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MIP-1α enhances Jurkat cell transendothelial migration by up-regulating endothelial adhesion molecules VCAM-1 and ICAM-1

Yi-Ran Ma[a,*], Ying-Huan Ma[b]

[a] Department of Transfusion, The First Hospital of China Medical University, Shenyang, Liaoning, China
[b] Department of Cancer, The 436rd Hospital of Chinese People's Liberation Army, Shenyang, Liaoning, China

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ABSTRACT

The aim of this study is to evaluate the expression of macrophage inflammatory protein-1α (MIP-1α) in Jurkat cells and its effect on transendothelial migration. In the present study, human acute lymphoblastic leukemia Jurkat cells (Jurkat cells) were used as a model of T cells in human T-cell acute lymphoblastic leukemia (T-ALL), which demonstrated significantly higher MIP-1α expression compared with that in normal T-cell controls. The ability of Jurkat cells to cross a human brain microvascular endothelial cell (HBMEC) monolayer was almost completely abrogated by MIP-1α siRNA. In addition, the overexpression of MIP-1α resulted in the up-regulated expression of endothelial adhesion molecules, which enhanced the migration of Jurkat cells through a monolayer of HBMEC. MIP-1α levels in Jurkat cells appeared to be an important factor for its transendothelial migration, which may provide the theoretical basis to understand the mechanisms of brain metastases of T-ALL at cellular and molecular levels.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive neoplastic disorder that arises from T-cell progenitors. It progresses rapidly and relapses in approximately 30% of patients after 2 years, and brain metastases are common in the advanced stage. The incidence of central nervous system (CNS) involvement in T-ALL is estimated to be approximately 25–81% and is associated with high mortality [6,17,25]. Despite its clinical importance, the way in which T-cells in T-ALL enter the CNS is poorly understood. The resulting CNS leukemia is associated with the migration of leukemia/lymphoma T-cells in T-ALL into the brain [2,16]. However, there is much to be learned about how the circulating leukemia/lymphoma T-cells in T-ALL invades the CNS.

It is known that blood brain barrier (BBB) breakdown is associated with tumor metastasis to the brain. The interaction of transendothelial migration between leukemia/lymphoma T-cells in T-ALL and vascular endothelial cells is the first step in tumor brain metastasis. Some leukocyte-endothelial adhesion molecules, such as ICAM-1 and VCAM-1 [3,8,14,15,18,19,23], in brain endothelial cells were involved in the transendothelial migration of T-cells [4,10]. Here, we try to explore the potential novel role of ICAM-1 and VCAM-1 on brain endothelial cells, that is, whether they contribute to T cells' transendothelial migration and promote brain metastases of T-cells in T-ALL.

Some members of the chemokine MIP-1α and MCP-1α are known to be associated with T cells' transendothelial migration in some pathological states [13,20]. A recent study showed that T cells, such as in primary adult T-cell leukemia cells, can also secrete MIP-1α [7]. Moreover, our previous study showed that the peripheral T-cells of Alzheimer's disease patients overexpress macrophage inflammatory protein-1α (MIP-1α), which contributes to T-cell migration into the brain [13]. We have thus hypothesized that MIP-1α can enhance Jurkat cells' transendothelial migration by upregulating the endothelial adhesion molecules VCAM-1 and ICAM-1.

To investigate this hypothesis, we examined the role of MIP-1α secreted by Jurkat cells as a model of leukemia/lymphoma T-cells in T-ALL and endothelial adhesion molecules VCAM-1 and ICAM-1 in HBMEC during the transendothelial migration of Jurkat cells. We show that Jurkat cells can overexpress MIP-1α and MIP-1α siRNA to reduce the adherence of Jurkat cells to HBMEC. Expression of ICAM-1 and VCAM-1 in HBMEC correlates with MIP-1α expression and the transendothelial migration of Jurkat cells are blocked by MIP-1α siRNA. These data suggests that MIP-1α, ICAM-1 and VCAM-1 mediate the transendothelial migration of Jurkat cells, which contributes

* Corresponding author at: Department of Transfusion, The First Hospital of China Medical University, North Nanjing Street, No. 155, Shenyang, Liaoning, China. Tel.: +86 24 83283036; fax: +86 24 83283038.
E-mail address: 13898807150@163.com (Y.-R. Ma).

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Table 1
Primers and probes for human MIP-1α detection by real-time RT-PCR.

Target	Amplicon length (bp)	Oligonucleotide sequence, 5′–3′, forward, probe, reverse	Nucleotide position	Genbank accession no.
MIP-1α	279	ATGCAGGTCTCCACTGCTG	84–102	NM002983
		TCGGCGTGTCAGCAGCAAGT	155–174	
		TCAGGCACTCAGCTCCAG	345–362	
GAPDH	225	GAAGGTGAAGGTCGGAGTC	81–99	NM002046
		CAAGCTTCCCGTTCTCAGCC	258–277	
		GAAGATGGTGATGGGATTTC	287–306	

The amplification conditions were as follows: for MIP-1α, 94 °C for 2 min and 40 cycles of 94 °C for 15 s and 58 °C for 1 min; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 40 cycles of 95 °C for 15 s and 64 °C for 1 min. pGEM T vectors (Promega) containing MIP-1α or GAPDH cDNA were used to construct standard curves. The initial copy number of the target mRNA was calculated from the standard curve. The amount of MIP-1α transcripts of individual samples was normalized to that of GAPDH.

to a further understanding of the transendothelial migration mechanism of circulating leukemia/lymphoma T-cells in T-ALL, possibly providing a novel intervention target for the prevention and treatment of brain metastases.

2. Materials and methods

2.1. Cells

HBMEC were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 10% Nu-serum (BD Biosciences, Bedford, MA, USA), 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 1× non-essential amino acids, and 1× MEM vitamins. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

T-cell acute lymphoblastic leukemia cell line Jurkat cells purchased from the ATCC (395 Oyster Point Blvd., Suite, CA, USA) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA). Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Normal human T lymphocytes, originally from healthy subjects, were recruited from the 463rd Hospital of the Chinese People's Liberation Army under an International Review Board-approved human studies protocol. Human T lymphocytes were separated with Fluorobeads Isolation Reagent (One Lambda, Canoga Park, CA). Written informed consent was obtained from all of the healthy subjects, and the study was approved by the 463rd Hospital of the Chinese People's Liberation Army Ethics Committee.

2.2. RNA interference and transfection of Jurkat cells with siRNA

MIP-1α siRNA was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and generally consisted of three to five target-specific 19–25 nt siRNAs designed to knock down gene expression. MIP-1α siRNA was electrotransferred into Jurkat cells using a Nucleofector™ system (Amaxa, Cologne, Germany) and reagent with the D32 program at 295 V, 1180 μF, and 5000 resistance, and knockdown was verified by real-time RT-PCR at 24 h after transfection. Approximately 70% of the cells were successfully transfected.

2.3. Real-time RT-PCR

Total RNA was treated with RNase-free DNase I (TaKaRa Bio Inc., Shiga, Japan) and reverse transcribed with AMV reverse transcriptase (Promega, Madison, WI, USA). Real-time PCR was performed with a PE5700 real-time PCR system (Perkin-Elmer, Norwalk, CT, USA) and ExTaq R-PCR V2.1 (TaKaRa Bio Inc.) according to the manufacturer's protocol. The primers and probes are listed in Table 1.

2.4. In vitro BBB model and endothelial permeability assay

An in vitro BBB model composed of HBMEC was developed on a transwell insert with a 3-μm pore size (Corning-Costar, New York, NY, USA) in a 24-well plate. HBMEC (2×10^5) were seeded in the upper chamber of a transwell insert in a 24-well plate. After the cells were incubated for 4–5 d to allow for cell attachment, we carried out our experiments. The integrity of the HBMEC monolayer was monitored by daily transendothelial electrical resistance measurements using a Millicell-ERS endothelia volt-ohmmeter (World Precision Instruments Inc., Sarasota, FL, USA). Horseradish peroxidase (HRP) flux measurements were performed as follows to judge the permeability of the barrier and the formation of the monolayer. HRP dissolved in serum-free RPMI-1640 was added to transwells at a final concentration of 0.5 μM. After 2 h, the medium in the lower chamber was collected and the HRP content was evaluated spectrophotometrically by assaying the peroxidase activity in buffer containing 0.5 mM ortho-phenylenediamine. Absorbance at 492 nm was measured. When the HRP became absent in the lower compartment, the model was used to set experiments.

2.5. Transendothelial migration

Jurkat cells (2×10^5) without or with MIP-1α siRNA [Jurkat cells (MIP-1α siRNA)] were loaded into the upper chambers of transwells. After incubation for 20 h, the cells that had transmigrated into the lower chamber were harvested and counted using a hemocytometer.

2.6. Cell adherence experiment

HBMEC were seeded on Transwell membranes. At confluency, 2×10^5 CM-Dil (Invitrogen) of untreated Jurkat cells or Jurkat cells (MIP-1α siRNA) were added to the upper chamber. Unattached Jurkat cells were washed out at 1, 2,3, and 4 h. Transwell membranes were collected, and the cells were fixed. The number of Jurkat cells in 10 randomly selected fields of view (×200) was counted.

2.7. Western blot

HBMEC were washed twice with ice-cold PBS and then lysed. Samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA) using a semi-dry transfer cell (Bio-Rad). The membrane was blocked with 5% non-fat milk and then incubated with rabbit anti-intercellular adhesion molecule (ICAM)-1, rabbit anti-vascular cell adhesion molecule (VCAM)-1, and rabbit anti-GAPDH (Cell Signaling Tech., Beverly, MA, USA) antibodies overnight at 4 °C. Then, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz) for 1 hour at room temperature. Immunoreactive protein bands were visualized using an ECL Plus detection system (GE Healthcare) with a luminescent image analyzer (LAS-1000 UV mini; Fujifilm, Tokyo, Japan). The mean density of each band was measured by Multi Gauge V 3.1 software, and the band density of the activated form of the protein was divided by the density of the corresponding total protein band to obtain the normalized band density.

3. Results

3.1. Jurkat cells overexpress MIP-1α and MIP-1α siRNA reduces the adherence of Jurkat cells to HBMEC

To identify the factors in Jurkat cells that were involved in transendothelial migration, we performed real-time RT-PCR analysis and found that the MIP-1α expression level in Jurkat cells was significantly higher than that in normal T-cell controls ($^*P < 0.001$; Fig. 1A). siRNA against MIP-1α was designed and transfected into Jurkat cells, and successfully transfected cells were analyzed by real-time RT-PCR. The results showed that the expression of MIP-1α were down-regulated in the siRNA MIP-1α T cells compared to the no-silence control (Fig. 1B; $^*P < 0.05$). Untreated Jurkat cells and Jurkat cells (MIP-1α siRNA) were incubated on a HBMEC monolayer in transwells for 20 h, and then, the number of Jurkat cells that crossed the HBMEC monolayer was also counted (Fig. 1C; $^{**}P < 0.01$). Jurkat cells or Jurkat cells (MIP-1α siRNA) were added to the upper chamber of Transwells with an HBMEC monolayer for 20 h, and then, the transendothelial electrical resistance (TEER) was measured. The TEER value of the Jurkat cells (MIP-1α siRNA) group was higher than the Jurkat cells group (Fig. 1D; $^*P < 0.05$). The HRP flux of the Jurkat cell (MIP-1α siRNA) group was lower than that of the Jurkat group (Fig. 1E; $^*P < 0.05$). MIP-1α siRNA affected the transendothelial migration rate of Jurkat cells and decreased Jurkat cells adhesion to HBMEC (Fig. 1F; $^*P < 0.05$, $^{**}P < 0.01$).