Cells Tissues Organs

Cells Tissues Organs 2007;185:48–50
DOI: 10.1159/000101302

Epithelial-Mesenchymal Transition in Rhesus Monkey Embryonic Stem Cell Colonies: A Model for Processes Involved in Gastrulation?

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Key Words

Embryonic stem cells · Epithelial-mesenchymal transition · Mesoderm · Ingression · Primate

Abstract

A characteristic feature of embryonic stem (ES) cells is their ability to give rise to differentiated cell types that are derived from all three primary germ layers. In the embryo of higher vertebrates, formation of mesoderm and definitive endoderm (gastrulation) occurs at the primitive streak through a spatially highly ordered process of cell ingression, combined with epithelial-mesenchymal transition (EMT). With respect to ES cell differentiation in vitro, however, germ layer derivative formation has not been studied in much detail, and data on any degree of spatial order that may be attained here are lacking. In the investigations to be reviewed here, rhesus monkey ES cells (line R366.4) were grown on mouse embryonic fibroblast feeder layers for up to 10 days during which time they formed multilayered disc-like colonies with an upper epithelial and a lower mesenchymal cell layer. Processes of epithelialization as well as EMT were studied by transmission electron microscopy, immunohistochemistry combined with confocal laser scanning microscopy, and marker mRNA expression (in situ hybridization, RT-PCR). It was found that under the culture conditions used most of the ES cell colonies developed transitorily a central pit where the epithelial upper layer cells underwent an EMT-like process and appeared to ingress to form the lower, mesenchymal layer, accompanied by appropriate changes of morphology and molecular markers. Similarities and differences in comparison with gastrulation/primitive streak formation in vivo are briefly discussed, as are ethical implications with respect to human ES cells. It is concluded that this rhesus ES cell colony system may be an interesting in vitro model for studies on some basic processes involved in early embryogenesis such as EMT/gastrulation and may open new ways to study the regulation of these processes experimentally in vitro in non-human primates.

Abbreviations used in this paper

EMT	epithelial-mesenchymal transition
ES cells	embryonic stem cells
MEF	mouse embryonic fibroblasts
ZO-1	zonula occludens-1

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Embryonic stem (ES) cells are derived from the inner cell mass (embryoblast) of mammalian blastocysts and have epiblast-like properties [reviewed by Denker, 2004]. Correspondingly, a characteristic feature of ES cells is their ability to give rise to differentiated cell types that are derived from all three primary germ layers. However, knowledge about details of the processes of germ layer (derivative) formation in ES cell colonies is still fragmentary. In the embryo of higher vertebrates, formation of mesoderm and definitive endoderm (gastrulation) occurs at the primitive streak through a spatially highly ordered process of cell ingression, combined with epithelial-mesenchymal transition (EMT) [Eakin and Behringer, 2004; Tam and Gad, 2004; Viebahn, 2004]. With respect to ES cell differentiation in vitro, however, germ layer derivative formation has not been studied in much detail, and specifically it is not known whether in this case processes of ingression and EMT may attain some degree of order reminiscent of processes occurring in the primitive streak of embryos. That this may indeed be the case, at least with certain primate ES cell lines and under certain culture conditions, was suggested by observations made on colonies of marmoset monkey ES cells [Thomson et al., 1996]. Any culture system that would allow to observe such phenomena regularly might, therefore, potentially serve as a very interesting in vitro model system for studying the cell biology of these processes. This would be particularly attractive in species like primates in which research on real embryos is impractical.

In the investigations to be reviewed here, rhesus monkey ES cells (line R366.4 obtained from the WiCell Research Institute, Madison, Wisc., USA [Thomson et al., 1995]) were grown on mouse embryonic fibroblast (MEF) feeder layers for up to 10 days during which time they formed multilayered disc-like colonies [Behr et al., 2005]. Processes of epithelialization as well as EMT were studied by transmission electron microscopy, immunohistochemistry combined with confocal laser scanning microscopy, and marker mRNA expression (in situ hybridization, RT-PCR).

Typically, differentiated colonies were found to be composed of an upper and a lower layer, the former growing on top of the layer of MEF feeder cells whereas the latter spread out underneath MEF [Behr et al., 2005]. Interestingly, cells in the upper layer formed an epithelium with typical apicobasal polarity and tight junctions, whereas cells in the lower layer expressed a mesenchymal phenotype. Most remarkably, in the central part of most of the colonies, a roundish pit developed and upper layer cells seemed to ingress here through a defect in the MEF layer to form the lower layer while undergoing an EMT. This was regularly seen during the first days of culture and was accompanied by a loss of epithelial apicobasal polarity and of tight and adherens junctions, as well as by downregulation of epithelial markers (E-cadherin, ZO-1). Likewise connexin 43 (in vivo an epiblast marker) was downregulated as demonstrated before in rabbit and mouse gastrulation [for literature see Behr et al., 2005]. As RT-PCR revealed, expression of *snail2*, a key regulator of EMT [Savagner, 2001], was more than 10-fold upregulated in these colonies during the time period of pit formation. *MesP2* [Saga et al., 1996] was also, although only slightly, upregulated. Expression of *Brachyury* and *goosecoid* was high from the start of colony formation on. These latter two are genes that, in embryogenesis, start being expressed already in the epiblast shortly before EMT sets in, i.e. in the presumptive primitive streak-forming cells [Blum et al., 1992; Viebahn et al., 2002]. Accordingly, the described observations are consistent with the interpretation that, under the culture conditions used, these rhesus monkey ES cells first form an epithelium with properties of pregastrulation stage epiblast, more specifically those parts of epiblast that are close to the presumptive primitive streak (expression of *Brachyury* and *goosecoid*), and that they subsequently undergo an EMT-like process by forming a central pit region and ingressing here as in the primitive streak of a normal embryo. The mesenchymal cell layer thus formed underneath may indeed be mesoderm-like, which, however, would have to be confirmed by additional marker studies. The ES cells appear to be pretriggered for gastrulation already from the start of the culture on. On the other hand, the morphology of the roundish pit was different from that of an in vivo primitive streak, and molecular marker expression showed no evidence of hypoblast formation.

These observations are on one hand consistent with the known fact that ES cells can form mesoderm derivatives (as one aspect of their pluripotency). In addition, however, they also show a remarkable pattern formation potential that has so far not been studied in detail nor exploited for experimental purposes, although the potential usefulness of ES cells for embryological studies was originally suggested [Thomson et al., 1996; Thomson and Marshall, 1998]. That a gastrulation potential is present in (and seems to be a peculiarity of) ES cells is also demonstrated by their potential to form normal, viable embryos in the environment of helper cells at tetraploid

complementation [Nagy et al., 1993]. The pattern formation potential (mimicking processes involved in gastrulation) in vitro, while offering a fascinating model with nonhuman primate ES cells, poses on the other hand an ethical problem with respect to human ES cell culture that has been largely overlooked so far [Denker, 1999; Pera, 2001; Denker, 2006].

In conclusion, this rhesus ES cell colony system may be an interesting in vitro model for studies on some basic processes involved in early embryogenesis such as EMT/gastrulation and may open new ways to study the regulation of these processes experimentally in vitro in nonhuman primates.

Acknowledgements

Thanks are due to B. Gobs-Hevelke, I. Kromberg, B. Maranca-Hüwel, D. Schünke and R. Brand for their skilful technical assistance. These investigations were supported by a grant from the Kompetenznetzwerk Stammzellforschung NRW.

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