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Adenoviral delivery of RNA decoys restores cellular proapoptotic protein PUMA expression by silencing Epstein-Barr virus-encoded miR-BART5 in nasopharyngeal carcinoma cells

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Epstein-Barr virus (EBV) encodes 48 mature microRNAs that play important roles in viral maintenance and promote host cell survival by regulating viral transcripts expression, inhibiting apoptosis or facilitating to evade cell immune surveillance. We have previously shown that EBV-encoded miR-BART5 targets and downregulates cellular pro-apoptotic protein p53-upregulated modulator of apoptosis (PUMA) to promote cellular survival of EBV-infected nasopharyngeal carcinoma (NPC) cells. Since compromising miR-BART5 might induce apoptosis of EBV-infected NPC cell, in this study we have established an adenoviral expression system to deliver anti-miR-BART5 decoys to NPC cells. The anti-miR-BART5 decoys comprised 6 tandem repeats of miR-BART5 binding sites and their expression was driven by EBV-EBER2 promoter. They were designed to serve as a competitive inhibitor of miR-BART5 to reverse miR-BART5's inhibitory effects on PUMA in EBV-infected NPC cells. The RNA polymerase III-dependent EBER2 promoter is particularly strong in EBV-infected cells due to viral activation of transcription machinery. From this study, we found that the adenovirus-delivered anti-miR-BART5 decoys were successfully expressed in EBV+ C666-1, HK1/EBV and AGS/BX1 epithelial cell lines. The expression was more robust in EBV-infected cells. Moreover, these decoys effectively counteracted the function of miR-BART5 and restored PUMA expression in transduced EBV+ epithelial cell lines. Furthermore, restoration of PUMA expression rendered EBV-infected cells more susceptible to apoptosis. Our work provides the foundation for a new strategy of therapeutic intervention in EBV-associated epithelial carcinoma. This work was supported by Hong Kong Innovation and Technology Fund (ITS/136/09), Hong Kong Research Grants Council (HKU 7668/09M), SK Yee Medical Foundation (2011) and Hong Kong University Grants Committee (AoE/M-06/08).