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Abstracts

Stem cells and tissue regeneration

Program/Abstract # 398

Stochastic response of mES cells toward BMP signaling is improved under dynamic microfluidic conditions

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In order to study how BMP signaling influences ES cell differentiation, a mouse ES cell line harboring a BMP responsive element (BRE) driving lacZ expression was generated. When the BRE-lacZ mES cells (BRE-lacZ-mESC) were grown in conventional petri dish culture conditions, ES cells responded heterogeneously to BMP signaling. This stochastic responsiveness to BMPs is not due to a pre-existing heterogeneity in our ES cells prior to growth factor exposure, but is instead a property intrinsic to ES cells grown in static cultures. We suggest that cells grown in petri dishes rapidly form microenvironments that alter their ability to respond to BMP signaling. Interestingly, under a microfluidic platform to deliver BMP4 to ES cells in a controlled manner to minimize paracrine and autocrine effects on ES cells, we found a significantly improved efficacy of ES cells response toward BMP signaling. This suggests an efficient method for directing the differentiation of ES cells. We will report on the usage of the fluidic device for ES cell differentiation.

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Program/Abstract # 399

Characterization of novel genes involved in early neurogenesis in the developing neural tube

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In mammals, it is assumed that neurogenesis in the developing neural tube ensues following its closure. Thus, we hypothesized that genes, whose expression in the embryonic neural tube is "turned on" following tube closure, might play a role in neurogenesis and neuronal cell differentiation. Using high-throughput gene expression analysis (Affymetrix Mouse Genome 430 2.0 expression arrays), we identified novel genes that are differentially expressed in open and closed sections of the embryonic mouse neural tube both at days E8.5 and E9.5. Whole mount in-situ hybridization studies identified within this group of genes four previously unstudied genes, whose expression is specific to the neural tube and/or the developing brain in mouse embryos at E8.5 and E9.5. Furthermore, there was a gradual increase of the transcript levels of the human orthologues of these four genes during in-vitro neuronal differentiation of human embryonic stem cells. We hypothesize that these genes are likely to be involved in the early stages of neurogenesis during neural tube development. Generation of null mutant mice of these genes is underway.

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Program/Abstract # 400

Targeting CNS integration of mouse ES cells using Eph/ephrins

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Eph family members including Eph receptor tyrosine kinases (RTKs) and their ephrin ligands interact through cell–cell contacts to regulate multiple events during neural development including cell migration, axon pathfinding and cell survival. We have used the Sleeping Beauty (SB) transposon system to mediate stable integration and reliable long-term expression of Eph family members in mouse and human ESCs. An SB transposon (pT2/ EF1α-EphA4-Hygro) was constructed that contains an EF1α promoter-hEphA4-IRES-Hygromycin-GCSF p(A). Mouse ES cells were co-transfected with pT2/ EF1α-hEphA4-Hygro and pPGK-SB11 (i.e. transposase) and individual colonies isolated to establish stable cell lines expressing hEphA4. These cells have an increased ability to grow as neurospheres compared to control cells, and culture in neuronal differentiation medium induces widespread neuronal differentiation. Recent microarray analyses show that these RTKs and ligands exhibit dynamic fold changes in mouse embryonic stem cells (mESCs) expressing neural-inducing genes, compared to control cells. Using cell type-specific markers, experiments are in progress to determine if EphA4-expressing cells maintain pluripotency or differentiate into cells from all germ layers in our cultures. Additionally, we have initiated experiments to transplant EphA4+ mESCs into the chick spinal cord to test their integration into ephrin-expressing domains. In the future, ESC lines will be developed as therapeutic strategies for mouse and rat models of spinal cord injury and stroke.

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