All for One and One for All: Herpesviral MicroRNAs Close in on Their Prey

Dölken, Lars; Jonjić, Stipan

The observed differences in immune response gene expression could be due to various receptors activating the p38/PMK-1 MAP kinase cascade in addition to parallel alternative immune pathways.

ACKNOWLEDGMENTS

The authors wish to thank C. Rubin and J. Ewbank for comments and unpublished data. Support was provided by National Institutes of Health R01 Award AI075286 (to E.M.).

REFERENCES

Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J.F., Kohara, Y., and Ewbank, J.J. (2004). Nat. Immunol. *5*, 488–494.

Kim, D.H., Feinbaum, R., Alloing, G., Emerson, F.E., Garsin, D.A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M.-W., et al. (2002). Science *297*, 623–626.

Kubo-Murai, M., Hazeki, K., Sukenobu, N., Yoshikawa, K., Nigorikawa, K., Inoue, K., Yamamoto, T., Matsumoto, M., Seya, T., Inoue, N., et al. (2007). Mol. Immunol. *44*, 2257–2264.

Means, T.K., Mylonakis, E., Tampakakis, E., Puckett, L., Stewart, C.R., Pukkila-Worley, R., Hickman, S.E., Moore, K.J., Calderwood, S.B., Hacohen, N., et al. (2009). J. Exp. Med. *206*, 637–653.

Mylonakis, E., and Aballay, A. (2005). Infect. Immun. *73*, 3833–3841.

Powell, J.R., Kim, D.H., and Ausubel, F.M. (2009). Proc. Natl. Acad. Sci. USA *106*, 2782–2787.

Reddy, K.C., Andersen, E.C., Kruglyak, L., and Kim, D.H. (2009). Science *323*, 382–384.

Ren, M., Feng, H., Fu, Y., Land, M., and Rubin, C.S. (2009). Immunity *30*, 521–532.

Steinberg, S.F. (2004). Biochem. J. *384*, 449–459.

Styer, K.L., Singh, V., Macosko, E., Steele, S.E., Bargmann, C.I., and Aballay, A. (2008). Science *322*, 460–464.

Wong, D., Bazopoulou, D., Pujol, N., Tavernarakis, N., and Ewbank, J. (2007). Genome Biol. *8*, R194.

Ziegler, K., Kurz, C.L., Cypowyj, S., Couillault, C., Pophillat, M., Pujol, N., and Ewbank, J.J. (2009). Cell Host Microbe *5*, this issue, 341–352.

All for One and One for All: Herpesviral MicroRNAs Close in on Their Prey

Lars Dölken[1] and Stipan Jonjic[2,*]
[1]Max von Pettenkofer-Institut, Pettenkoferstrasse 9a, D-80336 München, Germany
[2]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, B. Branchetta 20, 51000 Rijeka, Croatia
*Correspondence: jstipan@medri.hr
DOI 10.1016/j.chom.2009.03.009

Herpesviruses subvert immune cell activation by inhibiting NK cell receptor (NKG2D)-activating ligands such as MICB. A human cytomegalovirus microRNA was recently shown to repress MICB expression. Nachmani et al. (2009) extend this finding to two other human herpesviruses, providing evidence for a conserved functional role of viral microRNAs despite no sequence conservation among them.

MicroRNAs (miRNAs) are small noncoding RNAs of ~22 nucleotides expressed by virtually all multicellular organisms. They provide an ancient but continuously adapting mechanism for the regulation of gene expression. Recently, they have been identified as key regulators in nearly all biological processes, including cellular differentiation, proliferation, apoptosis, metabolism, and regulation of the immune response. In humans, they are believed to regulate ~30% of all cellular genes (for a review, see Bartel, 2004).

Herpesviruses are large DNA viruses that have coevolved with their hosts over millions of years. Humans can be infected with eight different herpesviruses, which, upon primary infection, establish lifelong latency. The fact that the immune system fails to efficiently control viral reactivation from latency often leads to recurrent infections and disease. Herpesviruses have evolved a robust set of strategies to counteract the vast repertoire of immune response mechanisms. Since the discovery of virally encoded miRNAs in members of the herpesvirus family (Pfeffer et al., 2004), it has been tempting to assume that they regulate not only the life cycle of the virus, but also the interaction between viruses and their hosts, including immune control of virus infection. So far, more than 50 viral miRNAs have been identified in 5 human herpesviruses, including human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV), as well as in herpes simplex virus 1 and 2 (HSV-1/-2).

Since miRNAs can be efficiently targeted with chemically modified antisense oligonucleotides, so-called antagomirs (Krutzfeldt et al., 2005), viral miRNAs present themselves as interesting candidates for novel antiviral drugs. However, in order to develop antiviral drugs based on viral miRNAs, a detailed understanding of their targets and function is required. Since only a few bases of a miRNA define its target specificity, identification of miRNA targets by computational means is often difficult. For cellular miRNAs, such bioinformatic algorithms rely heavily on evolutionary conservation of both miRNAs and their targets. Since viral miRNAs show no or very minimal sequence conservation, only very few targets of herpesvirus miRNAs have so

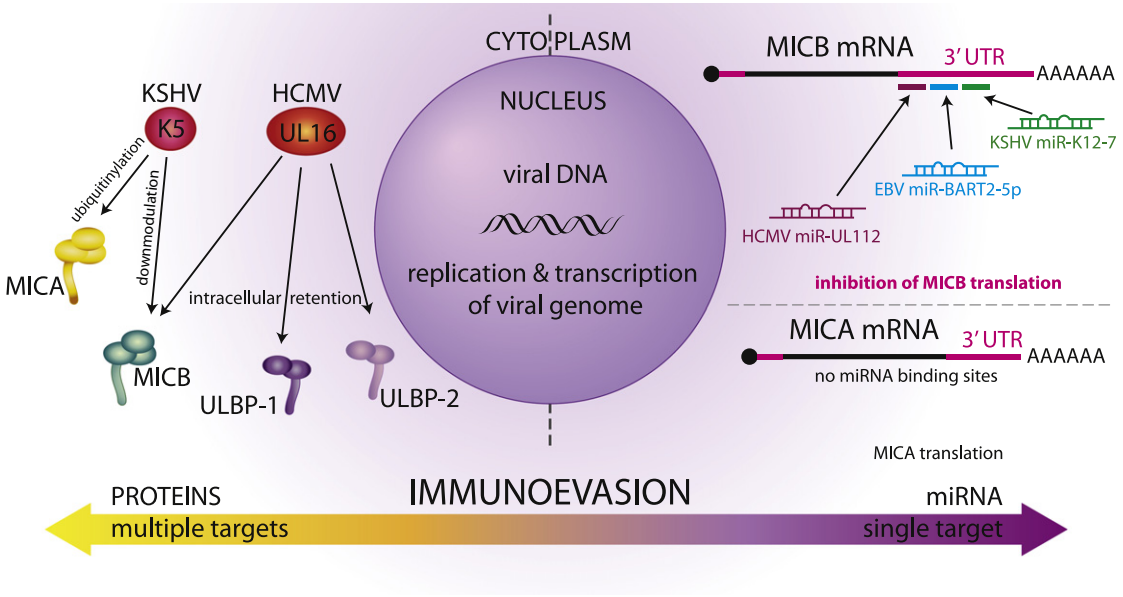

Figure 1. Selective Targeting of NKG2D Ligand MICB by Herpesviral MiRNAs
Human ligands for NKG2D receptor are targeted by herpesviruses, which leads to prevention of their expression on the cell surface and avoidance of immune cell activation. Usually, more than one NKG2D ligand is targeted by herpesviral immunoevasion proteins. In contrast to the immunoevasion proteins, three herpesviruses—HCMV, EBV, and KSHV—encode miRNAs, which selectively affect translation of MICB mRNA. All three miRNAs (HCMV miR-UL112, EBV miR-BART2-5p, and KSHV miR-K12-7) target the 3′-untranslated region (3′UTR) of MICB at unique nonoverlapping sites in close proximity to the coding sequence. Unlike cellular miRNAs (data not shown), viral miRNAs do not target MICA.

far been identified (reviewed by Gottwein and Cullen, 2008).

NKG2D is a potent activating receptor on NK cells and a costimulatory receptor on $CD8^+$ T cells. Through its ability to recognize a panel of ligands inducible by stress, transformation, or infection, it plays an important role in antitumor and antiviral immune responses. In humans, the NKG2D ligands are a diverse group of proteins of the major histocompatibility complex (MHC) class I-related chains A (MICA) and B (MICB) and ULBP/RAET families (Gonzalez et al., 2008). To avoid NKG2D-dependent immune cell activation, herpesviruses encode proteins that mediate the retention and sequestration of NKG2D ligands, thus preventing their surface expression. Previously, Mandelboim and his group reported that the stress-induced NKG2D ligand MICB is efficiently targeted by HCMV miRNA miR-UL112, resulting in significantly reduced susceptibility of infected cells to NK cell killing (Stern-Ginossar et al., 2007) (Figure 1). MiR-UL112 targets not only MICB but also the viral transactivator IE72 (Grey et al., 2007). Therefore, a single viral miRNA regulates both viral and host gene expression. Further on, they demonstrated that the two NK cell ligands, MICB and MICA, are both also a major target for multiple cellular miRNAs (Stern-Ginossar et al., 2008). Thus, they elucidated a cellular mechanism by which cells regulate and prevent accidental NK cell-mediated killing, indicating that HCMV has usurped this cellular mechanism in order to target these ligands. While both MICA and MICB are regulated by cellular miRNAs, HCMV miR-UL112 revealed a striking preference for regulating MICB.

In this issue of *Cell Host & Microbe* (Nachmani et al., 2009), Mandelboim and colleagues extend their study to miRNAs of two other human herpesviruses: EBV and KSHV. Using a bioinformatic and functional approach, they identified EBV miR-BART2-5p and KSHV miR-K12-7 as both selectively targeting MICB but not MICA (Figure 1). The expression of these two miRNAs resulted in a dramatic reduction of MICB levels on the cell surface and a significantly reduced NK cell killing of these cells. Most importantly, their study provides the first example of a functional conservation among various herpesvirus miRNAs downregulating a single host immune gene. None of the miRNAs show any sequence homology. However, remarkably, all three miRNAs target the 3′-untranslated region (3′UTR) of MICB and do so at unique nonoverlapping sites in close proximity to the coding sequence. The lack of sequence homology indicates that targeting a single host immune gene might represent both coevolution and individual convergent evolution of various herpesviruses. Also, this marks MICB as a key player in NK cell-mediated killing during herpesviral infection. This is further emphasized by the fact that both KSHV and HCMV encode proteins to downregulate MICB expression by posttranslational means. However, these proteins, K5 (of KSHV) and UL16 (of HCMV), are less restricted compared to the viral miRNAs and mediate the sequestration not only of MICB, but also of other NKG2D ligands. It will be interesting to study whether EBV also shares this strategy.

In addition, orthologous targeting of the same host gene by two independent mechanisms evokes further questions, such as:

1. Why do these viruses put so much effort in targeting the same gene by at least partially redundant mechanisms?
2. What is so special about MICB within the large group of stress-induced NKG2D ligands that three

different herpesviruses independently recognized the need to target it with a miRNA?

The picture of a conserved functional role of viral miRNAs is emerging. Three human herpesviruses prevent NK cell-mediated killing of infected cells by targeting the same stress-induced NK cell ligand. In addition, a number of herpesviruses have been found or predicted to target their major transactivators using viral miRNAs (reviewed in Gottwein and Cullen, 2008). Low-level expression of these proteins in latently infected cells might trigger an immune response. Therefore, one is tempted to speculate that one functional role of viral miRNAs is to prevent both NK and T cells from recognizing infected cells during a time when the expression of viral proteins preventing this recognition would lead to antigen presentation and elimination of the infected cells by cytotoxic T cells. Compared to viral immunoregulatory proteins, the advantage of viral miRNAs may be their poor immunogenicity, which could be of particular importance in cells undergoing reactivation of latent virus in immunocompetent host.

A better understanding of the linkage between immune response functions and viral miRNAs requires additional considerations. As for the viral immunoevasion proteins, major insights still have to come from the analysis of the role of viral immunoregulatory miRNAs in vivo. In addition, we need to understand the meaning of redundancy of viral immunoevasion of NKG2D. The answers to these important questions will be clarified by animal models looking at the transition from acute infection to viral latency and reactivation thereof.

REFERENCES

Bartel, D.P. (2004). Cell *116*, 281–297.

Gonzalez, S., Lopez-Soto, A., Suarez-Alvarez, B., Lopez-Vazquez, A., and Lopez-Larrea, C. (2008). Trends Immunol. *29*, 397–403.

Gottwein, E., and Cullen, B.R. (2008). Cell Host Microbe *3*, 375–387.

Grey, F., Meyers, H., White, E.A., Spector, D.H., and Nelson, J. (2007). PLoS Pathog. *3*, e163.

Krutzfeldt, J., Rajewsky, N., Braich, R., Rajeev, K.G., Tuschl, T., Manoharan, M., and Stoffel, M. (2005). Nature *438*, 685–689.

Nachmani, D., Stern-Ginossar, N., Sarid, R., and Mandelboim, O. (2009). Cell Host & Microbe *5*, this issue, 376–385.

Pfeffer, S., Zavolan, M., Grasser, F.A., Chien, M., Russo, J.J., Ju, J., John, B., Enright, A.J., Marks, D., Sander, C., et al. (2004). Science *304*, 734–736.

Stern-Ginossar, N., Elefant, N., Zimmermann, A., Wolf, D.G., Saleh, N., Biton, M., Horwitz, E., Prokocimer, Z., Prichard, M., Hahn, G., et al. (2007). Science *317*, 376–381.

Stern-Ginossar, N., Gur, C., Biton, M., Horwitz, E., Elboim, M., Stanietsky, N., Mandelboim, M., and Mandelboim, O. (2008). Nat. Immunol. *9*, 1065–1073.