Identification of genes involved in epithelial differentiation

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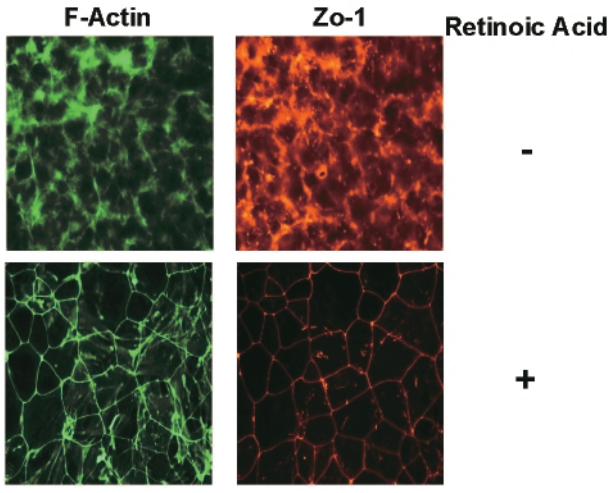
Molecular mechanisms regulating the epithelial differentiation have not been fully understood. One of the attractive approaches for understanding epithelial differentiation is to enrich the catalog of genes whose expression is specifically changed during epithelial expression. To this end, we decided to employ cDNA microarray analysis to trace the expression profile of the genes during the time course of differentiation. We first tried to set up an *in vitro* epithelial differentiation model using F9 mouse teratocarcinoma cells. F9 cells are known to differentiate into epithelial cells in the presence of retinoic acid. This is observed only when they are cultured as aggregates in suspension, but not when cultured on a solid surface such as a plastic ware. Even in the aggregate culture system, however, only the cells in the outermost single layer of aggregates can differentiate into epithelial cells, remaining the larger number of cells inside the aggregates undifferentiated. This is a disadvantage for the microarray analysis by compromising the detection of specific gene expression with epithelial differentiation by the undifferentiated population.

Epithelial cells generally face the extracellular milieu through their basolateral plasma membrane, where they exchange metabolites. To mimic the situation, we cultured F9 cells on a filter membrane. Interestingly, virtually all the cells on the membrane differentiated into epithelial cells, measured by the formation of tight junction with immunofluorescence microscopy, in the presence, but not in the absence, of retinoic acid.



F9 subclone cultured on plasics

In the course of the experiments, we found that retinoic acid induced differentiation of a small fraction of F9 cells into epithelial cells on a plastic ware. This prompted us to search for a subclone of F9 cells in which all the cells could differentiate into epithelial cells. As expected, limiting dilution of F9 cells gave rise to a subclone, F9.3, which differentiated into epithelial cells on a plastic ware in the presence of retinoic acid (figure). F9 and F9.3 cells were cultured on a filter membrane or on a plastic ware in the presence or absence of retinoic acid, and RNA was extracted at a various time points during the culture. Amount of the transcript of occludin, a tight junction component, and cytokeratin-18 was quantified with a Taqman real-time PCR method. In F9 cells, both transcripts were detected only when cultured on a filter membrane in the presence of retinoic acid. By contrast, retinoic acid induced the expression of both transcripts in F9.3 cells cultured on either a filter membrane or on a plastic ware. These changes were in good accordance with morphological changes by these cells upon epithelial differentiation. Microarray analysis is now underway using the RNA samples extracted as above.