論　文　内　容　の　要　旨

Synopsis of Thesis

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論文題名 Title	Extracellular vesicles from adipose-derived mesenchymal stem cells promote colony formation ability and EMT of corneal limbal epithelial cells （脂肪由来間葉系幹細胞からの細胞外小胞による角膜輪部上皮細胞のコロニー形成能と上皮間葉転換の促進）

論文内容の要旨

〔目　的(Objective)〕

To investigate the effects of extracellular vesicles from adipose-derived mesenchymal stem cells (AdMSC-EVs) on limbal epithelial cells (LECs), and to determine the functional factors within AdMSC-EVs affecting the cellular activities of LECs.

〔方法ならびに成績(Methods/Results)〕

In this study, we investigated the therapeutic potential of AdMSC-EVs on LECs, which are crucial for regenerating the corneal surface. AdMSC-EVs were isolated from AdMSC-conditioned medium by differential ultracentrifugation. These EVs were characterized by scanning electron microscopy and western blotting, confirming their exosome-like morphology and expression of canonical exosomal markers such as CD63, CD81, and TSG101.

To assess the effects of AdMSC-EVs on stem cell properties of LECs, we performed colony formation assays using LECs co-cultured with mitomycin C-treated NIH 3T3 feeder cells. LECs treated with AdMSC-EVs exhibited a significant increase in colony-forming efficiency (CFE) compared to the control group. This indicated that the AdMSC-EVs enhanced the stem cell properties of LECs. And this effect persisted even when LECs were pretreated with AdMSC-EVs prior to co-culture, suggesting a direct impact of the AdMSC-EVs on the LECs.

Further functional assays revealed that AdMSC-EVs accelerated cell migration in scratch assays while simultaneously reducing cell proliferation in proliferation assay. RNA sequencing analysis of EV-treated LECs showed upregulation of processes related to cell motility, cell migration and extracellular matrix organization-hallmarks of EMT. Gene expression of key EMT markers such as *WNT5A*, vimentin (*VIM*), and fibronectin (*FN1*) increased significantly, while differentiation markers like cytokeratin (*KRT*) 12 and *KRT* 13 were downregulated. The stem cell marker *TP63* remained stable, suggesting that AdMSC-EVs helped maintain an undifferentiated state in LECs.

To explore the molecular mediators of these effects, we profiled the microRNAs (miRNAs) within AdMSC-EVs. Among the most abundant miRNAs, miR-25, miR-191, and miR-335 were selected for functional validation as transfection of these individual miRNA mimics significantly enhanced CFE of LECs in colony formation assay. LECs transfected with these miRNA mimics showed promoted expression of mesenchymal markers at both the gene and protein levels. Importantly, TP63 expression remained unchanged, while KRT12 decreased and VIM and FN1 increased, supporting the hypothesis that these miRNAs contribute to the maintenance of an undifferentiated state of LECs and enhancement of the EMT phenotype induced by AdMSC-EVs.

〔総　括(Conclusion)〕

Our study highlighted that AdMSC-EVs could exert a positive influence on both the colony formation and EMT of LECs through miRNAs. Notably, miR-25, miR-191, and miR-335 were the most effective of highly expressed contributors to these effects.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究では、脂肪由来幹細胞（AdMSCs）が分泌する「細胞外小胞（EVs）」が、ヒトの角膜上皮細胞（LECs）にどのような影響を与えるかを調べました。EVsは細胞間で情報を伝える“メッセージカプセル”のような存在で、近年、再生医療で注目されています。本研究では、AdMSC-EVsをLECsに与えると、細胞の増殖や遊走能力が高まり、一部の遺伝子の発現が変化することを確認しました。これは「EMT」と呼ばれる細胞の性質変化に関係しており、再生や傷の修復に重要な現象です。また、EVsに多く含まれていたmicroRNAという小さな遺伝子制御因子が、この変化を引き起こす鍵であることも分かりました。本研究の成果は、将来的にEVsを使った角膜再生医療への応用に役立つ可能性があります。上記のような理由から、本研究は学位の授与に値すると考える。