widespread adaptation of ramp sequences in a variety of future applications.

The Ramp Atlas will help researchers better understand how ramp sequences affect gene expression, which may lead to more specific, targeted therapeutics. Tissue-specific gene and protein expression can

affect tissue-specific responses to drug therapies^{99,100}. For example, gene and protein expression profiles collected from tumor tissue samples can accurately predict patient responses to different cancer therapies^{101–106}. Because ramp sequence usage differs significantly between tissues and tissue-stratified cell types and can be highly correlated with local protein expression, genetic variation affecting ramp sequences may also affect drug response and overall gene expression. Therefore, clinical trials targeting gene expression may need to incorporate the impact of tissue-specific ramp sequences for optimal results. The Ramp Atlas is a hypothesis-generating platform that provides a framework for researchers to incorporate ramp sequences in creative protein regulation and expression analyses.

Methods

Identifying Ramp Sequences

Ramp sequences are calculated by identifying statistical outliers of codon efficiencies concentrated at the 5' end of gene coding sequences using the software package, ExtRamp⁷¹. Codon efficiencies are measured by the relative synonymous codon usage metric, which ranges from 0-1 and is calculated by dividing the number of occurrences for a specific codon by the number of occurrences for the most common codon that encodes the same amino acid. We used pre-computed relative synonymous codon usage values calculated in April 2021 for 43 tissues in the Human Protein Atlas⁸³⁻⁸⁵, 34 tissues in the GTEx Project⁷, 45 tissues in the FANTOM5 datasets^{8,9}, and 62 tissues in the consensus dataset found in the Human Protein Atlas, which combines gene expression from all three databases. We also used pre-computed synonymous codon usages spanning 66 tissue-stratified cell types, which were also downloaded in April 2021. We used default parameters in ExtRamp to identify ramp sequences in each tissue and cell type for 18,388 genes, where all annotated gene isoforms were required to have similar gene expression (i.e., gene expression was within the same quartile for each isoform). The longest isoform from the GRCh38 reference genome was considered representative for each gene and was used to identify ramp sequences in each tissue or cell type. Ramp sequence identification was performed for each tissue and cell type separately. Two output files were generated for each run: 1) ramp sequences identified by ExtRamp, and 2) genes that did not contain a predicted ramp sequence within the tissue or cell type. The original data files downloaded from the Human Protein Atlas were modified to include an extra column, "Ramp presence," with values of "Ramp" or "No ramp" for each gene in each tissue and cell type. These comma-separated values (CSV) files are available at <https://ramps.byu.edu/Downloads>. We also present these data in Tableau charts on the "Search Database" page of the Ramp Atlas, which allows users to view tissues where ramp sequences exist for a gene and compare the presence or absence of a ramp sequence to the normalized gene expression for each tissue.

Determining the Correlation Between Ramp Sequences and Gene Expression

We performed chi-squared analyses of ramp sequence presence across all datasets and then calculated the odds of both overall and tissue-specific increased expression if a gene contains a ramp sequence.

To determine if genes with ramp sequences tend to be highly expressed, we first grouped all genes across all tissues by whether or not they contained a ramp sequence (e.g., if gene A had a ramp sequence in 30 tissues and did not have a ramp sequence in 32 tissues, then it would be included 30 times in the "ramp" group and 32 times in the "no ramp" group with the corresponding gene expression for each tissue). We then calculated the odds of having "medium" or "high" expression versus "not detected" or "low" expression for each group. We calculated the relative odds of having increased gene expression with a ramp by dividing the odds of having "medium" or "high" expression in the "ramp" group by the odds of having "medium" or "high" expression in the "no ramp" group.

We performed the following calculations in each dataset to determine the extent to which genes with a ramp sequence in some tissues and not others are differentially expressed. First, we ensured that each gene had a ramp sequence in at least 5% of the tissues and did not have a ramp sequence at least 5% of the tissues. The average gene expression in the "ramps" and "no ramps" groups were calculated for each gene separately to limit the potential effects of sampling. The expression bins were transformed to follow a linear progression between "not detected" and "high expression" (i.e., "not detected" was given a weight of "1" and "high expression" was given a weight of "4"), and the average expression for each group (e.g., "ramps" and "no ramps") was calculated as follows:

$$\text{Average Expression} = \frac{(1 * \text{not detected}) + (2 * \text{low}) + (3 * \text{medium}) + (4 * \text{high})}{\text{total number of sequences}}$$

Finally, we counted the number of genes that had a higher tissue-specific gene expression when they had a ramp sequence compared to when they did not have a ramp sequence and calculated the odds of tissue-specific variable ramp sequences correlating with increased gene expression (see Table 2).

Comparing Ramp Presence across Tissue and Cell Types

We compared the proportion of genes with ramp sequences across different tissues or cell types for each dataset (e.g., FANTOM5, GTEx, Human Protein Atlas, consensus, and Human Protein Atlas cell types) and determined if the same genes always contained ramp sequences.

We conducted a single chi-squared test for each dataset to determine the extent to which ramp sequences are distributed across tissue and cell types. Each tissue contained at least 2,425 ramp sequences (i.e., 14% of sampled genes) and each cell type contained at least 1,521 ramp sequences (i.e., 15% of sampled genes), which was a large enough sample size to adequately assess the distribution using the chi-squared test.

We followed these dataset-wide chi-squared tests with more specific pairwise, two-proportion z-tests between each tissue type to identify which tissues had the most unique ramp sequences. We applied a Bonferroni correction for multiple tests (FANTOM5 $\alpha=5.05 \times 10^{-5}$, GTEx $\alpha=8.91 \times 10^{-5}$, Human Protein Atlas $\alpha=5.54 \times 10^{-5}$, consensus $\alpha=2.64 \times 10^{-5}$, cell type $\alpha=2.33 \times 10^{-5}$). Using Tableau, we created bar charts to display the total number of significant tests for each tissue/cell type, highlighting tissues/cell types with more unique proportions of ramp sequences.

Data Visualization

We used Tableau Software (www.tableau.com) to create The Ramp Atlas, which generates interactive graphics for users to query ramp sequence data across tissues and cell types. All Tableau charts are available at <https://ramps.byu.edu/SearchDatabase>. The bulk data files used to generate these Tableau charts are available for download at <https://ramps.byu.edu/Downloads>.

SARS-CoV-2 Ramp Sequence Analysis

We downloaded all SARS-CoV-2 nucleotide coding sequences from the National Center for Biotechnology Information (NCBI) SARS-CoV-2 Resources in November 2020. We analyzed 406,882 available SARS-CoV-2 gene sequences and overlapping gene bodies. Since SARS-CoV-2 utilizes the host machinery for translation, the optimal codon usage for SARS-CoV-2 should be based on the relative synonymous codon usage of the host tissue. Therefore, we used ExtRamp coupled with the relative synonymous codon usage values calculated from 62 tissue-specific highly expressed genes in the Human Protein Atlas, GTEx, FANTOM5, and consensus datasets to determine the extent to which ramp sequences were present in SARS-CoV-2 genes for each dataset. We then determined the frequency of ramp sequences in SARS-CoV-2 genes across each tissue and identified the number of ramp sequences shared between any two tissues. We also identified tissue-specific ramp sequences in human genes that encode SARS-CoV-2 entry factors, as reported in Singh, et al. ⁸⁶. We then ranked all tissues according to the total number of ramp sequences present in both SARS-CoV-2 and associated human entry factor genes. Using these data, we performed a one-tailed t-test to compare the mean number of SARS-CoV-2 and human entry factor genes

with ramp sequences in tissues that show high SARS-CoV-2 proliferation^{87,107} against those tissues that do not show high SARS-CoV-2 proliferation. All analyses are freely available and distributed using an ASP.NET web server hosted at Brigham Young University (<https://ramps.byu.edu>).

Declarations

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Tables

Table 1: Odds of Ramp Sequence Presence Correlating with Increased Gene Expression

Dataset	Chi-square	P-value	Odds Ratio
FANTOM5	459.3981	3.00x10 ⁻⁹⁹	1.1152
GTEx	715.4278	9.48x10 ⁻¹⁵⁵	1.1578
Human Protein Atlas	1415.5104	1.27x10 ⁻³⁰⁶	1.1947
Consensus	1176.3328	1.00x10 ⁻²⁵⁴	1.1477

For each dataset, the chi-squared value and p-value for increased expression if a gene has a ramp sequence as well as the odds of having higher expression if ramp sequence present vs not present. The odds ratio shows a significant difference in gene expression if a gene has a ramp sequence.

Table 2: Odds of Ramp Sequence Presence Correlating with Tissue-Specific Gene Expression

Dataset	Genes with Decreased Tissue Expression	Genes with Increased Tissue Expression	Chi-square	P-value	Odds Ratio
FANTOM5	936	1059	7.583	0.00589	1.1314
GTEx	686	634	2.0485	0.15236	0.9242
Human Protein Atlas	1070	1273	17.5881	2.74×10^{-5}	1.1897
Consensus	950	1134	16.2457	5.56×10^{-5}	1.1937

For each dataset, the chi-squared value and p-value for expression if a gene has a ramp sequence as well as the odds of having higher expression in a tissue where a ramp sequence is present. This odds ratio shows a significant difference in tissue-specific gene expression if a gene has a ramp sequence in that specific tissue.

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Figures

Tissue Ramp Chi-Square Pairs

Figure 1

Percentage of Significant Chi-Square Pairs in Tissue Types. We performed chi-square pair comparisons of ramp sequences across all tissues in each dataset and then pairwise two-proportion z-tests to identify which tissues have the most unique proportions of ramp sequences per gene. This figure shows, for each tissue, the percentage of pairwise z-tests that are significant, averaged across all datasets. Each tissue bar is colored based on the difference between the proportion of genes with ramp sequences and the average proportion of genes with ramp sequences across all tissues. Blue colored tissues have more ramp sequences than average while red colored tissues have fewer ramp sequences than average.

Cell Type Ramp Chi-Square Pairs

Figure 2

Percentage of Significant Chi-Square Pairs in Tissue-Stratified Cell Types. We performed chi-square pair comparisons of ramp sequences across all tissue-stratified cell types in each dataset and then pairwise two-proportion z-tests to identify which cell types have the most unique proportions of ramp sequences per gene. This figure shows, for each cell type, the percentage of pairwise z-tests that are significant, averaged across all datasets. Each tissue-stratified cell type bar is colored based on the difference between the proportion of genes with ramp sequences in the cell type and the average proportion of genes with ramp sequences across all cell types. Blue colored tissue-stratified cell types have more ramp sequences than average while red colored cell types have fewer ramp sequences than average.

Shared Ramps (Consensus)

Figure 3

Heat Map of Percent of Ramp Sequences Shared between Tissues. The percent of ramp sequences present in one tissue that are also present in another in the consensus dataset. Red indicates fewer shared ramp sequences and thus a more tissue-specific usage of ramp sequences. Tissue comparisons that are blue have more shared ramp sequences.

Shared Ramps (HPA Cell Types)

Figure 4

Heat Map of Percent of Ramp Sequences Shared between Tissue-Stratified Cell Types. The percent of ramp sequences present in one tissue-stratified cell type that are also present in another cell type. Red indicates fewer shared ramp sequences (i.e., more cell type-specific usage of ramp sequences). Tissue-stratified cell type comparisons that are blue have more shared ramp sequences.

Figure 5

Percentage of Tissues with a Ramp Sequence in SARS-CoV-2 Genes. The percentage of 62 tissues in the consensus dataset in which seven SARS-CoV-2 genes have a ramp sequence. Error bars show the standard error between the four different datasets. In genes marked with an asterisk, less than one percent of all variants contained a ramp sequence. Notably, the other four genes had a ramp sequence in the one and only variant provided in the SARS-CoV-2 genome dataset. More exact data are available on the Ramp Atlas SARS-CoV-2 page: <https://ramps.byu.edu/Covid>.

Figure 6

Percentage of Tissues with a Ramp Sequence in Human Genes for SARS-CoV-2 Entry Factors. The percentage of the 62 tissues in the consensus dataset in which human genes that code for SARS-CoV-2 entry factors have a ramp sequence. Error bars show the standard error between the four different datasets.

Figure 7

The Total Number of SARS-CoV-2 and Associated Human Entry Factor Genes with a Ramp Sequence in 62 Human Tissues. An aggregation of the data presented in Figures 5 and 6, organized by tissue, and showing the total number of genes, both in the SARS-CoV-2 genome and associated human entry factors, with ramp sequences. Tissues where SARS-CoV-2 is found in higher amounts are marked in red boxes. These tissues had a significantly greater number of genes with ramp sequences (p-value=0.0099).

Shared Ramps in SARS-CoV-2

Figure 8

Heat Map of Percentage of SARS-CoV-2 Ramp Sequences Shared Between Tissues. The percentage of ramp sequences in SARS-CoV-2 genes that are present in both tissues being compared. Calculated using the consensus dataset. The redder the tissue comparison, the smaller the percentage of ramp sequences were present in both tissues. The bluer the comparison, the higher percentage of shared ramp sequences.