Research article

Codon usage similarity between viral and some host genes suggests a codon-specific translational regulation

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**ABSTRACT**

The codon usage pattern is a specific characteristic of each species; however, the codon usage of all of the genes in a genome is not uniform. Intriguingly, most viruses have codon usage patterns that are vastly different from the optimal codon usage of their hosts. How viral genes with different codon usage patterns are efficiently expressed during a viral infection is unclear. An analysis of the similarity between viral codon usage and the codon usage of the individual genes of a host genome has never been performed. In this study, we demonstrated that the codon usage of human RNA viruses is similar to that of some human genes, especially those involved in the cell cycle. This finding was substantiated by its concordance with previous reports of an upregulation at the protein level of some of these biological processes. It therefore suggests that some suboptimal viral codon usage patterns may actually be compatible with cellular translational machineries in infected conditions.

1. Introduction

The genetic code is degenerate. There are 61 triplet codons coding for 20 amino acids and 3 stop codons [1]. Therefore, each amino acid is encoded by several codons, with the exception of two amino acids (methionine and tryptophan). This codon redundancy results in synonymous codon usage, whereby one amino acid is encoded by 2, 4, or 6 codons [2]. Several previous studies have revealed that synonymous codons are utilized with different frequencies and are not randomly used by different genomes or genes. This non-randomness is referred to as codon usage bias [3, 4]. Each species preferentially uses different synonymous codons [5]. This results in a species-specific codon usage bias.

Similarly, there are several tRNA species that carry the same amino acid. These tRNA species are called isoacceptors [6]. Codons and anti-codons in tRNA do not interact in a one-to-one fashion [7]. Base pairing at the third codon position is wobble; for example, G can pair with both cytosine (C) and uracil (U) [8]. It has been demonstrated that tRNA modification directly affects tRNA and mRNA wobble base pairing [9, 10]. Both the available tRNA isoacceptors and tRNA modification change with the cell cycle, and they can be altered by cellular stresses [11, 12]. For efficient protein translation, the codon usage pattern should correlate with the population of available tRNA isoacceptors [13]. In the cellular stress-response, the alteration could enhance the expression of stress-response genes, with the codon usage patterns compatible with the changed tRNA modifications. Those genes shown to be regulated by this codon-specific manner are called Modification Tunable Transcripts (MoTTs) [12, 14].

Two major models have been proposed to explain the causes of codon usage bias: mutation pressure, and translational selection [15]. As to mutation pressure, it is believed that GC content is the major factor driving codon usage bias [16, 17]. The high mutation rates of some nucleotides or codons result in nucleotide substitution that might contribute to lower frequencies of some nucleotides and codons [15]. Mutation pressure has been suggested to be the most important factor determining...
the codon usage bias in human RNA viruses [18, 19, 20]. However, there are correlations between the codon usage bias and other factors related to translation efficiency (such as available tRNAs, mRNA secondary structure, translation elongation rate, and the intragenic and intergenic codon bias) that cannot be explained by mutation pressure. This suggests that translational selection also influences codon usage bias [15]. The translational selection acts on codon usage bias to achieve efficient and accurate translation. The use of codons correlates with abundant tRNAs, resulting in a higher translation rate [21, 22, 23]. A correlation between codon usage bias and abundant tRNAs has been found in prokaryotes (such as E. coli [24]) and in some eukaryotes (such as S. cerevisiae [25], C. elegans [22], Drosophila [23], and human [26]). However, rare codons are preferred to encode some specific sets of genes or regions of genes, for instance, to enable protein oscillation in different phases of the cell cycle, slow down protein translation across the membrane, and reduce ribosome jamming and mRNA secondary structure at the 5’ end of coding sequences [11, 27, 28, 29]. Therefore, the translational selection acting on the optimization of frequent and rare codon utilization is important in appropriate gene translation.

Viral replication is dependent on the cellular machineries of the host cells. Thus, one would intuitively think that the codon usage of a viral genome should match that of its host in order to be efficiently expressed. Surprisingly, however, most viruses have codon usage patterns that are different from the codon usage preference of their hosts [30, 31, 32, 33]. A previous study indicated an alteration in the cellular tRNA level after the infection of human immunodeficiency virus type 1 (HIV-1) [34]. In contrast, the cellular tRNA level was found to be unchanged following vaccinia and influenza A virus (IAV) infection, whereas an alteration in the polysome-associated tRNA population was observed, particularly the population of polysome tRNA isoacceptors correlated with viral codon usage [35]. These findings suggest that the codon usage pattern and the regulation of translational machineries may influence gene expression in some viruses.

In this study, we investigated the relationship between the codon usage bias of human genes and human RNA viruses. It is generally believed that the codon usage bias of viruses differs from that of human genes; however, various human genes possess various codon usage patterns [19, 26]. In addition, intragenic codon biases had previously been reported in humans and mice [36]. A comparison of the codon usage at the genome level is therefore too generalized; a more precise comparison at the single-gene level may provide a better insight into the viral codon usage bias.

2. Results

2.1. Principal component analysis of the relative synonymous codon usage (PCA of RSCU)

A total of 20,190 major transcript variants of human protein coding sequences were recruited from the GENCODE database (version 26). The protein coding sequences of 77 human RNA viruses were downloaded from the NCBI database. The open reading frames (ORFs) of the protein coding sequences were rechecked by ORFfinder (NCBI) before performing the RSCU calculation. The RSCU is a simple parameter that represents the codon usage bias of synonymous codons in a coding sequence. In our analysis, the RSCU was calculated from the protein coding sequences of the human and RNA viruses. The RSCU of each gene consists of 59 values corresponding to 59 synonymous codons; thus, the PCA was performed to simplify the data to a smaller number of principal factors as a summary feature of the codon usage pattern of each gene. The PCA successfully reduced the 59 values of each RSCU into two significant components. The RSCUs of the human genes and RNA viruses were represented by the coordinates of principal component 1 (PC1, x-axis) and principal component 2 (PC2, y-axis) plotted on the PCA of an RSCU graph (Figure 1). The RSCU and PCA of the RSCUs of the human genes and human RNA viruses are shown in Supplementary File 1.

In Figure 1, the PCAs of the RSCUs of the human genes were represented with a transparent black dot; genes with a similar RSCU were located in the same area of the graph. The number of human gene located in each quadrant was counted: upper left, 6,020 genes; lower left, 4,227 genes; upper right, 4,664 genes; and lower right, 5,281 genes. Interestingly, many human genes were located densely in the right quadrants, specifically, between (x = 0.95 to 1.7) and (y = -0.7 to 0.6). Most RNA viruses were also located in the right quadrants. Additionally, negative sense-single strand RNA viruses (-ssRNA), ambisense RNA viruses (Ambi), and HIV-1 viruses were located in the area of the right quadrants.
where the human genes were densely located. However, a great variation was observed in some groups of RNA viruses, especially positive-sense single strand RNA viruses (þssRNA), double strand RNA viruses (dsRNA), and retroviruses (Retro).

The relationship between the PCA of the RSCU analysis and the codon adaptive index (CAI) was investigated. CAI is a common parameter used to assess the codon usage bias of a gene. It is calculated from the frequency of the overall codons in a given protein coding sequence with respect to a reference set of genes [37]. In our analysis, the human codon usage table, which is the average codon frequency of a human genome, was used as the reference set. From the results (Figure 1), the PCA of the RSCU of the human codon usage table was plotted near the x and y intercepts, showing the average codon usage pattern of all human genes. A number of human genes in the PCA of the RSCU graph were selected and subjected to the CAI calculation. The graphs of PC1 and CAI, of PC2 and CAI, were plotted; the linear regression and Pearson correlation coefficient (PCC) were subsequently analyzed. From Figure 2A, it was found that the CAI of genes gradually decreased with an increase in PC1 (R² = 0.7958, PCC = -0.8921, p-value < 0.0001), while a positive correlation was observed between CAI and PC2 (R² = 0.5824, PCC = 0.7631, p-value < 0.0001). The percentages of the GC content at the third position of the codon (%G+C(3)) of the human genes were also determined. In a similar way to CAI (Figure 2B), %G+C(3) gradually decreased with an increase in PC1 (R² = 0.9459, PCC = -0.9726, p-value < 0.0001). A weak correlation between PC2 and %G+C(3) was observed (R² = 0.4373, PCC = 0.6613, p-value < 0.0001). Thus, the PCA of the RSCU analysis could be used to characterize the heterogeneity of the codon usage bias in the human genome, in which genes in the left-upper quadrant contain more optimal codon usage for high expression, whereas those in the right quadrants near or below the x-axis have less optimal codon usage.

The PCAs of RSCUs of the human genes that coded for highly expressed proteins were plotted on a graph. The highly expressed proteins of humans had been previously identified using the proteomic approach (Figure 1; see the gene list in Supplementary Table 1). [38]. From Figure 1, most of the highly expressed proteins were located in the left quadrants. This is in agreement with our analysis showing the relationship between PCA and CAI, and it supports the validity of using PCA to predict CAI.

### 2.2. Human genes with codon usage bias similar to RNA viruses

From the PCA of the RSCU graph (Figure 1), it was demonstrated that most of the human RNA viruses were located in the right quadrants. The degree of difference in the codon usage pattern varied among the groups of viruses. This feature was also observed intragroup. The highest PC1 (x) belonged to rotavirus, indicating a high degree of difference in codon usage pattern compared to human genes and other RNA viruses. In particular, there were a number of human genes with RSCUs similar to RNA viruses (Figure 1), especially -ssRNA, Ambi, and HIV viruses, which were located in the right quadrants, where human genes were also located densely. To investigate the kinds of human genes with codon usage patterns similar to RNA viruses and the contributions of those genes in important biological processes, the human genes plotted in the same area with each subgroup of RNA viruses were retrieved. The criteria for selection of the human genes with RSCUs similar to RNA viruses are described in the methods section.

Figure 3 represents the selected human genes with RSCUs similar to RNA viruses. These human genes were subjected to gene ontology (GO) enrichment analysis using GO-TermFinder to identify the over-represented GO terms in biological processes [39]. REVIGO was then used to reduce the number of GO terms and visualize the results in a more comprehensible manner.
used to categorize the redundant GO terms [40]. The number of human genes with RSCUs similar to RNA viruses are listed in Table 1. The results (Figure 4) show the significant GO terms in the biological processes of human genes with RSCUs similar to RNA viruses. Only human genes retrieved from six subgroups of RNA viruses resulted in significant enrichment, namely, +ssRNA (subgroups 6, 7), -ssRNA (subgroups 3, 4), Retro (HIV-1), and Ambi (subgroup 4), where the human genes recruited from +ssRNA (subgroups 6, 7), -ssRNA (subgroup 4), Retro (HIV-1), and Ambi (subgroup 4) were in the same or adjacent area. The human genes with RSCUs similar to +ssRNA (subgroup 6), -ssRNA (subgroup 4), Retro (HIV-1), and Ambi (subgroup 4) shared similar GO terms in biological processes, including the cell cycle, the regulation of the cell cycle process, cell division, microtubule cytoskeletal organization, chromosome segregation, DNA repair, macromolecule catabolism, and cellular localization (Fig. 4A, B, D, E, and F), while human genes with RSCUs similar to +ssRNA (subgroup 3) were related to RNA processing (Figure 4C). The list of enriched GO terms in biological processes is shown in Supplementary File 2.

2.3. Codon usage bias of cell cycle regulated genes is similar to that of RNA viruses

From the previous section, we demonstrated that human genes in the GO terms of the cell cycle and the regulation of the cell cycle process adopt codon usage patterns similar to those of +ssRNA (subgroups 6, 7), –ssRNA (subgroup 4), retrovirus (HIV-1), and ambisense (subgroup 4) viruses. To confirm that the codon usage patterns of these RNA viruses are similar to human genes in the cell cycle and the regulation of the cell cycle process, the cell cycle codon score (CCCS) was used to evaluate the similarity of the codon usage pattern between that of viral genes and a set of cell cycle-regulated human genes (top-600 set) [11]. The CCCS of human RNA viruses had been calculated and is detailed in Table 2. A positive CCCS indicates that a gene has a codon usage pattern similar to the top-600 set. The results revealed that most of the +ssRNA viruses had a negative CCCS score. Only some +ssRNA viruses had a positive CCCS score (such as dengue viruses [DENVs], MERS-coronavirus, SARS-coronavirus, human coronaviruses, human enterovirus 68, and the hepatitis A virus), whereas most of the –ssRNA, dsRNA, HIV-1, HIV-2, and ambisense viruses had a positive CCCS score. However, HTLV-1 and HTLV-2 in the retrovirus group gave a negative CCCS.

A previous study by Frenkel-Morgenstern et al. also demonstrated that the codon usage pattern of the cell cycle regulated genes (CCRs) influence the cell cycle-dependent protein expression [11]. Thus, the similarity of the codon usage pattern of the CCR genes and RNA viruses was investigated. CCR genes cycling at the protein level and non-cycle regulated genes (NCCRs) that were found not to cycle at the protein level were selected from previous studies (Table 3). The RSCUs of the CCR and NCCR genes were plotted on a graph and compared with the RSCUs of RNA viruses (Figure 5). The results showed that the CCR genes were located with -ssRNA, dsRNA, and some +ssRNA viruses, indicating similar codon usage patterns, while the RSCUs of the NCCR genes were distributed all over the graph with no resemblance to RNA viruses. This result corresponds with the CCCS of RNA viruses (Table 2).

2.4. Human genes with similar codon usage patterns to RNA virus were upregulated in viral infections

From the previous section, we demonstrated that human genes with codon usage patterns similar to RNA viruses contribute to some important biological processes, such as the cell cycle, the regulation of the cell cycle process, cell division, microtubule cytoskeletal organization, chromosome segregation, DNA repair, macromolecule catabolism, cellular localization, and RNA processing (Figure 4). When coupled with the fact that some viral infections can manipulate host cellular pathways (especially the translation machineries), this finding suggests that human genes with codon usage patterns similar to RNA viruses may be upregulated during viral infection [41].

To substantiate this hypothesis, sets of proteomics data of RNA virus infection were reanalyzed. The lists of upregulated protein profiles upon HIV-1 [42], IAV [43], Zika virus (ZIKV) [44], and dengue virus serotype 2 (DENV-2) [45] infections were obtained from previous studies. The lists of upregulated proteins upon viral infection were submitted to GO-TermFinder (Supplementary File 3), and the enriched GO terms were compared to the enriched GO terms of human genes with codon usage patterns similar to RNA viruses from every subgroup. Several enriched GO terms of upregulated protein profiles during viral infections were found to be identical to the GO terms of human genes with codon usage patterns similar to RNA viruses (Figure 4). In the case of HIV-1 and ZIKV, the identical GO terms included the cell cycle, the regulation of the cell cycle process, the mitotic cell cycle, organelle organization, cell division, microtubule-based process, and cellular localization. The identical GO terms of IAV and DENV-2 included macromolecule metabolic processes, nucleic acid metabolic process, chromosome organization, cellular stress response and RNA processing.
Table 1. Categories of human RNA viruses. RNA viruses were categorized based on the nucleic acid types of their genomes. The viruses in each group were divided into subgroups based on their PCA of RSCU. In subgroups containing more than one virus, the mean virus RSCU (mean vRSCU (a, b)) was calculated. The number of human genes with RSCUs similar to RNA viruses were also represented.

| RNA viruses | Subgroups | Members | PCA of RSCU component | No. of human genes with similar RSCU |
|-------------|-----------|---------|-----------------------|-------------------------------------|
|             |           |         | 1 (x)                 | 2 (y)                               |
| +ssRNA      | 1         | Rubella virus | -1.11152              | -2.38693                            | 67 |
|             | 2         | Hepatitis G virus | -0.20805              | -1.33632                           | |
|             |           | Hepatitis C virus | -0.38012              | -0.86880                           | |
|             |           | Mean vRSCU | -0.29409               | -1.10256                            | 192 |
|             | 3         | Hepatitis E virus | 0.05314               | -1.99911                           | |
|             |           | Ross river virus | 0.08755               | -1.48581                           | |
|             |           | Chikungunya virus | 0.21340               | -1.65931                           | |
|             |           | Sindbis virus | 0.25123                | -1.94371                           | |
|             |           | Eastern equine encephalitis virus | 0.48833               | -1.30690                           | |
|             |           | Western equine encephalitis virus | 0.47744               | -1.79137                           | |
|             |           | Venezuelan equine encephalitis virus | 0.42568               | -1.26628                           | |
|             |           | O’nyong nyong virus | 0.56417               | -1.63171                           | |
|             |           | Mean vRSCU | 0.32012               | -1.63553                            | 101 |
|             | 4         | Enterovirus 71 | 0.64889               | -0.88714                           | |
|             |           | Human coxsackievirus A9 | 0.54435               | -0.52889                           | |
|             |           | Human coxsackievirus B4 | 0.65238               | -0.59692                           | |
|             |           | Echoviruses | 0.57004                | -0.57533                           | |
|             |           | Polioviruses | 0.76484                | -0.35123                           | |
|             |           | Norwalk virus | 0.77184                | -0.52468                           | |
|             |           | Sapporo virus | 0.62754                | -0.94239                           | |
|             |           | Dengue virus type 1 | 0.91914               | -0.19005                           | |
|             |           | Dengue virus type 2 | 0.85638               | 0.1919                             | |
|             |           | Dengue virus type 3 | 0.88466               | -0.24134                           | |
|             |           | Dengue virus type 4 | 0.80321               | -0.02901                           | |
|             |           | Japanese encephalitis virus | 0.34886               | -0.42656                           | |
|             |           | Murray Valley encephalitis virus | 0.76079               | -0.71468                           | |
|             |           | St. Louis encephalitis virus | 0.61121               | -0.16698                           | |
|             |           | West Nile virus | 0.42713               | -0.40840                           | |
|             |           | Yellow fever virus | 0.55894               | -0.03048                           | |
|             |           | Zika virus | 0.46268                | 0.08730                            | |
|             |           | Kyasanur Forest disease virus | 0.26948               | -0.22710                           | |
|             |           | Omak hemorrhagic fever virus | 0.25430               | -0.25169                           | |
|             |           | Tick-borne encephalitis virus | 0.15574               | -0.30465                           | |
|             |           | Mean vRSCU | 0.59467               | -0.35595                            | 659 |
|             | 5         | SARS coronavirus | 1.38814               | -1.47854                           | |
|             |           | MERS coronavirus | 1.40490               | -1.39221                           | |
|             |           | Human astrovirus | 1.21324               | -1.94323                           | |
|             |           | Mean vRSCU | 1.33543               | -1.30499                            | 169 |
|             | 6         | Rhinovirus A,B and C | 1.46621               | -0.28559                           | |
|             |           | Human parechovirus | 1.48761               | -0.44531                           | |
|             |           | Enterovirus 68 | 1.50423                | -0.49488                           | |
|             |           | Mean vRSCU | 1.48662               | -0.40859                            | 946 |
|             | 7         | Hepatitis A virus | 1.96492               | -0.10146                           | 188 |
|             |           | Human coronaviruses | 1.83078               | -1.03063                           | 129 |
| -ssRNA      | 1         | Borna virus | 0.27188                | -1.00708                           | 233 |
|             | 2         | Rabies virus | 0.76128                | -0.25367                           | |
|             |           | Mokola virus | 0.79901                | -0.11880                           | |
|             |           | Measles virus | 0.58253               | -0.23633                           | |
|             |           | Mean vRSCU | 0.71427               | -0.20293                            | 837 |
|             | 3         | Vesicular stomatitis virus | 1.10766               | -0.23715                           | |
|             |           | Influenza A virus H1N2 | 0.98353               | -0.58886                           | |
|             |           | Marburg virus | 1.29734               | -0.55782                           | |
|             |           | Ebola viruses | 1.09920                | -0.67494                           | |
|             |           | Influenza B virus | 1.28533               | -0.26627                           | |
|             |           | Human parainfluenza virus type 1 | 1.09898               | -0.58691                           | |
|             |           | Human parainfluenza virus type 3 | 1.48386               | -0.16452                           | |

(continued on next page)
Furthermore, a difference in codon usage has been found to partially contribute to a G-to-A hypermutation in the HIV-1 genome [52, 53]. The G-to-A hypermutation in the HIV-1 genome has been attributed to viral reverse transcriptase (RT), which lacks 3′-to-5′ exonuclease proofreading activity, leading to the misincorporation of nucleotides [50, 51]. In addition, the function of host enzymes of the APOBEC3 (A3) family has been found to partially contribute to a G-to-A hypermutation [52, 53]. Moreover, there are significant variations of codon usage bias among different species, and even among genes in the same organism [5, 21]. Theoretically, two major factors shape the codon usage bias: mutation pressure and translational selection [18]. Mutation pressure can result in uneven frequencies of nucleotide content, which can in turn influence codon usage bias [15, 47]. As to translational optimization, the frequent codons are usually found correlated with the population of tRNA isoacceptors [13, 15]. Thus, the frequent or optimal codons would result in more rapid protein translation due to the greater availability of tRNAs corresponding to the frequently used codons [48].

Although the replication of viruses relies on the host cell machinery, several viruses possess different a codon usage pattern to the codon usage preferences of their host [31]. For instance, the HIV-1 genome has been found to be A-rich [49]. The G-to-A hypermutation in the HIV-1 genome has been attributed to viral reverse transcriptase (RT), which lacks 3′ to 5′ exonuclease proofreading activity, leading to the misincorporation of nucleotides [50, 51]. In addition, the function of host enzymes of the APOBEC3 (A3) family has been found to partially contribute to a G-to-A mutation [52, 53]. Furthermore, a difference in codon usage has been observed among individual genes of HIV-1 [51]. The HIV-1 gag gene, encoded for structural protein, adopts a great difference in codon usage pattern compared to human host cells. In contrast, the HIV-1 genes involved in the regulation of the replication cycle, tat and rev genes, have been demonstrated to be more similar to human codon usage bias [54]. In this analysis, the PCA of RSCU represented codon usage patterns of human genes and RNA viruses as a coordinate of PC1 (x) and PC2 (y) on a graph. We demonstrated that the PCA of RSCU analysis is compatible with a well-established index, CAI. This suggested that the PCA of RSCU could be used in assessing codon usage bias and comparing the difference in codon usage pattern. In particular, the PCA of RSCU allowed a comparison to be made of individual genes in the whole genome scale. Human genes possess various, different codon usage patterns, as observed in each quadrant of a graph. As mentioned earlier, there is a number of human genes located densely in the right quadrants; these genes adopt a non-optimal codon usage pattern similar to CCR genes. Although most of the RNA viruses have a non-optimal codon usage pattern, a great variation in codon usage patterns was observed among the groups of +ssRNA, dsRNA, and retroviruses. The greatest difference in the codon usage patterns belonged to rotavirus, as seen in the graph. By comparison, the rubella virus exhibited a more similar codon usage pattern to humans. These results correspond with those of another study which demonstrated that the codon usage patterns of +ssRNA viruses are closer to human than other RNA viruses, and that the lowest CAI belongs to dsRNA viruses. In more detail, rubella virus (+ssRNA) had the highest mean vRSCU (1.25862) and PC1 (−0.71004) and PC2 (0.11539) compared to other RNA viruses. In contrast, the HIV-1 genes with RSCUs similar to RNA viruses were retrieved and subjected to CAI at 0.773, and rotavirus had the lowest CAI at 0.683 [55].}

### Table 1 (continued)

| RNA viruses | Subgroups | Members | PCA of RSCU component | No. of human genes with similar RSCU |
|-------------|-----------|---------|-----------------------|-------------------------------------|
|             |           |         | 1 (x)                | 2 (y)                              |
|             |           |         |                       |                                     |
|             | Human parainfluenza virus type 2 | 1.25862 | -0.71004             |                                     |
|             | Mumps virus | 0.97594 | -1.15319             |                                     |
|             | Nipah virus | 1.21851 | -0.10109             |                                     |
|             | Hendra virus | 1.06209 | -0.98056             |                                     |
|             | Respiratory syncytial virus | 1.46890 | 0.17766              |                                     |
|             | Metapneumovirus | 1.35819 | -0.42793             |                                     |
|             | Mean vRSCU | 1.20763 | -0.41448             | 898                                 |
| dsRNA       | 1         | Influenza C virus | 1.72634 | -0.54316             | 586                                 |
|             | 2         | Rotaviruses | 2.23210 | -1.29556 | 7                      |
| Retro       | 1         | HTLV-1 | 0.25781 | -0.96125 | 261                                 |
|             | 2         | HTLV-2 | 0.11613 | 0.15063 | 790                                 |
|             | 3         | HIV-1  | 1.41668 | -0.26596 | 1037                                |
|             | 4         | HIV-2  | 0.94712 | -0.34939 | 812                                 |
| Ambi        | 1         | Sin nombre virus | 0.25123 | -1.94371 | 70                                  |
|             | 2         | Lymphocytic choriomeningitis virus | 0.77488 | 0.08308 |                                     |
|             |           | Lassa virus | 0.78719 | 0.24056 |                                     |
|             |           | Machupo virus | 0.90932 | -0.37322 |                                     |
|             |           | Rift valley fever virus | 0.81849 | -0.15444 |                                     |
|             |           | Junin virus | 0.96106 | -0.12456 |                                     |
|             | Mean vRSCU | 0.85019 | 0.08357              | 950                                 |
|             |           | Guanarito virus | 1.09370 | -0.71405 |                                     |
|             |           | La Crosse virus | 1.19379 | -0.84942 |                                     |
|             |           | Crimean-Congo virus | 1.05851 | -0.55100 |                                     |
|             | Mean vRSCU | 1.11533 | -0.70482             | 529                                 |
|             |           | Bunyamwera virus | 1.39643 | -0.85192 |                                     |
|             |           | Hantaan virus | 1.47251 | -0.11594 |                                     |
|             |           | Seoul virus | 1.41416 | -0.37791 |                                     |
|             | Mean vRSCU | 1.42770 | -0.44859             | 522                                 |

### 3. Discussions

Synonymous codons are distributed unequally and in a non-random fashion, which is referred to as codon usage bias [46]. Moreover, there are significant variations of codon usage bias among different species, and even among genes in the same organism [5, 21]. Theoretically, two major factors shape the codon usage bias: mutation pressure and translational selection [18]. Mutation pressure can result in uneven frequencies of nucleotide content, which can in turn influence codon usage bias [15, 47]. As to translational optimization, the frequent codons are usually found correlated with the population of tRNA isoacceptors [13, 15]. Thus, the frequent or optimal codons would result in more rapid protein translation due to the greater availability of tRNAs corresponding to the frequently used codons [48].

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In this analysis, the PCA of RSCU represented codon usage patterns of human genes and RNA viruses as a coordinate of PC1 (x) and PC2 (y) on a graph. We demonstrated that the PCA of RSCU analysis is compatible with a well-established index, CAI. This suggested that the PCA of RSCU could be used in assessing codon usage bias and comparing the difference in codon usage pattern. In particular, the PCA of RSCU allowed a comparison to be made of individual genes in the whole genome scale. Human genes possess various, different codon usage patterns, as observed in each quadrant of a graph. As mentioned earlier, there are a number of human genes located densely in the right quadrants; these genes adopt a non-optimal codon usage pattern similar to CCR genes. Although most of the RNA viruses have a non-optimal codon usage pattern, a great variation in codon usage patterns was observed among the groups of +ssRNA, dsRNA, and retroviruses. The greatest difference in the codon usage patterns belonged to rotavirus, as seen in the graph. By comparison, the rubella virus exhibited a more similar codon usage pattern to humans. These results correspond with those of another study which demonstrated that the codon usage patterns of +ssRNA viruses are closer to human than other RNA viruses, and that the lowest CAI belongs to dsRNA viruses. In more detail, rubella virus (+ssRNA) had the highest CAI at 0.773, and rotavirus had the lowest CAI at 0.683 [55].

A number of human genes with codon usage patterns similar to that of RNA viruses were found by the present study (Figure 3). The human genes with RSCUs similar to RNA viruses were retrieved and subjected to...
gene ontology enrichment analysis. Interestingly, it was found that only human genes similar to groups of viruses in the right quadrant resulted in significant enrichment, namely, the cell cycle, the cell cycle regulation process, cell division, microtubule cytoskeletal organization, chromosome segregation, DNA repair, macromolecule catabolism, and cellular localization.

The number of human genes with RSCUs similar to RNA viruses retrieved from each subgroup varied from only seven to a thousand genes (Table 1). However, the number of retrieved genes did not affect the significance level or the number of enriched GO terms in Figure 4; for instance, 898 genes for the +ssRNA subgroup 3 resulted in only 1 enriched GO term, whereas 198 genes for the +ssRNA subgroup 7 resulted in 5 GO terms. This suggests that the enriched GO terms did not result from a bias from the different numbers of retrieved genes among the virus groups. It is also possible that some virus groups with a limited number of retrieved genes might not have had sufficient statistical power to enable the detection of enriched GO terms. Among the retrieved human genes, only the genes that adopted a non-optimal codon usage pattern retrieved from the area where they were located densely in the right quadrants gave significant overrepresented GO terms. This

Figure 4. GO terms enrichment in the biological processes from human genes with RSCUs similar to RNA viruses. GO terms with $p \leq 0.01$ were taken as a significant enrichment and represented with $-\log_{10} (p$-value). The bold alphabets indicate GO terms that were found to be identical to the enriched GO terms of upregulated protein profiles in viral infections, determined by high-throughput quantitative proteomics; H (HIV-1), Z (Zika virus), F (influenza A virus), and D (dengue virus serotype 2).
## Table 2. Cell-Cycle Codon Score (CCCS) of human RNA viruses.

| Groups | Gene isoform or viruses         | PCA components | CCCS  |
|--------|---------------------------------|----------------|-------|
|        |                                 | PC1 (x)        | PC2 (y) |       |
|         |                                 |                |        |
| +ssRNA | Rubella virus                   | -1.11152       | -2.38693 | -0.041109 |
|         | Hepatitis G virus               | -0.20805       | -1.33632 | -0.012674 |
|         | Hepatitis C virus               | -0.39012       | -0.8688  | -0.015136 |
|         | Hepatitis E virus               | 0.05314        | -1.99911 | -0.006142 |
|         | Ross river virus                | 0.08755        | -1.48581 | -0.011565 |
|         | Chikungunya virus               | 0.2134         | -1.65931 | -0.008180 |
|         | Sindbis virus                   | 0.25123        | -1.94371 | -0.009176 |
|         | Eastern equine encephalitis virus | 0.48833    | -1.3069  | 0.002974 |
|         | Western equine encephalitis virus | 0.47744    | -1.79137 | -0.003173 |
|         | Venezuelan equine encephalitis virus | 0.42568  | -1.26628 | -0.003554 |
|         | Oroyong nyong virus             | 0.56417        | -1.63171 | -0.001119 |
|         | Entervoirus 71                  | 0.64889        | -0.88714 | -0.002627 |
|         | Human coxsackievirus A9         | 0.54435        | -0.52889 | -0.002991 |
|         | Human coxsackievirus B4         | 0.65238        | -0.59692 | 0.000998 |
|         | Echoviruses                     | 0.57004        | -0.57533 | -0.002082 |
|         | Polioviruses                    | 0.76484        | -0.35123 | 0.001193 |
|         | Norwalk virus                   | 0.77184        | -0.52468 | 0.001137 |
|         | Sapporo virus                   | 0.62275        | -0.94239 | 0.004792 |
|         | Dengue virus type 1             | 0.91914        | -0.19005 | 0.005112 |
|         | Dengue virus type 2             | 0.85638        | 0.19119  | 0.005403 |
|         | Dengue virus type 3             | 0.88466        | -0.24134 | 0.004578 |
|         | Dengue virus type 4             | 0.80321        | -0.02901 | 0.003912 |
|         | Japanese encephalitis virus     | 0.34886        | -0.42656 | -0.003955 |
|         | Murray Valley encephalitis virus | 0.76079     | -0.71468 | 0.000022 |
|         | St.Louis encephalitis virus     | 0.61121        | -0.16698 | 0.000160 |
|         | West Nile virus                 | 0.42713        | -0.4084  | -0.003391 |
|         | Yellow fever virus              | 0.55894        | -0.03048 | -0.000690 |
|         | Zika virus                      | 0.46368        | 0.0873   | -0.002752 |
|         | Kyasanur Forest disease virus   | 0.26948        | -0.2271  | -0.006086 |
|         | Omsk hemorrhagic fever virus    | 0.2543         | -0.25169 | -0.005482 |
|         | Tick-borne encephalitis virus   | 0.15574        | -0.30465 | -0.007427 |
|         | SARS coronavirus                | 1.38814        | -1.47854 | 0.027135 |
|         | MERS coronavirus                | 1.4049         | -1.39321 | 0.031220 |
|         | Human astrovirus                | 1.21324        | -1.04323 | 0.014968 |
|         | Rhinovirus A,B and C            | 1.46621        | -0.28559 | 0.013209 |
|         | Human parechovirus              | 1.48761        | -0.44531 | 0.013904 |
|         | Enterovirus 68                  | 1.50423        | -0.49488 | 0.013644 |
|         | Hepatitis A virus               | 1.96492        | -0.10146 | 0.022827 |
|         | Human coronaviruses             | 1.83078        | -1.03636 | 0.024501 |
| -ssRNA  | Borna virus                     | 0.27188        | -1.00708 | -0.002858 |
|         | Rabies virus                    | 0.76128        | -0.25367 | 0.000205 |
|         | Mokola virus                    | 0.79901        | -0.1188  | 0.001436 |
|         | Meadles virus                   | 0.58253        | -0.23633 | 0.000810 |
|         | Vesicular stomatitis virus      | 1.10766        | -0.23715 | 0.006431 |
|         | Influenza A virus H3N2          | 0.98353        | -0.58886 | 0.008199 |
|         | Marburg virus                   | 1.29734        | -0.55782 | 0.000675 |
|         | Ebola viruses                   | 1.0992         | -0.67494 | 0.008390 |
|         | Influenza B virus               | 1.28533        | -0.26627 | 0.014146 |
|         | Human parainfluenza virus type 1| 1.09898        | -0.58691 | 0.015018 |
|         | Human parainfluenza virus type 3| 1.48386        | -0.16452 | 0.020038 |
|         | Human parainfluenza virus type 2| 1.25962        | -0.71004 | 0.016267 |
|         | Mumps virus                     | 0.97594        | -1.15319 | 0.009273 |
|         | Nipah virus                     | 1.21851        | -0.10109 | 0.011326 |
|         | Hendra virus                    | 1.06209        | -0.09805 | 0.009457 |
|         | Respiratory syncytial virus     | 1.4689         | 0.17766  | 0.020368 |
|         | Metapneumovirus                 | 1.35819        | -0.42703 | 0.016291 |
|         | Influenza C virus               | 1.72654        | -0.54316 | 0.021730 |

(continued on next page)
suggested that the difference in codon usage bias in human genes might have specific functions. The contribution of non-optimal codon usage bias in human genes on the regulation of protein expression has been investigated in previous research [11, 56, 57]. One study on CCR genes revealed that the non-optimal codon usage pattern generates the oscillation in protein expression during cell cycle progression [11]. We demonstrated that the RSCUs of RNA viruses were similar to the RSCUs of CCR genes, using both the CCCS calculation and the PCA of RSCU.

Table 2. (continued)

| Groups | Gene isoform or viruses | PCA components | CCCS |
|--------|-------------------------|----------------|------|
|        |                         | PC1 (x)        | PC2 (y) | |
| dsRNA  | Colorado tick fever virus | 1.29334        | -1.66493 | -0.005517 |
|        | Mammalian orthoreovirus  | 1.05591        | -1.47831 | 0.006214  |
|        | Human picobirnavirus     | 0.95531        | -1.1807 | 0.003846  |
|        | Rotaviruses              | 2.2321         | -1.29556 | 0.028856  |
| Retro  | HTLV-1                   | 0.25781        | -0.96125 | -0.006770 |
|        | HTLV-2                   | 0.11613        | 0.15063 | -0.007187 |
|        | HIV-1                    | 1.41668        | -0.26596 | 0.008663  |
|        | HIV-2                    | 0.94712        | -0.34939 | 0.007142  |
| Ambi   | Sin nombre virus         | 0.25123        | -1.94371 | 0.017737  |
|        | Lymphocytic choriomeningitis virus | 0.77488 | 0.08308 | 0.006150 |
|        | Lassa virus              | 0.78719        | 0.24056 | 0.007772  |
|        | Machupo virus            | 0.90932        | 0.37322 | 0.008089  |
|        | Rift valley fever virus  | 0.81849        | -0.15444 | 0.003661 |
|        | Junin virus              | 0.96106        | -0.12456 | 0.009001  |
|        | Guanarito virus          | 1.0937         | -0.71405 | 0.009082  |
|        | La Crosse virus          | 1.19379        | -0.84942 | 0.007769  |
|        | Crimean-Congo virus      | 1.05851        | -0.551  | 0.006304  |
|        | Bunyamwera virus         | 1.39643        | -0.85192 | 0.018548  |
|        | Hantaan virus            | 1.47251        | -0.11594 | 0.015166  |
|        | Seoul virus              | 1.41416        | -0.37791 | 0.015762  |

Table 3. List of CCR and NCCR genes.

| Gene symbols | Descriptions | References |
|--------------|--------------|------------|
| CCR          | TRANS2B      | transformer 2 beta homolog [70, 71, 72, 73]|
|              | TOP1         | topoisomerase |
|              | E2F5         | E2F transcription factor 5 |
|              | H2AFV        | H2A histone family member V |
|              | ANP32E       | acidic nuclear phosphoprotein 32 family member E |
|              | STAG1        | stromal antigen 1 |
|              | USP7         | ubiquitin specific peptidase 7 |
|              | EZH2         | enhancer of zeste 2 polycomb repressive complex 2 subunit |
|              | RBBP7        | RB binding protein 7, chromatin remodeling factor |
|              | DDX5         | DEAD-box helicase 5 |
|              | GTT2F2       | general transcription factor IF subunit 2 |
|              | GARS         | glycyl-tRNA synthetase |
|              | TARS         | threonyl-tRNA synthetase |
|              | EPBS         | glutamyl-prolyl-tRNA synthetase |
| NCCR         | EFHD2        | EF-hand domain family member D2 [74] |
|              | ZNF433       | zinc finger protein 433 |
|              | STAG3        | stromal antigen 3 |
|              | LMNA         | lamin A/C |
|              | E2F4         | E2F transcription factor 4 |
|              | HMGA1        | high mobility group AT-hook 1 |
|              | YPEL1        | yippee like 1 |
|              | SET          | SET nuclear proto-oncogene |
|              | DOX46        | DEAD-box helicase 46 |
|              | EZH1         | enhancer of zeste 1 polycomb repressive complex 2 subunit |
|              | HMGA2        | high mobility group AT-hook 2 |
|              | SAE1         | SUMO1 activating enzyme subunit 1 |
|              | FHAD1        | forkhead associated phosphopeptide binding domain 1 |
|              | GAPDH        | glyceraldehyde-3-phosphate dehydrogenase [11] |
|              | WARS         | tryptophyl-tRNA synthetase |
results showed that ssRNA, dsRNA, HIV-1, HIV-2, ambisense viruses, and a few viruses in the ssRNA group exhibited non-optimal codon usage similar to that of the CCR genes. This suggests that despite having a non-optimal codon usage bias, viral genes might be efficiently expressed during the specific phase of the cell cycle correlated with the available tRNA population in that period.

The tRNA population is tissue specific and varies with cellular conditions [58]. Alteration of the tRNA population depends on the level of aminoacyl tRNA synthetase and cellular ATP concentration [59]. In yeast cells, oscillation of the aminoacyl tRNA synthetase and ATP during the cell cycle has been found to result in an increase in tRNA levels in the G2/M phase, but a low tRNA level was observed toward the end of the G1 phase [11]. Therefore, with a low-charged tRNA concentration, the genes expressed during G1 prefer optimal codon usage bias, whereas the genes with non-optimal codon usage bias are highly expressed in the other phases of the cell cycle with a high-charged tRNA concentration [11]. The availability of charged tRNAs during the cell cycle may regulate protein translation in a codon usage-specific manner. Several studies have revealed viral subversion of the cell cycle by arresting via various mechanisms to generate the resources and favorable environment for viral replication and viral protein production [60]. Cell cycle arrest has been observed in both DNA and RNA virus infections. In some RNA viruses, cell cycle arrest at a specific phase may lead to an increase in viral protein translation [61]. During the G2/M phase, the expression of many proteins has been found to fluctuate by arresting at G2/M, viruses may use this mechanism to regulate protein expression [62]. Another study found that HIV-1 was more transcriptionally active during the G2 phase, and that arresting of the cell cycle may help limit the host immune response [63]. Moreover, an HIV-1 infection also causes an alteration at the cellular tRNA level [34]. As to avian coronavirus infections, G2/M arrest has been found with an increased viral protein expression [64]. Furthermore, rotavirus infection arrests the cell cycle in the S/G2 phase, favoring viral protein expression [61], while influenza A virus infection arrests the cell cycle in the G0/G1 phase, resulting in increased viral protein expression [65, 66]. These findings suggest that viruses may manipulate the cell cycle and cellular translation machinery to create available tRNA population favoring the viral codon usage pattern.

Several GO terms of human genes with codon usage patterns similar to RNA viruses have been found by previous studies to be identical to the GO terms of upregulated protein profiles in viral infections. Global proteomic and phosphoproteomic changes in HIV-1 infected CD4+ T cells revealed that HIV-1 affected transcriptional and translational regulation, and targeted RNA or protein degradation in order to modulate biological processes (including signal transduction, cell cycle, metabolic processes, and the immune system) [42]. In addition, a study of ZIKV infected human neurospheres also found an upregulation profile of proteins involving cell cycle arrest. This resulted in an alteration of the cell cycle in order to regulate the transcription and translation of the host cells [44]. IAV infection targeted several cellular pathways to favor its replication, including aminoacyl-tRNA biosynthesis, glycolysis, fatty acid biosynthesis, and spliceosome [43]. Moreover, regulated proteins and phosphoproteins in DENV-infected cells were related to cellular macromolecule biosynthesis, RNA splicing, chromatin modification, and cell stress response, and these regulations help facilitate viral protein expression [45]. This suggests that human genes with codon usage patterns similar to that of viruses may be upregulated at the translational level in a viral infection. It is unclear whether viruses evolved to regulate the translational machinery in order to accommodate the codon usage pattern that had already been shaped by mutational pressure, or whether they adapted their codon usage pattern to match the cellular translational machinery condition in infected cells. These two possibilities are not mutually exclusive. Further studies are required to gain more insight into this new aspect of the virus-host interaction.

4. Materials and methods

4.1. Protein coding sequences of human genomes

The protein coding sequences of human genomes were downloaded from the GENCODE database (version 26) in FASTA format [67]. The data set provided the nucleotide sequences of the coding transcripts on the reference chromosomes, including multiple transcript variants for each gene. Thus, only the major transcript variants were selected for analysis. The major transcript variant is the longest transcript variant of the gene with a complete ORF. For each gene, one representative as a major transcript variant with the longest sequence length was selected using a custom python script (Supplementary File 4). The ORFs of the protein coding sequences were rechecked by the ORFfinder tool at the
4.2. Sequences of human RNA virus genomes

The data set for the sequences of the human RNA virus genome utilized by a previous study was used [55]. A total of 77 RNA viruses that can cause diseases in humans were selected. The protein coding sequences of those RNA viruses were downloaded in FASTA format from the Nucleotide database (RefSeq, NCBI). For each virus species, the protein coding sequences from different isolations of the same virus species were downloaded. The sequences were selected based on their availability in the database (some viral protein coding sequences were only available in a few numbers). Any coding sequence with unidentified nucleotides that could not be translated or with an incomplete ORF was excluded. The selected human RNA viruses were categorized by their family, genus, and genome polarity. They comprised 39 positive-sense single strand RNA (+ssRNA) viruses; 18 negative-sense single strand RNA (−ssRNA) viruses; 4 double strand RNA (dsRNA) viruses; 4 retroviruses (retro); and 12 ambisense (+/−, ambi) viruses. Supplementary File 5 presents a list of the human RNA viruses used in this study, the accession numbers of the sequences, and the number of coding sequences of each virus.

4.3. The RSCU analysis

The RSCU is the ratio of the observed frequency of the codon in a gene to the expected frequency of the codon under the condition that all the synonymous codons are equally used. The three stop codons (TAA, TAG, TGA), Met (ATG), and Trp (TGG) were excluded from the analysis. The observed frequency of the codons in the genes was counted. The FASTA sequences were parsed, and the codons of the coding sequences for each transcript variant were counted by a python script and the Biopython library (Python version 3.5.2, with Biopython version 1.66; for the scripts, see Supplementary File 6). Then, the RSCU was calculated as follows:

\[
RSCU_i = \frac{X_i}{\sum_{i=1}^{n} X_i}
\]

where \( n \) is the number of synonymous codons (1 ≤ \( n \) ≤ 6) for the amino acid, and \( X_i \) is the number of occurrences of codon \( i \). The synonymous codons with RSCU values greater than 1.0 had a positive codon usage bias and were defined as abundant codons, while those with RSCU values less than 1.0 had a negative codon usage bias and were defined as less-abundant codons. In the case of RSCU values that were exactly 1.0, it meant that there was no codon usage bias, and the codons were chosen equally [68]. The RSCU of human RNA viruses were calculated using the CAIcal server, which is available at http://genomes.urv.es/CAIcal [37]. The multiple protein coding sequences from different isolations of the virus in the same species were submitted to the CAIcal server for the RSCU calculation. After that, the average RSCU was calculated to represent the RSCU of each RNA virus species. The RSCUs of the human genes and RNA viruses are provided in Supplementary File 1.

4.4. The calculation of CAI

The CAI of a specific gene was calculated using the CAI calculator on the CAIcal server [37]. The reference human codon usage table was obtained from the Codon Usage Database (http://www.kazusa.or.jp/codon/) [69].

4.5. PCA of RSCUs

The RSCUs of the protein coding sequences of 20,190 human genes and 77 human RNA viruses were input to the PCA. The PCA was performed using PASW Statistics for Windows, version 18 (SPSS Inc., Chicago, Ill., USA). The Kaiser–Meyer–Olkin Measure of Sampling Adequacy test (KMO–MSA) was also analyzed. The overall KMO–MSA was 0.799, which was greater than a cut off of 0.5, indicating that the sample size was adequate. The principle components were successfully extracted using covariance matrix and Quartimax rotation, which reduced the high dimensions of the dataset to a smaller number of dimensions. The selection of the significant components was based on a scree plot and the proportions of variance. The scree plot was a plot of the component numbers and eigenvalues; only the first two components had eigenvalues greater than 1, and they accounted for 45.5% of the total variance. The RSCU of the gene was represented by the coordinate of PC1 and PC2 (\( x, y \)) on the graph. The PCAs of the RSCU graphs were plotted and analyzed using GraphPad Prism 7 (GraphPad Software, Inc., CA).

4.6. The CCCS calculation

The CCCS of a gene evaluates the similarity of the codon usage between that of a specific gene and a set of cell cycle regulated human genes. The calculation has been previously described [11]. Briefly, the CCCS is the sum of the codon preference (CP) values of the cell cycle regulated human genes (top-600 set) over all codons in the coding sequence of a gene, normalized by the length of the cDNA. The CCCS of a specific gene was calculated as follows:

\[
CCCS(g) = \sum_{codon[gene]} CP^{top-600}(codon) / length(g)
\]

where \( g \) is every codon of a gene, and CP top-600(codon) is the CP in the top-600 gene set (see cited reference, Table 1).

4.7. GO enrichment

On the PCA of the RSCU graph, the human genes located in the same area of RNA viruses were taken as human genes with RSCUs similar to RNA viruses. To select these human genes, the average RSCU of each group of RNA viruses was initially calculated. However, a great variation of RSCU was observed in each group of RNA virus categorized by the nucleic acid types of their genomes. Hence, the viruses in each group were divided into subgroups by their RSCU before calculation of the average PC1 (\( x \)) and PC2 (\( y \)); these RNA-virus subgroups are listed in Table 1. Subsequently, the average RSCU of the RNA viruses in each subgroup (mean vRSCU, coordinate [\( a, b \)]) were set as the circle center of a circle with a radius of 0.3 units (Figure 3). The radius was calculated to be minimal, to not exceed the standard deviation of the distance between the circle center and the human genes, and to cover most of the viruses in each subgroup. The human genes located within the circle were taken as the human genes with RSCUs similar to RNA viruses. The distance (\( r \)) from the circle center (\( a, b \)) was measured as follows:

\[
(a-x)^2+(b-y)^2 = r^2
\]

where \( a \) and \( b \) are the coordinates of the mean vRSCU of each subgroup of RNA viruses, while \( x \) and \( y \) are the coordinates of the RSCUs of the human genes.

The human genes with an RSCU similar to each group of RNA viruses were analyzed for GO enrichment using GoTermFinder, and REVIGO was used to categorize the redundant GO terms [39, 40]. The whole genome of Homo sapiens was used as a background list. The over-represented GO terms in the biological process were investigated in both analyses, with \( p \leq 0.01 \) taken as a significant enrichment.
4.8. Statistical analysis

The simple linear regression analysis and Pearson correlation coefficient (PCC) were determined using GraphPad Prism 7 with \( p < 0.05 \) was taken as significant.

Declarations

Author contribution statement

Kunlakanya Jitobaom: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Supinya Phakaratsakul: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Thanyporn Sirisomthong: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Additional information

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