Spatial location of Epiplakin to Outer Surface layer of Hela Cells Clustered on Matrigel.

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ABSTRACT

Epiplakin, a new member of plakin family, has unique characteristics of domain structure, but the precise role in cells remains obscure yet. Epiplakin has been found to be expressed in several tissues including the epithelium of digestive organs and in cultured cells such as HeLa cells. Results from immunofluorescence staining indicated that HeLa cells cultured in polystyrene dishes expressed a high level of epiplakin compared to mesenchymal cells such as fibroblasts and hepatic stellate cells, and epiplakin was at least in part colocalized with vimentin, plectin, or desmoplakin in HeLa cells. When cultured on Matrigel containing the basement membrane components, HeLa cells formed multicellular clusters. Interestingly, epiplakin was localized exclusively to the outer surface layer of HeLa cell clusters. Other epithelial cells including HepG2 cells and HEp2 cells, but not mesenchymal cells including fibroblasts and hepatic stellate cells, also formed clusters after culturing on Matrigel. Also in HepG2 and HEp2 cell clusters, a similar localization of epiplakin to the outer surface layer was observed, suggesting a pivotal role of epiplakin in spatial organization and/or polarization of epithelial cells. In HeLa cells of the outer surface layer, epiplakin was mainly located to the subapical membrane region and appeared to surround vesicles. Since epiplakin belongs to a cross-linker protein family involved in cytoskeleton organization, we searched using dual immunofluorescence staining for whether or not epiplakin is colocalized with other cytoskeletal proteins such as cytokeratin, vimentin, plectin, BPAG1, or desmoplakin to explore a role of epiplakin in cellular organization.

INTRODUCTION

Epiplakin is a new member of the plakin (cytolinker) family with cytoskeletal linker proteins including bullous pemphigoid antigen 1 (BPAG1), plectin, and desmoplakin, which have a common domain structure and cross-link intermediate filaments to F-actin, microtubules and/or adhesion complexes (1). Epiplakin has been identified and cloned using an autoimmune serum from the patient with a subepidermal blistering disease resembling bullous pemphigoid (1). Of members of plakin family, epiplakin with a 450 kDa molecular mass has an unique domain structure comprising of 13 repeated B domains and lacking a coiled-coil rod domain and an amino-
terminal domain, both of which are common to all other members of plakin family. Epiplakin has been found to be expressed in the epithelium of digestive and glandular organs, as well as in the epidermis (1, 2), although the precise role in cells and tissues remains to be clarified.

In this study, we examined an epiplakin localization in cultured HeLa cells and compared to localization of intermediate filaments and related proteins, F-actin, and microtubules. As well as other epithelial cells, HeLa cells formed multicellular clusters when cultured on Matrigel containing the basement membrane components. Epiplakin was localized exclusively to the outer surface layer of HeLa cell clusters, as shown by immunofluorescence staining. We compared the epiplakin localization in the HeLa cell clusters with the distribution of other cytoskeletal proteins to explore a role of epiplakin in epithelial cells.

MATERIALS AND METHODS
HeLa cells derived from cervical carcinoma, HepG2 cells derived from hepatocellular carcinoma, HEP2 cells derived from larynx epidermoid carcinoma, fibroblasts, and hepatic stellate cells were cultured in DMEM containing 10% FBS using polystyrene dishes, type I collagen-coated dishes, type I collagen gel, and Matrigel. Cultured cells were immunofluorescently stained for epiplakin and other cytoskeleton-related proteins including vimentin, cytokeratin, desmoplakin, plectin, BPAG1, F-actin, and microtubules, followed by detection of fluorescence signals using confocal laser scanning microscopy.

RESULTS AND DISCUSSION
The results from dual immunofluorescence staining showed that epiplakin was at least in part colocalized with vimentin, plectin, or desmoplakin, suggesting epiplakin is closely associated with intermediate filaments and has a common role as cytoskeleton cross-linker. However, the unique domain structure of epiplakin with 13 repeated B domains and lacking actin- and microtubule-binding domains is suggestive of the peculiar function. When cultured on Matrigel containing the basement membrane components, HeLa cells, as well as other epithelial cells such as HepG2 cells and HEP2 cells, aggregated to form multicellular clusters. In HeLa cell clusters epiplakin but not other cytoskeletal proteins including cytokeratin, vimentin, plectin, and desmoplakin was exclusively localized to the outer surface layer, within the HeLa cells of which epiplakin was mainly located in the subapical membrane area. The specific localization of epiplakin in multicellular clusters suggests an important role in a spatial recognition and cell-cell interaction of epithelium, and/or in polarization of epithelial cells.

REFERENCES