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研究課題名（和文）Modified mesenchymal stem cells for brain remodeling and motor recovery in a rodent stroke model

研究課題名（英文）Modified mesenchymal stem cells for brain remodeling and motor recovery in a rodent stroke model

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研究成果の概要（和文）：この研究は、脳卒中後の脳回復に向けて修飾された間葉系幹細胞（MSC）の潜在的効果を評価しました。MSCは多能性と適応性により組織回復の源とされますが、移動能力が課題です。一方、内皮前駆細胞（EPC）は移動能力に優れ、本研究ではEPCのマイクロベシクルでMSCの移動性を向上させました。げっ歯類モデルでの実験では、若年MSC-MV処理群が最良の結果を示し、行動回復と炎症反応の低減が観察されました。

研究成果の学術的意義や社会的意義
Stroke is a devastating disease affecting millions. Despite improved care, interventions save lives but fail to prevent brain ischemia’s lasting motor disabilities. The economic and mental burden is significant. Our research emphasizes a novel cell population's potential in stroke immunomodulation.

研究成果の概要（英文）：This project evaluated the potential effect of modified mesenchymal stem cells (MSCs) for brain recovery after stroke. MSCs are known to be a source of tissue recovery because of their innate pluripotency and adaptability. However, it has been observed that these cells have poor migration skills. In contrast, endothelial progenitor cells (EPCs) are known for their migration abilities. For this study, we modified MSCs derived from human fat with EPCs microvesicles, increasing their mobility. To test the ability of this modified population in vivo, a rodent model of stroke was used, and different cell populations were transplanted 24 hours after stroke onset. Groups were randomly split into young MSC, adult MSC (with and without modification), EPCs, microvesicles only, and bovine serum albumin (BSA) as control. The highest mortality rate was found for BSA. The best results were observed in young MSC-MV-treated animals, showing behavioral recovery, and reduced the inflammatory response.

研究分野：Neurosciences

キーワード：Mesenchymal stem cells　Stroke　Neuroprotection　Microglial modulation

１．研究開始当初の背景

Mesenchymal stem cells (MSCs) are multipotent cells with significant clinical therapy potential thanks to their ability to differentiate into various cell types and their immunomodulatory properties. These characteristics make MSCs promising candidates for regenerative medicine applications, including tissue repair and modulation of the inflammatory response. However, one of the major challenges in MSC-based therapies is the low efficiency of MSC migration and engraftment at the targeted sites following transplantation. This inefficiency limits the therapeutic outcomes and necessitates the development of strategies to enhance the homing and integration of MSCs into the desired tissues.
Recent research has explored various methods to improve the migratory capacity of MSCs. Among these, the use of microvesicles (MVs) has shown promising results. Microvesicles are small extracellular vesicles that play a critical role in intercellular communication and can influence the behavior of recipient cells. Specifically, microvesicles derived from endothelial progenitor cells with low aldehyde dehydrogenase activity (Alde-Low EPCs) have been identified as potential enhancers of MSC migration. Studies have demonstrated that these MVs can facilitate the homing of MSCs to hypoxic tissues, which are often the sites of injury and inflammation. In a wound healing model, the application of MVs from Alde-Low EPCs not only improved the migration of MSCs to the hypoxic regions but also modulated the inflammatory response. This dual action is crucial for effective tissue repair, as it involves both the regeneration of damaged tissue and the reduction of inflammation. The modulation of the inflammatory response by MVs contributes to a favorable environment for tissue regeneration, thereby enhancing the overall therapeutic efficacy of MSCs.

２．研究の目的

The present study proposes the modification of MSCs internalized with Alde-Low EPC-derived microvesicles (MVs) and examines their ability to modulate the inflammatory response *in-vitro*. Additionally, the potential of these modified MSCs to improve outcomes *in-vivo*, in a rodent model of stroke was evaluated by assessing their effectiveness in enhancing behavioral recovery and modulating microglial polarization.

３．研究の方法

(1) *In-vitro* Experiments
To investigate the incorporation and effects of endothelial progenitor cell-derived extracellular vesicles (EPC-MV) on MSCs, EPC-MVs were first prepared and characterized for size and marker expression, and their incorporation into MSCs was assessed using flow cytometry. Cell growth assays determined the effects of EPC-EV on the proliferation of adipose-derived mesenchymal stem cells (AAM) and induced adipose-derived mesenchymal stem cells (IAM). The differentiation potential of AAM and IAM was evaluated via adipogenesis and osteogenesis assays. The expression of anti-inflammatory cytokines and angiogenic factors in MSCs post-EPC-MV incorporation was analyzed, as was the migratory ability towards SDF1 signaling.
Anti-inflammatory abilities of IAM-MV were tested using LPS-induced monocytes (THP1 cells) cocultured with MSCs, where inflammatory cytokine expression was measured. Additionally, THP1 monocytes were differentiated into M0 macrophages with PMA, then trans-differentiated into M1 macrophages with LPS, and inflammatory cytokine expression was assessed.

(2) *In-vivo* Experiments
Transient Middle Cerebral Artery Occlusion (tMCAO) was induced in 8-week-old mice using the filament method via the common carotid artery (1h occlusion, 24 h reperfusion). Fifty-five animals were randomly divided into 7 groups for treatment as follows: Four groups used MSC were derived from human adipose tissue from adults (AAM) and infants (IAM), transfected with Alde-Low EPCs MVs (AAM-MV and IAM-MV). Three control groups used EPCs, MVs, or phosphate buffer saline (PBS). Twenty-four hours after treatment, animals were euthanized, and samples

were collected for histology. Animals with subarachnoid hemorrhage found after euthanasia were not included. Behavioral analysis was done using the Longa score after MCAO, before treatment, and before euthanasia. After the experiment ended, brain samples were collected for histochemistry to collect data regarding infarct size, and cell counting for microglia (all), polarized M1 and M2 microglia, blood vessels, astrocytes, and neuronal count in multiple areas involving the core and penumbra areas (9 microscope fields in 3 different levels for each animal). The present study was approved by the Ethical Committee of the University of Tsukuba (22-112) and conducted following the guidelines for laboratory animal use.

４．研究成果

(1) *In-vitro* Experiments

Incorporation of IAM with EPC-MV resulted in upregulation of anti-inflammatory cytokines, and angiogenic factors, and enhanced migratory ability towards SDF1 signaling. Size and marker expression analyses confirmed the identity and purity of EPC-MV. Flow cytometry demonstrated efficient EPC-MV incorporation into MSCs. EPC-MV incorporation did not affect AAM and IAM proliferation or their differentiation into adipocytes and osteocytes. However, it induced the expression of anti-inflammatory cytokines and angiogenic factors and enhanced migratory ability towards SDF1. In anti-inflammatory assays, IAM-MV significantly reduced inflammatory cytokine expression in LPS-induced monocytes. Differentiation of THP1 monocytes into M0 macrophages and their subsequent trans-differentiation into M1 macrophages was significantly reduced when co-cultured with IAM-MVs. These results highlight the cytokine-mediated immunomodulation abilities of IAM-MV and their potential effect in delaying M1 polarization of microglia.

(2) *In-vivo* Experiments

The behavioral analysis showed different trends depending on the provided treatment. For animals treated with PBS, A-AM, A-AM-MV, and MVs, the score worsened after treatment. However, scores forEPCs and I-AM were mostly unchanged, but improvement was found for IAM-MV (P-Value = 0.04 when compared to PBS).

The brain infarct was characterized by measuring the infarct volume ratio and edema ratio (comparing 4 different levels per brain sample) when compared to the contralateral hemisphere. For IAM-MVs the lowest ratios were found showing the preservation of the architecture and reduced infarct size after treatment when compared to other groups.

To explore microglial modulation, double staining identifying the entire microglial population (Iba-1) and the neurotoxic microglia (CD16-32) was done and counting was performed in 9 areas, including the core and penumbra of the infarct area. The highest neurotoxic microglial ratio was found for PBS, whereas the lower ratio was for IAM-MV-treated animals.

Other histochemistry showed a slight increase in blood vessels for MVs and PBS groups when counting vessels and branches stained by CD-31 markers. Neuron count was also performed using the NeuN marker, which found higher values for IAM, IAM-MV, and EPCs in contrast to PBS. Regarding glial cells, astrocyte numbers were more elevated in IAM-MV transplanted animals when compared to PBS; however, the morphology of the astrocytes differed since PBS injected animals had reactive astrocytes with large bodies and several ramifications in contrast to IAM-MV injected animals where the aspect of the astrocytic population did not suggest reactivity.

The findings of the treated stroke model highlighted the potential beneficial effect of IAM-MV, 24 hours after transplantation. The behavior of animals only improved in the group treated with IAM-MV. Also, these animals exhibited a reduced polarization towards M1 microglia, which is known to induce neurotoxicity. Additionally, cell counting for neuronal markers showed lowest values for PBS, while cell-transplated animals exhibited similar countings. And regarding the glial response, despite the increase number of astrocytes found for IAM-MV in contrast to PBS-treated animals, astrocyte morphology suggested reactive astrocytes for the PBS group. Without regarding the cell count, reactive astrocytes are more associated to neuroinflammation and neurotoxicity. Despite its general function supporting neurogenesis and remodelation, reactive

astrocytes are more associated with glial scar formation and deleterious outcomes from the perspective of movement recovery.

This project highlights the potential of modified MSCs as a potential therapeutic alternative for stroke treatment. Further investigation should extend to the extension of the observation period to evaluate dynamic changes at different time intervals in microglia polarization, vascularization, glial changes, tissue remodeling and neurogenesis.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Puentes Sandra, Khanh Vuong Cat; Osamu Ohneda; Aiki Marushima
２．発表標題 Infant-Modified Adipose-Derived Mesenchymal Stem Cells Modulates Microglial Response in a Rodent Stroke Model
３．学会等名 IEEE 45th Annual International Conference of the Engineering in Medicine and Biology Society (EMBC) 2023（国際学会）
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関