Epiplakin Is a Paraneoplastic Pemphigus Autoantigen and Related to Bronchiolitis Obliterans in Japanese Patients

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All plakin family proteins are known to be autoantigens in paraneoplastic pemphigus (PNP). In this study, we first examined whether PNP sera also react with epiplakin, another plakin protein, by various immunological methods using 48 Japanese PNP sera. Immunofluorescence confirmed that cultured keratinocytes expressed epiplakin. Epiplakin was detected by 72.9% of PNP sera by immunoprecipitation-immunoblotting with KU-8 cell extract, but not by immunoblotting of either normal human epidermal extract or KU-8 cell extract. Epiplakin was essentially not detected by 95 disease and normal control sera. Statistical analyses of various clinical and immunological findings revealed a significant correlation of the presence of anti-epiplakin antibodies with both bronchiolitis obliterans and mortality. No epiplakin-negative PNP case developed bronchiolitis obliterans. However, although 29.4% of European patients with PNP had bronchiolitis obliterans, significant correlation with anti-epiplakin autoantibodies was not observed. In further studies for lung, immunofluorescence showed the presence of epiplakin in normal human lung, particularly respiratory bronchiole, immunoprecipitation-immunoblotting showed that PNP sera reacted with epiplakin in cultured lung cells, and mice injected with polyclonal antibody specific to epiplakin histopathologically showed abnormal changes in small airway epithelia. These results indicated that epiplakin is one of the major PNP autoantigens and is related to PNP-related bronchiolitis obliterans.

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INTRODUCTION

Paraneoplastic pemphigus (PNP), or paraneoplastic autoimmune multiorgan syndrome (Nguyen et al., 2001), is an autoimmune blistering skin disease with severe mucocutaneous lesions. PNP is commonly associated with hematological malignancies (Anhalt, 1997; Anhalt et al., 1990). Histopathology shows acantholysis and apoptotic cells in epidermis and interface changes (Anhalt, 1997; Anhalt et al., 1990; Billet et al., 2006; Sehgal et al., 2009).

Prognosis of PNP is poor with approximately 68% mortality (Leger et al., 2012). Particularly, all PNP cases with chronic respiratory disease show fatal outcome (Nousari et al., 1999; Takahashi et al., 2000). Severe inflammation occurring in respiratory bronchioles leads to irreversible fibrotic reaction, resembling bronchiolitis obliterans (BO). In a previous study, BO occurred in 6% of 53 European patients with PNP (Leger et al., 2012), whereas the prevalence was 25% in our recent study for 107 Japanese patients with PNP. However, pathogenesis in PNP-related BO is currently unknown.

PNP develops autoantibodies to various antigens, mainly plakin family proteins. Immunoprecipitation (IP) first detected the 250-kDa desmoplakin I, the 230-kDa bullous pemphigoid 230 (BP230), the 210-kDa doublet of desmoplakin II and unknown protein, and the 190-kDa and 170-kDa unknown proteins (Anhalt et al., 1990). Then, immunoblotting (IB) of normal human epidermal extract showed that all PNP sera reacted with the 210-kDa and 190-kDa doublet proteins (Borradori et al., 1998; Hashimoto, 2001; Hashimoto et al., 1995). Thereafter, the 210-kDa and 190-kDa proteins were identified as envoplakin (EPL) and periplakin (PPL), respectively (Kiyokawa et al., 1998). This reactivity is very useful for

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Abbreviations: BO, bronchiolitis obliterans; BP, bullous pemphigoid; Dsg, desmoglein; EPL, envoplakin; EPPK, epiplakin; IB, immunoblotting; IF, immunofluorescence; IHC, immunohistochemistry; IP, immunoprecipitation; NHSAE, normal human small airway epithelial; pAb, polyclonal antibody; PNP, paraneoplastic pemphigus; PPL, periplakin; RP, recombinant protein

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diagnosis of PNP (Joly et al., 2000; Mouquet et al., 2008; Poot et al., 2013). Plectin, another plakin protein, was also found to be PNP autoantigen (Proby et al., 1999). Thus, all known plakin proteins are PNP autoantigens.

Non-plakin proteins are also detected by PNP sera. ELISAs detected antibodies to desmoglein 3 (Dsg3) and/or Dsg1 in PNP sera (Amagai et al., 1998; Brandt et al., 2012). We also found that the unknown 170-kDa protein was alpha-2-macroglobulin-like-1 (Numata et al., 2013; Schepens et al., 2010), and BP180 was frequently reacted by PNP sera (Tsuchisaka et al., 2014).

Epiplakin (EPPK) was originally identified as an autoantigen in a patient (Fujiwara et al., 1996), and is expressed in entire epidermis, skin appendices, and other intestinal epithelia (Fujiwara et al., 2001). Subsequent studies showed that EPPK is a plakin protein with many repeats of plakin-specific B-domain and linker-domain (Spazierer et al., 2003; Takeo et al., 2003). EPPK connects intermediate filaments (Spazierer et al., 2006, 2008; Wang et al., 2006). Despite abundant expression of EPPK in stratified and simple epithelia, gene-targeted mice showed no abnormality in either phenotype or keratin filament organization, except for mild delay of wound healing (Goto et al., 2006, Ishikawa et al., 2010).

Because EPPK is a plakin protein, we speculated that EPPK might also be a PNP autoantigen. In this study, we investigated whether anti-EPPK antibodies were present in PNP sera by various analyses. Immunoprecipitation-immunoblotting (IP-IB) detected anti-EPPK autoantibodies in 35 of 48 PNP sera. Statistical analysis indicated correlations of anti-EPPK antibodies with PNP-related BO and mortality. Additional studies indicated that EPPK is expressed in respiratory cells and tissues and was reacted by PNP sera, suggesting that anti-EPPK autoantibodies may develop BO.

RESULTS

Clinical and immunological parameters for all 48 Japanese patients with PNP are summarized in Supplementary Table S1 online.

Immunohistochemistry (IHC) and immunofluorescence (IF) of normal human skin, KU-8 cells, and rat bladder

By IHC and IF of normal human skin, rabbit polyclonal antibody (pAb) for human EPPK (anti-EPPK pAb) (Figure 1a and c), but not normal rabbit IgG (Figure 1b and d), showed cytoplasmic staining in entire epidermis. By IF, anti-EPPK pAb (Figure 1e), but not normal rabbit IgG (Figure 1f), showed perinuclear cytoplasmic staining in cultured KU-8 cells. Finally, by IF using the rat bladder, anti-EPPK pAb (Figure 1g), but not normal rabbit IgG (Figure 1h), showed cell surface and cytoplasmic reactivity.

IB of normal human epidermal extract

We first attempted to detect anti-EPPK antibodies in 48 Japanese PNP sera by IB of normal human epidermal extract. Anti-EPPK pAb detected approximately 500-kDa EPPK (Supplementary Figure S1 online). However, whereas all PNP sera detected the 210-kDa EPL and 190-kDa PPL, neither PNP nor normal sera reacted with EPPK.

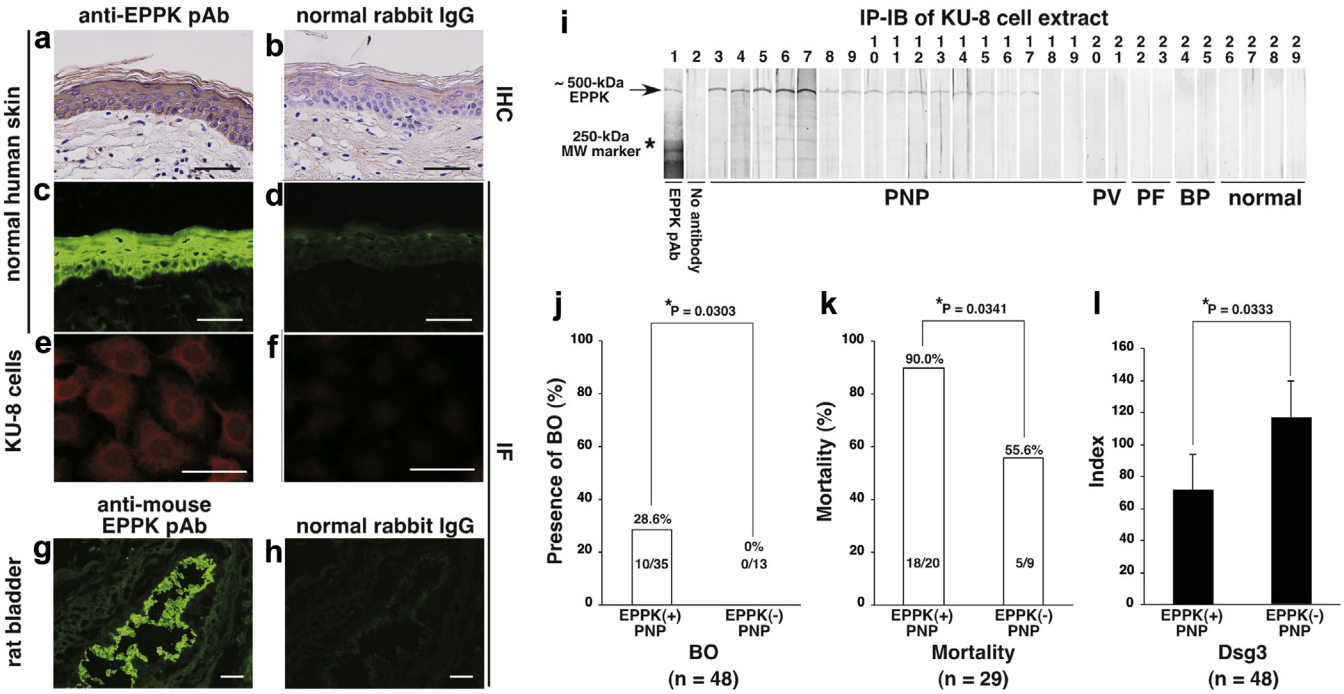

Figure 1. EPPK detection in normal human skin and KU-8 cells. (**a, b**) IHC of normal human skin with anti-EPPK pAb and normal rabbit IgG. (**c**–**h**) IF of normal human skin (**c, d**), KU-8 cells (**e, f**), and rat bladder (**g, h**). Bars = 50 μm. (**i**) IP-IB of KU-8 cell extract for EPPK with anti-EPPK pAb (lane 1), no antibody (lane 2), PNP (lanes 3–19), PV (lanes 20, 21), PF (lanes 22, 23), BP (lanes 24, 25), and normal sera (lanes 26–29). The positions of the approximately 500-kDa EPPK and the 250-kDa molecular marker are shown on the left. (**j**–**l**) Statistical analyses for association of BO (**j**), mortality (**k**), and ELISA indices for Dsg3 (**l**). Asterisks (*) indicate statistically significant differences between EPPK(+) PNP and EPPK(−) PNP ($P < 0.05$). BO, bronchiolitis obliterans; BP, bullous pemphigoid; Dsg, desmoglein; EPPK, epiplakin; IF, immunofluorescence; IHC, immunohistochemistry; IP-IB immunoprecipitation-immunoblotting; pAb, polyclonal antibody; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris.