The α and β Subunits of the Metalloprotease Meprin Are Expressed in Separate Layers of Human Epidermis, Revealing Different Functions in Keratinocyte Proliferation and Differentiation

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The zinc endopeptidase meprin (EC 3.4.24.18) is expressed in brush border membranes of intestine and kidney tubules, intestinal leukocytes, and certain cancer cells, suggesting a role in epithelial differentiation and cell migration. Here we show by RT-PCR and immunoblotting that meprin is also expressed in human skin. As visualized by immunohistochemistry, the two meprin subunits are localized in separate cell layers of the human epidermis. Meprin α is expressed in the stratum basale, whereas meprin β is found in cells of the stratum granulosum just beneath the stratum corneum. In hyperproliferative epidermis such as in psoriasis vulgaris, meprin α showed a marked shift of expression from the basal to the uppermost layers of the epidermis. The expression patterns suggest distinct functions for the two subunits in skin. This assumption is supported by diverse effects of recombinant meprin α and β on human adult low-calcium high-temperature keratinocytes. Here, β induced a dramatic change in cell morphology and reduced the cell number, indicating a function in terminal differentiation, whereas meprin α did not affect cell viability, and may play a role in basal keratinocyte proliferation.

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INTRODUCTION

Meprin (EC 3.4.24.18) is a zinc endopeptidase of the astacin family and the metzincin superfamily, originally found in intestinal and renal brush border membranes of humans, mice, and rats (Sterchi *et al.*, 1982, 1983, 1988). Meprins consist of two homologous subunits, α and β, which assemble to homo- or heterooligomeric complexes, whose basic unit is a disulfide-linked dimer (Becker *et al.*, 2003; Bertenshaw *et al.*, 2003; Ishmael *et al.*, 2005). Both subunits are multidomain type 1 membrane proteins composed of an amino-terminal propeptide, an astacin-like protease domain with the extended zinc binding active site motif HEXXHXXGFXHE and a conserved methionine residue in a β-1,4-turn (Met-*turn*) (Stöcker *et al.*, 1993, 1995; Bond and Beynon, 1995), a MAM domain (meprin A5 protein tyrosine phosphatase μ), and a TRAF domain (tumor necrosis factor receptor associated factor), which are thought to mediate protein–protein interactions, followed by an epidermal growth factor-like module, the C-terminal transmembrane domain, and a cytosolic tail. An inserted (I) domain in the α subunit is cleaved on the secretory pathway, resulting in the loss of the membrane anchor and subsequently in the secretion of the enzyme in those tissues, where α is not co-expressed with β, whereas the β subunit predominantly remains membrane bound (Marchand *et al.*, 1995; Eldering *et al.*, 1997). In contrast to the rodent orthologs, the human meprin β subunit contains several *O*-glycosylation sites in the extracellular part near the membrane. Human meprin oligomers may be shed from the cell surface by proteolytic cleavage in this region (Hahn *et al.*, 2003; Leuenberger *et al.*, 2003). Activation of meprins requires the removal of the N-terminal pro-peptide, and is catalyzed by trypsin in the intestinal lumen. Plasmin has also been identified as a meprin α activating enzyme (Rösmann *et al.*, 2002). However, this does not apply to promeprin β, whose activation site appears to be inaccessible for peptidases larger than trypsin (Becker *et al.*, 2003).

There are various *in vitro* observations of a cleavage of basement membrane proteins (e.g., collagen IV, nidogen-1, and fibronectin), protein kinases, growth factors, cytokines

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Abbreviations: HaCaT, human adult low calcium high temperature

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(interleukin-1β), and other bioactive peptides by meprin, but both subunits exhibit markedly different cleavage specificities (Choudry and Kenny, 1991; Kaushal *et al.*, 1994; Chestukhin *et al.*, 1997; Köhler *et al.*, 2000; Bertenshaw *et al.*, 2001; Kruse *et al.*, 2004; Herzog *et al.*, 2005).

Meanwhile, a broader range of sites of meprin expression other than kidney and intestine has been discovered, which varies between species and between developmental stages within a species. The subunits may be co-expressed or expressed individually as shown for the human intestine, where meprin β is expressed in the small intestine only, but meprin α is expressed in both the small and large intestine (Lottaz *et al.*, 1999a). Contrarily, in newborn mice, a more ubiquitous distribution of meprin subunits in the intestine and kidney has been reported (Kumar and Bond, 2001). However, after weaning the mRNA of meprin α was barely detectable in the intestine of ICR and C3H/He mice (Kumar and Bond, 2001). Less described expression sites of meprin are the salivary glands of mice (Craig *et al.*, 1991), the plexus choroideus, the inner ear, the nasal epithelium, and smooth muscle cells of rats (Bunnett *et al.*, 1993; Spencer-Dene *et al.*, 1994). In humans, meprin mRNA was also detected in the liver, skeletal muscle, stomach, and pancreas (http://merops.sanger.ac.uk). The observation that intestinal leukocytes express meprin (Lottaz *et al.*, 1999a) suggests a function in innate immunity. This assumption is supported by the analysis of meprin$^{-/-}$ mice (Crisman *et al.*, 2004), in which these lymphoid cells exhibited a restricted ability to migrate through the extracellular matrix.

Additionally, meprin has also been detected in certain epithelial carcinomas such as colorectal cancer (Matters and Bond, 1999; Lottaz *et al.*, 1999b), where soluble human meprin α is secreted not only apically but also basolaterally, thereby increasing the proteolytic potential of tumor cells for the destruction of the basement membrane (Kruse *et al.*, 2004). An abnormal meprin secretion has also been observed in rats with experimentally induced acute renal failure (Trachtman *et al.*, 1995; Carmago *et al.*, 2002). Taken together, these findings suggest a contribution of meprin to epithelial differentiation, matrix remodelling and cell migration, and also to inflammatory processes, tumor growth and metastasis.

Much less is known on alternative sites of meprin expression in adult mammalian tissues besides the tissues mentioned above. Here we present human skin as a new expression locus of meprin α and β. The fact that each subunit is expressed in a distinct cell layer of the epidermis, namely the stratum basale and the stratum granulosum, demonstrates the uniqueness of these proteases and reveals different physiological functions.

RESULTS

The antisera used for meprin detection are subunit specific and allow for a clear distinction between meprin α and β

To examine the specificity of the polyclonal antibodies used for the immunofluorescences, we tested meprin expressing insect cells (*Sf*9, *Spodoptera frugiperda*). These cells were infected with *Baculo* viruses containing human meprin α or β cDNA as described before (Becker *et al.*, 2003). Non-infected cells were used as a control for unspecific signals. Meprin α and β-expressing cells were detected by the α and β antisera, respectively (Figure 1). Furthermore, an antiserum directed against both subunits provided signals in both transfected cell lines. These results comply with the specificity of the antisera observed in the Western blot analysis (Figure 2j). Not all insect cells show fluorescence signals, which is because not all host cells are infected simultaneously resulting in different meprin levels. However, the selectivity of the antibodies used for the different meprin subunits is clearly evident. To exclude the fact that the antisera bind non-specifically in human tissues, cryosections from human ileum were analyzed. Fluorescence signals could be detected only on the apical side of the intestinal epithelium and on certain cells in the lamina propria, corresponding to leukocytes (data not shown), which is in accordance with published data (Lottaz *et al.*, 1999a).

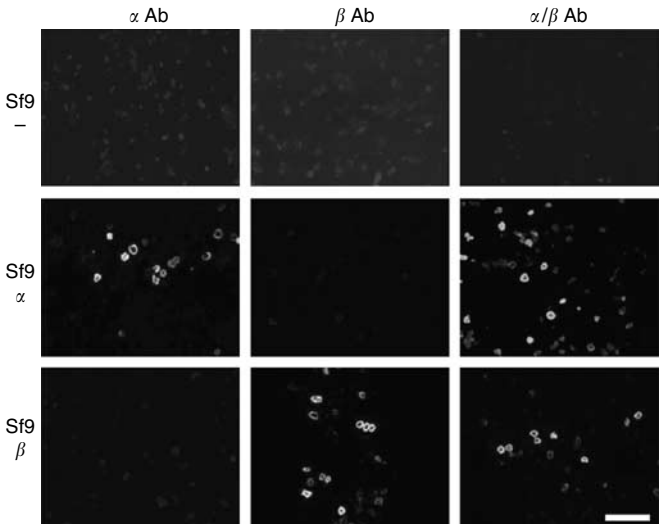

Figure 1. Control assay to test the specificity of the used antisera. Meprin transfected and nontransfected insect cells (*Spodoptera frugiperda, Sf*9) were incubated with antibodies (Abs) generated against meprin α, β, and both subunits. Bar = 50 μm.

Both meprin subunits are detected in human epidermal skin, albeit in different cell layers

The antisera were then used to analyze cryosections of human skin for meprin expression (Figure 2a and b). The fluorescence signals obtained by the α-specific antiserum localize exclusively to keratinocytes of the stratum basale (Figure 2a), the epidermal cell layer, where cell proliferation occurs. The signals appear strictly perinuclear. No fluorescence is observed at the cell membranes or in the extracellular space. By contrast, the data obtained for meprin β show an expression pattern restricted to the stratum granulosum displaying a linear pericellular staining of 3–4 cell layers (Figure 2b). Hence, both meprin subunits are expressed in the epidermis, but they are strictly separated, which confirms their different functionality. To prove further specificity of the antisera, recombinant meprin α and β were preincubated with the used antisera, which resulted in the