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論　文　内　容　の　要　旨

〔　題　　名　〕 Regulation of anterior neural plate development studied using an epiblast stem cell model

(エピブラスト幹細胞（EpiSC）を利用した前部神経板の発生機構の研究)

The first major somatic tissue derived from the epiblast is the neural plate. To investigate the regulation of the neural plate development from the epiblast, I took advantage of an epiblast stem cell (EpiSC) line. The epiblastic state of EpiSCs was maintained under the culture condition with addition of activin (Nodal substitute). When this signal was removed, EpiSCs developed into the anterior neural plate cells equivalent to those of ~E7.5 mouse embryos in 1 day and to those of ~E8.25 embryos in 2 days. I confirmed this by immunocytochemistry and microarray analysis.

The anterior neural plate cells are further regionalized after E7.5. In E6.5~7 mouse embryos. Wnt antagonist Dkk1 is expressed in the visceral endoderm underlying the anterior-most part of the epiblast and continue to repress Wnt signal activity. This suggests that the Dkk1-dependent inhibition of Wnt signal primes the epiblast cells to develop into the anterior-most part of the neural plate (anterior forebrain precursor). In order to test this model using EpiSC culture, I inhibited endogenous Wnt signal activity by addition of Dkk1 or a chemical Wnt inhibitor. The inhibition of Wnt signal activated the *Hesx1* 5' enhancer and also the expression of *Hesx1* and *Six3*, which are characteristic of the anterior-most region of the neural plate, confirming the model.

Dkk1 is expressed in the anterior visceral endoderm in E6.5 to E7.5, and then expressed in the anterior mesendoderm. I lastly examined at which stage the effect of Dkk1 is more important on the anterior forebrain precursor development, and concluded that the Dkk1 action at the initial stage is more important.

From this study, I drew following main conclusions. (1) EpiSCs cultured under an activin-free condition mimic the anterior neural plate development in mouse embryos. (2) Inhibition of Wnt signal in EpiSCs under the activin-free culture condition activates the *Hesx1* expression and promotes the development of anterior forebrain precursor. (3) This effect involves the activation of the *Hesx1* 5' enhancer.

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論文審査の結果の要旨

本研究は、２つの内容によって構成されている。申請者は、まず、エピブラスト幹細胞を着床後胚における体細胞系列の発生の研究に活用した。培養からActivin/Nodalシグナルを除去することによって、エピブラスト幹細胞がマウス胚の前部神経板に相当する細胞に発生することを示した。次いで、この条件下でDkk1によって早期にWntシグナルを抑制すると、エピブラスト幹細胞が、前部神経板の小領域である前脳先端部の前駆体を生み出すことを示した。この実験系を駆使することによって、マウス胚自身を用いては解明が困難であった、神経板の領域化の初期過程を明らかにできた。エピブラスト幹細胞を用いた実験系の開発をも含めて、新規性に富んだ研究成果であり、学位の授与に相応しいと判断される。