Cell checkpoints and enterocyte differentiation: a recipe for sequential stages

Focus on “Caco-2 intestinal cell differentiation is associated with G₁ arrest and suppression of CDK2 and CDK4”

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The complicated process of maturation and differentiation of cells has been difficult to unravel, and the absorptive intestinal epithelial cell (also known as the enterocyte) is no exception. Enterocytes proliferate from anchored stem cells in the crypts of Lieberkuhn by mitosis and migrate up the crypt while simultaneously maturing, expressing enzymes for luminal and intracellular digestion and absorption. Enterocytes later become senescent near the villus tips and are subsequently sloughed into the lumen of the intestine. This process is completed in 3 to 7 days in humans. The billions of cells that comprise our absorptive interface are rapidly turned over to resupply critical proteins by cell renewal that are important for effective nutrient assimilation.

Curiously, the process of proliferation, differentiation, and programmed senescence in the enterocyte is not well understood. A complex mix of cell-cell and cell-matrix interactions likely play a role, modulated by growth factors, luminal factors, and perhaps hormones. The physiological presence of pancreatic enzymes and bile can also hasten the life span of the enterocyte. Indeed, after the advent of adverse conditions or injury, the enormous proliferative potential of the small intestine is awakened, summoning replacement enterocytes to populate damaged villi. This plasticity allows for complete recovery from the injury. An understanding of the control mechanisms for enterocyte proliferation and maturation is in its infancy. One reason for this is the inability to maintain normal enterocytes in culture for research study.

What clues are there from cell models that will enhance our understanding of maturation mechanisms of the enterocyte? In the current article in focus (Ref. 2; see p. C1193 in this issue), Ding and colleagues address the timing of cell cycle checkpoint activation and differentiation of Caco-2 intestinal cells. Caco-2 cells spontaneously differentiate to an enterocyte phenotype when grown to confluence and have been previously shown to accumulate at the G₁ cell cycle checkpoint by 3 days after reaching confluency. In a series of experiments comparing “preconfluent,” confluent, and postconfluent cells after 3, 6, and 12 days, the authors discovered that accumulation of cells at the G₁ checkpoint at day 3 postconfluency preceded the ascertainment of sucrase and alkaline phosphatase activity, measurements of differentiation, which occurred by day 6 postconfluency. The events that lead to cells accumulating at the G₁ checkpoint on day 3 appear to be partially the result of increased expression of the universal cyclin-dependent kinase (CDK) inhibitor, p21Waf1/Cip1, binding to CDK2, a critical kinase that allows transition through G₁ in association with cyclin E. Immunodepletion experiments suggest that another CDK inhibitor is present, but this inhibition is not due to p27Kip1/Pic2 and p57Kip2. Continued accumulation at the G₁ phase on days 6 and 12 postconfluency was due to decreased levels of cyclin E. Interestingly, CDK4 kinase levels also decrease on day 3 postconfluency. This reduction does not appear to be due to inhibitors but is more likely due to decreased levels of the partners of CDK4 kinase, the D cyclins, and is sustained through days 6 and 12 postconfluency.

For immature enterocytes to differentiate, it appears that they must be in the G₁ phase of the cell cycle. Logically, a cell cannot devote energy to proliferation while simultaneously performing complex physiological processes. This is the opposite of neoplastic cells, in which proliferation is selected for, and cells often regress from a terminally differentiated state. During the G₁ phase of the cell cycle, a trigger occurs that activates gene transcription in the enterocyte for terminally differentiated proteins. Whether differentiation is specifically triggered by the increased expression of p21Waf1/Cip1 or other inhibitory proteins or by a decrease in cyclin expression is not known. Potential candidates for activation of differentiation include homeobox genes, such as Cdx2 (4). One potential area for study would be to assess Cdx2 expression in the Caco-2 cell model. Indeed, by conditional expression of Cdx2, IEC-6 cells, another undifferentiated intestinal cell line, arrested cellular proliferation for several days, followed by a period of growth and differentiation (4). Fetal intestinal cells transformed by simian virus 40 (SV40) large T antigen displayed an irreversible growth arrest mediated by p21Waf1/Cip1 and 1 to 2 days later acquired increases in brush-border enzymes (3). Transgenic mice for wild-type and mutant SV40 T antigen linked to an intestinal fatty acid-binding protein gene promoter...
suggests that downregulation of CDK2 and/or cyclin D1 expression is important for control of the proliferation status of the enterocyte and commencement of terminal differentiation (1). These other reports are consistent with the findings of Ding et al. (2) but they utilize transforming proteins or overexpress genes in their models. At this time, the Caco-2 model of confluency may be the best model that simulates the neighboring contact and interaction between enterocytes. Although it now appears that we know the cell cycle state and timing of differentiation in this model, we still do not know the trigger. A better understanding of interaction between G1 phase regulators and transcription activators of intestinal differentiation might be fruitful in the future.

REFERENCES


