



Title	Development and evaluation of a novel xeno-free culture medium for human-induced pluripotent stem cells
Author(s)	花, 瑩
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論文内容の要旨
Synopsis of Thesis

氏 名 Name	HUA YING
論文題名 Title	Development and evaluation of a novel xeno-free culture medium for human-induced pluripotent stem cells (ヒト人工多能性幹細胞のための新規ゼノンフリー培養液の開発と評価)
論文内容の要旨 〔目的(Purpose)〕 Human-induced pluripotent stem cells (hiPSCs) are considered an ideal resource for regenerative medicine because of their ease of access and infinite expansion ability. To satisfy the sizable requirement for clinical applications of hiPSCs, large-scale, expansion-oriented, xeno-free, and cost-effective media are critical. Although several xeno-free media for hiPSCs have been generated over the past decades, few of them are suitable for scalable expansion of cultured hiPSCs because of their modest potential for proliferation and high cost. In order to develop an efficient but cost-effective xeno-free medium for hiPSCs, this study was conducted. 〔方法ならびに成績(Methods/Results)〕 In this study, we developed a xeno-free ON2/AscleStem PSC medium (ON2) and cultured 253G1 hiPSCs on different matrices, including iMatrix-511 and gelatin nanofiber (GNF) in ON2. Over 20 passages, we evaluated cell proliferation by doubling times; pluripotency by flow cytometry, immunofluorescence staining and qRT-PCR; and differentiation ability by three germ layer differentiation in vitro and teratoma formation in severe combined immunodeficiency mice, followed by histological analysis. In addition, we compared the maintenance effect of ON2 on hiPSCs with StemFit® AK02 (AK02N) and Essential 8™ (E8). Besides 253G1 hiPSCs, we cultivated different hiPSC lines, including Ff-101 hiPSCs, ATCC® ACS-1020™ hiPSCs, and Down's syndrome patient-specific ATCC® ACS-1003™ hiPSCs in ON2. We found that 253G1 hiPSCs in ON2 demonstrated normal morphology and karyotype and high selfrenewal and differentiation abilities on the tested matrices for over 20 passages. Moreover, 253G1 hiPSCs kept on GNF showed higher growth and stemness, as verified by the shorter doubling time and higher expression levels of pluripotent markers. Compared to AK02N and E8 media, 253G1 hiPSCs grown in ON2 showed higher pluripotency, as demonstrated by the increased expression level of pluripotent factors. In addition, all hiPSC lines cultivated in ON2 were able to grow for at least 10 passages with compact clonal morphology and were positive for all detected pluripotent markers. 〔総括(Conclusion)〕 Our xeno-free ON2 was compatible with various matrices and ideal for long-term expansion and maintenance of not only healthy-derived hiPSCs but also patient-specific hiPSCs. This highly efficient medium enabled the rapid expansion of hiPSCs in a reliable and cost-effective manner and could act as a promising tool for disease modeling and large-scale production for regenerative medicine in the future.	

論文審査の結果の要旨及び担当者

(申請者氏名) HUA YING (花 瑩)				
論文審査担当者	(職)	氏 名		
	主 査	大阪大学教授	宮川 繁	署 名
	副 査	大阪大学教授	新谷 康	署 名
	副 査	大阪大学教授	下村 修一郎	署 名
論文審査の結果の要旨				
iPS細胞培養における微細環境は、培地と細胞外基質の両方を最適化する必要があるが、現行の培養方法においては培地コスト、異種由来成分の添加、未分化の維持の不安定性などが課題である。本研究では、再生医療、病態モデル構築や創薬などiPS細胞研究においては必要不可欠であるヒトiPS細胞の未分化維持する培地の開発を行った。本培地を健常人および疾患患者由来の複数株を用いて検討したところ、既存の培地と同等以上に、iPS細胞の未分化状態を長期間安定して維持することが可能であった。本培地を用いて、種々の培養基材上で培養したところ、iMatrix-511、Fibronectin、Vitronectin、ならびに新規培養基材であるGelatin Nanofiber上でも安定して未分化状態を維持できることを示した。本培地は動物由来成分を排除したゼノフリー培地であり、既存培地と比較してコストが削減されていることが特徴である。 本研究はiPS細胞を用いたトランスレーショナルリサーチにおける細胞培養方法確立に寄与するものと考えられ、学位の授与に値すると考えられる。				