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Commentary

Presence of Viral microRNA in Extracellular Environments

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Lifelong occult infection is made possible through the establishment of viral latency. Both RNA and DNA viral genomes can remain in the infected host cell either by converting to DNA and integrating as a provirus, or as an episome, respectively. Reactivation of the virus via cellular stress stimuli contribute to morbidity and mortality in infected individuals. Latently infected cells produce virally encoded microRNAs (miRNAs) which are small, non-coding, single-stranded RNAs that can exert regulatory effects on target mRNAs. This can contribute to the associated pathogenesis and therefore miRNAs are of interest as potential biomarkers and predictors of clinical outcomes.

Kaposi's sarcoma-associated herpesvirus (KSHV), a gammaherpesvirus, is the etiologic agent of several pathologies including, Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and several subtypes of multicentric Castleman's disease (MCD), all of which are common in immunosuppressed patients, specifically those with AIDS. KSHV is capable of establishing lifelong latent infection in several cell types including dendritic cells, monocytes, endothelial cells, and B-lymphocytes. Depending on the latently-infected cell, KSHV can produce 25 miRNAs with various functions including immune evasion through the modulation of cytokines, promotion of cell cycle progression via avoidance of apoptosis, maintenance of latency, and tumorigenesis (Piedade and Azevedo-Pereira, 2016; Louten et al., 2015). Another gammaherpesvirus, the Epstein-Barr virus (EBV) can establish persistent latent infection through the maintenance of genomic DNA as episomes in resting memory B-lymphocytes. Occasionally these cells are reactivated and are capable of infecting and transforming new B-cells, thereby contributing to several lymphomas including Burkitt's, Hodgkin's disease and immunoblastic lymphoma. EBV encodes 44 mature miRNAs which contribute to EBV's oncogenic properties (Piedade and Azevedo-Pereira, 2016; Louten et al., 2015).

Early detection of these viruses plays a critical role in preventing transmission and improving patient outcomes. Advancements in laboratory diagnostics, such as qPCR and ELISA, have been successful in providing earlier diagnoses. However, the sensitivity of these assays may limit the effectiveness of these methods and thereby underrepresent the prevalence of infections such as KSHV and EBV in the human population. Currently, the most common method is the detection of antibodies produced in response to viral infection (i.e. seropositivity). Regardless, the robustness and stability of miRNAs makes them the ideal choice for diagnostic targets.

In a recent article published in *EBioMedicine*, Fuentes-Mattei and colleagues examined plasma samples from four independent patient cohorts with post-surgical/post-chemotherapy sepsis, chronic lymphocytic leukemia, and post-abdominal surgery were examined to compare the prevalence of KSHV as determined by seropositivity vs. prevalence determined by viral miRNA qPCR (Ct>35) (Fuentes-Mattei et al., 2017). Overall analysis of all four independent cohorts ($n = 214$) showed qPCR-detectable expression of a single KSHV miRNA, either KSHV-miR-K12-10b, KSHV-miR-K12-12, or KSHV-miR-K12-4-3p, in 78.50% of patients though 27.57% of patients tested seropositive for KSHV IgG. For a better correlation to clinical outcomes, viral miRNAs were detected in 90.48% of patients with a low WBC count (WBC < 400 cells/μl) and in 92.59% of patients with a low lymphocyte count (LYM < 1000 cells/μl), suggesting the detection power was significantly higher than ELISA detection of KSHV IgG antigen (14.29% and 18.52% respectively). The study concluded that the prevalence of KSHV infection is much greater than previously thought when utilizing ELISA diagnostic methods, and that use of miRNAs for diagnosis was significantly more accurate (Fuentes-Mattei et al., 2017).

To confirm significance of the discrepancy between these two diagnostic methods, Fuentes-Mattei et al. went on to analyze the same independent patient cohorts for the prevalence of EBV, as this virus has been shown to have a higher prevalence in the human population (Fuentes-Mattei et al., 2017). Analysis showed detectable expression of a single EBV miRNA, either EBV-miR-BART4 or EBV-miR-BHRF1-1, in 98.99% of patients while Epstein-Barr virus nuclear antigen 1 (EBNA-1) IgG seropositivity was found in 95.97% of the cohort. However, when patients with cases of low lymphocyte counts were tested, 92.59% of cases tested positive for EBV miRNAs, while only 62.96% tested positive for EBNA-1 IgG, further supporting the increased diagnostic resolution of miRNAs over seropositivity methods (Fuentes-Mattei et al., 2017).

Many human viruses of clinical importance including polyomaviruses, hepatitis B virus, hepatitis C virus, human papillomavirus, human immunodeficiency virus produce viral miRNAs (Louten et al., 2015). These specific viral miRNAs have likewise been explored as potential diagnostic platforms (Lagatie et al., 2014; Zhu et al., 2017; Takahashi et al., 2013;

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Kumar Gupta and Kumar, 2015; Dahiya and Atreya, 2014). Additionally, these viral miRNAs may become packaged into extracellular vesicles, including exosomes, from virally infected cells, contributing to increased stability and prolonged half-life, thereby promoting their utility as a potential biomarker (Schwab et al., 2015; Harwig et al., 2016). Therefore, the detection of several viruses, including KSHV and EBV, in human body fluids via qPCR analysis of viral miRNAs is a strong diagnostic platform and should be considered as a new gold standard for diagnosis of occult viral infections.

Disclosure

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