Title	間葉系幹細胞/間質細胞株 (ASCL) を用いた新しい肝臓再生法の開発
Sub Title	Therapeutic potential of adipose tissue-derived stem cell lines in liver regeneration
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Abstract	背景/目的：本研究では、in vitro肝虚血再灌流障害モデルにおける間葉系幹細胞/間質細胞株(Adipose stem/stromal cell line, ASCL)の細胞増殖・再生・修復機能を検証する。方法として、Wister系ラットの肝細胞をASCLとともに共培養し、低酸素培養による細胞障害および細胞生存率をLDH測定およびTUNEL法により評価を行う。また、低酸素障害後の細胞を回収しAlb、IL-6、IL-1α、HMGB-1、NF-κB、ERK1/2、HGF、SODの遺伝子及びタンパク発現を測定する。 結果：ヒトASCLの技術を応用し、ラット皮下脂肪組織より間葉系幹細胞(Adipose stem cell, ASC)を単離し成熟脂肪細胞へ分化させ、再び脱分化を誘導しASCLの樹立に成功した。ASCLの治療効果を検討するため、肝虚血再灌流モデルラットにそれぞれ生理食塩水、ASC、ASCLを脾臓に投与し3群に分けて比較検討した。結果は、ASCL投与群のラットは生理食塩水とASC群と比較して、AST・ALTの上昇が抑制されていた。肝臓の免疫組織化学染色による病理組織学的検討では、生理食塩水群のラットと比べ、ASC群とASCL群において活性化Caspase-1の発現量が著明に抑制されていた。肝細胞低酸素障害実験では、単離したラット肝細胞をASCもしくはASCLとそれぞれと共培養し、単独培養群と共に1%酸素濃度で3時間の低酸素ストレスを加えた。低酸素障害後、TUNEL染色法によりアポトーシスの評価を行った結果、ASC及びASCL共培養群におけるTUNEL発現量は単独培養群に比べ有意に低発現であった。培養上清中のLDHを測定した結果、同様にASC及びASCL共培養群において有意に低値であり細胞障害が抑制されることが示唆された。また、遺伝子発現解析では単独培養群に比べASC及びASCL共培養群において抗炎症作用を有するTGF-β、IL-10、IL-1RAのmRNA発現の増加が確認された。 以上の結果より、ASC及びASCLは肝臓虚血再灌流損害を抑制する働きを有することが示唆された。 In this study, we purpose to find out evidence of adipose stem/stromal cell line (ASCL) suppressing the apoptosis process in in-vitro liver ischemia/reperfusion injury model. We also aim to verify ASCLs can attenuate liver ischemia/reperfusion injury by promoting cell proliferation, regeneration and repair function. Hepatocytes are isolated from liver tissue of Wistar rats and co-cultured with ASCLs as a method. By incubating in hypoxia environment, co-culture groups are induced to cell damage. The hypoxia injury is assessed by cell viability and apoptosis progression respectively with LDH and TUNEL measurement. To analyze more details in mechanism of ASCLs cell therapy in liver injury, co-cultured cells are collected after hypoxia for assessing gene and protein level expression such as ALB, IL-6, IL-1α, HMGB-1, NF-κB, ERK1/2, HGF, SOD. The procedure of establishing rat-derived ASCLs was referred to the protocol of human-derived ASCLs' establishment. Briefly, adipose stem cells (ASC) were extracted from Wistar rats' lateral abdominal fat and induced its differentiation so that extracted ASCs were differentiated into mature adipocytes. ASCLs were produced by de-differentiation of mature adipocytes eventually. To assess therapeutic potential of ASCLs, rats induced to hepatic ischemia/reperfusion were divided into control, ASC and ASCL injecting groups and injected with normal saline, ASCs and ASCLs respectively. As a result, increases in serum concentrations of AST and ALT in ASCL group were significantly suppressed comparing to ones in the control and ASC group. To observe the pathological changes in liver tissue, immunohistology staining was analyzed in each group of individuals. Activated Caspase-1 was highly expressed in control group's liver tissue while suppressed in ASC and ASCL's one. In hepatocytes' hypoxia/reoxygenation experiment, isolated hepatocytes were co-cultured with ASCs or ASCLs respectively, these co-culture groups and single culture of hepatocytes as control group were incubated in the environment of 1% O2 and 5% CO2 for 3 hours so as to add hypoxia stress. TUNEL staining was used for assessing apoptosis progression after hypoxia injury. As a result, TUNEL expression was significantly low expressed in ASC and ASCL co-culture group. Similar result was showed in LDH level of each group's culture medium which suggest both ASCs and ASCLs are able to alleviate cell damage. In gene expression analyze, mRNA expressions of anti-inflammatory cytokines such as TGF-β, IL-10, IL-1RA were increased in ASC and ASCL co-culture group comparing to control group. In conclusion, both ASCs and ASCLs have ability to suppress injury from hepatic

	ischemia/reperfusion according to current results.
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研究課題（日本語）

間葉系幹細胞/間質細胞株（ASCL）を用いた新しい肝臓再生法の開発

研究課題（英訳）

Therapeutic Potential of Adipose Tissue-Derived Stem Cell Lines in Liver regeneration

１．研究成果実績の概要

背景/目的：本研究では、in vitro 肝虚血再灌流障害モデルにおける間葉系幹細胞/間質細胞株(Adipose stem/stromal cell line, ASCL)の細胞増殖・再生・修復機能を検証する。方法として、Wister 系ラットの肝細胞を ASCL とともに共培養し、低酸素培養による細胞障害および細胞生存率を LDH 測定および TUNEL 法により評価を行う。また、低酸素障害後の細胞を回収し Alb、IL-6、IL-1α、HMGB-1、NF-κB、ERK1/2、HGF、SOD の遺伝子及びタンパク発現を測定する。
結果：ヒト ASCL の技術を応用し、ラット皮下脂肪組織より間葉系幹細胞(Adipose stem cell, ASC)を単離し成熟脂肪細胞へ分化させ、再び脱分化を誘導し ASCL の樹立に成功した。ASCL の治療効果を検討するため、肝虚血再灌流モデルラットにそれぞれ生理食塩水、ASC、ASCL を脾臓に投与し 3 群に分けて比較検討した。結果は、ASCL 投与群のラットは生理食塩水と ASC 群と比較して、AST・ALT の上昇が抑制されていた。肝臓の免疫組織化学染色による病理組織学的検討では、生理食塩水群のラットと比べ、ASC 群と ASCL 群において活性化 Caspase-1 の発現量が著明に抑制されていた。肝細胞低酸素障害実験では、単離したラット肝細胞を ASC もしくは ASCL とそれぞれと共培養し、単独培養群と共に 1%酸素濃度で 3 時間の低酸素ストレスを加えた。低酸素障害後、TUNEL 染色法によりアポトーシスの評価を行った結果、ASC 及び ASCL 共培養群における TUNEL 発現量は単独培養群に比べ有意に低発現であった。培養上清中の LDH を測定した結果、同様に ASC 及び ASCL 共培養群において有意に低値であり細胞障害が抑制されることが示唆された。また、遺伝子発現解析では単独培養群に比べ ASC 及び ASCL 共培養群において抗炎症作用を有する TGF-β、IL-10、IL-1RA の mRNA 発現の増加が確認された。
以上の結果より、ASC 及び ASCL は肝臓虚血再灌流損害を抑制する働きを有することが示唆された。

２．研究成果実績の概要（英訳）

In this study, we purpose to find out evidence of adipose stem/stromal cell line (ASCL) suppressing the apoptosis process in in-vitro liver ischemia/reperfusion injury model. We also aim to verify ASCLs can attenuate liver ischemia/reperfusion injury by promoting cell proliferation, regeneration and repair function. Hepatocytes are isolated from liver tissue of Wistar rats and co-cultured with ASCLs as a method. By incubating in hypoxia environment, co-culture groups are induced to cell damage. The hypoxia injury is assessed by cell viability and apoptosis progression respectively with LDH and TUNEL measurement. To analyze more details in mechanism of ASCLs cell therapy in liver injury, co-cultured cells are collected after hypoxia for assessing gene and protein level expression such as ALB, IL-6, IL-1α, HMGB-1, NF-κB, ERK1/2, HGF, SOD.
The procedure of establishing rat-derived ASCLs was referred to the protocol of human-derived ASCLs' establishment. Briefly, adipose stem cells (ASC) were extracted from Wistar rats' lateral abdominal fat and induced its differentiation so that extracted ASCs were differentiated into mature adipocytes. ASCLs were produced by de-differentiation of mature adipocytes eventually. To assess therapeutic potential of ASCLs, rats induced to hepatic ischemia/reperfusion were divided into control, ASC and ASCL injecting groups and injected with normal saline, ASCs and ASCLs respectively. As a result, increases in serum concentrations of AST and ALT in ASCL group were significantly suppressed comparing to ones in the control and ASC group. To observe the pathological changes in liver tissue, immunohistology staining was analyzed in each group of individuals. Activated Caspase-1 was highly expressed in control group's liver tissue while suppressed in ASC and ASCL's one. In hepatocytes' hypoxia/reoxygenation experiment, isolated hepatocytes were co-cultured with ASCs or ASCLs respectively, these co-culture groups and single culture of hepatocytes as control group were incubated in the environment of 1% O2 and 5% CO2 for 3 hours so as to add hypoxia stress. TUNEL staining was used for assessing apoptosis progression after hypoxia injury. As a result, TUNEL expression was significantly low expressed in ASC and ASCL co-culture group. Similar result was showed in LDH level of each group's culture medium which suggest both ASCs and ASCLs are able to alleviate cell damage. In gene expression analyze, mRNA expressions of anti-inflammatory cytokines such as TGF-β, IL-10, IL-1RA were increased in ASC and ASCL co-culture group comparing to control group.
In conclusion, both ASCs and ASCLs have ability to suppress injury from hepatic ischemia/reperfusion according to current results.

３．本研究課題に関する発表

発表者氏名 （著者・講演者）	発表課題名 （著書名・演題）	発表学術誌名 （著書発行所・講演学会）	学術誌発行年月 （著書発行年月・講演年月）