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

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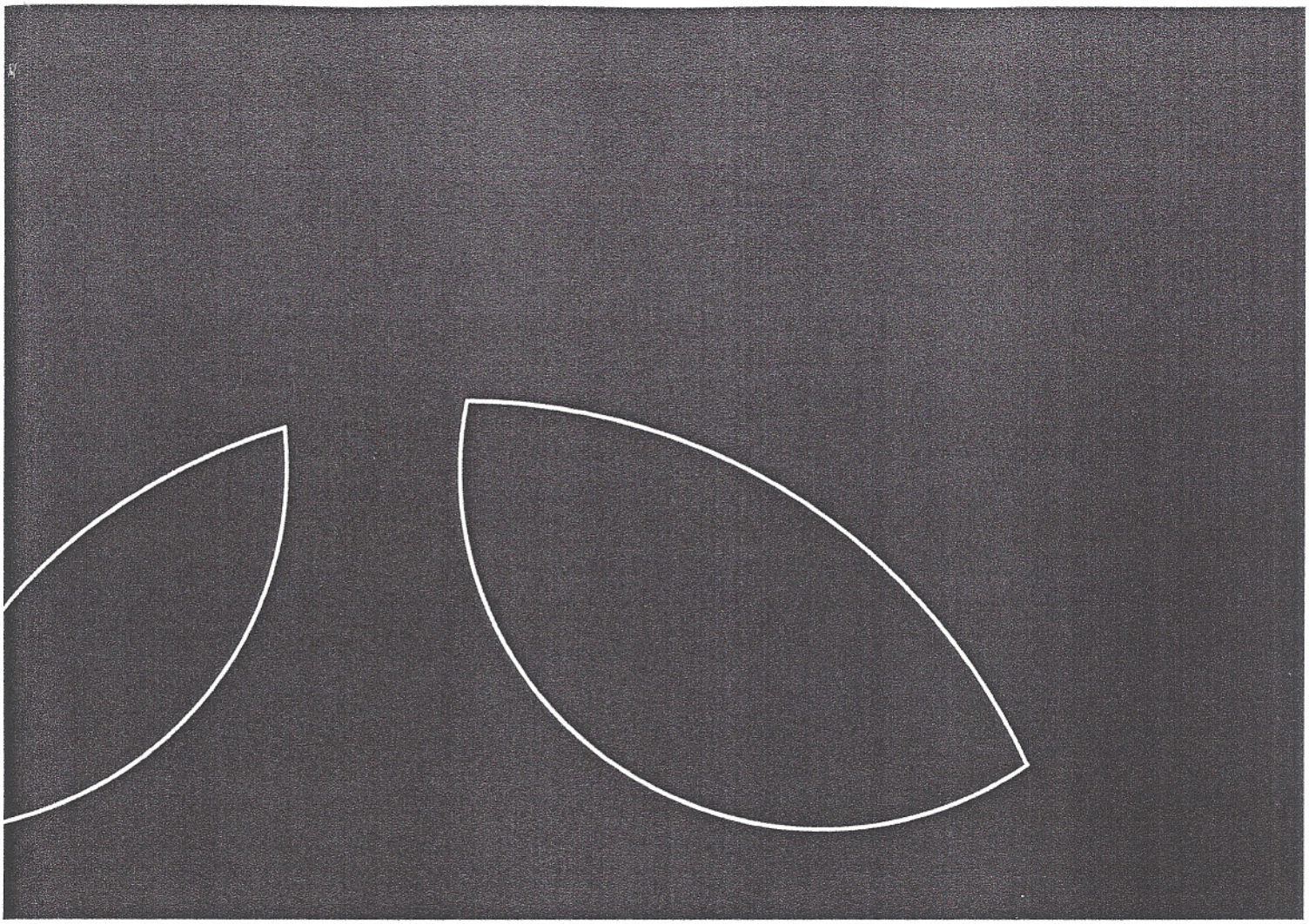
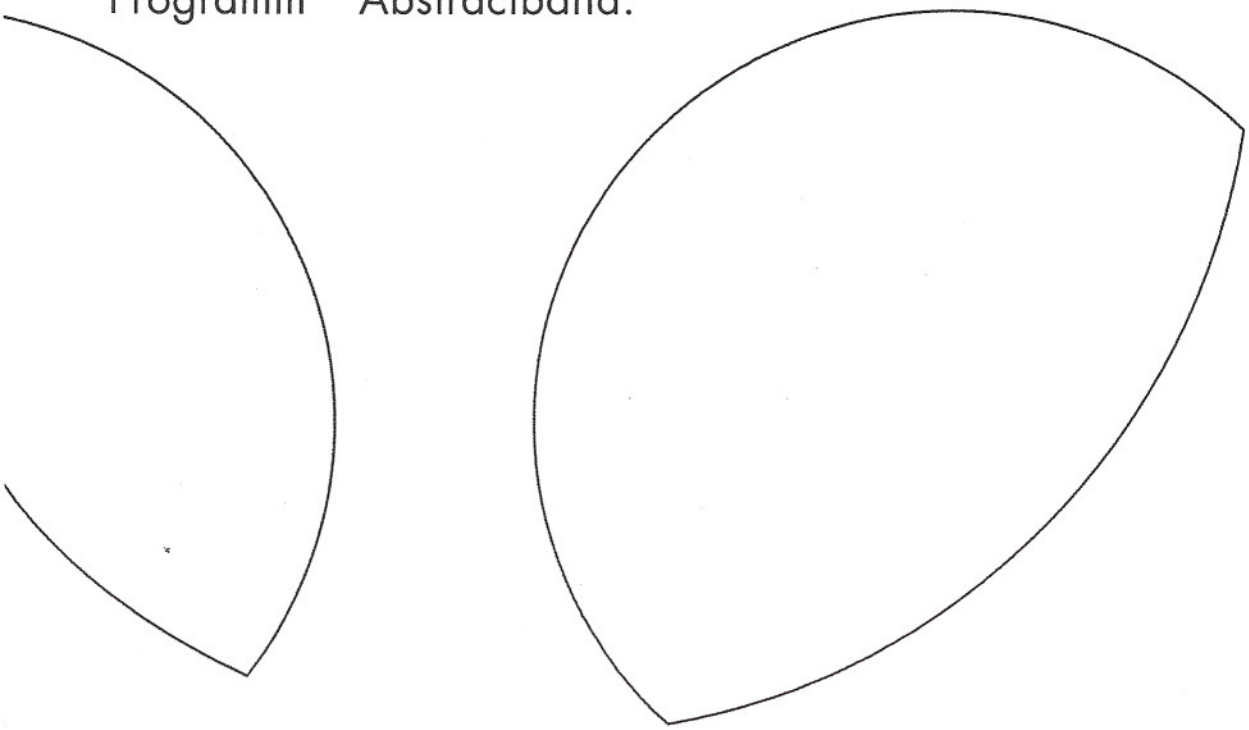
Our project focuses on the identification of specific molecular and cell biological properties that characterize differentiating ES cell colonies as opposed to other cell aggregates.
Rhesus monkey embryonic stem (rhES) cells (line R366.4) were grown on mouse embryonic fibroblast (MEF) feeder layers for up to ten days to form multilayered colonies. Within this period, stem cell colonies were found to differentiate spontanously into more complex structures with a disc-like morphology. These complex colonies were characterised by morphology, immunohistochemistry (ZO-1; Cx43) and marker mRNA expression (*Oct-4, Tert, brachyury; goosecoid, snail2, MesP1*) in order to identify processes of epithelialisation as well as epithelial-mesenchymal transition (EMT). Typically, differentiated colonies were composed of an upper and a lower layer, the former growing on top of the layer of mouse feeder cells whereas the lower ES cell layer spread out underneath the MEF feeder cells. Morphology and immunohistochemistry revealed that cells in the upper layer formed an epithelium whereas cells in the lower layer expressed a mesenchymal phenotype. Interestingly, in the central part of the colonies, a roundish pit developed. Here the feeder layer disappeared, and upper layer cells seemed to ingress here to form the lower layer. In most colonies only one pit was found, and it was positioned approximately in the center of the colony. Cell morphology as well immunohistochemical findings were consistent with the view that cells of the upper layer migrated 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. This switch of phenotype was indicated by a loss of the protein ZO-1, which is a marker for epithelial cells, as well as of Connexin 43, which is an embryonic marker for epiblast. Morphology indicated a concomitant loss of epithelial apico-basal polarity. Phenotypic changes of this type are known as a characteristic of the EMT that takes place at vertebrate gastrulation. Accordingly we found a concomitant more than 10-fold up-regulation of the gene *snail2*, which is a key regulator of the process of EMT and suppresses the epithelial phenotype. Conversion of epiblast to mesoderm was also indicated by the regulated expression of the mesoderm markers *brachyury* and *goosecoid* during rhES cell colony differentiation. In contrast, there was no evidence for hypoblast formation. 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.

(Supported by Kompetenznetzwerk Stammzellforschung NRW)



Interne Klausurtagung Herne 19. - 20. November 2004.

Programm Abstractband.



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