ABSTRACT

MOLECULAR AND FUNCTIONAL STUDIES OF PARTHENOGENETIC EMBRYONIC STEM CELLS

By

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Parthenogenetic Embryonic Stem Cells (P-ESCs) are derived from Parthenogenetically activated embryos (PgEs) at the blastocyst stage. PgEs are created without sperm contribution, followed by inhibition of the second polar body extrusion which renders the PgE genome diploid, consisting of duplication of only the maternal genomic complement. Due to their exclusively maternal genomic complement, PgEs cannot develop to term. However, P-ESCs can be successfully derived. P-ESCs are morphologically indistinguishable from normal fertilized biparental embryonic stem cell (B-ESCs), they are pluripotent, i.e. can be propagated in vitro for prolong period of time while still keeping their embryonic stem cells characteristics, and when differentiated in vivo can give rise to tissues from the three germ layers – ectoderm, endoderm and mesoderm. Despite their broad differentiation plasticity, P-ESCs, exhibit some limitations to contribute to endoderm and mesoderm muscle tissues. This phenomenon has been attributed to deregulation of imprinted genes. Imprinted genes are subset of genes which are differentially expressed from the maternal and paternal alleles. H19 is a paternally imprinted gene whose expression in P-ESCs is highly upregulated. A great body of literature suggests that H19 is critical for proper development of PgEs since parthenogenetic offspring has been successfully

derived by deleting the H19 coding region from one of the maternal chromosomes. H19 has been found to play an important role for P-ESCs differentiation potential as well. Therefore we hypothesized that modulation of H19 gene expression in P-ESCs by using a small hairpin RNA (shRNA) approach, will enhance the ability of these cells to give rise to endoderm and mesoderm-muscle derivatives in in vivo and in vitro assays.

Our study focuses on developing a model for stable and efficient downregulation of H19 in human, primate and mouse P-ESCs. We further follow the differentiation potential of mouse P-ESCs using an in vivo teratoma formation assay and an in vitro study whereby the P-ESCs are induced to give rise to beating cardiomyocytes.

Our data revealed that P-ESCs with stable H19 downregulation can be derived from human, primate and mouse P-ESCs. Moreover, we showed for the first time, to our knowledge that suppression of H19 in P-ESCs resulted in an increased propensity of the cells to give rise to endoderm and mesoderm muscle derivatives in vivo as well as to higher incidence of beating embryo bodies in an in vitro differentiation assay.

In conclusion, our study provides further knowledge to the biology of P-ESCs and the effect of the imprinted genes, H19 in particular. Future studies would elucidate the molecular mechanism through which H19 exerts its effect and the potential applicability of P-ESCs for autologous stem cell therapy.