Epithelium and fibroblast-like phenotypes derived from HPV16 E6/E7-immortalized human gingival keratinocytes following chronic ethanol treatment

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Abstract

Epithelial-mesenchymal transition (EMT) may be critical for neoplastic progression and its eventual tumorigenicity of epithelia. In this context, we investigated whether EMT and EMT-associated features occurred after chronic ethanol treatment of human gingival keratinocytes immortalized with the E6/E7 oncogenes of human papillomavirus (HPV) type 16. Following a nine-week treatment of cells with 30 mMethanol in keratinocyte growth medium, they were cultured in normal DMEM with 10% serum. These cell populations were able to proliferate in this medium gradually exhibiting elongated morphology indicating that these cells underwent EMT. Control cells without ethanol treatment did not survive subcultures in DMEM. Upon long-term subcultures of ethanol-treated cells, two phenotypes were obtained exhibiting epithelium-like and spindle-shape fibroblast-like morphology (respectively, termed as EPI and FIB cells), the latter indicating EMT. In comparison to EPI cells, the phenotypic transition to FIB cells was concomitant with a decrease in the expression of keratins, desmoplakins and a complete loss of K14. Moreover, FIB cell transition strongly correlates with an increase in the expression of vimentin and simple epithelial keratinK18. These alterations in FIB cells were associated with the ability of these cells to exhibit anchorage-independent growth, while EPI cells exhibited anchorage-dependent growth. Concerning the transformation stage, FIB cells represent a progressively more advanced transformed phenotype which may reflect an early step during HPV- and ethanol-dependent multi-step carcinogenesis.

Keywords: Human gingival keratinocytes, human papillomavirus type 16, anchorage independence, intermediate filaments, ethanol