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Kaposi's Sarcoma Herpes Virus Taps into a Host MicroRNA Regulatory Network

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Kaposi's sarcoma-associated herpes virus (KSHV), the causative agent of several neoplasms, has recently been shown to encode 12 microRNAs. mir-155 is a host-encoded microRNA associated with lymphomagenesis. Two new papers provide strong evidence that a KSHV-encoded microRNA and mir-155 share a common set of mRNA targets and binding sites, implying a possible link between viral- and non-viral-mediated tumorigenesis.

The most recently discovered human herpesvirus, Kaposi's sarcoma-associated herpes virus (KSHV), is a large DNA virus that encodes over 80 different proteins. KSHV is the causative agent of several diseases including Kaposi's sarcoma (KS, a highly vascularized skin lesion) and the hyperproliferative B cell disorders primary effusion lymphoma (PEL) and multicentric Castleman's disease. Numerous studies of individual proteins have explored KSHV-mediated tumorigenesis; however, a comprehensive understanding of the mechanism remains obscure. The recent discovery that KSHV encodes 12 microRNAs (miRNAs) raises the possibility that these non-protein-coding gene products may contribute to viral-induced tumorigenesis (Cai et al., 2005; Grundhoff et al., 2006; Pfeffer et al., 2005; Samols et al., 2005).

miRNAs are small, ~22 nt RNAs that are processed from longer hairpin precursor transcripts into an effector complex able to bind to and prevent protein expression from specific mRNAs (Figure 1). Through both computational sequence matching and experimental observations, it is clear that the 5′ end of the miRNA, the so-called seed region (nucleotides 2–8), plays a critical role in mRNA target recognition. Binding of a miRNA to a mRNA typically results in reduced translation. Some targeted transcripts also have lower steady state levels, thereby allowing for experimental determination of some miRNA targets via cDNA microarray expression analysis. Each miRNA may bind to numerous (estimated to be over 100) mRNA targets involved in a particular biological process. For example, miRNAs that are involved in regulation of muscle and neu-

ronal cell development have been elucidated, and experimental determination of their mRNA targets identified numerous mRNAs with seed complements in their 3′UTR (Lim et al., 2005). These data imply the existence of regulatory networks in which miRNAs act as nodal effectors that target specific sequences found in the 3′UTRs of a set of transcripts. Presumably, each set is involved in a particular biological process that requires similar temporal or spatial regulation of member transcripts.

At last count, over 120 viral-encoded miRNAs have been reported, mostly

from members of the herpesvirus and polyomavirus families. The functions of the majority are unknown; however, molecular targets have been identified for a few. Some viral miRNAs target viral transcripts by directing their cleavage or translational repression. In addition, some viral miRNAs direct translational repression of host transcripts by binding to viral-specific sites. However, until now, no viral miRNAs have been reported that bind to mRNA targets via sites utilized by host miRNAs. Two recent reports now show that at least one viral miRNA, KSHV mir-K12-11, has the potential to regulate multiple transcripts
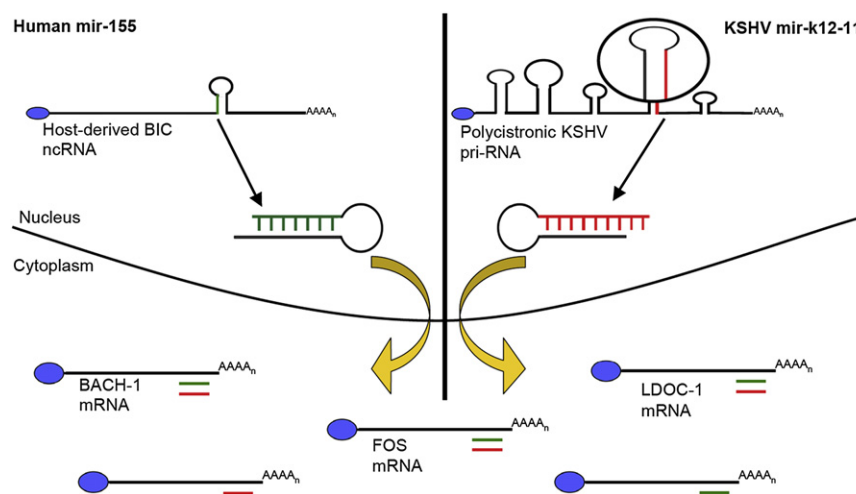

Figure 1. Human mir-155 and KSHV mir-k12-11 Regulate a Shared Set of mRNA Targets
The precursor hairpin RNA (pre-miRNA) that gives rise to mir-155 is contained within the noncoding RNA BIC (B cell integration complex). mir-k12-11 is generated from a primary transcript (pri-miRNA) that gives rise to multiple KSHV miRNAs. Drosha cleavage in the nucleus of both pri-miRNA transcripts releases a short-hairpin structured pre-miRNA, which is exported to the cytoplasm. Subsequently, Dicer cleavage of the pre-miRNA into the ~22 nucleotide miRNA occurs. This is followed by assembly of the miRNA into the multi-protein RNA-induced silencing complex (RISC), which allows for targeted downregulation of specific host mRNA transcripts. When exogenously expressed, mir-155 and KSHV mir-k12-11 share several common mRNA targets and binding sites, such as the transcriptional regulators BACH-1 and FOS, as well as the proapoptotic effector LDOC-1.

via the same binding sites utilized by the host miRNA, mir-155 (Gottwein et al., 2007; Skalsky et al., 2007).

Mir-155 is a miRNA with a particularly interesting history. It is contained within the noncoding RNA product of the B cell integration complex (BIC) gene, which had been a suspected proto-oncogene before the discovery of miRNAs. BIC was originally identified as a common integration site of avian leukosis virus (ALV)-induced lymphomas and has been shown in co-overexpression studies to cooperate with c-*myc* to enhance lymphomagenesis (Tam et al., 1997). Mir-155 is upregulated in some human lymphomas and plays a role in the adaptive immune response, likely by controlling cytokine production (Thai et al., 2007). KSHV is the causative agent of PEL and KS, both of which are associated with aberrant cytokine production. Thus, the realization of a conserved seed sequence raises the exciting possibility that mir-k12-11 may be contributing to tumorigenesis by tapping into the same network of transcripts that mir-155 regulates.

The observation that mir-k12-11 shares sequence identity in the seed region with mir-155 was first noted in a review article by Nair and Zavolan (2006). These authors suggested this shared seed identity could be an example of convergent evolution, thus implying a common set of targets and a shared function. However, it was alternatively possible that this shared seed sequence identity was inconsequential, and the differences in the remaining, nonseed nucleotides would confer differential target specificity. Now, both Skalsky et al. and Gottwein et al. clearly show that when exogenously expressed, mir-k12-11 and mir-155 downregulate the expression of a common set of shared cellular mRNA targets.

Both groups expressed mir-k12-11 and performed cDNA microarray gene expression analysis to identify candidate mRNA targets whose steady state levels are reduced in the presence of mir-k12-11. Skalsky et al. (2007) analyzed stable pools of human embryonic kidney cells (HEK293) that were transfected with a plasmid driving expression via the cytomegalovirus (CMV) promoter. Utilizing a different cell type and expression system, Gottwein et al. (2007) profiled a B cell lymphoma line transduced with a lentiviral vector expressing a chimeric pre-miRNA hairpin
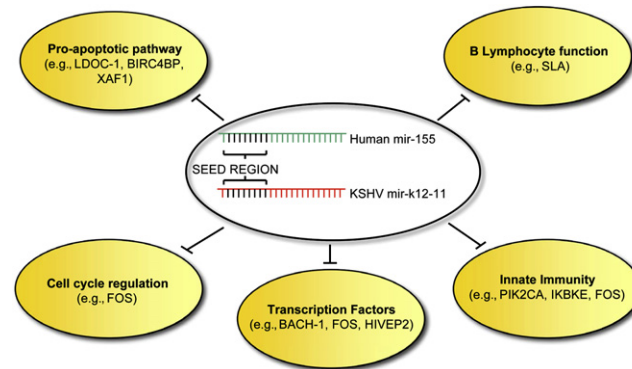

Figure 2. Common mRNA Targets of Both Human mir-155 and mir-k12-11 Are Involved in Cellular Processes with Potential Relevance to Tumorigenesis
Identical seed sequence (nucleotides 2–8) allows both mir-155 and mir-k12-11 to regulate a common set of mRNA targets. Skalsky et al. (2007) and Gottwein et al. (2007) used a combination of computational prediction and microarray cDNA expression analysis to identify candidate targets.

designed to generate expression of the fully processed mir-k12-11. Both reports combined prediction algorithms with microarray analysis to generate a list of candidate mRNA targets regulated by mir-k12-11. Next, Skalsky et al. (2007) additionally applied microarray analysis to cells exogenously expressing mir-155, while Gottwein et al. (2007) selected several mir-k12-11 candidate targets and subjected them to real-time PCR expression analysis in cells exogenously expressing mir-155. Each group independently concluded that both mir-k12-11 and mir-155 have the ability to regulate a common set of mRNA targets. It is notable, though, that there is little overlap among the targets identified in each study. Importantly, however, the targets from both studies are enriched for complements to the mir-k12-11 and mir-155 seed region and selected targets were shown by each group to be direct targets in heterologous luciferase reporter assays.

Interestingly, both groups identified BACH-1, a transcriptional repressor involved in hypoxia signaling, as a target for exogenously expressed mir-k12-11 and mir-155. Computational methods were used to identify BACH-1 as a candidate target, yet neither group detected robust changes in BACH-1 mRNA steady state levels upon expression of mir-k12-11 or mir-155. However, heterologous luciferase reporters containing portions of the BACH-1 3′ UTR were specifically downregulated by exogenous expression of either mir-k12-11 or mir-155, implying a majority of the regulation occurs at the level of translation. Furthermore, immunoblot analysis

showed BACH-1 steady state protein levels decreased several fold by exogenous expression of either mir-k12-11 or mir-155 in monocyte or B lymphocyte cells (Gottwein et al., 2007; Skalsky et al., 2007). These results strongly implicate BACH-1 as a target for downregulation by exogenous expression of either mir-k12-11 or mir-155.

Although these experimental approaches are standard for the field, one potential caveat is that exogenous expression may somehow bias which candidate mRNA targets are identified. Therefore, a higher level of confidence can be obtained when antisense inhibitors of endogenous miRNAs are used in conjunction with exogenous expression studies. Gottwein et al. (2007) showed that at least one candidate, FOS (part of the AP-1 transcriptional activator complex) is targeted by endogenous mir-k12-11; PEL cells that uptake a cholesterol-modified antisense inhibitor of mir-k12-11 show an increase in endogenous FOS protein levels. Surprisingly, BACH-1 showed little increase in expression under the same experimental conditions. The authors suggest this is likely due to "masking" of the mir-k12-11 inhibitory effects, since BACH-1 is also targeted by two other KSHV-encoded miRNAs (mir-k12-1 and mir-k12-6). This is one of the only points of contention between the two reports, since Skalsky et al. (2007) also tested mir-k12-6, yet observed no effect on BACH-1 3′ UTR reporters. One possible explanation for this apparent discrepancy is that mir-k12-1, which was not tested by Skalsky et al. (2007), has a more dramatic effect on

BACH-1 expression. Similar antisense strategies directed against both endogenous mir-k12-11 and mir-155 could help identify which candidate targets are subjects for more in depth study.

To better understand KSHV-associated pathogenesis, it will be important to test the most promising candidate miRNA targets in other relevant models, such as cultured primary endothelial cells as well as in vivo models. KSHV is particularly adept at "pirating" host protein coding genes into its genome; thus, it makes sense that KSHV would also tap into an existing host miRNA regulatory pathway. From a viral perspective, the ability to target many genes involved in an existing regulatory network may provide more "evolutionary bang for your buck." Indeed, it is likely that this strategy is not unique to KSHV, as other viral miRNAs display various degrees of seed identity with host miRNAs (Gottwein et al., 2007; Skalsky et al., 2007).

On the whole, these two studies suggest that mir-k12-11 and mir-155 share a set of common mRNA targets that form a part of a regulatory network important for B cell function, cell-cycle regulation, and apoptosis. All of these pathways could play an important role in the lymphomagenesis associated with misexpression of mir-155 or KSHV infection (Figure 2). This is an exciting time in the world of viral miRNAs, where the field is poised for an avalanche of miRNA target discoveries. The next challenge will be to integrate these findings into a better understanding of viral life cycle and pathogenesis.

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Defensins' Offensive Play: Exploiting a Viral Achilles' Heel

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Vertebrates rely on antimicrobial peptides as a front-line defense against invading pathogens. Certain cationic antimicrobial peptides, such as human α-defensins, have traditionally been thought to destroy invading microbes by disrupting their lipid membranes. In this issue of *Cell Host & Microbe*, Smith and Nemerow reveal that α-defensins can inactivate adenoviruses, which lack lipid membranes, through direct binding of the defensin to the virus's naked protein shell.

Vertebrates employ an impressive arsenal of molecular weapons to defend themselves against invading microbes. One defense mechanism involves the local release of noxious chemicals, such as reactive oxygen intermediates, that are capable of directly destroying microbial structures. Obviously, the use of such broadly reactive chemicals must be tightly controlled to avoid catastrophic damage to host tissues. Control over these systems typically involves a variety of signal-

ing proteins, for example, the toll-like receptors, which are specifically engaged by broadly conserved pathogen-associated molecular patterns (PAMPs). Fortunately, the vertebrate antimicrobial arsenal also includes a wide variety of bactericidal and virucidal peptides that are relatively harmless to host cells. Because of their low toxicity to host cells, antimicrobial peptides can be produced constitutively at relatively high concentrations in microenvironments such as the outer layers of

the skin, the crypts of the small intestines, and the phagosomes of neutrophils, which typically serve as immunological first responders at sites of infection.

A group of antimicrobial peptides aptly named defensins were first discovered more than 40 years ago as a bactericidal component abundant in rabbit neutrophils. Defensins and defensin-like peptides have since been identified in a broad range of organisms ranging from fungi to insects to humans (reviewed in Lehrer,