HHS Public Access

Author manuscript

J Endod. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

J Endod. 2017 January ; 43(1): 84–89. doi:10.1016/j.joen.2016.10.006.

Viral microRNAs Identified in Human Dental Pulp

Sheng Zhong, DDS, MS[1,2,#], **Afsar Naqvi, PhD**[3,#], **Eric Bair, PhD**[2], **Salvador Nares, PhD, DDS**[3], and **Asma Khan, BDS, PhD**[2]

[1]Endodontic Associates, MN

[2]Department of Endodontics, University of North Carolina, Chapel Hill, NC

[3]Department of Periodontics, College of Dentistry, University of Illinois at Chicago, Chicago, IL

Abstract

Introduction—MicroRNAs (miRs) are a family of non-coding RNAs that regulate gene expression. They are ubiquitous among multicellular eukaryotes and are also encoded by some viruses. Upon infection, viral miRs (vmiRs) can potentially target gene expression in the host and alter the immune response. While prior studies have reported viral infections in human pulps, the role of vmiRs in pulpal disease is yet to be explored. The purpose of this study was to examine the expression of vmiRs in normal and diseased pulps and to identify potential target genes.

Methods—Total RNA was extracted and quantified from normal and inflamed human pulps (N=28). Expression profiles of vmiRs were then interrogated using miRNA Microarrays (V3) and the miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Santa Clara, CA). To identify vmiRs that were differentially expressed, we applied a permutation test.

Results—Of the 12 vmiRs detected in the pulp, 4 vmiRs (including those from herpes virus and human cytomegalovirus) were differentially expressed in inflamed pulps as compared to normal pulps ($p<.05$). Using bioinformatics we identified potential target genes for the differentially expressed vmiRs. They included key mediators involved in the detection of microbial ligands, chemotaxis, proteolysis, cytokines and signal transduction molecules.

Corresponding Author: Asma A Khan, BDS, PhD, 1170 First Dental Building, CB #7450, School of Dentistry, UNC Chapel Hill, Chapel Hill, NC 27599-7450, Asma_Khan@unc.edu.

[#]Co-first authors

The authors deny any conflict of interest.

Statement regarding conflict of interest

We affirm that we have no financial affiliations (e.g., employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria) or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript not have any such arrangements existed in the past three years.

Protection of human subjects

The informed consent of all human subjects who participated in the experimental investigation reported or described in this manuscript was obtained after the nature of the procedure and possible discomforts and risks had been fully explained.

Conclusions—This data suggests that miRs may play a role in interspecies regulation of pulpal health and disease. Further research is needed to elucidate the mechanisms by which vmiRs can potentially modulate the host response in pulpal disease.

Introduction

MicroRNAs (miRs) are 21-24 nucleotides (nt) long, single stranded, non-coding RNAs. By fine-tuning the transcriptome, they regulate most fundamental biological processes including cell differentiation, signaling, cell death and pathogen response (1,2). miRs are transcribed as long precursor transcripts called primary-miRs (pri-miRs) by RNA polymerase II. The localized stem-loop structure on pri-miR is recognized by Drosha to release ~60-80 nt pre-miR in the nucleus. These imperfectly paired stem-loops are exported to the cytoplasm and are processed by Dicer to enzymatically generate miR duplexes. Of the two strands, the mature miR strand is finally loaded onto RNA-induced silencing complexes (mi-RISC) while the passenger (star) strand is generally degraded. The mature miR guides the protein machinery to regulate mRNAs and eventually affects translation.

Recent studies show that viruses, especially those with DNA genome, encode miRs in order to regulate their life cycle inside the host (3-5). The viral transcripts generated in the nucleus are recognized by the host proteins and processed in a way similar to the canonical miR pathway. Viral pri-miRs are primarily transcribed by polymerase II; however, it has been shown that polymerase III generates tRNA-like miR precursors in murine gamma herpesvirus type 68 (6). Following the discovery of vmiRs in Epstein-Barr virus (EBV), several herpesvirus families were later found to encode miRs. To date, more than 250 different miRs of viral origin have been identified and the list continues to expand (7).

Herpesviruses are large, double-stranded DNA viruses that infect a range of invertebrate and vertebrate animals. There are 8 types that infect humans: herpes simplex viruses 1 and 2 (HSV 1 and 2), varicella-zoster virus, EBV, human cytomegalovirus (HCMV), human herpesvirus 6, human herpesvirus 7, and Kaposi's sarcoma-associated herpesvirus (KSHV). The genomic locations of alpha (e.g. HSV 1 and 2) and gamma (e.g. EBV, KSHV) herpesviruses miRs are clustered generally in the vicinity of latency associated regions, however, beta herpesvirus (e.g. HCMV) are scattered throughout the viral genome. The expression profiles of vmiRs are restricted to specific stages of the virus life cycle. For instance, most of the miRs in KSHV and EBV are expressed during latency while HSV-1 miR-H1 and few HCMV miRs are expressed during the productive stages of the infection.

VmiRs may regulate both viral and/or host transcripts. Several vmiRs target viral genes to which they are antisense, *e.g.,* EBV miR-BART2 targeting BALF5 gene while, others control transcripts emanating from other genomic locations, *e.g.,* HSV-1 miR-H6 targeting ICP4 (3,8). Target identification reveals that several vmiRs regulate the same target, a feature commonly observed in mammalian system. For instance, HSV-1 miR-H3 and miR-H4 regulate the level of pathogenesis factor ICP34.5 while EBV latent membrane protein 1 (LMP1) is controlled by three different vmiRs (3).

Modulation of host genes is another function of viral miRs. Given the capacity to regulate hundreds of probable host transcripts, vmiRs have the potential to significantly influence the host transcriptome. Global transcriptome profiling has revealed the set of host genes under direct influence of vmiRs. Unlike metazoan miRs, vmiRs exhibit limited sequence conservation even in closely related families indicating their functional divergence (9). Furthermore, emerging evidence shows that KSHV miRs exert a protective function by targeting IKBA and NF-κB pathways and thus rescuing cell cycle progression and inhibiting apoptosis. Intricate interactions of KSHV miRs with other viral oncogenes are likely to maintain or break the fine balance between uncontrolled cell growth and cell homeostasis, and are therefore, essential for KSHV-induced cellular transformation (10).

The oral cavity is an ecological niche of diverse microbial species. Several bacterial and viral species are known to coexist in the same oral environment. Both gram positive and gram negative bacteria are known as causal agents for various oral diseases. Herpesviruses are the predominantly detected family of viruses in such infections. Under “healthy” conditions, this indigenous microflora remains benign and assists the host by maintaining basal host immunity, thereby establishing symbiotic association. Influenced by various external and internal factors, however, this equilibrium may be disturbed leading to modulated immune responses (11).

Although viruses have been found in diseased pulps and periapical tissues, a causal relationship between viral infection and endodontic disease is yet to be established. Ferriera et al. reported that 48% of apical abscesses samples had KSHV infections, whereas Chen et al. detected HCMV in 29% of the patients with similar disease (12,13). A more recent study suggested that B cells and plasma cells in inflamed granulomas are a major source of EBV infection, and that EBV could play a pivotal role in controlling immune cell responses in periapical granulomas (11). The prevalence of herpesviruses varies across different reports, nonetheless, it is recognized that they may act as opportunistic infections. The presence of herpesviruses in HIV-infected patients further supports the notion that these pathogens are activated when the hosts’ immunity is compromised (14).

Although the role of these viruses in augmenting the disease has been proposed, there is little information on how they contribute towards pathogenesis. It is likely that one mechanism by which they augment pulpal and periapical disease is by reshaping the host immune response via vmiRs. To explore this, we first compared the viral encoded miR profiles in healthy and diseased human pulps. We then used in silico target prediction of the differentially expressed vmiRs to identify the potential host target genes that are vital in defense against pathogens.

Materials and Methods

Study participants and sample collection

This study was approved by the Institutional Review Board, at the University of North Carolina at Chapel Hill and conforms to the STROBE guidelines for reporting observational studies (http://www.strobe-statement.org). Written informed consent was obtained from 28 participants who were recruited from the School of Dentistry, University of North Carolina

at Chapel Hill. The inclusion criteria were ≥12 years old and American Association of Anesthesiologists status I or II. Patients who had a compromised immune system or those who were taking medications known to influence the immune response were excluded from the study. Participants were enrolled into two groups based on the pulpal status of the teeth being treated. Normal pulps were extirpated from healthy third molars or teeth extracted for orthodontic purpose. These did not include teeth with carious lesions or deep (≥5mm) probing defects. Diseased pulps were extirpated from carious teeth diagnosed with irreversible pulpitis defined with either carious pulpal exposures or the presence of spontaneous pain and an exaggerated and lingering response to cold (1,1,1,2-tetrafluoroethane) (15).

After obtaining informed consent, local anesthesia was administered. For teeth diagnosed with normal pulps, the teeth were extracted and the root canal system immediately accessed. The pulp was then extirpated using a sterilized barbed broach or Hedstrom hand file. For teeth diagnosed with diseased pulps, rubber dam isolation was obtained and the tooth and rubber dam were disinfected with 0.2% chlorhexidine gluconate. The carious tooth structure was removed and then the root canal system was accessed. Pulp tissue was collected using a sterilized barbed broach or Hedstrom hand file. Pulp tissue was gently separated from the instrument and placed in a sterile eppendorf tube with 0.5ml RNAsafer Stabilizer Reagent (VWR, Bridgeport, NJ). All samples were stored at −80° C until processing.

RNA isolation and miRNA microarray

Samples were thawed on ice and centrifuged at 4°C for 2 minutes at 12,000 rpm to remove the stabilizer reagent. Total RNA was extracted using the miRNeasy Mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The RNA was quantitated using the NanoDrop (Thermo Scientific, Wilmington, DE) and RNA integrity assessed using the 2100 Bioanalyzer (Agilent, Foster City, CA). The miRNA expression profiles of normal and diseased pulps were interrogated using Human miRNA Microarrays (V3) and the miRNA Complete Labeling and Hyb Kit (both from Agilent Technologies, Santa Clara, CA). The microarrays consist of glass slides containing 8 identical 15K oligonucleotide microarrays incorporating probes for 866 human and 89 human viral miRNAs represented from the Sanger miRBase 12.0. The procedure was performed as described previously (16). Briefly, 300 ng of total RNA samples were dephosphorylated, denatured by dimethyl sulfoxide, and then immediately transferred to ice-water bath for ligation. The samples were incubated with T4 RNA ligase at 16°C in a circulating water bath for 2 hours. Labeled miRNAs were desalted through Micro Bio-spin 6 columns (Bio-Rad, Hercules, CA) for purification. Samples were hybridized at 55° C for 20 hours at 20 rpm in a rotating hybridization oven. The microarray slides were then washed using fresh wash buffer. Finally, the slides were scanned using the Agilent Microarray Scanner and the Agilent Feature Extraction Software version 10.5.1.1 (Agilent, Foster City, CA).

Statistical analysis

For microarray data analysis, any expression value that was lower than the reported error for that particular gene (which includes negative expression values) was set to be equal to the estimated error rate. Quantile normalization was applied to the expression data. To identify

genes that were differentially expressed in each group, we applied a permutation test to test the null hypothesis that the mean expression of each gene was the same in both groups. An exact hypothesis test was used since the sample size was small. We used the resulting p-values to estimate the false discovery rate q-value when the differential expression of each miR is called "significant". Since we performed multiple hypothesis tests, the usual $p<0.05$ threshold for significance was too liberal for this analysis. However, using the Bonferroni-adjusted significance threshold ($p<0.05/335$) would be too conservative and result in a less of power given our small sample size. Thus, for each p-value, we computed the q-value, which is defined to be the false discovery rate when all tests with a p-value less than or equal to the given p-value are called "significant". This allows us to control type I error while avoiding the power loss that would result from using a Bonferroni correction.

Bioinformatics miRNA analysis and target selection

To identify the viral miRNA targets, we used viR-miR database (http://alk.ibms.sinica.edu.tw). Viral miR sequences were entered into the search database and list of genes were procured as an output file. As an alternate, we also searched the 3′ UTR of some important genes employing the RNA hybrid algorithm that is used by viR-miR database.

Results

Twenty-eight patients were recruited into the study and from whom thirty teeth were used to collect dental pulps. The demographic data of the study subjects is summarized in Table 1. No difference was noted in gender distribution between the two groups although subjects from whom diseased pulps were collected were significantly older than those from whom normal pulps were collected ($p<0.001$). The latter group consisted primarily of younger individuals undergoing tooth extraction for orthodontic reasons or for removal of 3rd molars. None of the teeth in the normal pulp group were extracted due to carious or periodontal pathology. In the diseased group 12 teeth had carious pulpal exposure with no recent history of spontaneous pain or thermal sensitivity while the remaining 6 teeth had a carious lesion and were associated with spontaneous pain and/or lingering response to cold.

In our study, we identified four viral miRNAs that were expressed at higher level in diseased human dental pulp. HSV1-miR-H1, HCMV-miR-US4, HCMV-miR-UL70-3p, and KSHV-miR-K12-3 were increased 3.84, 4.64, 5.36 and 3.81 folds respectively ($p<0.05$). We also detected HSV-miR-H6 and KSHV-miR-K12-10b that were expressed at higher level in diseased dental pulp; however, their levels were not statistically significant. We have also listed a few other viral miRNAs that were detected in our samples but did not show statistically significant changes.

To identify potential targets for the vmiRs differentially expressed in diseased pulps, we used viR-miR database (http://alk.ibms.sinica.edu.tw). We also searched PUBMED and confirmed the role of these vmiRs in inflammation, immunity and angiogenesis. The target genes comprise key mediators involved in the detection of microbial ligands (toll-like receptor 1{TLR1}), chemotaxis (Chemokine ligand 25), proteolysis (MMP24, matrix metalloprotease 24), pro-inflammatory and anti-inflammatory cytokines (IL8, IL-10) and

signal transduction molecules (Inducible T cell co-stimulator). The results of *in silico* studies are summarized in Table 3.

Discussion

Although the vast majority of our knowledge on pulpal disease is centered on bacterial infection, we are now increasingly aware of the potential role of viral infections or co-infections in pulpal pathosis. The presence of viral infections in healthy and diseased pulps have been reported before (17,18,19). An observational study on viral infections in the pulp and periapical tissues reported the presence of HCMV in healthy and inflamed pulps (17). The same study found EBV in inflamed but not in normal pulps. Studies on HIV patients report detecting the virus in healthy pulp tissues as well as in pulp fibroblasts (17,18). A study on patients with chronic hepatitis C infections noted that the pulps had altered cellularity and vasculature. The tissue also contained increased mucosubstances and decreased fibronectin glycoprotein (20). A recent case report on the presence of Varicella Zoster virus in the root canal systems of teeth in a patient with trigeminal herpes zoster suggested an etiological role of the virus in pulpal and periapical disease (21).

The route by which viruses and their microRNA infect pulp cells is yet to be explored. As in bacterial infections, it may occur via a carious lesion. Another source may be from infected cells elsewhere in the body. Viruses secrete significant quantities of vmiRs into exosomes which merge and deliver their contents into recipient cells. In this manner vmiRs are disseminated to distant non-permissive cells including immune cells and modulate target cell functions. A third potential source for pulpal infection is via infected nerves as suggested in the recent case report on the presence of Varicella Zoster virus in the root canal systems (21).

To our knowledge, this is the first report on viral microRNA (vmiRs) in healthy and diseased pulps. By using microarray analysis, we were able to profile virus-derived miRs in both healthy and diseased human dental pulp. In our study, we detected twelve vmiRs with four of them significantly changed in diseased pulps. Prior studies show that vmiRs have the capacity to regulate hundreds of probable host transcripts and in this way can alter the hosts immune response (22, 23, 24).

Herpesvirus miRs were the predominantly identified group of vmiRs in our study. Herpes simplex viruses 1 and 2 (HSV-1 and -2) are members of the *alpha* subfamily and are important human pathogens, widely known as the causative agents of cold sores and genital herpes respectively. The initial herpesvirus infection is followed by a latent phase in host cells, which ensures the survival of the viral genome throughout the lifetime of infected individuals. Typically, the primary site of infection is mucosal epithelium, where the virus enters cells and starts its productive cycle. The productive cycle entails a cascade of gene expression, viral DNA replication, and assembly and egress of infectious virus. In the host, virus can enter sensory neurons that innervate the site of primary infection and establish lifelong latent infection in which no infectious virus is detected, but from which virus can reactivate and cause recurrent disease. Study by Cui et al experimentally detected HSV1-miR-H1 that was expressed during productive infection from HSV1-infected Vero cells (25).

In our study, HSV1-miR-H1 was expressed at an induced level (3.84 fold higher) in diseased human dental pulp indicating either increased viral replication in the infected cells or accumulation of latently infected cells.

Interestingly, we also detected some vmiRs in control tissues from healthy subjects, albeit at low levels. However, it is not very surprising considering that human herpes virus persists as latent lifelong subclinical infection and that several viral miRs are either expressed exclusively or at higher levels during latency which is the predominant phase of viral life cycle. Indeed, in our study we identified several EBV BART miRs that are derived from latency associated BART transcript. Moreover, vmiRs, similar to host miRNAs, can target exosomes which are nanovesicles (~40-100 nm) secreted by various cells. These extracellular vesicles, through endocytic pathway, can merge and deliver their contents including proteins, mRNA and miRNAs into recipient cells (26, 27). Upon endocytosis, the exosomes release their contents to recipient cells that can modulate their functions or responses. Recent studies have demonstrated vmiRs in the exosomes of EBV and KSHV infected cells (27, 28). As mentioned before, viruses can secrete significant quantities of vmiRs into exosomes to be disseminated across distant non-permissive cells including immune cells. Evidently, several reports demonstrate that vmiRs specifically downregulate genes of innate as well as adaptive immunity. For instance, KSHV-miRK10 directly target TNF-like weak inducer of apoptosis receptor (TWEAKR) leading to reduced expression of IL-8 and monocyte chemoattractant protein 1 (MCP1) (29). EBV, HCMV and KSHV encoded miRs can target the stress induced immune ligand MHC class I polypeptide-related sequence B (MICB) to escape recognition by natural killer cells (30). Similarly, in Primary Effusion Lymphoma patients, exosomes carrying KSHV vmiRs promote endothelial cell migration and thus augment angiogenesis (28).

Epstein–Barr virus (EBV) infects more than 90 % of the population and establishes lifelong persistence in the host despite immune responses (31). Innate and adaptive immunities, therefore, need to cope with primary and secondary encounters with EBV, in addition to its latency and reactivation. In a minority of infected individuals, EBV is strongly linked to a remarkable variety of nonmalignant and malignant tumors in humans (32). This successful lifelong persistence and, in particular, its ability to establish latency despite specific immune responses show that EBV has developed powerful strategies and mechanisms to exploit, evade, abolish, or downsize effective immune responses to ensure its own survival. EBV encodes at least 40 miRs that map to two regions of the viral genome. The BHRF1 miRs are located immediately upstream and downstream of the BHRF1 open reading frame, while the BART miRs lie within the intronic regions of the BARTs. Our study detected six different BART miRNAs viz., BART-10, -12, -14, -16, -19-3p and -3*. Among these, BART-12, -13 and -19-3p showed higher expression levels. Compared to healthy control only BART-19-3p were downregulated, albeit not significant. Remaining BART miRs showed similar expression profiles in healthy and diseased pulp. Therefore, the potential clinical implications of the detected EBV BART miRNAs in pulpitis is highly remote.

Human cytomegalovirus belongs to the beta subfamily of herpesviridae and is a highly prevalent human pathogen, usually acquired at an early age. Following primary infection, the virus also establishes life-long latent infection with episodes of reactivation. Unlike most

of the herpesviridae, the miRs of HCMV are scattered along both strands of its genome, and are expressed through productive infection (33). We detected increased expression of miR-US4 and miR-UL70 in inflamed pulps indicating a role of these vmiRs in immunity. Consistent with this, Kim et al., demonstrated the suppression of MHC class I antigen presentation by miR-US4 through targeting of antigen peptide generating enzyme endoplasmic reticulum aminopeptidase 1 (ERAP1) (34).

KSHV-miR-K12-3 is expressed during latent infection and is detected in the exosomes of infected human brain microvascular endothelial cells. As with other herpes virus encoded vmiRs, KSHV-miR-K12-3 regulates both viral and cellular genes. KSHV-miR-K12-3 modulates the expression of replication and transcription activator (RTA) gene which is crucial for viral replication during lytic phase. This is achieved by repressing expression of Nuclear factor I/B (NFIB), an activator of RTA. Viral dissemination is promoted by KSHV-miR-K12-3 through downregulation of C/EBPβ (CCAAT/enhancer-binding protein β), a transcriptional repressor of IL-6 and IL-10 that promote growth of KSHV-infected cells (35). KSHV-miR-K12-3 directly represses caspase 3 expression thereby preventing apoptosis of infected cells (36). Another important gene targeted by miR_K12-3-3p is thrombospondin 1 (THBS1) which functions as an anti-angiogenic, anti-proliferative protein (37). THBS1 also induce immune response by recruiting monocytes and facilitate T cell migration (38, 39). In macrophages and dendritic cells, ectopic expression of KSHV-miR-K12-3 exhibit impaired phagocytosis and pro-inflammatory cytokines secretion upon challenge with heat-killed gram negative bacteria (Naqvi et al., Unpublished results). Together, these findings suggest diverse functional role of KSHV-miR-K12-3 in regulating viral life cycle, survival of infected cells and subversion of host immune responses.

The discovery of viral miRs has generated considerable attention into their functional relevance in processes such as cell death, viral proliferation, and oncogenesis. Could the vmiRs contribute to the endodontic disease pathogenesis? To address this question, we examined the miRNAs gene profile from both healthy and diseased human dental pulp. We found considerable vmiRs expressed in both groups and some of them are significantly changed in the diseased human dental pulp. Although the functions of these viral miRNAs are yet to be explored, our study provides potential direction for the future research.

Acknowledgments

This study was funded by the American Association of Endodontists Foundation and NIH R01DE021052

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–97. [PubMed: 14744438]
2. Ambros V. The functions of animal microRNAs. Nature. 2004; 431:350–5. [PubMed: 15372042]
3. Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature. 2008; 454:780–3. [PubMed: 18596690]
4. Pfeffer S. Identification of virally encoded microRNAs. Methods Enzymol. 2007; 427:51–63. [PubMed: 17720478]

5. Pfeffer S, Sewer A, Lagos-Quintana M, et al. Identification of microRNAs of the herpesvirus family. Nat Methods. 2005; 2:269–76. [PubMed: 15782219]
6. Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR. Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci U S A. 2005; 102:5570–5. [PubMed: 15800047]
7. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 2011; 39(Database issue):D152–7. [PubMed: 21037258]
8. Barth S, Pfuhl T, Mamiani A, Ehses C, et al. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. Nucleic Acids Res. 2008; 36:666–75. [PubMed: 18073197]
9. Yao Y, Zhao Y, Xu H, et al. Marek's disease virus type 2 (MDV-2)-encoded microRNAs show no sequence conservation with those encoded by MDV-1. J Virol. 2007; 81:7164–70. [PubMed: 17459919]
10. Moody R, Zhu Y, Huang Y, et al. KSHV microRNAs mediate cellular transformation and tumorigenesis by redundantly targeting cell growth and survival pathways. PLoS Pathog. 2013; 9:e1003857. [PubMed: 24385912]
11. Makino K, Takeichi O, Hatori K, Imai K, Ochiai K, Ogiso B. Epstein-Barr virus infection in chronically inflamed periapical granulomas. PLoS One. 2015; 10:e0121548. [PubMed: 25884725]
12. Ferreira DC, Paiva SS, Carmo FL, Rocas IN, Rosado AS, Santos KR, et al. Identification of herpesviruses types 1 to 8 and human papillomavirus in acute apical abscesses. J Endod. 2011; 37:10–6. [PubMed: 21146068]
13. Chen V, Chen Y, Li H, Kent K, Baumgartner JC, Machida CA. Herpesviruses in abscesses and cellulitis of endodontic origin. J Endod. 2009; 35:182–8. [PubMed: 19166769]
14. Saboia-Dantas CJ, Coutrin de Toledo LF, Sampaio-Filho HR, Siqueira JF Jr. Herpesviruses in asymptomatic apical periodontitis lesions: an immunohistochemical approach. Oral Microbiol Immunol. 2007; 22:320–5. [PubMed: 17803629]
15. Trope, M.; Sigurdsson, A., editors. Essential Endodontology. Blackwell, Inc.; London: 1998.
16. Zhong S, Zhang S, Bair E, Nares S, Khan AA. Differential expression of microRNAs in normal and inflamed human pulps. J Endod. 2012; 38:746–52. [PubMed: 22595106]
17. Li H, Chen V, Chen Y, Baumgartner JC, Machida CA. Herpesviruses in endodontic pathoses: association of Epstein-Barr virus with irreversible pulpitis and apical periodontitis. J Endod. 2009; 35:23–9. [PubMed: 19084119]
18. Sabeti M, Simon JH, Slots J. Cytomegalovirus and Epstein-Barr virus are associated with symptomatic periapical pathosis. Oral Microbiol Immunol. 2003; 18:327–8. [PubMed: 12930527]
19. Glick M, Trope M, Bagasra O, Pliskin ME. Human immunodeficiency virus infection of fibroblasts of dental pulp in seropositive patients. Oral Surg Oral Med Oral Pathol. 1991; 71:733–6. [PubMed: 1676502]
20. Grawish, Mel-A.; Khounganian, R.; Hamam, MK.; Zaher, AR.; Hegazy, D.; El-Negoly, SA.; Hassan, G.; Zyada, MM. Altered coronal tissue of the human dental pulp in chronic hepatitis C virus infected patients. J Endod. 2013; 39:752–8. [PubMed: 23683274]
21. Patel K, Schirru E, Niazi S, Mitchell P, Mannocci F. Multiple apical radiolucencies and external cervical resorption associated with Varicella Zoster Virus: A case report. J Endod. 2016; 42:978–83. [PubMed: 27133503]
22. Malterer G, Dölken L, Haas J. The miRNA-targetome of KSHV and EBV in human B-cells. RNA Biol. 2011; 8:30–4. [PubMed: 21301209]
23. Stern-Ginossar N, Saleh N, Goldberg MD, Prichard M, Wolf DG, Mandelboim O. Analysis of human cytomegalovirus-encoded microRNA activity during infection. J Virol. 2009; 83:10684–93. [PubMed: 19656885]
24. Cullen BR. Viral and cellular messenger RNA targets of viral microRNAs. Nature. 2009; 457:421–5. [PubMed: 19158788]
25. Cui C, Griffiths A, Li G, et al. Prediction and identification of herpes simplex virus 1-encoded microRNAs. J Virol. 2006; 80:5499–508. [PubMed: 16699030]

26. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9:654–9. [PubMed: 17486113]
27. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, et al. Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci U S A. 2010; 107:6328–33. [PubMed: 20304794]
28. Chugh PE, Sin SH, Ozgur S, et al. Systemically circulating viral and tumor-derived microRNAs in KSHV-associated malignancies. PLoS Pathog. 2013; 9:e1003484. [PubMed: 23874201]
29. Abend JR, Uldrick T, Ziegelbauer JM. Regulation of tumor necrosis factor-like weak inducer of apoptosis receptor protein (TWEAKR) expression by Kaposi's sarcoma-associated herpesvirus microRNA prevents TWEAK-induced apoptosis and inflammatory cytokine expression. J Virol. 2010; 84:12139–51. [PubMed: 20844036]
30. Nachmani D, Lankry D, Wolf DG, Mandelboim O. The human cytomegalovirus microRNA miR-UL112 acts synergistically with a cellular microRNA to escape immune elimination. Nat Immunol. 2010; 11:806–13. [PubMed: 20694010]
31. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. Nat Rev Cancer. 2004; 4:757–768. [PubMed: 15510157]
32. Munz C. Viral infections in mice with reconstituted human immune system components. Immunol Lett. 2014; 161:118–124. [PubMed: 24953718]
33. Dhuruvasan K, Sivasubramanian G, Pellett PE. Roles of host and viral microRNAs in human cytomegalovirus biology. Virus Res. 2011; 157:180–92. [PubMed: 20969901]
34. Kim S, Lee S, Shin J, et al. Human cytomegalovirus microRNA miR-US4-1 inhibits CD8(+) T cell responses by targeting the aminopeptidase ERAP1. Nat Immunol. 2011; 12:984–91. [PubMed: 21892175]
35. Lu CC, Li Z, Chu CY, et al. MicroRNAs encoded by Kaposi's sarcoma-associated herpesvirus regulate viral life cycle. EMBO Rep. 2010; 11:784–90. [PubMed: 20847741]
36. Suffert G, Malterer G, Hausser J, et al. Kaposi's sarcoma herpesvirus microRNAs target caspase 3 and regulate apoptosis. PLoS Pathog. 2011; 7:e1002405. [PubMed: 22174674]
37. Lawler PR, Lawler J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. Cold Spring Harb Perspect Med. 2012; 2:a006627. [PubMed: 22553494]
38. Narizhneva NV, Razorenova OV, Podrez EA, et al. Thrombospondin-1 up-regulates expression of cell adhesion molecules and promotes monocyte binding to endothelium. FASEB J. 2005; 19:1158–60. [PubMed: 15833768]
39. Li SS, Forslöw A, Sundqvist KG. Autocrine regulation of T cell motility by calreticulin-thrombospondin-1 interaction. J Immunol. 2005; 174:654–61. [PubMed: 15634883]

Table 1

Study participant demographics. Mann-Whitney U test was used to compare age and McNemar's Chi-square test was used to compare gender distribution between the two groups. Age is presented as mean ± standard deviation. Pain history is defined as patients' report of spontaneous and/or thermally evoked pain in the past 14 days.

Variables	Normal pulp (n=12)	Diseased pulp (n=18)	p value
Mean Age (StdDev)	37 (14.42)	18 (3.00)	<.0001
Gender			
Female	5 (42%)	10 (56%)	0.71
Male	7 (48%)	8 (44%)	
Pain History	0%	6 (30%)	

Table 2

vmiRs expressed in normal and diseased human dental pulp.

miRNA	p value	q value	Healthy	Diseased	Fold change
ebv-miR-BART10	1.00	0.94	4.51	4.55	1.01
ebv-miR-BART12	0.88	0.94	37.50	42.21	1.13
ebv-miR-BART13	0.93	0.94	127.51	139.94	1.10
ebv-miR-BART16	1.00	0.94	18.31	18.14	0.99
ebv-miR-BART19-3p	0.17	0.42	104.01	59.41	0.57
ebv-miR-BART3*	0.95	0.94	8.02	8.48	1.06
hcmv-miR-UL70-3p	0.02	0.12	98.26	526.22	5.36
hcmv-miR-US4	0.01	0.05	19.67	91.24	4.64
hsv1-miR-H1	0.03	0.17	34.72	133.40	3.84
hsv1-miR-H6	0.24	0.53	12.93	26.57	2.05
kshv-miR-K12-10b	0.10	0.33	1.68	6.22	3.69
kshv-miR-K12-3	0.02	0.10	291.49	1110.32	3.81

Table 3

Potential vmiR target genes were identified using viR-miR database http://alk.ibms.sinica.edu.tw

v-miRNA	Target gene Abbrev	Target Gene	Gene Product Function	GO term (accession, ontology)
hsv1 miR-H1	HOXC10	Homeobox C10	Plays a role in the morphogenesis in multi-cellular organisms.	GO:0003677, Molecular Function
hsv1 miR-H1	MYD88	Myeloid differentiation primary response gene 88	Involved with immunity and inflammatory response	GO:0006954, Biological Process
hsv1-miR-H1-5p	THBS1	Thrombospondin 1	Acts in immediate response to infection or injury causing local vasodilation, extravasation of plasma into intercellular spaces and production of white blood cells and macrophages	GO:0006954, Biological Process
hsv1-miR-H1-5p	IL10	Interleukin 10	Pleiotropic effects in immunoregulation and inflammation, B-cell survival, proliferation, and antibody production, block nuclear factor kappa B activity	GO:0019969, Molecular Function
hsv1-miR-H1-5p	IL8	Interleukin 8	Aids in chemotaxis in response to an external stimulus	GO:0006935, Biological Process
hsv1-miR-H1-5p	ICOS	Inducible T-Cell Co-Stimulator	Plays an important role in cell-cell signaling, immune responses, and regulation of cell proliferation	GO:0052156, Biological Process
hsv1-miR-H1-5p	FOS	FBJ murine osteosarcoma viral oncogene homolog	Acts in regulation of transcription	GO:0006355, Biological Process
hsv1-miR-H1-5p	CCR9	C-C chemokine receptor type 9	Acts with receptor to act as a chemokine	GO:0031734, Biological Process
hsv1-miR-H1-5p	CCR7	C-C chemokine receptor type 7	Expressed to activate B and T lymphocytes, stimulate dendritic cell maturation, and thought to mediate EBV effects on B lymphocytes	GO:0031732, Molecular Function
hsv1-miR-H1-5p	PLCB1	Phospholipase C, beta 1	Involved in the metabolism of lipids	GO:0006629, Biological Process
hcmv-miR-UL70-3p	ADAM15	ADAM metallopeptidase domain 15	Involved in angiogenesis, negative regulation of cell-matrix adhesion, and negative regulation of cell growth	GO:0004896, Molecular Function
hcmv-miR-UL70-3p	ATG4A	Autophagy related 4A, cysteine peptidase	Involved with autophagy, transport of cells, and cell homeostasis and remodeling	GO:0006914, Biological Process
hcmv-miR-UL70-3p	CCL25	Chemokine (C-C motif) ligand 25	Involved with the immune response, cell chemotaxis and chemokine activity	GO:0006955, Biological Process

v-miRNA	Target gene Abbrev	Target Gene	Gene Product Function	GO term (accession, ontology)
hcmv-miR-UL70-3p	CCR9	Chemokine (C-C motif) receptor 9	Acts with receptor to act as a chemokine	GO:0031734, Biological Process
hcmv-miR-UL70-3p	FOS	FBJ murine osteosarcoma viral oncogene homolog	Implicated in regulation of transcription, differentiation, and transformation.	GO:0035556, Biological Process
hcmv-miR-UL70-3p	GPR64	G protein-coupled receptor 64	Involved with cell surface receptor signaling and componentry with the cell membrane	GO:0007166, Biological Process
hcmv-miR-UL70-3p	IL10	Interleukin 10	Pleiotropic effects in immunoregulation and inflammation, B-cell survival, proliferation, and antibody production, block nuclear factor kappa B activity	GO:0019969, Molecular Function
hcmv-miR-UL70-3p	IL8	Interleukin 8	Involved in chemotaxis, neutrophil activation, and immune response	GO:0006955, Biological Process
hcmv-miR-UL70-3p	IL1a	Interleukin 1, alpha	Involved in generation of fever in response to infection	GO:0001660, Biological Process
hcmv-miR-UL70-3p	ICOS	Inducible T cell co-stimulator	Plays an important role in cell-cell signaling, immune responses, and regulation of cell proliferation	GO:0052156, Biological Process
hcmv-miR-UL70-3p	ICAM1	Intercellular adhesion molecule 1	Plays a role in cell membrane componentry	GO:0016021, Cellular Component
hcmv-miR-UL70-3p	IL6R	Interleukin 6 receptor	Acts as a cytokine receptor activator	GO:0006954, Biological Process
hcmv-miR-UL70-3p	MMP24	Matrix metalloproteinase 24	Involved in cell-cell adhesion and cadherin adhesion	GO:0006355, Biological Process
hcmv-miR-UL70-3p	PRKCA	Protein kinase C, alpha	Inloved in signal transduction pathways related to cell adhesion and transformation	GO:0001525, Biological Process
hcmv-miR-UL70-3p	THBS1	Thrombospondin 1	Acts in immediate response to infection or injury causing local vasodilation, extravasation of plasma into intercellular spaces and production of white blood cells and macrophages	GO:0007155: Biological Process
kshv-miR-K12-3-5p	ICAM1	Intercellular adhesion molecule 1	Plays a role in cell membrane componentry	GO:0016021, Cellular Component
kshv-miR-K12-3-5p	ICOS	Inducible T cell co-stimulator	Plays an important role in cell-cell signaling, immune responses, and regulation of cell proliferation	GO:0052156, Biological Process
kshv-miR-K12-3-5p	GPR64	G protein-coupled receptor 64	Acts as in cell surface signaling pathways and in membrane componentry	GO:0007166, Biological Process

v-miRNA	Target gene Abbrev	Target Gene	Gene Product Function	GO term (accession, ontology)
kshv-miR-K12-3-5p	FOS	FBJ murine osteosarcoma viral oncogene homolog	Implicated in regulation of transcription, differentiation, and transformation.	GO:0006355, Biological Process
kshv-miR-K12-3-5p	MRC1	Mannose receptor, C type 1	Involved in the recognition of carbohydrates importation in cell-cell recognition and neutralization of pathogens, and viral entry into the host cell	GO:0046718, Biological Process
kshv-miR-K12-3-5p	PLCB2	Phospholipase C, beta 2	Involved in calcium ion binding and signal transduction	GO:0035556, Biological Process
kshv-miR-K12-3-5p	PLCB4	Phospholipase C, beta 4	Involved in calcium ion binding and signal transduction	GO:0035556, Biological Process
kshv-miR-K12-3-5p	TLR-1	Toll-like receptor 1	Associated with regulation of signaling pathways related to innate immunity, and initiate adaptive immunity	GO:0034130, Biological Process