Normalized Proliferation of Normal and Psoriatic Keratinocytes by Suppression of sAPPα-Release

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The soluble form of the β-amyloid precursor protein (sAPPβ) is known to function in the autocrine regulation of epidermal growth and repair. Here we show that its proteolytic release by β-secretase in normal human keratinocytes is susceptible to hydroxamic-acid-based zinc metalloproteinase inhibitors and suppressed by these inhibitors by 80%–90%. As various other growth factors participate in regulating epidermal growth we investigated whether the inhibitor-induced sAPPβ-deficiency would affect keratinocyte proliferation. At optimal inhibitor concentrations the suppression of sAPPβ-release was followed by a decline in proliferation by 50%–60%, indicating that sAPPβ is a major growth factor that cannot be compensated for by other growth factors. This finding was the basis for the treatment of human lesional psoriatic keratinocytes with these inhibitors, which resulted in the normalization of their increased proliferation rates. The reversibility of these effects and the lack of toxicity underline the value of these inhibitors and suggest their therapeutic application in psoriatic skin diseases.

Key words: β-amyloid precursor protein/growth inhibition/keratinocytes/psoriasis/β-secretase


Normal growth, differentiation and wound repair of the skin are regulated by a variety of ions, cytokines and growth factors (Martin, 1997; Fuchs and Raghavan, 2002; Werner and Grose, 2003). Disturbances of these factors or changes, particularly in the expression of growth factors may, therefore, entail hyper- or hypotrophic skin anomalies. Although the dermis participates in most diseased states of the skin, the visible hallmarks of hyperproliferative skin diseases such as psoriasis are excessive production and exfoliation of epidermal cells (Krueger et al., 1990). Activated T cells appear to play a central role pointing to a primary immunological basis of the disease. These activated T cells are known to secrete cytokines such as interferon-γ and interleukin (IL)-2 which induce keratinocytes to proliferate (Lebwohl, 2003). Keratinocytes, however, are able to stimulate their own proliferation by an autocrine loop. This is accomplished by the production of a variety of cytokines and growth factors such as IL-1, IL-3, IL-6 and IL-8, transforming growth factor alpha (TGFα), TGFβ as well as platelet-derived growth factor (PDGF) (Ansel et al., 1990). The strongly increased keratinocyte proliferation is an important feature of psoriasis and remains, therefore, an additional target in the therapy of the disease (Lebwohl, 2003). Lesional psoriatic keratinocytes are characterized by the overexpression of a group of specific proteins including psoriasis (Ruse et al., 2003), cytokeratins 6, 10, 16 and 17, the antiflammatory protein SKALP/elafin (Pol et al., 2002a, b) and TGFβ (Elder et al., 1989).

Recently, the β-amyloid precursor protein (APP) has been reported to fulfill a variety of biological purposes, including its role as a growth factor precursor mainly for epithelial cells including keratinocytes (Schmitz et al., 2002). Human keratinocytes synthesize three APP isoforms of which the isoform APP751 and APP770 are expressed at levels about eight to 10 times above APP695 (Hoffmann et al., 2000; Kummer et al., 2002). APP exerts a striking dual function which is brought about by its differential cleavage leading to the release of at least two peptides with fundamentally different properties: The largely insoluble Aβ1-42 peptide, the main constituent of plaques in Alzheimer’s disease (Sisodia and Price, 1995), is the product of the amyloidogenic cleavage by β- and γ-secretases, whereas sAPPβ, the soluble N-terminal portion of APP, is generated in the non-amyloidogenic pathway by the proteolytic action of α-secretase. α-secretase is a plasma membrane associated protease (Sisodia, 1992; Parvathy et al., 1999) that cleaves APP within the Aβ1-42 sequence thereby precluding the formation of the Aβ1-42 peptide (De Strooper and Annaert, 2000). Several members of the ADAM (a disintegrin and metalloproteinase) family of proteinases (Blobel, 1997) have been shown to possess α-secretase activity, including ADAM9, ADAM10 and tumor necrosis factor-alpha convertase (TACE or ADAM17) (Black and White, 1998; Lammich et al., 1999). From overexpression and knockout studies it appears that these three enzymes, and possibly others, contribute to the α-secretase cleavage of APP to varying extents depending on the cell type (Allinson et al., 2003). Hydroxamic-acid-based zinc metalloproteinase inhibitors such as Batimatstat, SB244000 and SB251626 bind to the active site of the α-secretase and coordinate with the essential zinc ion (Botos et al., 1996), inhibiting the cleavage of APP and

Abbreviations: APP, β-amyloid precursor protein; NBK, normal human breast skin keratinocytes; PK, lesional psoriatic keratinocytes; sAPPβ, soluble form of APP

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blocking the production of sAPPα (Parvathy et al., 1998). In neuronal cells, these inhibitors exert also effects on TNFα-convertase and ACE-secretase (Parkin et al., 2002), which, however, appear to be absent from keratinocytes.

In keratinocytes and epidermal wound healing model systems, increased expression of APP and release of sAPPα are followed by elevated rates of proliferation (Kummer et al., 2002). Because sAPPα can be regarded as a major epidermal growth factor, inhibition of its release would be a promising approach for studies on its role in the regulation of pivotal keratinocyte functions.

In this report, we show that in psoriatic epidermis the expression of APP is not limited to the basal cell layer and is also observed in supra-basally located cells suggesting that inhibition of α-secretase activity might have a particularly strong effect on psoriatic epidermis. We have, therefore, analyzed the inhibition of α-secretase and its effect on keratinocyte proliferation in cell culture and in a newly developed epidermal organ culture system. The results show that keratinocyte proliferation is significantly reduced when Batimastat or other structurally related inhibitors are applied and that this effect is directly linked to suppressed sAPPα-secretion. Because these effects are reversible and the inhibitors do not exert detectable toxic effects on cells they are valuable tools for experiments in which the deficiency of endogenous sAPPα is required. Due to the strong proliferation-reducing effect on psoriatic keratinocytes, the inhibition of α-secretase is of potential therapeutic use.

**Results**

In this study, the proteolytic release of sAPPα from APP and the effects of α-secretase inhibitors were determined. Recombinant sAPPα751 (rec-sAPPα751; Fig 1a) was prepared, purified (Fig 1b) and applied to compensate for the inhibitor induced sAPPα-deficiency and its consequences on keratinocyte proliferation. Two distinct in vitro systems were employed, the cell culture of keratinocytes, using human adult low calcium, high-temperature keratinocytes (HaCaT) cells, normal human keratinocytes (NHK), isolated human breast skin keratinocytes (NBK) as well as lesional psoriatic keratinocytes (PK) and the organ culture of isolated human epidermis.

**Inhibition of sAPPα-release without effects on the expression of full-length APP** Optimal concentrations of the hydroxamic-acid-based zinc metalloproteinase inhibitors at which the release of sAPPα by keratinocytes was efficiently inhibited but toxic side effects were excluded were determined. Necrotic cells were marked with propidium iodide whereas apoptotic cells were detected by the binding of Annexin-V to phosphatidyl serine. The presence of up to 100 μM of inhibitor in the culture medium of HaCaT and NHK’s after 7 h revealed neither an effect on the number of necrotic cells nor of apoptotic cells (data not shown).

Analysis of the APP expression in cell lysates revealed that none of the inhibitors had any effect on the expression of APP as shown for the amount of mature or immature APP (Fig 2a). The small proportion of total cellular APP residing in the plasma membrane (Selkoe, 1999), where most of the sAPPα-release is known to occur, explains why the inhibition of sAPPα-release did not result in a detectable increase of total cellular APP levels.

The application of the inhibitors caused a suppression of sAPPα-release into the culture medium in a concentration-dependent manner reaching strong inhibition at 20 μM (shown for HaCaT cells) (Fig 2a). Quantitation of this inhibitory effect revealed that all inhibitors significantly suppressed the release of sAPPα at concentrations of 20 μM by about 80% (Fig 2b) or up to 90% (Fig 2d). At 30 μM, complete inhibition was observed. To use the lowest possible concentration resulting in sufficient inhibition, however, all subsequent experiments were performed at inhibitor concentrations of 20 μM. At this concentration highest inhibition was reached at 7 h after the addition of the respective inhibitor (Fig 2c, d).

**Reversibility of the inhibitory effect** To investigate the duration of the inhibitory effect on sAPPα-release, keratinocytes were incubated in the presence of 20 μM of each one inhibitor for various periods of time. The densitometric analysis of the immunoblots indicated that the effect of each of the three inhibitors on the sAPPα-release lasted for about 24 h. After this period the α-secretase activity was regained and at 48 h the sAPPα-release increased to 20%–60% (here shown for HaCaT cells) (Fig 2c, d) and at 72 h to 40%–90% of the original levels (not shown). The inhibitory periods were prolonged when inhibitors were replenished again after 24 h of incubation (data not shown). Inhibitor induced suppression of keratinocyte proliferation and re-establishment by exogenously added rec-sAPPα751 The proportion of proliferation competent keratinocytes was determined immunocytochemically by

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**Figure 1** Preparation of recombinant sAPPα (rec-sAPPα751). (a) structure of recombinant sAPPα (rec-sAPPα751) carrying a His-tag (blue) for purification purposes. The scheme depicts also the distribution of the three heparin-binding sites (HBS, green), the Kunitz-type serine protease inhibitor domain by which it is distinguished from sAPP695 (KPI, black) and the putative proliferation-stimulatory region (Jin et al., 1994) (yellow). (b) Coomassie blue stained SDS gel of recombinant sAPPα751, indicating the purity of the preparation.
of the inhibitor showed a similar decline. The reduction in proliferation of keratinocytes after treatment with the inhibitors (here SB244000) was overcome by the addition of rec-sAPP2751 (Fig 3c). These results showed that sAPPα was indeed the growth-promoting agent. Moreover, the full reversibility of inhibition strongly argues against the occurrence of toxic side effects.

There was a clear sequence of events with maximum inhibition of sAPPα-release at 7 h (see Fig 2d), maximum reduction of keratinocyte proliferation was observed at 24 h (see Fig 3a) after addition of the respective inhibitors and complete compensation by exogenously added rec-sAPP2751 (Fig 3d). The difference of 17 h between both events corresponds to previous observations of sAPPα-induced proliferation which is noted after a period of about this length (Hoffmann et al, 2000).

To detect whether the reduced proliferation in the presence of the inhibitors was also detectable in keratinocytes in situ, epidermis from surgically removed normal female breast skin was cultured for 48 h in the absence or presence of 20 μM of each one of the inhibitors. The epidermal structure (Fig 4a), particularly that of the basal cell layer remained intact as shown by the immunocytochemical detection of β1-integrin (Fig 4b). The number of proliferation competent keratinocytes in whole-mount preparations as shown by immunocytochemical visualization of Ki67 (Fig 4c, d) was significantly reduced in the presence of each of the inhibitors, shown for SB244000 (Fig 4d), but regained former levels after the addition of 10 nM rec-sAPP2751. When such epidermal preparations were incubated in the presence of [3H]thymidine, the incorporation was clearly diminished after application of the inhibitors and re-established following the addition of rec-sAPP2751 (Fig 4e).

Effect of the inhibitors on the expression of APP and the release of sAPPα in lesional psoriatic keratinocytes

Comparison of immunocytochemical sections of normal human breast and lesional psoriatic skin revealed that in lesional psoriatic skin the APP-expression was not restricted to the basal cell layer as shown for normal human breast skin (Fig 5a) but located also in supra-basal cell layers (Fig 5b). Thus, the APP-expression and, presumably, also the sAPPα-release was strongly increased in lesional psoriatic as compared to normal human breast epidermis (Fig 5c). In contrast, APP-expression did not differ significantly in isolated keratinocytes from normal human breast (Fig 5d) and lesional psoriatic (Fig 5e) skin, indicating that the amount of APP per single cell was nearly the same in both healthy and lesional psoriatic keratinocyte subtypes.

Similar to HaCaT cells (Fig 2), NHK or NBK (Fig 5d) the release of sAPPα by PK’s was significantly reduced after 7 h at 20 μM of each one of the inhibitors (here Batimastat), whereas the expression of APP remained constant (Fig 5d, e). The PK’s differed from NBK’s in that they showed an early recovery of the sAPPα-release after 24 h, already.

Proliferation rates of lesional psoriatic keratinocytes

Due to the pronounced papillary structure of lesional psoriatic skin (Fig 5b), whole-mount cultures of the epidermis were not suitable to determine the proliferation rates by the immunocytochemical Ki67-, BrdU- or the radiochemical
[3H]-thymidine-assays. This made necessary the establishment of lesional psoriatic keratinocyte cultures which required greater care than that of NBK’s. Despite optimal conditions in keratinocyte growth medium (KGM)-2 and the avoidance of all centrifugation steps, 40% of all psoriatic cultures did not survive. All successful cultures, however, maintained their highly increased proliferation rates and the typical cytokeratin expression for at least three passages. Thus PK’s exhibited a highly elevated expression of CK5, 6 and 10 which were barely detectable in NBK’s (Fig 6a), whereas CK18 was equally expressed in both systems. The immunocytochemical detection of proliferation competent keratinocytes by the Ki67- and BrdU-assays revealed that in contrast to NBK’s the proliferation of PK’s remained increased about threefold when transferred to normal culture medium (Fig 6b).

After 24 h of incubation in the presence of 20 μM of each one of the inhibitors (shown for Batimastat, BrdU-assay), the proliferation rate was significantly reduced by ~ 50% thereby reaching the proliferation rates of NBK. The reduction in proliferation was completely compensated for in NBK and by 70% in PK by the addition of rec-sAPPα751 (Fig 6c). The proportion of proliferation competent cells was greater in cultured lesional psoriatic than in NBK’s (not shown). This may explain why the inhibitors exerted their effect on more lesional psoriatic than on normal keratinocytes. Indirect reduction of sAPPα-release by antisense inhibition of APP-expression was comparable to the inhibition of α-secretase in NBK’s but less efficient in PK’s reaching 60% of control values (data not shown).

**Discussion**

Inflammatory cells such as activated T cells are known to be involved in the manifestation of psoriasis (Ghoreschi et al., 2003; Lebwohl, 2003), whereas the notion that keratinocytes themselves also contribute to their enhanced
proliferation rate is not uniformly supported (Kadunce and Krueger, 1995). Indeed, initial studies on cultured keratinocytes from lesional psoriatic skin failed to demonstrate high proliferation rate in the presence of the cytokines from lesional psoriatic skin (Krueger, 1995). Indeed, initial studies on cultured keratinocytes such as TNFα, which are known to be highly expressed in lesional psoriatic keratinocytes (Harper et al., 1987; Van Ruissen et al., 1988; Nanney et al., 1996), have shown that other growth factors are unable to fully make up for the loss of TNFα-deficiency is novel and the basis for the anti-psoriatic treatment with these inhibitors. Some growth factors are known to be highly expressed in lesional psoriatic keratinocytes such as TNFα (Ansel et al., 1990; Gröne, 2002), TGFα (Elder et al., 1989), heparin binding EGF and amphiregulin (Cook et al., 1992; Piepknorn, 1996). The release of these growth factors might be sensitive to metalloproteinase inhibitors as well (Piepknorn et al., 1998). The inhibition of their release might account for the incomplete compensation of lesional psoriatic keratinocyte proliferation by rec-sAPPα27-51. The less efficient reduction in the proliferation rate of PK using the specific inhibition of APP-secretase cleavage. After about 48 h of incubation however, in the presence of each of the inhibitors, the constitutive release of sAPPα is partially restored. As the repletion of the inhibitors shortly before the end of this period prolonged the inhibition of sAPPα-release, it can be assumed that with time the inhibitor concentrations decrease due to degradation or irreversible binding to the α-secretase and clearance, e.g., by endocytosis. Judging by the efficient block of sAPPα-release the inhibitors appear to be most suitable experimental tools to efficiently hamper cell-type specific functions of sAPPα.

**Advantage of α-secretase inhibition above existing procedures** Our study shows that hydroxamic-acid-based zinc metalloproteinase inhibitors can be applied at optimal concentrations of 20 μM to epidermal organ- and keratinocyte-cultures for prolonged periods of time without toxic side-effects and that this application results in an inhibition of sAPPα-release by about 80%–90%. As α-secretases are known to cleave all members of the APP protein family including the APP homologues APLP1 (Paliga et al., 1997) and APLP2 (Slunt et al., 1994), their inhibition is superior to the knockout of an APP isoform or the blocking by antisense procedures. This view is supported by the observation that the expression of APP is not suppressed by the inhibitors and that total cellular APP accumulation does not increase despite the suppressed α-secretase cleavage. After about 48 h of incubation however, in the presence of each of the inhibitors, the constitutive release of sAPPα is partially restored. As the repletion of the inhibitors shortly before the end of this period prolonged the inhibition of sAPPα-release, it can be assumed that with time the inhibitor concentrations decrease due to degradation or irreversible binding to the α-secretase and clearance, e.g., by endocytosis. Judging by the efficient block of sAPPα-release the inhibitors appear to be most suitable experimental tools to efficiently hamper cell-type specific functions of sAPPα.

**Normalisation of keratinocyte proliferation as an additional anti-psoriatic treatment** Presumed key regulators of keratinocyte proliferation include PDGF, EGF (Nanney et al., 1996), TGFα from basal keratinocytes (Rappolee et al., 1988; Nanney et al., 1996), KGF from dermal fibroblasts (Werner et al., 1992) and activin (Munz et al., 1999). Inhibition of these growth factors or blockade of receptor signaling, however, revealed that none of these factors is absolutely essential for epidermal growth (Grose and Werner, 2002). In contrast, although numerous other factors contribute to the regulation of keratinocyte proliferation (Martin, 1997; Fuchs and Raghavan, 2002), the almost complete block of sAPPα-release results in a significant reduction in the proliferation rates in both keratinocyte- and epidermal organ cultures by 50%–60%. Recent observations have shown that the proliferation rates of keratinocytes from non-lesional psoriatic skin are between the rates of keratinocytes from lesional psoriatic and normal skin (unpublished observations). The higher proliferation rates characteristic of PK’s are reduced to approximately normal levels following the inhibition of α-secretase. Hence, cytokines and other epidermal growth factors synthesized and released by keratinocytes such as TGFα (Barrandon and Green, 1987; Elder et al., 1989) can compensate only partially for the lack of sAPPα. The finding that other growth factors are unable to fully make up for the sAPPα deficiency is novel and the basis for the in vitro treatment of PK’s with these inhibitors. Some growth factors are known to be highly expressed in lesional psoriatic keratinocytes such as TNFα (Ansel et al., 1990; Gröne, 2002), TGFα (Elder et al., 1989), heparin binding EGF and amphiregulin (Cook et al., 1992; Piepknorn, 1996). The release of these growth factors might be sensitive to metalloproteinase inhibitors as well (Piepknorn et al., 1998). The inhibition of their release might account for the incomplete compensation of lesional psoriatic keratinocyte proliferation by rec-sAPPα27-51. The less efficient reduction in the proliferation rate of PK using the specific inhibition of APP-expression by antisense constructs supports this possibility.
The three inhibitors differ considerably in length and composition of their side chains (Gearing et al., 1994), but no significant differences exist in their inhibitory effects on sAPPα-release and on keratinocyte proliferation. Differences may exist, however, in the recovery of suppressed sAPPα-release with Batimastat showing prolonged periods of suppression. The lack of major differences in their specific function allows to select the most suitable inhibitor for its ability to reach the basal epidermal cell layer. The decrease in proliferation can be effectively overcome by exogenously added rec-sAPPα751, supporting our view that the inhibitory proliferation rates indeed result from the strongly suppressed sAPPα-secretion and that toxic side effects do not occur. This is important as long-term safety and tolerability are major selection criteria for optimal antipsoriatic therapies.

Methods are being developed to apply these inhibitors to the epidermis in situ. In further experiments, nude mice transplanted with lesional psoriatic skin will allow to study in detail the effects of the inhibitors in an in vivo model system.

The most successful existing therapies for psoriasis include photochemotherapy (Zanoli, 2003) as well as methotrexate (Heydendael et al., 2003) and immunomodulatory treatment (Asadullah et al., 2002). Our results suggest an additional therapeutic approach in the treatment of psoriatic skin diseases by the application of hydroxamic-acid-based zinc metalloproteinase inhibitors.

Materials and Methods

Material Biopsies of lesional skin of five psoriatic patients (aged between 27 and 78 years) were taken under local anesthesia after obtaining informed consent. The patients suffered from clinically and histopathologically confirmed psoriasis and were left without treatment for at least 4 wk. Experimentation with human tissue samples was conducted in accordance with the ethical principles and guidelines of the Medical Faculty of the University of Bonn as approved by the governmental authorities. Hydroxamic-acid-based zinc metalloproteinase inhibitors (Batimastat, SB244000, SB251626), were kindly provided by GlaxoSmithKline-Pharmaceuticals (Harlow, UK). APP synthesis was specifically inhibited by the preparation and application of antisense constructs as previously described (Hoffmann et al., 2000). The secondary antibodies were...
from Dianova (Hamburg, Germany). Recombinant sAPPα (rec- sAPPα751) was prepared as described (Popp et al., 1996) using EBNA293 cells and the cDNA of APP751.

**Culture of human epidermis** Human skin (Dr K. Jaeger, Marien-hospital, Brühl, Germany) was digested with 10 mg per mL di-sperse-II (Roche Molecular-Biochemicals, Mannheim, Germany) in KGM (BioWiThaker, Verviers, Belgium) without EGF/BPE (KGM*) for 15 h at 4°C, before epidermis and dermis were separated. For organ culture, the epidermal sheets were placed on porous polycarbonate membranes (3 μm pore size) of tissue culture inserts (Nunc, Naperville, Illinois) and cultured in KGM, which allowed “air-exposed” culture. To visualize the structure of normal and psoriatic skin and to test the preservation of isolated epidermis 5 μm sections were stained with hematoxylin and eosin.

**Cell culture** In all experiments, three keratinocyte cell-culture systems were used. HaCaT cells cultured in Dulbecco’s minimal essential medium (DMEM; BioWiThaker) containing 10% fetal calf serum (FCS), NHK obtained from BioWiThaker and isolated NBK (Leigh et al., 1994) cultured in KGM. In all studies, the cells were cultured during the experiments in serum-free DMEM (DMEM**) or KGM**. Keratinocytes from lesional psoriatic skin biopsies were prepared by the same technique. Care was taken that the dermal papillae remained intact which was controlled by scanning electron microscopy (not shown). Lesional psoriatic keratinocytes (PK) were cultured in KGM-2 (BioWiThaker) containing 10% FCS. 48 h later the medium was replaced by serum-free medium, which was changed every two days. Under these conditions, PK’s maintained their high proliferation rates and their characteristic overexpression of different cytokeratines (monoclonal cytokeratine 5, 6, 10 and 18 antibodies were from Chemicon, International, Hofheim, Germany). Because of the decline of the hyperproliferative characteristics of PK’s after multiple passages all experiments were performed after the second or third passage.

**Proliferation assays** To determine the proliferation rate the culture of HaCaT cells, NHK, NBK, PK or epidermal organ culture were performed as described above. After the respective incubations, the cells were stained either with the monoclonal anti-Ki67 antibody (DAKO, Hamburg, Germany) or after BrdU-incorporation performed as described above. After the respective incubations, proliferation indexes were calculated from the ratio of the Ki67- or fluorescence microscope (Axiophot, Zeiss, Jena, Germany). The proliferation index was calculated from the ratio of the Ki67- or BrdU-positive cells and the total number of cells marked with propidium iodide (2 μg per mL in anti-fade reagent; Biomeda Corporation, Foster City, California). For morphometric quantitation, the imaging software ImageProPlus (Media Cybernetics, Silver Spring, Maryland) was used. The integrity of basal cells was determined by the monoclonal antibody P52D against β1-integrin (Chemicon).

**[3H]thymidine-incorporation assay** Human epidermis was cut into equal pieces using a 6 mM biopsy punch (pfm GmbH, Cologne, Germany) and placed on membranes of tissue culture inserts (Nunc). For the last three hours of incubation 20 μCi per mL [3H]thymidine (Amersham-Bioscience, Little Chalfont, UK) was added to the medium (DMEM*). The epidermal sheets, together with the membrane, were cut out using an 8 mM biopsy punch, washed and transferred into vials with scintillation cocktail (Lumac-LSC B.V., Groningen, The Netherlands). The radioactivity was determined in a TriCarb®-scintillation counter (Packard,Burhach, Germany).

**Immunoelectrophoretic blot analysis** To analyze the expression of APP in keratinocytes and the sAPPα-release into culture medium, cell lysates and media, concentrated 50-fold (Centricon tubes; Amicon, Bredford, MA), were run on a 10% polyacrylamide SDS-gel and blotted onto a nitrocellulose-membrane (Protan-Nitrocellulose, Schleicher-Schuell, Dassel, Germany). Subsequently the proteins were detected using the rabbit anti-APP antisemur 2189, raised as described (Popp et al., 1996), or the monoclonal anti- sAPPα antibody 1560 (Chemicon), visualized by chemiluminescence (ECL, Amersham, Braunschweig, Germany), documented on XAR-5 films (Kodak, Stuttgart, Germany) and evaluated by digital image analysis.

**Immunocytochemistry** Cryostat sections, 7 μm thick, of cultured epidermis were stained with the rabbit anti-APP antisemur 2189 and the monoclonal anti-human epiligrin (laminin-5) antibody MAB1949 (Chemicon). Sections were viewed with an LSM510 (Zeiss).

**Statistical analysis** The results were analyzed for statistical differences between the groups by a one-way ANOVA. In general, the differences were taken to be statistically significant at p < 0.05.

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