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論文題目	CDH18 is a fetal epicardial biomarker regulating differentiation towards vascular smooth muscle cells （CDH18 は血管平滑筋細胞への分化を制御する胎児心外膜バイオマーカーである）		

（論文内容の要旨）

The epicardium is a mesothelial layer that covers the myocardium and serves as a progenitor source supporting cardiac development, repair and regeneration. During development the epicardium is derived from the pro-epicardium (PE), a transient structure that spreads over the heart tube, and contributes to different cardiac lineage descendants, making it essential for cardiogenesis. Epicardial cell plasticity is governed by finely tuned signaling that regulates the epicardial-to-mesenchymal transition (EMT) and the cell-fate decisions of epicardial-derived cells (EPDCs). Interestingly, the epicardium reactivates upon cardiac injury promoting cardiac repair and regeneration. In recent years, the epicardium has emerged as a therapeutic target. However, the biology of the epicardium as well as its repair and regeneration processes are still poorly understood as powerful tools to investigate epicardial function, including markers with pivotal roles in developmental signaling, are lacking. In this study, human induced pluripotent stem cells (hiPSCs) were used to recapitulate epicardiogenesis and successfully generate hiPSC-epicardial-like (EPI) cells with high efficiency. By a retro-perspective analysis of public available RNA-seq datasets, type II classical cadherin CDH18 was identified as a biomarker defining lineage specification in human active epicardium, and its expression in EPI cells as well as in explant cells derived from mouse embryonic hearts at E14 was demonstrated. The exclusiveness of *CDH18* expression to epicardial cells and its absence in EPDCs were confirmed, thus strengthening the importance of CDH18 as a biomarker compared to other common epicardial marker genes. The knockdown of *CDH18* led to the loss of epicardial identity accompanied by the onset of EMT. The loss of CDH18 induced the activation of the Wnt signaling pathway and in combination with loss of TCF21 resulted in a cell-fate-specific differentiation towards cardiac smooth muscle cells (SMCs). This effect was found to be more apparent in cells representing a more PE-like state compared to cells representing a fetal-like epicardial stage. While the ectopic expression of *CDH18* was not sufficient to inhibit the directed differentiation of SMCs, it did reduce marker expression and decreased the invasive potential of SMCs derived from hiPSCs. Furthermore, the correlation of *GATA4* expression with epicardial *CDH18* expression was shown, hinting at a potential regulator role. These results highlight the importance of tracing *CDH18* expression in hiPSC-derived epicardial cells, providing a model for investigating epicardial function in human development and disease and enabling new possibilities for regenerative medicine.

（論文審査の結果の要旨）

心外膜は心筋を覆う上皮層であるが、心臓の発生において非心筋細胞のソースとなり重要な役割を果たしている。心外膜は心臓の損傷時に再活性化し、心臓の修復と再生を促進するが、心外膜細胞の生物学的特性については、まだ十分に理解されていない。本研究ではヒト人工多能性幹細胞（iPSC）を用いて心外膜細胞を高効率で作製することに成功した。網羅的遺伝子発現解析から心外膜細胞のマーカーとして II 型カドヘリン CDH18 が同定された。CDH18 の発現は心臓においては心外膜細胞においてのみ認められ、心外膜細胞から分化した心外膜由来細胞(EPDC)には存在しないことが確認された。CDH18 をノックダウンすると、Wnt シグナル経路の活性化を引き起こし、TCF21 の発現低下を引き起こし、血管平滑筋細胞（SMC）への特異的な分化を引き起こすことが明らかになった。この効果は、胎児心外膜細胞と比べて、より早期の心外膜原基でより著明であった。さらに CDH18 の過剰発現により SMC のマーカーの発現が低下しその遊走能を低下させた。また、心外膜細胞において GATA4 と CDH18 の発現が相関しており、GATA4 が CDH18 の発現を制御していることが示唆された。これらの結果は、ヒトの発生や疾患における心外膜の機能解析に貢献し、ヒトの心臓の発生メカニズムの解明に寄与するところが多い。

したがって、本論文は博士（医科学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 4 年 2 月 14 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降