Abstract

The MAPK/ERK pathway, which is evolutionarily conserved in eukaryotes is one of the most intensively studied signaling pathways and consists of a three-tier cascade of Raf-MEK-ERK protein kinases. A variety of extracellular signals are transduced from receptors to hundreds of substrates by a series of sequential phosphorylations leading from Raf to MEK to ERK. The ERK pathway regulates a plethora of cell- and extracellular signal-specific responses such as gene expression, proliferation, differentiation, migration, and apoptosis. The proper execution of these physiological processes requires a precise temporal and spatial regulation of the pathway and disruption of the regulatory mechanisms leads to pathological consequence such as tumor transformation. Specificity and regulation of signal transduction are provided in part by the presence of isoforms at each level of the ERK signaling pathway. The functional differences between the effector protein kinases ERK1 and ERK2 have been controversial for a long time, but it is still unclear how important they are in achieving an appropriate cellular response.

In this work, we focused on the functional characterization of ERK1 and ERK2 isoforms in MDCK epithelial cells. Specifically, we examined the effects of ERK2 inactivation on cell morphology and expression of its selected substrates. We found that inactivation of the ERK2 leads to defects in epithelial formation as cells lose their cuboidal shape, spread over a larger area, and are often multinucleated. Though the colony-forming ability of ERK2 inactivated cells is not affected, the colonies are less compact and irregularly shaped. We demonstrated that there are significant changes in the expression of EGR1 and c-Fos at both protein and mRNA levels. Both the morphological and expression changes induced by loss of the ERK2 can be reversed by ectopic expression of ERK2 as well as ERK1 in ERK2-deficient cells. In conclusion, our results reveal a novel role of the ERK signaling pathway in the formation and maintenance of epithelial characteristics in MDCK cells. They also suggest that both isoforms are functionally interchangeable in these processes and that the observed changes depend on the total amount of activated ERK rather than on the presence of a particular isoform.

Key words: ERK1, ERK2, protein kinase, phosphorylation, gene expression, immediate early genes, transcription factors, epithelium, apical-basal polarity