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LETTER TO THE EDITOR

Human herpesvirus miRNAs statistically preferentially target host genes involved in cell signaling and adhesion/junction pathways

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Dear Editor,

MicroRNAs (miRNAs) are small non-coding RNAs that play key roles in the regulation of major biological processes in plants and animals. Recent research has revealed that viruses also encode their own miRNAs [1]. Ninety-five mature human viral miRNAs have been reported so far, eighty-five of which are encoded by five large double-stranded DNA human herpesviruses, including human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), herpes simplex virus 1 (HSV-1), HSV-2 and Kaposi's sarcoma-associated herpesvirus (KSHV) [2]. Studies of individual human viral miRNAs have shown that they can regulate both viral and human genes to suppress host anti-viral response and modify behaviors of infected cells [1, 3]. However, to date, there has been no systematic study of whether viral miRNAs preferentially target any particular pathways in human. Here, we identified the human genes putatively targeted by known miRNAs encoded by the five human herpesvirus and analyzed the pathways underlying these genes. We found that these viral miRNA-regulated human genes were statistically significantly enriched in cell signaling and adhesion/junction pathways.

To identify the putative human target genes of viral miRNAs, we used PITA, an effective *in silico* algorithm that identifies miRNA targets based on a thermodynamic miRNA-target interaction model [4]. For stringent quality control, we performed Monte Carlo permutations to set a rigorous *target score* threshold that limited False Discovery Rate (FDR) at a low level of 0.0026 (see details in Supplementary information, Data S1). We scanned the 3′ UTR regions of all known human mRNAs and identified 1228, 534, 1133, 579 and 185 candidate human mRNA targets for miRNAs encoded by HCMV, EBV, HSV-1, HSV-2 and KSHV, respectively (Figure 1A). The complete list of candidate target genes can be downloaded from http://vmirna.cbi.pku.edu.cn/.

We analyzed the pathways underlying the human genes regulated by miRNAs encoded by each of the herpesviruses. We mapped the human target genes to pathway knowledgebase Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/). As some pathways were naturally larger and might involve more genes by chance alone, it was important to identify the statistically enriched pathways that were more likely to be biologically meaningful. For each pathway, a *p*-value derived from hyper-geometric distribution was computed to assess whether the number of candidate target genes associated with the pathway was larger than expected in the whole genome background. The *P*-value was further adjusted for multiple hypothesis testing to get a corresponding FDR-corrected *P*-value [5, 6]. The enriched target pathways for each herpesvirus were listed in Supplementary information, Table S1.

Close inspection revealed that these pathways fell into predominantly two categories: cell signaling pathways and cell adhesion/junction pathways (highlighted in light yellow and light green in Supplementary information, Table S1, and summarized in Figure 1B). We then repeated the above pathway analysis by considering, as one group, all human genes targeted by known miRNAs encoded in all five herpesviruses. As shown in Supplementary information, Table S2, similar patterns were observed — cell signaling pathways and cell adhesion/junction pathways were statistically significantly preferentially targeted. Moreover, similar statistical enrichment of cell signaling and adhesion/junction pathways was also observed in the downregulated genes identified by a recently published microarray-based viral miRNA target identification experiment [7] (Supplementary information, Table S3).

Several enriched cell signaling pathways were related to cell proliferation, differentiation and cell death. Previous reports indicated that some viruses modulate host immune and apoptosis-related pathways, mostly as a kind of immunoevasion strategy [1, 3, 8]. Our statistical

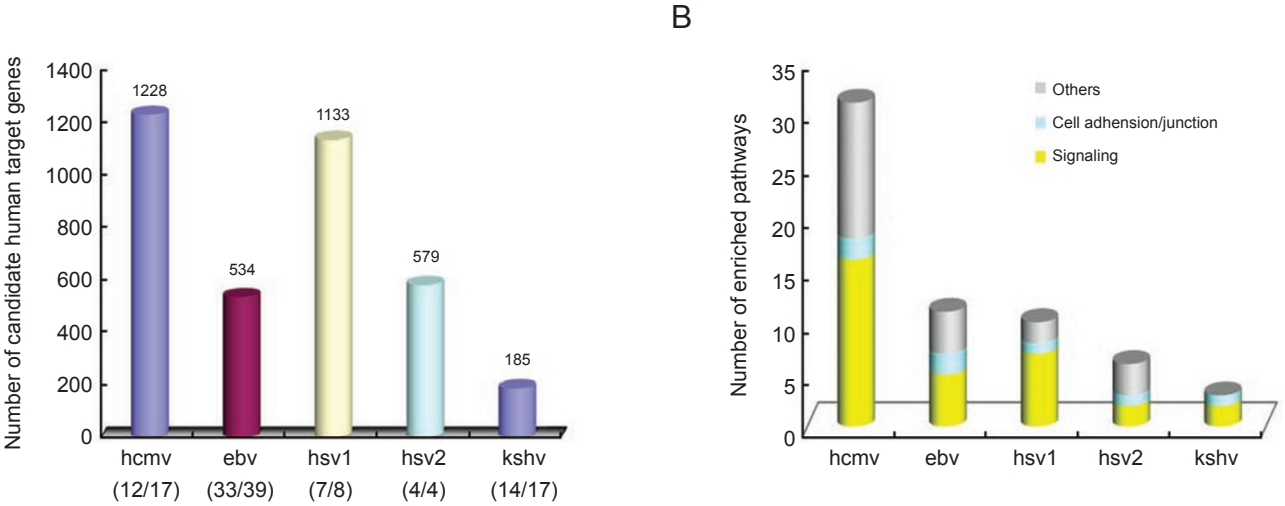

Figure 1 Summary of the number of human genes and pathways targeted by known mature miRNAs encoded in each of the human herpesviruses. **(A)** Number of candidate human target genes for miRNAs encoded by each human herpesvirus. Targets were predicted by PITA with a low False Discovery Rate (FDR) of less than 0.0026. In parenthesis under each virus name we listed the number of miRNAs with targets predicted over the total number of miRNAs encoded by that herpesvirus. **(B)** Summary of human pathways preferentially targeted by miRNAs encoded by each human herpesvirus. The pathways fell into predominantly two categories: cell signaling pathways and cell adhesion/junction pathways (highlighted in light yellow and light green, respectively).

findings were consistent with these reports, while suggesting additional new signaling pathways. Of particular interest was the Wnt pathway, which was preferentially targeted by miRNAs encoded in three herpesviruses (except HCMV and HSV-2), as well as when they were considered as one group. Wnt is a multifunctional signaling pathway that involves a number of genes responding to a variety of developmental signals and cross-talking with other pathways such as MAPK, adherens junction and focal adhesion pathways [9]. Recent literature suggested that herpesviruses intervene with the Wnt pathway [10]. Our pathway analysis revealed that the human Wnt pathway included as many as 25 candidate viral-miRNA targets, one-quarter of which were targeted by more than one virus (Supplementary information, Figure S1A).

The enriched cell adhesion/junction-related pathways included tight junction, focal adhesion and adherens junction pathways. Cell-cell adhesion/junction plays key roles in the immune system [11]. The components of junctional complexes and other adhesion proteins could also be used by viruses as receptors for entering or exiting the host cell [12]. Recently, KSHV-encoded miRNAs were reported to suppress a major regulator of cell adhesion and migration [7]. Two receptors in the adherens junction pathways, Nectin and ErbB1/2, were reported to be downregulated in infected cells, but the exact mechanisms remained unclear [13, 14]. Our analysis showed that Nectin and ErbB1/2 were putatively targeted by miRNAs encoded by multiple herpesviruses (Supplementary information, Figure S1B).

To our knowledge, this is the first work to systematically investigate the functional characteristics of human genes regulated by herpesvirus miRNAs as a group based on rigorous statistical analysis. The consistent finding that human cell signaling and cell adhesion/junction pathways were preferentially targeted by multiple herpesviruses is especially striking when considering the fact that there are large differences between the miRNAs encoded in different viruses [15]. Our results revealed that different miRNAs in different herpesviruses could target different genes within the similar cell signaling or cell adhesion/junction pathways, or target different 3′UTR sites within the same mRNA (a full list of candidate target genes in human is available at http://vmirna.cbi.pku.edu.cn/).

Interestingly, several human pathways preferentially regulated by herpesviruses could be inter-connected into a "cell communication" network based on KEGG annotations, as shown in Supplementary information, Figure S2. Our observations could provide new clues for further understanding the complex mechanisms of host-virus interactions.

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(**Supplementary information** is linked to the online version of the paper on the Cell Research website.)