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Xiaohong Shi and Richard M. Elliott

Generation and analysis of recombinant Bunyamwera orthobunyayiruses expressing V5 epitope-tagged L proteins

ABSTRACT

Introns from the Epstein-Barr virus (EBV) BART RNAs produce up to 20 micro RNAs (miRNAs) but the spliced exons of the BART RNAs have also been investigated as possible mRNAs, with the potential to express the RPMS1 and A73 proteins. Recombinant RPMS1 and A73 proteins were expressed in Escherichia coli and used to make new monoclonal antibodies that reacted specifically with artificially expressed RPMS1 and A73. These antibodies did not detect endogenous expression of A73 and RPMS1 proteins in a panel of EBV-infected cell lines representing the different known types of EBV infection. BART

TOP ABSTRACT INTRODUCTION METHODS RESULTS DISCUSSION REFERENCES

RNA could not be detected on Northern blots of cytoplasmic poly(A)⁺ RNA from the C666.1 NPC cell line and BART RNA was found to be mainly in the nucleus of C666.1 cells, arguing against an mRNA role for BART RNAs. In contrast, some early lytic cycle EBV mRNAs were found to be expressed in C666.1 cells. Artificially expressed A73 protein was known to be able to bind to the cellular RACK1 protein and has now also been shown to be able to regulate calcium flux, presumably via RACK1. Overall, the results support the conclusion that the miRNAs are functionally important products of BART transcription in the cell lines studied because the A73 and RPMS1 proteins could not be detected in natural EBV

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The BART RNAs are a heterogeneously spliced group of Epstein-Barr virus (EBV) RNAs transcribed rightward from position ABSTRACT INTRODUCTION 138 352 to 160 531 on the EBV wild-type genetic map (Sadler & Raab-Traub, 1995); Smith et al., 2000; de Jesus et al., 2003. METHODS RESULTS). BART RNAs have been detected in peripheral blood of normal EBV carriers (Chen et al., 1999) and in all EBV-associated DISCUSSION diseases that have been examined, including Burkitt's lymphoma (Tao et al., 1998), gastric carcinoma (Sugiura et al., 1996), REFERENCES salivary gland carcinomas (Raab-Traub et al., 1991), oral hairy leukoplakia (Webster-Cyriaque & Raab-Traub, 1998), nasal natural killer and T cell lymphomas (Chiang et al., 1996); van Gorp et al., 1996), Hodgkin's lymphoma (Deacon et al., 1993) and hepatocellular carcinomas (Sugaware et al., 1999). Most of the viral micro RNAs (miRNAs) that are expressed in EBV latent infections are derived from the BART RNAs (Cast al., 2006); Griffiths-Jones et al., 2006); Grundhoff et al., 2006); Pfeffer et al., 2004). The BART miRNAs are thought to be derived mainly from introns prior to splicing of the BART primary transcripts (Edwards et al., 2008). Few functional targets have yet been identified for the EBV miRNAs but there is evidence that miR BART2 can regulate the EBV DNA polymerase gene (Barth et al., 2008) and miR BART 1-5p and 17-5p can regulate EBV LMP1 (Lo et al., 2007.).

The BART RNAs (also known as complementary strand transcripts or BARF0 RNAs) were originally identified by analysis of cDNA libraries established from the nude-mouse-passaged nasopharyngeal carcinoma (NPC) cell line C15 (Gilligan et al., 1990); Hitt et al., 1989). A 4.0 kb cDNA clone (RPMS1, Smith et al., 2000) containing six exons was isolated that spanned the whole BART region but many other spliced partial cDNA clones of BART RNA containing combinations of the 16 different alternate exons have been described previously (Sadler & Raab-Traub, 1995). Prior to the discovery of the EBV miRNAs, some open reading frames (ORFs) in the spliced BART cDNAs were investigated as potential proteincoding sequences, if the BARTs were acting as mRNA. We have previously studied the RPMS1 and A73 ORFs, which could be translated in vitro



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