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Epstein–Barr virus growth/latency III program alters cellular microRNA expression

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ABSTRACT

The Epstein–Barr virus (EBV) is associated with lymphoid and epithelial cancers. Initial EBV infection alters lymphocyte gene expression, inducing cellular proliferation and differentiation as the virus transitions through consecutive latency transcription programs. Cellular microRNAs (miRNAs) are important regulators of signaling pathways and are implicated in carcinogenesis. The extent to which EBV exploits cellular miRNAs is unknown. Using micro-array analysis and quantitative PCR, we demonstrate differential expression of cellular miRNAs in type III versus type I EBV latency including elevated expression of miR-21, miR-23a, miR-24, miR-27a, miR-34a, miR-146a and b, and miR-155. In contrast, miR-28 expression was found to be lower in type III latency. The EBV-mediated regulation of cellular miRNAs may contribute to EBV signaling and associated cancers.

Introduction

The Epstein–Barr virus (EBV) has been implicated in a variety of lymphoid and epithelial cancers, including Burkitt's lymphoma, Hodgkin's disease, post-transplant lymphoproliferative disease, AIDS-associated immunoblastic lymphoma, and nasopharyngeal carcinoma. This ubiquitous human herpes virus persists for the life of its host by establishing latent infection predominantly in resting memory B lymphocytes (Babcock et al., 1998; Miyashita et al., 1997). The process by which EBV establishes latency in memory B cells is not fully understood. In the model proposed by Thorley-Lawson and colleagues (Thorley-Lawson, 2005), EBV establishes infection in naïve B cells, and through successive viral transcription programs, drives the proliferation and differentiation of the cell into a memory B cell. During this differentiation process, EBV expresses proteins that mimic antigen activation of B cells and effectively bypass the normal host signals that drive B cell differentiation. In a newly-infected naïve B cell, EBV initiates the growth program (also referred to as latency type III), and expresses a total of nine protein-coding genes (Kieff and Rickinson, 2007). These proteins — Epstein–Barr nuclear antigen (EBNA)-1, -2, -3a, -3b, -3c, and -LP, and latent membrane protein (LMP)-1, -2a, and -2b, — contribute the surrogate proliferation, migration, and survival signals that antigen-activated naïve B cells receive. Escape of growth program-associated lymphoblasts from normal immune surveillance in post-transplant and HIV-associated immune-suppressed individuals leads to lymphoproliferative diseases, demonstrating the oncogenic potential of this EBV transcriptional program. The oncogenic potential of the EBV growth/latency III program is also demonstrated by its expression in primary B cells transformed in vitro by infection with EBV, which generates long-lived replicating cell lines referred to as lymphoblastoid cell lines (LCLs).

According to Thorley-Lawson's model, as the EBV-infected cell matures into a memory B cell, both the virus and the cell enter a quiescent state. The virus becomes transcriptionally silent, allowing it to

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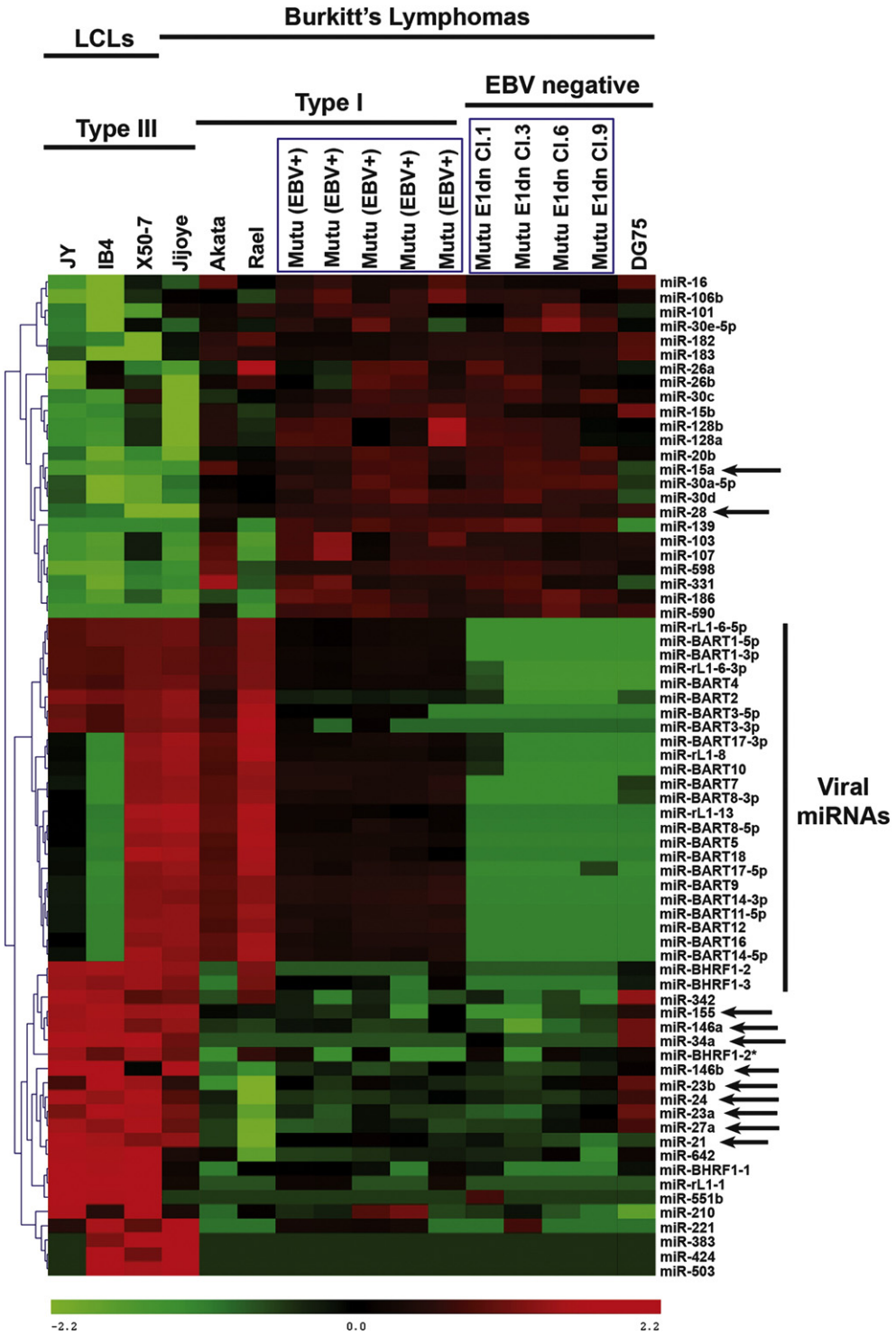

Fig. 1. EBV latency gene expression pattern correlates with distinct cellular and viral miRNA expression profiles. Micro-array analysis of miRNA expression was performed on RNA isolated from EBV infected, immortalized cell lines expressing viral latency patterns I (Akata, Rael, Mutu) or III (JY, IB4, Jijoye), and EBV negative cell lines (Mutu EBNA-1 dominant negative clones, DG75). Eight separate total RNA pairs were hybridized to miRNA arrays, and the data was grouped for analysis. Cluster analysis of miRNAs differentially expressed (ANOVA, $p<0.01$) among EBV negative, EBV positive/latency I and EBV positive/latency III cell lines is represented by heat map, with red elements representing higher expression and green elements representing lower expression.

escape immune recognition and preventing the subsequent immune-mediated destruction of the host cell (Hochberg and Thorley-Lawson, 2005). Homeostatic maintenance of the memory cell population drives expression of EBNA-1 alone (latency program, or latency type I) during cell replication in order to concurrently replicate the viral genome and appropriately segregate the viral episomes into the daughter cells (Hochberg et al., 2004; Yates, Warren, and Sugden, 1985).

Both antigen-driven B cell activation and EBV-driven activation are tightly regulated. Recently a new class of cellular regulatory elements, the small non-coding microRNAs (miRNAs), has been shown to play critical roles in a variety of cell signaling pathways. Through incorporation of the ~22 nucleotide single-stranded mature miRNA into the RNA-induced silencing complex (RISC) and subsequent imperfect base pairing within the 3′ untranslated region (UTR) of