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EBV-Specific Micro-RNA Via Exosome: A Key Inter-Cellular machinery between EBV$^+$ Tumor and Tumor-Surrounding Immune Cells?

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Abstract

The Epstein-Barr virus (EBV) is one of the most major human pathogen that establish long-term latent, or chronic infections, which is associated with a heterogeneous group of lymphoma, including Burkitt's lymphoma, Hodgkin's lymphoma (HL), NK-T lymphomas and lymphoproliferative disease. These malignancies are subdivided into in terms of EBV latent infection pattern, with typical three types of latency: type I to type III. HL is characterized by a minority of neoplastic Hodgkin and Reed-Sternberg (HRS), which are embedded in non-neoplastic bystanders, mostly B and T cells, but also macrophages. Without these bystander cells, these HL cells are incapable of being engrafted in immunodeficient mice. In this context, the non-tumor immune cells are tumor-supportive "inflammatory niche". Because of the complexity of interplay between tumor and tumor surrounding immune cells, the detailed mechanism and how tumor cells escape from the attack of host immune cells remains an open question. Small RNAs including miRNAs are well known intra-cellular regulatory elements of gene expression. Recently, it was reported that they are conjugated in exosomes and transferred to cells and are involved in tumor metastasis by educating tumor surrounding niche. Moreover, it was also reported that EBV-infected lymphocytes produce exosomes that contain viral

encoded, EBV specific miRNAs (BART-miRNA) and that these could be transferred in host cells and decrease the levels of known cellular targets. Accordingly, we hypothesized that EBV+ tumor derived exosomal BART-miRNA might redirect tumor surrounding immune cells from tumor reactive into tumor- -supportive "inflammatory niche", which ultimately leads to tumor progression. To this aim, first, we evaluated tumor derived viral encoded BART-miRNA in EBV+HL clinical specimens by using BART-miRNA specific probe *in situ* hybridization. As expected, these EBV specific BART-miRNA could be detected in HRS as well as in tumor surrounding inflammatory niche, especially macrophage. This result indicated that tumor derived EBV specific BART-miRNA could transfer to the non-tumor cells in the tumor inflammatory niche, supporting the in vivo relevance of secretary EBV specific miRNA. Next, we evaluated the properties of exosomes produced by EBV^+ cells (EBV-Ex). To this aim, EBV-Ex was harvested either from the media of the type III or type I EBV-transformed lymphoid cell line. Then, by using transwell co-culture system, we tested the delivery and the effect of EBV-Ex on human peripheral blood mononuclear cells (PBMC) derived monocyte/macrophage (Mo/Mf). As a result, we detected uptake of fluorochrome dye-labeled EBV-Exo in Mo/Mf. We also confirmed exosomal BART miRNA transfer in Mo/Mf. Surprisingly, exosome from Type III latency (Type III-Ex) was relatively enriched in BART miRNA, and were potent on Mo/Mf in inducing surface CD69 expression . This is in contrast to that of exosome from Type I latency (Type I-Ex), in which BART miRNA were relatively vacant and were weak in inducing surface CD69 expression. Panels of cytokine analysis by Q-PCR revealed that type III-Ex treated Mo/Mf displayed an anti-inflammatory/immunosuppressive cytokine rich signature, especially IL-10, compared to type I-Ex treated Mo/Mf, suggesting the possibility that type III-Ex might polarize macrophage into immunosuppressive M2-like phenotype. Intriguingly, type III-Ex from BART miRNA deletion mutant derivative cell lines totally lack the type III -Ex signature. Moreover, ectopic expression of a part of BART in Type I cells changed the EBV-Ex signature from type III to type I , suggesting the importance of specific BART lesion in functional EBV-Ex production in terms of Mo/Mf polarization. Taken these together, secretary tumor derived miRNAs in EBV associated malignancy, specifically in EBV^+HL, might play a certain role in tumor inflammation niche. EBV might utilize the exosomal machinery to secrete key viral-encoded miRNAs, through which a small number of neoplastic EBV^+ cells could modulate the tumor microenvironment.