Synopsis of Original Research Paper

Development of in vitro teratogenicity test method by imitating macroscopic pattern of the human embryo

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Safety is one of the most fundamental criteria that cosmetics must fulfill. People, who may become pregnant, are concerned about the safety of themselves and the fetus. Recently, human pluripotent stem cells have gained attention for developing in vitro tests of teratogenicity and malformation because using animals for testing cosmetics is prohibited now. However, the effect of toxicity on the whole body (teratogenicity) is not easy to assess because it is difficult to form a macroscopic spatial pattern from human pluripotent stem cells (hPSCs). The cause of the difficulty is that the movement of morphogens, which are responsible for the cell-cell interaction and cell differentiation, cannot be controlled by a standard culture dish. We previously applied unidirectional-microfluidics to control the advection-diffusion of the morphogen and succeeded in making a macroscopic pattern where the upstream cells differentiated but downstream cells did not. However, the mechanism is not well understood.

We made an "in vitro organizer formation hypothesis" and tested it. In vivo, the organizer secretes inhibitors of mesoderm induction signal, which form a concentration gradient of signal, resulting in a macroscopic differentiation pattern. To test the hypothesis, we measured the signaling inhibitors in the supernatant of the cultured hPSCs. We found that the cells secrete a BMP inhibitor, Noggin, secreted from the organizer. However, the concentration of secreted Noggin is too low to inhibit BMP action. Thus, we assumed that diffusion on the cell surface is slow because proteoglycans such as heparan sulfate bind Noggin and BMP. Based on this assumption, experimental results could be explained by the reaction advection-diffusion model, supporting our hypothesis. Moreover, using a potent teratogen, thalidomide, we found that many genes, including cardiomyocyte marker ACTC1, were up- and down-regulated, suggesting these genes could be used as teratogenicity markers.

Our findings may use to develop in vitro tools that help the safety test of cosmetics.