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Review Article

Significance of trogocytosis and exosome-mediated transport in establishing and maintaining the tumor microenvironment in lymphoid malignancies

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It is widely accepted that the tumor microenvironment plays an important role in the progression of lymphoid malignancies. Interaction between the tumor and its surrounding immune cells is considered a potential therapeutic target. For example, anti-programmed cell death 1 (PD-1) antibody stimulates the surrounding exhausted immune cells to release PD-1/PD-L1, thereby leading to the regression of PD-L1-positive tumors. Recently, biological phenomena, such as trogocytosis and exosome-mediated transport were demonstrated to be involved in establishing and maintaining the tumor microenvironment. We found that trogocytosis-mediated PD-L1/L2 transfer from tumor cells to monocytes/macrophages is involved in immune dysfunction in classic Hodgkin lymphoma. Exosomes derived from Epstein-Barr virus (EBV)-associated lymphoma cells induce lymphoma tumorigenesis by transferring the EBV-coding microRNAs from the infected cells to macrophages. In this review, we summarized these biological phenomena based on our findings.

Keywords: Exosome, lymphoid malignancy, trogocytosis, tumor microenvironment

INTRODUCTION

The tumor immune microenvironment has been focused on in both solid and hematological tumors. Bystander cells in lymphoid malignancy are important for the progression of tumors.[1] Immune cells around the tumor contribute to the pro-tumor immunity, and support the survival and progression of the tumor as a niche. Immune cells are currently targeted for immunotherapy, for example, chimeric antigen receptor (CAR) -T cell therapy,[2] and immune checkpoint inhibitors such as anti-programmed cell death 1 (PD-1) antibody.[3] CAR-T cell therapy is particularly effective against hematological tumors. In the case of classic Hodgkin lymphoma (cHL), anti-PD-1 antibody is highly effective. This suggests that the immune interactions between the tumor and its surrounding cells are important for the progression of hematological tumors and their therapy.

Intercellular communication tools, such as cytokines and chemokines, are important for educating tumor biology. Furthermore, intercellular transfer of molecules, such as via trogocytosis and exosomes, was recently reported to be involved in facilitating the interaction between the tumor and surrounding immune cells.[4-7] Although cytokines and chemokines induce gene expression of target cells, they must undergo signal transduction. Trogocytosis and exosomes mediate direct transfer of molecules from donor cells to target cells.[8] They may be able to control the function of target cells more rapidly than the induction of gene expression by cytokines and chemokines.

In this article, we reviewed these biological phenomena functioning in lymphoid malignancy based on our findings.

TROGOCYTOSIS

Trogocytosis is a phenomenon characterized by the directional movement of molecules between the interacting cells or towards the cells connected to a donor cell via the interchanging junctions of the plasma membrane.[9,10] In the 1970s, the proteins that were specifically expressed in one cell type were detected from the surface of other cell types.[11,12] The transfer of major histocompatibility complex (MHC) molecule from antigen-presenting cells (APCs) to

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T-cells has also been observed.[13] Using *in vitro* and *in vivo* experiments, trogocytosis was demonstrated to occur in the membrane of lymphocytes such as B-, T-, and NK cells. Lymphocytes exhibit the potential to extract the surface molecules via the "immunological synapse" from APCs through conjugation. Physiologically, trogocytosis may influence immune regulation. $CD8^+$ T-cells, which acquire MHC from APCs via trogocytosis, are sensitive to peptide-specific lysis mediated by the neighboring T-cells.[14] The transfer of CD80/86 from dendritic cells to T-cells helps to regulate T-cells. Transferred CD80/86 are impaired in cytotoxic T-lymphocyte antigen 4 (CTLA4)-expressing cells.[15] "Cross-dressing" involves the transfer of MHC complexes from the surface of donor cells via intercellular transfer. After viral infection, dendritic cells acquire MHC of the donor cell through cross-dressing via trogocytosis, leading to the activation of drive memory $CD8^+$ T-cells.[16] Trogocytosis is characterized by the rapid movement of the membrane. In a previous report, trogocytosis occurred within minutes, which can be distinguished from other intercellular transfer mechanisms such as exocytosis.[17]

Some tumor cells were reported to trogocytose immune cells. One molecule of interest is human leukocyte antigen G (HLA-G). HLA-G is a potent immune inhibitory molecule that impairs the functions of NK, T-, and B-cells.[18] The transfer of HLA-G from the tumor to NK cells leads to the suppression of the anti-tumor effects.[4] Another report revealed that the transfer of natural killer group 2 membrane D ligand (NK2GDL) from the tumor cells pays a role in NK cell-cell fratricide via the NKG2D-NKG2DL axis.[19] However, the biological significance of other molecules remains unknown.

Lymphoid malignancy and trogocytosis

A previous report demonstrated that several cell lines of lymphoid malignancies trogocytose immune cells,[20] suggesting that trogocytosis is involved in the biology of lymphoid malignancies. In particular, cHL was assumed to be involved in this phenomenon.[7] Tumor cells, such as Hodgkin and Reed-Sternberg (HRS) cells, account for only 1% in tumor tissue, and surrounding immune cells, such as B-, T-cells, and macrophages, play an important role in cHL.[21] HRS cells release numerous chemokines, such as CCL5, CCL17, CCL22, and CCL28, which attract the immune cells.[22]

We focused on PD-L1, which is highly upregulated in HRS cells. It is widely accepted that anti-PD-1 antibody is strongly effective against cHL, which was reported to exhibit an overall response rate of 65-87%.[3,23-26] PD-L1 in monocyte/tumor-associated macrophages (TAMs) is also upregulated in the tumor tissue of cHL.[27,28] Moreover, the proportion of monocyte/TAMs in the tumor tissue or peripheral blood is associated with a poor prognosis of cHL.[29,30] We hypothesized that the direct contact between HRS cells and monocytes is important for the survival of tumors, and found that PD-L1/L2 membrane transfer occurs from HRS cells to monocytes/TAMs via trogocytosis.[31] As the upregulation of PD-L1/L2 on monocytes/TAMs was confirmed to support immune evasion by tumors,[32-34] this transfer may function in cHL immune dysregulation.

To demonstrate that direct contact induces PD-L1/L2 upregulation, we co-cultured HRS cells with monocytes. Upregulation was induced just after 1 hour of co-culture and PD-L1/L2 membrane transfer from HRS cells to monocytes was detected using a confocal microscope. We next generated PD-L1/L2 knockout cell lines and revealed that these cell lines co-cultured with monocytes did not induce PD-L1/L2 upregulation in the recipient cells. Thus, the upregulation of PD-L1/L2 on monocytes in a short time may be attributed to trogocytosis.

We further confirmed that PD-L1/L2 upregulation via trogocytosis is observed in the tumor tissue of patients with cHL. We postulated that TAMs in contact with HRS cells (HRS-contacted TAMs) exhibit higher PD-L1/L2 levels than those not in contact (HRS-uncontacted TAMs). Indeed, HRS-contacted TAMs exhibited significantly upregulated PD-L1/L2 levels compared with HRS-uncontacted TAMs.[31] Images are presented in Figure 1. Furthermore, CD30, which is strongly expressed in HRS cells, was highly upregulated in HRS-contacted TAMs. This supports the idea that trogocytosis induces PD-L1/L2 upregulation on TAMs in cHL patients.

PD-L1/L2 upregulation on TAMs may play a role in the suppression of effector T-cells. HRS cells are often defected in MHC class I peptide molecules.[35] TAMs, which is one of the APCs, possess MHC class II molecules and can recognize $CD4^+$ T-cells. CHL tissue surrounding HRS cells has abundant $CD4^+$ regulatory and type 2 helper T-cells. $PD\text{-}1^+$ $CD4^+$ T-cells accumulate in close proximity to HRS cells.[36] As HRS cells originate from B cells, they possess MHC class II molecules, like APCs. However, more than 60% of patients with cHL exhibit negative or decreased expression of MHC class II molecules in HRS cells.[35] A recent report suggested that the anti-PD-1 antibody response in cHL is not due to the cytotoxic T-cells because no cytotoxic T-cell immune response was observed after anti-PD-1 antibody treatment. Conversely, a reduction in type 1 regulatory T-cells and $PD\text{-}L1^+$ TAMs was observed after this treatment.[37] Collectively, PD-L1/L2 upregulation on TAMs via trogocytosis functions in the induction of PD-1/PD-L signaling between TAMs and $CD4^+$ T-cells, and may affect cHL tumor progression. We summarized our findings in Figure 2. Trogocytosis is more effective in facilitating the rapid increase of PD-L1/L2 on the surface of TAMs than cytokines. Transferred PD-L1/L2 by trogocytosis may promptly induce immunosuppression.

Reports on other molecules that undergo trogocytosis in cHL are listed in Table 1. The transfer of CD137 from HRS cells to APCs, such as B-cells and monocytes, plays a role in immune suppression in cHL.[38] CD137 is a T-cell co-stimulatory molecule expressed on HRS cells. As a result of the transfer of CD137 to APCs, the CD137-CD137L complex is internalized, thereby leading to the disappearance of CD137L from APCs. In addition, the transfer of CD137 from HRS cells to the neighboring HRS cells is observed, which leads

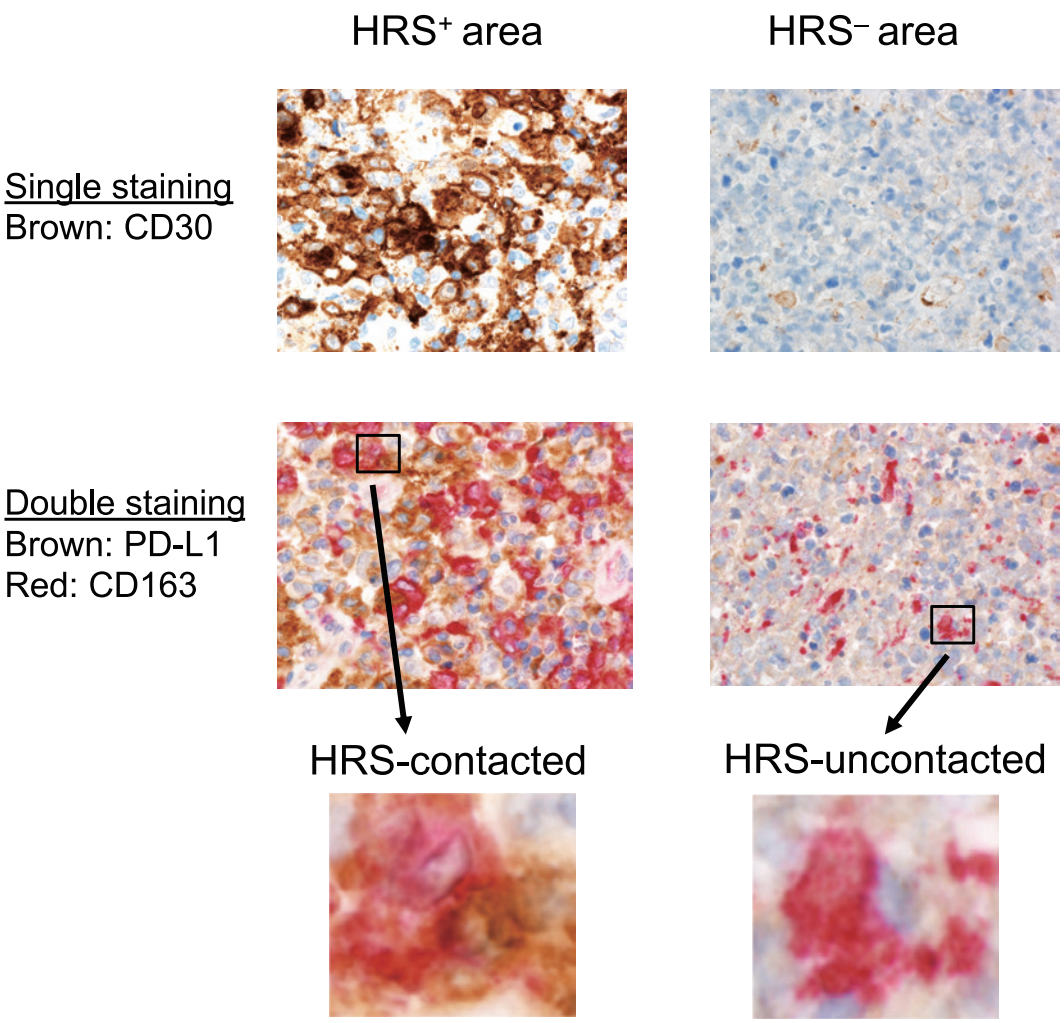

Fig. 1. Expression of PD-L1 was higher in TAMs in direct contact with HRS cells than in TAMs in not contact with HRS cells.
Using CD30 single staining, the HRS-abundant region was defined as the HRS^{+} area and the HRS-scarce region was the HRS^{-} area. PD-L1/CD163 double staining was performed in each area. Brown: PD-L1, Red: CD163.

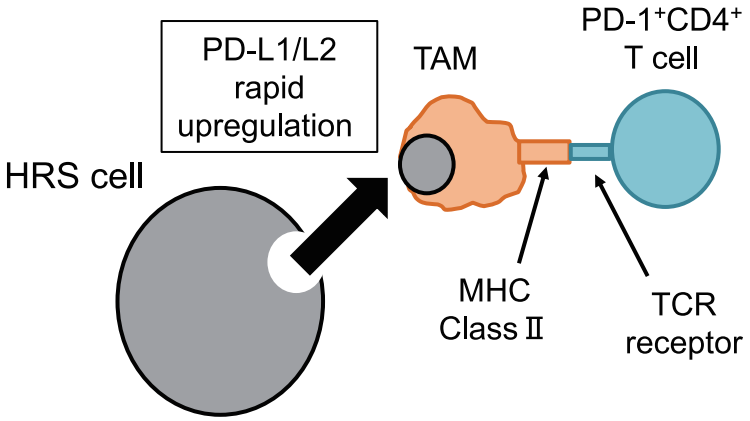

Fig. 2. Shema for the establishment of the tumor microenvironment in cHL.
Trogocytosis-mediated transfer from HRS cells lead to rapid PD-L1/L2 upregulation on TAMs. In cHL tissue, PD-1^{+}CD4^{+} T-cells are enriched, and they interact with PD-L1/L2 upregulated TAMs through PD-1 and PD-L1/L2 interaction via MHC presentation.

Table 1. Role for trogocytosis in cHL

Target molecules	Recipient cells	Suggested roles	References
PD-L1/L2	monocyte/TAMs	enhancement of T cell inhibition (particularly $CD4^+$ T cells)	31
CD137	APCs, HRS cells	inhibition of T cell activation	38
CD30	various cells	induce CD30-CD30L signaling	42
CD83	T cells	generation of suppressive $PD\text{-}1^+$ $CD83^+$ $CD4^+$ T cells	39

TAMs: tumor-associated macrophages, APCs: antigen-presenting cells, HRS: Hodgkin and Reed-Sternberg

to the disappearance of CD137L from HRS cells.

CD83 was recently demonstrated as a promising target molecule for the treatment of cHL.[39] CD83 exerts immunosuppressive effects and $PD\text{-}1^+$ $CD83^+$ T-cells may be implicated in unresponsiveness in the tumor microenvironment.[40] Li *et al*. provided evidence for the transfer of CD83 from HRS cells to T-cells, resulting in the generation of $PD\text{-}1^+$ $CD83^+$ suppressive $CD4^+$ T-cells.[39]

CD30 is involved in escaping immune surveillance via trogocytosis. CD30 is associated with recruiting TNFR-associated factors (TRAF) and TRAF-binding proteins, resulting in NF-κβ activation.[41] Nakashima *et al*. reported that the trogocytosis of CD30 from HRS cells to the surrounding cells leads to the expression of CD30L. Internalized CD30-CD30L complexes may be play an essential role in initiating the CD30 signaling pathway and this process can be inhibited via actin polymerization inhibitors.[42]

Other markers for immune checkpoint molecules in cHL may also play important roles. For example, CD86 on TAMs in close proximity to HRS cells is upregulated and involved in immune evasion mediated by the CD86-CTLA4 axis.[43] CD86 is upregulated in HRS cells.[44] Membrane transfer of CD86 from HRS cells to TAMs may occur and be related to the immune microenvironment of cHL.

Focusing on other lymphoid malignancies, Brown *et al*. demonstrated that the transfer of CD86 and HLA-G from the tumor cells to regulatory T-cells is associated with a poor prognosis of multiple myeloma (MM) by examining 168 MM patient samples of bone marrow and peripheral blood.[45] However, there are relatively few reports demonstrating the biological significance of trogocytosis, especially in the field of oncology; therefore, further studies are required.

Clinical application of trogocytosis

Reports of trogocytosis are relatively abundant regarding treatments based on immunotherapy. For example, treatment using the anti-CD20 antibody rituximab in B-cell lymphoid malignancies leads to the transfer of CD20 from the tumor cells to monocytes via the FcR receptor, thereby leading to antigen loss in the tumor cells.[46,47] Using chronic lymphocytic leukemia (CLL) B-cells, Valgardsdottir *et al*. investigated trogocytosis and phagocytosis mediated by human neutrophils following treatment using anti-CD20 antibodies such as rituximab and obinutuzumab. They revealed that human neutrophils mediate trogocytosis rather than phagocytosis of CLL cells opsonized with anti-CD20 antibodies. Trogocytosis can be observed more effectively upon treatment with rituximab than with obinutuzumab.[48] Similarly, MM treatment using the anti-CD38 antibody daratumumab results in the loss of CD38 protein due to its transfer from the tumor cells to monocytes and granulocytes.[49] Collectively, trogocytosis influences the effectiveness of molecular targeting therapy and some drugs are exclusively associated with this phenomenon.

Trogocytosis was reported to be involved in CAR-T cell therapy resistance.[50] The transfer of CD19 from acute lymphoid leukemia cells to infused CAR-T cells leads to CD19 loss from the tumor cells and CD19 upregulated CAR-T cells are attacked by other surrounding CAR-T cells. As trogocytosis is involved in inducing resistance to immune therapy in some cases, this phenomenon requires monitoring.

Therefore, regulating trogocytosis may improve the effectiveness of immunotherapy. Neutrophil antibody-dependent cellular cytotoxicity (ADCC) involves a trogocytosis-related necrotic procedure for inducing cancer cell death. Trogocytosis from antibody-opsonized cancer cells to neutrophils leads to cell death, and this process is promoted via CD47- SIRPα checkpoint inhibition.[51] Guideng *et al*. demonstrated a method for T-cell receptor ligand discovery using trogocytosis.[52] Trogocytosis from T-cells to target cells may be used to identify novel targets for immunotherapy. Furthermore, capturing the tumor cell membrane via trogocytosis results in increased cytotoxic T-cells.[53]

In contrast, we focused on trogocytosis-mediated transfer of molecules involved in the immune microenvironment from a biological viewpoint.[31] We revealed that inhibitors of trogocytosis may be used as anti-tumor agents. To date, trogocytosis have not been focused from this point of view. Although the detailed mechanisms of trogocytosis in human cells are not well classified, some agents have been reported to inhibit amebic trogocytosis, resulting in cell death.[54] Clinical applications of trogocytosis inhibitor must be assessed in the future.

The molecular targets associated with trogocytosis require further investigation. Considering the role of trogocytosis, the following points may be key factors: 1) recipient cells can acquire a protein from donor cells in as early as few minutes, 2) recipient cells can be deprived of donor proteins that are not originally expressed, and 3) the loss of proteins from donor cells occurs due to the transfer. These features are clues for investigating the novel molecules that play an important role in tumor progression via trogocytosis. Elucidating the mechanisms for trogocytosis may help identify new therapeutic targets for establishing the treatment of hematological tumors.

EXOSOMES

Exosomes are extracellular vesicles (EVs) that are produced in the endosomal component of cells and range from 50-200 nm in diameter. Exosomes were originally discovered in the 1960s, and are regarded as the mediators of extracellular excretion mechanisms to release unnecessary proteins and nucleic acids. However, after microRNAs (miRNAs) were reported to be present in exosomes in 2007, many researchers have investigated the biological significance of miRNA secretion via exosomes.[55] Exosomes function as intercellular communicators and are believed to be involved in the development of many diseases. Tumor cells were reported to secrete exosomes more actively than normal cells, and the tumor antigen expressing exosomes may be considered as a diagnostic and therapeutic target.

Moreover, tumor-derived exosomes affect the immune microenvironment, and play a role in the metastasis and progression of tumors. When the tumor metastasizes to other organs, the cells have to migrate between the stromal cells to reach the blood vessels. Exosomes promote tumor metastasis through activating tumor cell motility via fibronectin, degradation of extracellular matrix via protease MT1-MMP, and promotion of angiogenesis and vascular permeability.[56-59] Many tumor cells are known to establish a tumor microenvironment, which is known as a pre-metastasis niche, before metastasis. Peinado *et al*. demonstrated that the tumor-derived exosomes are essential for the migration of monocytes from the bone marrow, which leads to the formation of a pre-metastasis niche, and they also determined the orientation of metastatic tissue.[60] In addition, integrin, which is expressed on the surface of exosomes, was reported to position the metastatic tissue.[61]

There are several molecules (DNA, RNA, and protein) that can be transferred by exosomes. However, biological differences between the types of transferred molecules remain unknown. Zhang *et al*. classified exosomes by size; exomeres (~35 nm), small-exosomes (60-80 nm), and large-exosomes (90-120 nm).[62] These three subsets demonstrated unique biophysical properties by different expression patterns of proteins, lipids, DNA, RNA, and N-glycosylation. Further studies are needed to assess the biological differences in these molecules.

As mentioned earlier, miRNA secretion via exosomes has attracted the attention of many researchers. miRNA is 18–25-nt single-stranded non-coding RNA that binds complementarily to multiple arrays of target mRNA, resulting in the destabilization and inhibition of the translation of the gene.[63,64] Each miRNA targets several mRNAs, and miRNAs are implicated in many biological processes and phenomena.[65] We previously found a link between miRNAs and hematological malignancies, and demonstrated that miRNAs are involved in cell differentiation and tumor progression.[66-69] In the next section, we discuss the role of exosome-mediated transfer of miRNAs in the development of lymphoid malignancy based on our findings.

Lymphoid malignancy and exosomes

Epstein-Barr virus (EBV) is associated with several lymphoid malignancies. EBV can induce B- and NK/T-cell lymphoid malignancies in immunosuppressed patients.[70] We previously focused on the development of EBV-positive lymphoma[71,72] and investigated the functional role of exosomes secreted by lymphoma cells.[73] Patients with EBV-positive diffuse large B-cell lymphoma (DLBCL) and cHL exhibit a poorer prognosis than EBV-negative patients.[74] In particular, rituximab and CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP), which are a part of standard chemotherapy, were less effective against EBV-positive DLBCL.[75] Therefore, there is an urgent need to establish new treatments for EBV-positive lymphoma.

Of note, many immune cells infiltrate EBV-positive lymphoma tissue, suggesting that the survival of tumor cells depends on the inflammatory microenvironment. Pedal *et al*. reported that EBV-coding protein, latent membrane protein 1, is released from EBV-infected cells and incorporated into the dendritic cells, thereby leading to changes in their properties. In addition to proteins, EBV-derived miRNAs were detected in the exosomes. Of note, 40 miRNAs are clustered and transcribed from the BamHI fragment A rightward transcript (BART) region, known as BART miRNA.[76] Previous studies revealed that intracellular BART functions in the survival of tumors by inhibiting apoptosis.[77] Therefore, we hypothesized that the secreted BART miRNA is involved in the development of EBV-positive lymphoma.

We demonstrated that exosomes secreted from EBV-transfected cells are selectively incorporated into monocytes, but not lymphocytes, when peripheral blood mononuclear cells are treated with exosomes. Exosomes possessing high copy numbers of miRNAs induce TNF-α and IL-10 expression in monocytes, suggesting that the transfer of EBV-derived miRNA can induce a TAM-like phenotype.[78] This suggests that macrophages, which capture exosomes containing EBV miRNA, play an important role in the formation of tumors.

Next, we used an EBV-infected humanized mouse model, which was previously described.[79] Although EBV only infects primates, such as humans, this model enables infection by EBV and the development of lymphoma in infected mice through repopulating human $CD34^+$ hematopoietic stem cells in immunocompromised NOG (NOD/Shi-scid/IL-2Rγ^{null}) mice. We compared the lymphoma-forming capacity between the Akata and B95-8 EBV strains. Akata possesses a complete miRNA cluster, whereas B95-8 has a deletion in a large part of the miRNA cluster. Akata-infected mice developed lymphoma and died within 12 weeks after infection. Conversely, B95-8 infected mice did not develop lymphoma. We then intravenously administered miRNA-containing exosomes after B95-8 infection to examine the effects of exosome-mediated miRNA transfer. Transfer of miRNAs via exosomes significantly promoted the development of lymphoma. Moreover, there was marked macrophage infiltration of lymphoma tissue, suggesting that

exosomes influenced macrophage infiltration. We depleted the macrophages through clodronate liposome administration, which resulted in the depletion of lymphoma cells. This supports the hypothesis that EBV-derived exosomes mediate the formation of a microenvironment by increasing macrophage infiltration, as shown in Figure 3.[73]

We further investigated other potential EV-carried inflammatory factors associated with TAM formation in EBV-positive lymphoma. Mass spectrometric and phospholipidomic analysis revealed that several immunomodulatory proteins and lipid mediators containing EVs are important for inducing the tumor microenvironment in EBV-positive lymphoma.[80]

Exosome-mediated establishment of a tumor-supporting environment has been reported. Waldenström macroglobulinemia, which is characterized by a mutation in the innate immune-signaling adaptor myeloid differentiation response 88 (MyD88), was reported to secrete a mutant of MyD88 in EVs. The mutant of MyD88 transferred via EVs induces signaling in the recipient cells and establishes a proinflammatory microenvironment in the bone marrow.[81] Exosomes from Burkitt lymphoma cell lines increased the proliferation, differentiation, and class switch recombination in recipient B-cells.[82] Another report demonstrated that exosomes are related to angiogenesis in MM. Hypoxia upregulated miR-135b secretion in MM-derived exosomes and miR-135b suppressed factor-inhibiting hypoxia-inducible factor-1 (HIF-1) in the recipient endothelial cells. These events are thought to induce angiogenesis under hypoxic conditions.[83]

Clinical application of exosomes

Exosomes are present in human body fluid. They are easy to extract through liquid biopsy, such as blood, urine, and saliva, and function as a novel diagnostic and prognostic tool.[84,85] They are stable and can be preserved for long duration. We suggest that BART miRNA in EBV-positive lymphoma be used as a novel marker for predicting a poor outcome. We investigated BART13 miRNA expression in EBV-positive DLBCL biopsy tissue at our institute. The high expression of BART13 in EBV-positive lymphoma is strongly correlated with a poor prognosis.[73] Although it should be noted that the data were from a small number of samples, EBV-positive lymphoma exhibiting high expression of BART miRNA may require a new therapeutic approach instead of standard chemotherapy, such as R-CHOP. However, further investigation of BART miRNA in liquid biopsy should be addressed. Circulating miRNAs are non-invasive, and they have been reported for the diagnosis and prognosis.[86,87] Thus, circulating BART miRNA may become an easy-to-use tool for the treatment of EBV-positive lymphoma.

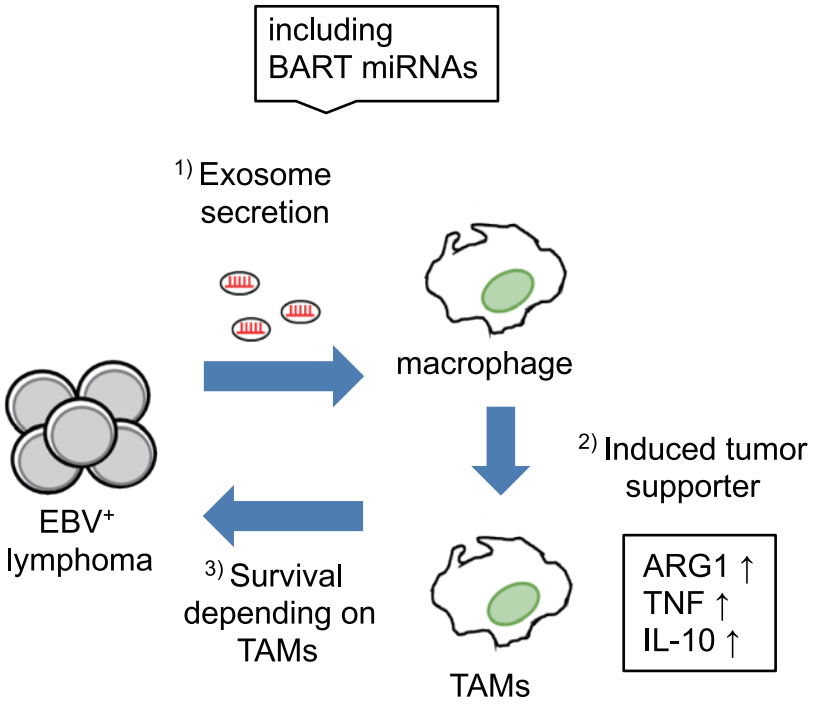

Fig. 3. Shema for the establishment of the EBV+ B cell lymphoma microenvironment.
1) EBV-infected cells release exosomes containing EBV miRNAs (including BART miRNAs), which are incorporated into the macrophages. 2) Lymphoma-derived exosomes alter the gene expression and convert the macrophages into "tumor-associated macrophages". The accumulation of BART miRNAs and upregulation of tumor-supporting molecules, TNFα, IL-10, and ARG1, promote the development of EBV+ B cell lymphoma. 3) The survival of EBV+ B cell lymphoma depends on TAMs and the deletion of TAMs leads to tumor death.

Reports on exosomes and its suggested roles in lymphoid malignancies are listed in Table 2.

Another report demonstrated that miRNAs are useful for the prognosis of hematological malignancies. In CLL, a few miRNAs, such as miR-21, miR-155, and miR-146a, were demonstrated to play key roles in cancer progression. The miRNA expression profile can be used as a biomarker for treatment and follow-up.[88] EVs possessing miRNAs, such as miR-24, miR-155, miR-127, and let-7, are considered useful biomarkers in cHL, which may help predict therapy response and relapse.[89] Furthermore, two circulating miRNAs, let-7b and miR-18a, are useful biomarkers for predicting favorable survival in patients with MM.[90] Let-7b was demonstrated as a tumor-suppressor miRNA in MM. miR-18a was reported to inhibit HIF-1α activity[91] and lead to the induction of M1 macrophages through targeting IRF2.[92] Thus, let-7b and miR-18a function in the inhibition of tumor progression in MM.

Exosomes can be used as the therapeutic targets for immunotherapies. In several DLBCL cell lines, exosome-mediated Wnt signaling controlled the transitions of cell states and the inhibition of exosomes strongly reduced tumor progression.[93] A recent report revealed that melanoma-derived exosomal PD-L1 plays an immuno-suppressive role in the tumor and may be associated with the response to anti-PD-1 antibody.[94] Further elucidation of the role of immune checkpoint molecules expressed in cancer-derived exosomes is warranted to improve the effectiveness of cancer immunotherapy. On the other hand, it has been reported that EVs are used in immunotherapy. Using acute lymphoid leukemia cell lines, even EV antigens alone can induce memory T lymphocytes via B-cell activation.[95]

In summary, studies on exosomes have made great progress in the past decade. However, there has been less progression of clinical trials for hematological tumors associated with EVs than those for solid tumors.[6] Therefore, we should focus on the clinical application of exosomes in this area.

Table 2. Clinical application for exosomes in lymphoid malignancies

Diseases	Exosome-releasing factors	Suggested roles	References
EBV+ lymphoma	BART13 miRNA	prediction of poor outcome	73
CLL	miR-21, miR-155, miR-146a	biomarker for treatment and follow-up	88
cHL	miR-24, miR-155, miR-127, let7	prediction of therapy response and relapse	89
MM	let-7b, miR-18a	prediction of favorable survival	90

EBV: Epstein-Barr virus, CLL: chronic lymphocytic leukemia, cHL: classical Hodgkin lymphoma, MM: multiple myeloma, BART: *Bam*HI fragment A rightward transcript

CONCLUSION

Although trogocytosis and exosome-mediated transport were initially considered as arbitrary phenomena, they have the potential to affect the surrounding immune cells to establish the tumor microenvironment, as described in this review. Bystander immune cells play an important role in the tumor microenvironment. As hematological malignancies originate from the immune cells, these phenomena affect the development and biology of tumors. Therefore, they may be used as markers for predicting outcome. In addition, elucidating these factors may help overcome the issue of chemotherapy resistance and improve cancer immunotherapy.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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