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A Mystery of AHNAK/Desmoyokin Still Goes On

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The recombinant technique for the development of gene knockout mice has played a critical role in clarifying the biological *in vivo* functions of the proteins of interest. This technique is powerful, but it requires labor-intensive and time-consuming processes. Therefore, gene target projects have a certain risk, especially for the young fellows who often actually do the work. It requires multiple steps: characterization of genomic sequences, construction of targeted vector, screening of embryonic stem (ES) cells with homologous recombination, injection of ES clones to blastocysts, screening for mutant mice, etc. The real difficulty, however, may start when the knockout mice do not have any apparent phenotype.

Desmoyokin was originally identified as a new desmosomal plaque protein that is found at the periphery of the cytoplasmic plaque of desmosomes in the stratified squamous epithelia (Hieda and Tsukita, 1989). Hashimoto and his colleagues isolated mouse monoclonal antibodies against a desmosome preparation made from bovine muzzle epidermis and found that one of them recognized desmoyokin. Using the monoclonal antibody as an antibody probe, they immunoscreened a mouse keratinocyte expression cDNA library and isolated cDNA clones encoding desmoyokin (Hashimoto *et al*, 1993). The deduced amino acid sequence revealed that desmoyokin is identical to AHNAK, which is a tumor-related protein of exceptionally large size (700 kDa) and downregulated in cell lines of neuroblastoma, small cell lung carcinomas, and Burkitt lymphomas (Shtivelman *et al*, 1992). AHNAK is encoded by an intronless gene located on human chromosome 11q12–13 and has three main structural regions: the N-terminal 251 amino acids, a large central region of 4390 amino acids with multiple repeated units of about 128 amino acids in length, and the C-terminal of 1002 amino acids. The central region is predicted to have antiparallel β-strands connected by intervening loops. Several putative regulatory elements are clustered within the C-terminal region, including nuclear export localization signals, a leucine zipper, and potential phosphorylation sites for protein kinase B and C (Nie *et al*, 2000; Sussman *et al*, 2001).

AHNAK/desmoyokin is a ubiquitous protein expressed in a variety of cell types. In epithelial cells AHNAK/desmoyokin is distributed mainly on the cell membranes, suggesting its role in cell–cell adhesion. On the other hand, AHNAK/desmoyokin is expressed in the nucleus and cytoplasm of nonepithelial cells. It also shuttles between nucleus and cytoplasm depending on extracellular calcium concentration, and the C-terminus is responsible for this translocation (Nie *et al*, 2000). In cardiomyocytes, AHNAK/desmoyokin is associated with the regulatory β2 subunit of the cardiac L-type Ca^{+2} channel through the C-terminal region (aa 5262–5643), suggesting its role in the subsarcolemmal cytoarchitecture as a linker protein between cardiac L-type Ca^{2+} channels and the actin-based cytoskeleton (Hohaus *et al*, 2002). At the plasma membrane, AHNAK/desmoyokin interacts with the annexin 2/S100A10 complex and regulates cortical actin cytoskeleton organization and cell membrane cytoarchitecture (Benaud *et al*, 2004). All of these experimental results are based on the findings *in vitro*, using cultured cells. The biological function of AHNAK/desmoyokin *in vivo* remains to be elucidated.

In this issue of the JID, Kouno *et al* (2004) generated AHNAK/desmoyokin-deficient mice by gene-targeting technique. The AHNAK/desmoyokin-deficient ES cells proliferated with similar doubling time to wild-type cells and showed the normal developmental potential to differentiate into several lineages including cardiac cells and neural cells. AHNAK/desmoyokin-deficient mice were viable and fertile and showed no gross developmental defects or macroscopic abnormalities. Histologically, skin sections of AHNAK/desmoyokin-deficient mice at different ages during appendage development showed no abnormalities of hair follicles and sebaceous glands. By immunohistochemistry, mutant epidermal cells expressed the epidermal differentiation markers laminin 5, keratin 5, keratin 1, and involucrin, as found in normal epidermis. The mutant epidermis treated with 12-o-tetradecanoyphorbol-13-acetate (TPA), which stimulates protein kinase C and induces the translocation of AHNAK/desmoyokin, showed similar increased numbers of BrdU-incorporated cells and similar thickness of the epidermis as found in TPA-treated normal epidermis. Electron microscopic studies revealed no abnormality in the ultrastructure of intercellular junctions or in the localization of desmocollin, a transmembrane component of desmosomes. There was no spontaneous tumor formation in the mutant mice, and two-stage carcinogenesis experiments showed no significant difference in tumor formation rates between wild-type and the mutant mice. Taken together, the authors concluded that the deficiency of AHNAK/desmoyokin in mice has only minimal, if any, effects on epidermal cell adhesion, tumorigenesis, cell proliferation, and differentiation.

Why no phenotype? No phenotype of mice deficient for a certain gene indicates that other molecules compensate for the loss of function caused by the defected gene product. Just recently, Benaud and his colleagues used small interfering RNA or siRNA, another method to see the effect by the loss of function, and demonstrated that downregulation of AHNAK/desmoyokin using specific siRNA prevented cortical actin cytoskeleton reorganization that is required to support cell height in Madin-Darby canine kidney cells