# Epithelial-mesenchymal transition in colonies of rhesus monkey embryonic stem cells: a model for gastrulation?

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## Introduction

Epithelial-mesenchymal transition (EMT) is the regulated phenotypic transition of an epithelial cell to a mesenchymal cell. This phenotypic modulation occurs, for example, during gastrulation and neural crest cell migration and is characterized by a concomitant loss of epithelial cell polarity and epithelial cell adhesion molecules. In contrast, mesenchymal cells adopt a migratory and rather flattened phenotype. A general key transcription factor for the process of EMT is the zinc finger protein Snail2.

The ability to give rise to differentiated cell types that are derived from all three primary germ layers of the embryo, i.e. endoderm, mesoderm and ectoderm, is what makes ES cells unique. However, our understanding of the developmental potential of embryonic stem cells is still fragmentary. We employed the rhesus monkey ES cell line R366.4 (Thomson et al., 1995) to identify processes of epithelialisation as well as EMT and to further analyse the potential of primate ES cell colonies to undergo processes of regulated self-organisation.

## Materials and Methods

Rhesus monkey embryonic stem (rhES) cells (line R366.4) were grown on mouse embryonic fibroblast (MEF) feeder layers for up to ten days to form multilayered colonies. The growth medium consisted of DMEM supplemented with 20 % Knockout Serum Replacement (Gibco), 0.1 mM b-mercaptoethanol, 1 % nonessential amino acids and 1 mM glutamine. The medium was changed daily. The colonies were characterised by morphology, immunohistochemistry (ZO-1; Cx43) and marker mRNA expression (*Oct-4*, *Tert, brachyury; goosecoid, snail2, MesP1*) as revealed by RT-PCR and in situ hybridisation.

## Results

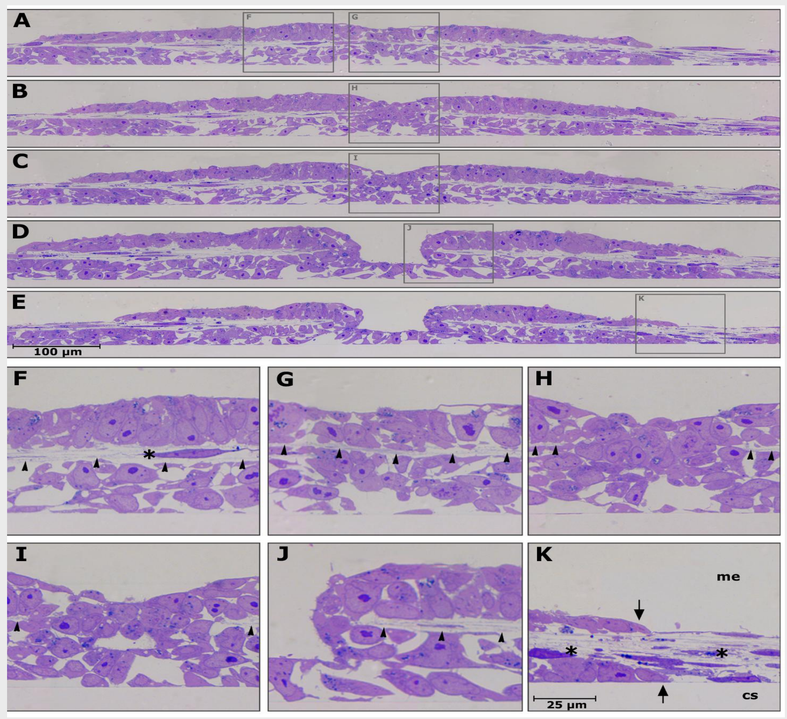


**Fig. 1: Differentiated ES cell colonies form two distinct layers and a central pit.** A typical differentiated colony of rhES cells in a series of cross sections starting from the periphery of the colony (A) and continuing towards the center (E). As the sections reveal, the differentiated colony is composed of an upper and a lower layer and between the two, the preexisting layer of mouse feeder cells (A). Note the lack of the feeder layer in the central region of the colony where a pit-like structure emerges (E). (F-K) are enlargements of the boxed areas in (A) to (E). Arrowheads = mouse feeder layer; asterisks = mouse embryonic fibroblasts embedded within the matrix of the feeder layer; arrows = stem cells of the upper and the lower layer, respectively, at the very periphery of the colony; me = medium; cs = cover slip

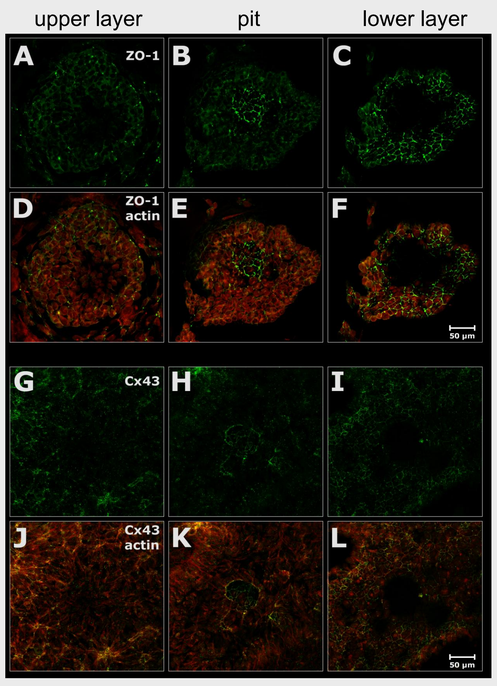


**Fig. 2: ZO-1 (maker for polarised epithelium) and Connexin 43 are strongly expressed in the upper layer.** ZO-1 (A-C) and the gap junction protein connexin Cx43 (G-I) and F-actin (D-F; J-L) in differentiated colonies of rhES cells (Confocal laser scanning micrograph images). xy-sections are from the upper layer of the colony (A, D; G, J), grazing the pit in the center of the colony (B, E; H, K) and the lower layer (C, F; I, L). Merger of the confocal images (D-F; J-L). Typical patterns are presented. Note the web-like staining pattern of both ZO-1 and Cx43 in the upper layer and the top cells in the pit, respectively. In contrast, cells of the lower layer showed only weak and rather irregular staining of ZO-1 and Cx43 (asterisks).

## Results

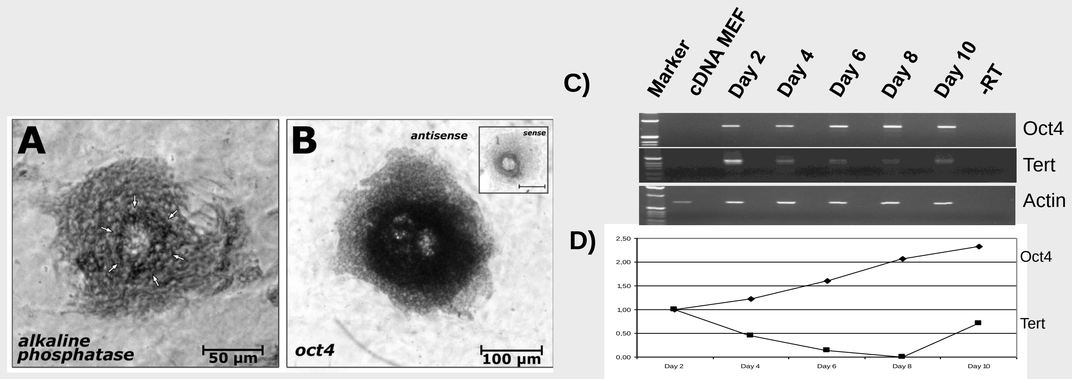


**Fig. 3: Analysis of stem cell marker expression** by alkaline phosphatase staining (A) and whole-mount in situ hybridization with Oct-4 (B), respectively. Alkaline phosphatase and oct-4 transcripts are detectable in almost all cells of the colony. (C) and (D) Quantification of undifferentiated stem cell marker mRNA expression in rhES cells after 2, 4, 6, 8, and 10 days of culture. (C) The mRNA for the transcription factor Oct-4 was clearly up-regulated whereas the telomerase reverse transcriptase (Tert) mRNA was down-regulated during the experimental period. (D) Quantification of the signals shown in (C).

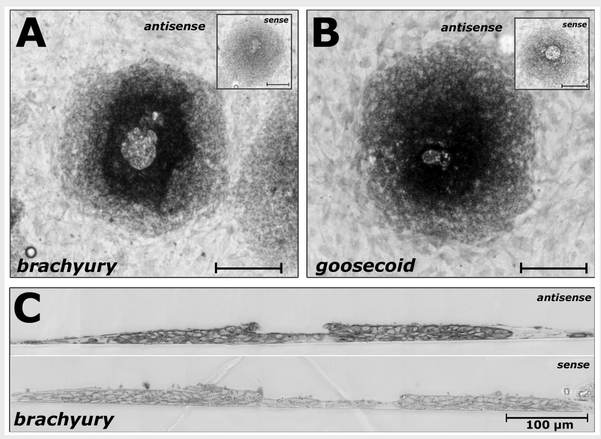


**Fig.4: *Brachyury* and *Goosecoid* are reliable marker genes for those cells that start the process of EMT during gastrulation.** *Brachyury* (A) and *goosecoid* expression (B) in differentiated colonies as revealed by in situ hybridization (full-face view). Cellular distribution of *Brachyury* expression as seen in cross sections (C).Note that *Brachyury* transcripts are not restricted to a specific layer or cell population of the colony but are found in all cells irrespective of their position. Mouse embryonic feeder cells show no staining signal.

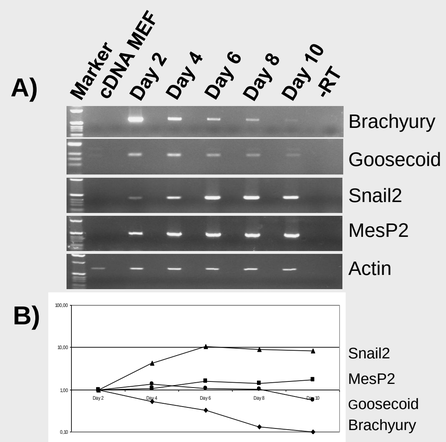


**Fig. 5: Quantification of marker mRNA expression** after 2, 4, 6, 8, and 10 days of culture. (A) The mRNAs for the transcription factors *Brachyury* and *goosecoid* are clearly down-regulated during the experimental period. In contrast, the transcription factors *Snail2* and *MesP1* are upregulated during the first days of culture. (B) Quantification of the signals shown in (A).
**Snail2**: key transcription factor for EMT
**MesP2**: transcrption factor induced at the site of gastrulation followed by high expression predominantly in mesodermal tissues

## Conclusion and Outlook

Cell morphology as well immunohistochemical findings were consistent with the view that cells of the upper layer of these colonies are migrating through the pit downwards, continuing through the defect in the feeder layer to form the lower cell layer while undergoing a phenotypic transition from the epithelial to the mesenchymal phenotype.

Phenotypic changes of this type are known as a characteristic of the EMT that takes place at vertebrate gastrulation.

Thus, these rhesus ES cell colonies may be an interesting model for studies on some basic processes involved in early primate embryogenesis such as EMT / gastrulation and may open new ways to study the regulation of these processes experimentally in vitro.

Outlook:

To manipulate the process of EMT by changed cell culture conditions.

To test whether the EMT also occurs in colonies of ES cells of other (primate) species.

NRW.

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