CDB SEMINAR

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16:00~17:00 C1F CDB Auditorium

Molecular analyses of mouse mesoderm formation and axial elongation morphogenesis using embryonal carcinoma cells

Various types of pluripotent stem cell lines have been established, such as embryonic stem (ES), embryonal carcinoma (EC), embryonal germ (EG), epiblast stem (EpiS), and induced pluripotent stem (iPS) cells. They can propagate indefinitely as undifferentiated cells, and can be induced to differentiate into various cell types in vitro. These pluripotent stem cell lines have been used as convenient and effective tools to study molecular and cellular mechanisms of embryonic cell differentiation. However, they have not been fully explored to investigate the mechanisms of complex morphogenetic processes, which are pivotal for constructing the three dimensional architecture of embryos.

Here, we demonstrate that embryoid bodies (EBs) of mouse P19 EC cell line can be used to recapitulate axial elongation morphogenesis similar to the one that takes place at the caudal end of normal embryos. P19 EC cells were aggregated to form EBs in hanging drops of culture medium. Upon aggregation, P19 EBs expressed various genes that are normally expressed in the primitive streak and caudal end of embryos. At 5-6 days of culture, over 90% of EBs exhibited elongation, which was apparently driven by convergent extension morphogenesis. Elongated EBs displayed distinct patterns of localized gene expressions, which were reminiscent of those along the anterior-posterior axis of normal embryos. Experiments using shRNA-mediated gene knockdown showed that elongation morphogenesis in P19 EBs were dependent on various genes, such as Wnt3a, Wnt5a, Brachyury, and Cdx2, which are known to be critical for caudal development during normal embryogenesis, suggesting that elongation morphogenesis of P19 EBs are controlled by the mechanisms that operate in vivo. Using this in vitro morphogenesis system, we also showed that knockdown of RhoA or pharmacological inhibition of ROCK interferes with the elongation morphogenesis without compromising the localized expressions of caudal genes.

This study demonstrates that a homogeneous population of cultured cells can recapitulate embryonic elongation morphogenesis in vitro, which can be used to identify specific genes and signaling pathways involved in this process.

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