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Thr308 determines Akt1 nuclear localization in insulin-stimulated keratinocytes

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ABSTRACT

Here, we determined the localization and activation of protein kinase B (Akt) in acute cutaneous wound tissue in mice. Akt1 represented the major Akt isoform that was expressed and activated in wound margin keratinocytes and also in the cultured human keratinocyte line HaCaT. Mutation of Akt1 protein, exchanging the activation-essential Ser473 and Thr308 residues for inactive Ala or phosphorylation-mimicking Asp and Glu residues, revealed that phosphorylation of Ser473 represented an essential prerequisite for auto-phosphorylation of Thr308 within the Akt1 protein in keratinocytes. Moreover, cell culture experiments and transfection studies using Thr308 mutated Akt1 proteins demonstrated that phosphorylation of Akt1 at Thr308 appeared to selectively exclude the active kinase from the nucleus and direct the kinase to the cytoplasmic compartment in keratinocytes upon insulin stimulation. In summary, our data show that phosphorylation of Thr308 during insulin-mediated Akt1 activation is an essential prerequisite to exclude Akt1 from the nuclear compartment.

At present there is growing evidence emphasizing a central role of protein kinase B (Akt) in skin and keratinocyte biology. Three highly conserved isoforms of Akt are known: Akt1, Akt2 and Akt3, all of them targets of active phosphatidylinositol 3-kinase (PI3K) [1]. Enzymatic activation of Akt occurs through phosphorylation of the kinase in its activation loop (Thr308) and carboxy-terminal tail (Ser473), respectively [2]. Activation of the PI3K/Akt signaling pathway controls cell survival in differentiating keratinocytes [3–5]. In addition, Akt1/Akt2 double knock-out mice displayed a translucent skin which could be attributed to a reduced proliferation of the basal keratinocytes [6]. These observations were supported by data showing that proliferating human primary keratinocytes expressed only Akt1 and Akt2 isoforms, from which only the targeted reduction of Akt1 was functionally connected to the induction of keratinocyte cell death and the disruption of an organized expression of differentiation markers in organotypic skin cultures [4]. These *in vitro* findings, suggesting Akt1 to promote keratinocyte survival, might find their *in vivo* counterparts during skin repair. Epithelial healing is driven by the response of wound margin keratinocytes toward a diverse multitude of protein-type mediators [7], most of them known to be potent activators of Akt [8]. Among these factors, the severe insulin resistance of chronic wound tissue of diabetic mice particularly suggested the Akt activating insulin [9] to participate in impaired healing [10]. In this study, we observed the Akt1 isoform to be activated in keratinocytes upon skin wounding and in cultured keratinocytes upon insulin stimulation. Moreover, we determined activation of Akt1 at Thr308 to be essential for auto-phosphorylation and cytoplasmic localization of the stimulated kinase in keratinocytes.

Materials and methods

Animals. Female C57BL/6J mice were obtained from Charles River (Sulzfeld, Germany). At the age of 12 weeks, mice were wounded as described below.

Wounding of mice. Wounding of mice was performed as described previously [11,12]. Briefly, six full-thickness wounds (5 mm in diameter, 3–4 mm apart) were made on the back of each mouse by excising the skin and the underlying panniculus carnosus. Skin biopsy specimens were obtained from the animals 5 days after injury. All animal experiments were performed according to the guidelines and approval of the local Ethics Animal Review Board.

Cell culture. Quiescent human HaCaT keratinocytes [13] were stimulated with insulin (2 μg/ml) for the indicated periods of time. Insulin was from Roche Biochemicals (Mannheim, Germany).

Preparation of protein lysates and immunoblot analysis. Skin and keratinocyte samples were homogenized as described [10,14]. Cytoplasmic and nuclear fractions were generated according to a published protocol by Schreiber et al. [15]. Specific proteins were detected using antisera directed against Akt1 (Epitomics,

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Hamburg, Germany), total Akt, phospho-Akt (Ser473), phospho-Akt (Thr308) (Cell Signaling, New England Biolabs, Frankfurt, Germany), Flag fusion proteins (Sigma, Taufkirchen, Germany), nucleolin and insulin receptor β-subunit (Santa Cruz, Heidelberg, Germany).

Immunoprecipitation. Two hundred micrograms of wound lysate were incubated overnight with the respective Akt1- (Epitomics), Akt2-, Akt3- (Cell Signaling, New England Biolabs) or Flag (Sigma)-specific antibodies according to instructions of the manufacturer. Immunoprecipitates were isolated using protein G Sepharose (Sigma). Flag-tagged Akt1 was immunoprecipitated using an anti-Flag M2 affinity gel (Sigma) in 500 μl of Flag buffer (25 mM Tris–Cl, pH 7.4, 150 mM NaCl, 1 mM CaCl2, and 1% Triton X-100). Protein G Sepharose- and Flag-bound precipitates were washed in TBST (150 mM NaCl, 10 mM Tris–Cl, pH 8.0, and 0.05% Tween 20) and protein was eluted using Laemmli buffer. Triton X-100 and Tween 20 were obtained from Sigma.

Immunohistochemistry. Complete 5-day wounds were isolated from the back, bisected, and frozen in tissue freezing medium. Six micrometer frozen sections were subsequently analyzed using immunohistochemistry as described [11]. Antisera against phospho-Akt (Ser473) and phospho-Akt (Thr308) (Cell Signaling) were used for immunodetection.

Immunofluorescence. Transfected HaCaT keratinocytes were grown on glass slides and stimulated with insulin (2 μg/ml). Control and stimulated cells were subsequently fixed using methanol/EDTA (0.02% w/v) or paraformaldehyde (2% w/v) for 15 min at −20 °C or room temperature, respectively. Fixed cells were blocked with 5% goat serum diluted in PBS/Triton (0.1% w/v). Antiserum against Flag fusion proteins (Sigma) was incubated for 1 h at room temperature. The fluorescence-coupled secondary antibody Alexa Fluor 488 (Molecular Probes, Leiden, The Netherlands) was diluted 1:250 in 5% goat serum/PBS and incubated in the dark for 30 min. Nuclei were counterstained using 4′,6-diamidino-2-phenylindole (DAPI) (Sigma).

Generation of mutated and Akt1-Flag fusion constructs. A full length human Akt1 cDNA was amplified from total HaCaT keratinocyte cDNA using Pfu polymerase (Promega, Mannheim, Germany) and 5′-GATAGAATTCGGGCACCATGAGCGACG-3′ and 5′-CTATGGATCCGGCCGTGCTGC TGGC-3′ as primers. Amplicons were cloned into EcoRI/BamHI sites of pCMV-Flag N3 [16]. The pCMV-NE-Akt1-Flag was used as a template for subsequent cloning strategies. Mutation of Akt1 Thr308 and Ser473 residues was performed using the pCMV-NE-Akt1-Flag vector as a template, the QuickChange Site-Directed Mutagenesis Kit (Stratagene, Heidelberg, Germany) and the following primers: 5′-GGT GCC ACC ATG AAG GCC TTT TGC GGC ACAC-3′ (for the Thr308 to Ala mutation; Akt1-T308A), 5′-CGG TGC CAC CAT GAA GGA ATT CTG CGG CAC ACC TGA GTA CC-3′ (for the Thr308 to Glu mutation; Akt1-T308E), 5′-CCC ACT TCC CCC AGT TCG CGT ACT CGG CCA GCA GCA CG-3′ (for the Ser473 to Ala mutation; Akt1-S473A) and 5′-CCC ACT TCC CCC AGT TCG ACT ACT CGG CCA GCA GCA CG-3′ (for the Ser473 to Asp mutation; Akt1-S473D).

Transfection experiments. HaCaT cells were transiently transfected with FuGene™ transfection reagent (Roche Biochemicals, Mannheim, Germany) and the appropriate plasmid DNA according to the instructions of the manufacturer.

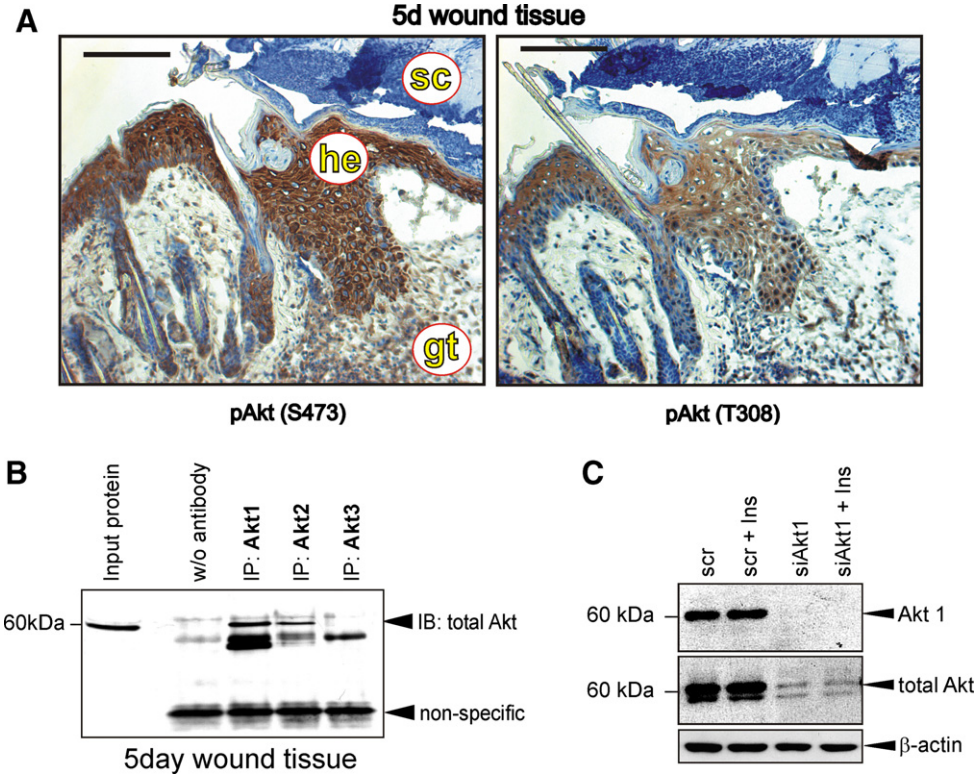

Fig. 1. Localization and isoform expression of Akt in skin wounds and keratinocytes. (A) Frozen serial sections from mouse 5-day wounds were incubated with antibodies directed against phosphorylated Akt (Ser473 or Thr308) as indicated. Nuclei were counterstained with hematoxylin. *gt*, granulation tissue; *he*, hyperproliferative epithelium, *sc*, scab. Bars, 50 μm. (B) Hundred micrograms of total protein from 5-day *wound tissue* were immunoprecipitated using Akt1-, Akt2-, and Akt3-specific antibodies. Immunoprecipitates were analyzed by immunoblot and a total-Akt-specific antibody. Wound lysates were obtained from 8 wounds ($n = 8$) isolated from 4 individual mice ($n = 4$) which have been pooled prior to analysis. (C) Akt1 protein depletion in HaCaT keratinocytes by specific siRNA transfection. Cells were treated with a scrambled (*scr*) or Akt1-specific (*siAkt1*) siRNA in the presence or absence of insulin. Akt1 depletion was controlled using a specific *Akt1* or a *total Akt* recognizing antibody. Loading of the immunoblot was controlled using β-actin.

Silencing of Akt1 expression by siRNA. HaCaT keratinocytes (2×10^5) were grown in 6-well plates to reach 40–60% confluency. Cells were subsequently transfected twice with small interfering RNA (siRNA, 50 nM final concentration) using oligofectamine® (Invitrogen, Karlsruhe, Germany) and OptiMEM (Invitrogen) as described by the manufacturer. The Akt1-specific siRNA was purchased from Applied Biosystems (Darmstadt, Germany).

Results and discussion

Akt1 is the predominant isoform in wound and cultured keratinocytes

Here we determined the localization of activated Akt during acute skin repair in mice. Keratinocytes located at the margins of the wound exhibited particularly strong signals for Akt phosphorylated at both Ser473 and Thr308 residues (Fig. 1A). This observation appears important for two reasons: first, the expression and presence of activated Akt was nearly completely restricted to the wound margin epithelium, which is built from highly proliferating keratinocytes as a consequence of the mitotic potency of growth factor action on the cells [7,17,18]. This finding again confirms the known role of the PI3K/Akt pathway for keratinocyte biology in the control of cell survival and differentiation [3–5]. In particular, Pankow et al. [5] described an expression of PI3K catalytic subunits and subsequent phosphorylation of Akt in hyperproliferative wound epidermis, which is in clear accordance with the here presented data and the basic finding that Akt is a target of active PI3K [19]. Second, co-localizing prominent signals for Ser473- and Thr308-phosphorylated Akt (Fig. 1A) in wound keratinocytes strongly suggested that indeed a full enzymatic activation of Akt, which has been reported to be dependent on phosphorylation of the kinase in its activation loop (Thr308) and carboxy-terminal tail (Ser473) [2], took place upon wounding. Although the S473 and T308 phospho-specific antibodies, that had been used for histologic (Fig. 1A) analyses, had been generated against the Akt1 phospho-motifs, we aimed to establish additional experimental evidence to determine the Akt isoform [1] responsible for the observed prominent signal in wound keratinocytes. Immunoprecipitation experiments from 5-day wound tissue lysates demonstrated that Akt1 indeed represented the predominant isoform in wounded skin tissue (Fig. 1B) and again argued for Akt1 as the activated kinase isoform in wound margin keratinocytes (Fig. 1A). To unequivocally prove Akt1 as the major isoform in keratinocytes, we depleted functional Akt1 protein from the cells using an Akt1-specific siRNA approach *in vitro*. As shown in Fig. 1C (upper panel), Akt1 siRNA transfection potently abrogated Akt1 protein from keratinocytes. Moreover, the also nearly complete loss of the total Akt protein signal (middle panel) upon Akt1 siRNA treatment clearly exhibited Akt1 as the dominant Akt isoform in the cells. Importantly, the remaining weak signal for total Akt in parallel to the Akt1 siRNA knock-down again reflected the low expression level of Akt2 in keratinocytes. Akt3 could not be detected in keratinocytes (data not shown). These experiments noting Akt1 as predominant in keratinocytes are in accordance to reports showing Akt1 as the major isoform in skin [6] and as a central regulator of keratinocyte survival during differentiation [4].

The role of Thr308 and Ser473 phosphorylation in insulin-mediated intracellular localization of Akt1

Next, we investigated a functional connection between the wound-derived mediator insulin [10] and activation of Akt1 in keratinocytes, as insulin resistance is implicated in diabetes-impaired wound healing and wound keratinocyte atrophy [10] and

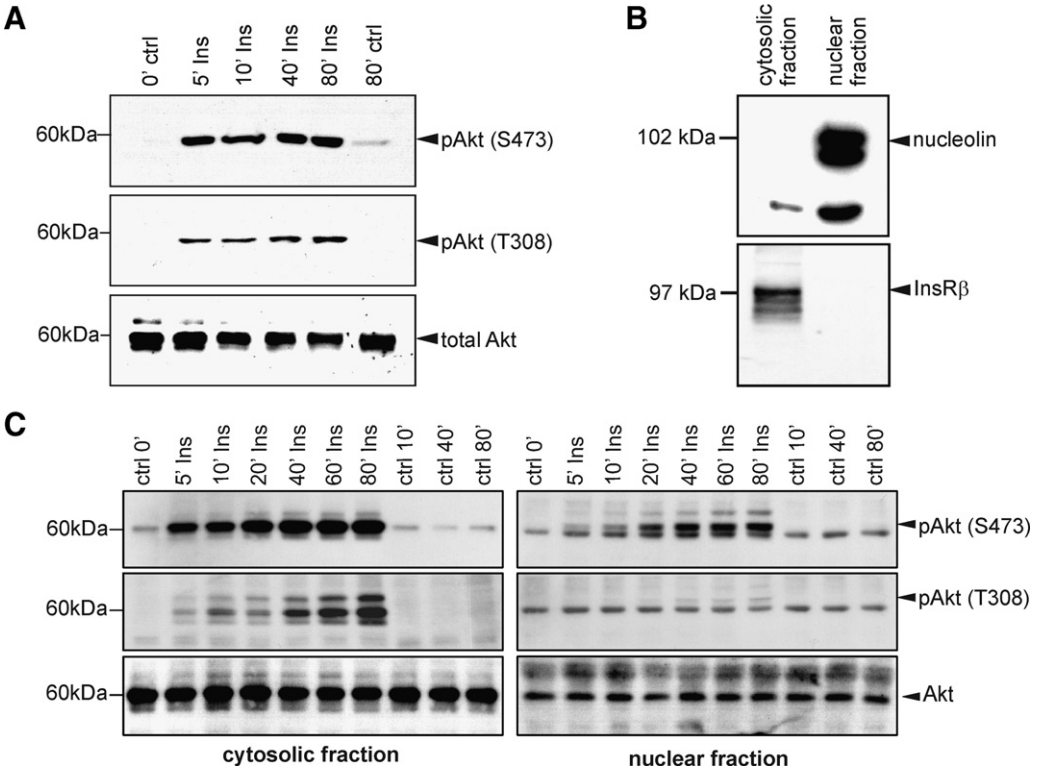

Fig. 2. Insulin-mediated Akt1 activation and localization in cultured keratinocytes. (A) Immunoblot showing Thr308- and Ser473-phosphorylated Akt protein in insulin-treated HaCaT keratinocytes as indicated. (B) Immunoblot to control the isolated cytosolic (*insulin receptor β-subunit*) and nuclear (*nucleolin*) fractions from insulin-stimulated keratinocytes. (C) Immunoblots of cytosolic (left panels) and nuclear (right panels) fractions isolated from non-stimulated (*ctrl*) and insulin-stimulated HaCaT keratinocytes (*Ins*) were analyzed at the indicated time points for endogenous Akt1 phosphorylated at Ser473 or Thr308. Total Akt was used as a loading control.

with respect to Akt1 as a critical signaling molecule upon insulin treatment in a variety of cells [9]. For this purpose, we stimulated quiescent cultured human HaCaT keratinocytes with insulin. As shown in Fig. 2A, insulin mediated a robust phosphorylation at both essential Ser473 and Thr308 residues and thus an activation of Akt 1 [2] in the cells. In recent years, a focus has not only been placed on Akt activation but also translocation of the activated kinase. Interestingly, insulin-like growth factor-(IGF)-1 as well as insulin itself have been reported to mediate Akt activation and translocation into the nucleus, resulting in a distinct nuclear localization of Akt in diverse cell types [20–22]. To further allow a more detailed view into Akt1 movements upon activation in keratinocytes, we separated the cytosolic from the nuclear compartment (Fig. 2B). Following insulin stimulation, we found a rapid phosphorylation of Akt1 at Ser473 in the cytoplasm, which preceded phosphorylation of the kinase at Thr308 (Fig. 2C, left panels). Although the nuclear appearance of Ser473-phosphorylated Akt1 was delayed, suggesting the translocation of Akt1 from the cytoplasmic into the nuclear compartment (Fig. 2C, left panels), it is important to note here that we failed to detect Thr308-phosphorylated Akt in the nuclear compartment (Fig. 2C, right panels).

Differential function of Thr308 and Ser473 residues for intrinsic activation of Akt1

Obviously, our data from insulin-stimulated Akt1 phosphorylation suggested a preceding Ser473 phosphorylation as a prerequisite for phosphorylation of Thr308 within the kinase (Fig. 2C) [2]. To assess our hypothesis, we generated a set of Akt1-Flag mutants. Consistent with the known ability of negatively charged amino acids (Glu, Asp) to mimick phosphorylated residues and thus activation of target proteins such as guanylyl cyclase, 6-phosphofructo-2-kinase or PI3K [23–25], we replaced either Thr308 for Glu or Ser473 for Asp residues within the Akt1-Flag fusion construct. Additionally, we mutated Akt1-Flag fusion proteins at Thr308 or Ser473 for an Ala residue to prevent a phosphorylation of the enzyme at the respective sites. Transfection of Akt1 mutants into exponentially growing keratinocytes revealed that phosphorylation of Ser473 represented an essential prerequisite for phosphorylation of Thr308 within the Akt1 protein. This could be convincingly shown by the presence of the negatively charged amino acid Asp, which has been replaced for Ser at position 473, that enables auto-phosphorylation of Thr308 in the mutated Akt1 protein (Fig. 3, middle panel). By contrast, Ser473 phosphorylation occurred independently from the presence or absence of a functional Thr308 residue (Fig. 3, upper panel).

Thr308 functions in the control of Akt1 nuclear localization in insulin-treated keratinocytes

Phosphorylated Thr308 appeared to exclude Akt1 from the nuclear compartment of insulin-treated keratinocytes (Fig. 2C). To further strengthen our observation, we transfected HaCaT keratinocytes using the respective Ser473 and Thr308 mutant Akt1-Flag fusion constructs. As shown in Fig. 4, we observed the overexpressed wild-type Akt1-Flag fusion protein to be preferentially localized within the cytoplasmic compartment of transfected cells. However, addition of insulin mediated an accumulation of Akt1 in the nucleus (panel 1), which could be shown to be dependent on Ser473, as the functional inactivation of the Ser473 phosphorylation site by Ala excluded the Akt-Flag fusion protein from the nucleus upon insulin treatment of transfected cells (panel 2). In

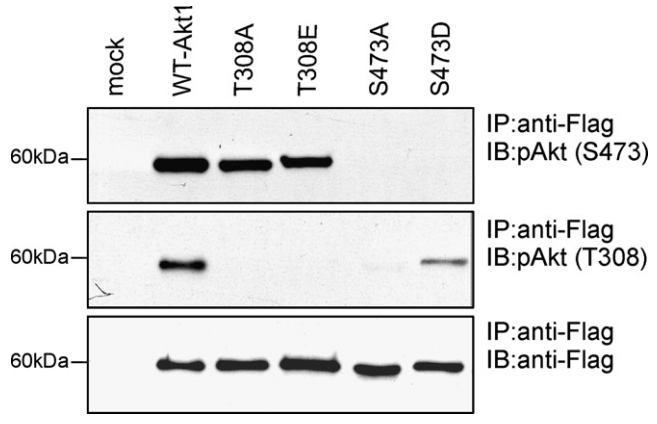

Fig. 3. The role of Ser473 and Thr308 for activation of Akt1. Keratinocytes were transfected with pCMV expression plasmid (*mock*), wild-type Akt1-Flag (*WT-Akt1*) and mutated Akt1-Flag constructs (Thr308 to Ala: *T308A*; Thr308 to Glu: *T308E*; Ser473 to Ala: *S473A*; Ser473 to Asp: *Ser473D*) as indicated. Recombinant Akt proteins were precipitated from total cellular lysates using anti-Flag agarose and precipitates were subsequently analyzed for phosphorylated Ser473 (upper panel) and Thr308 (middle panel) residues by immunoblot. Equal loading was controlled using an anti-Flag antibody (lower panel).

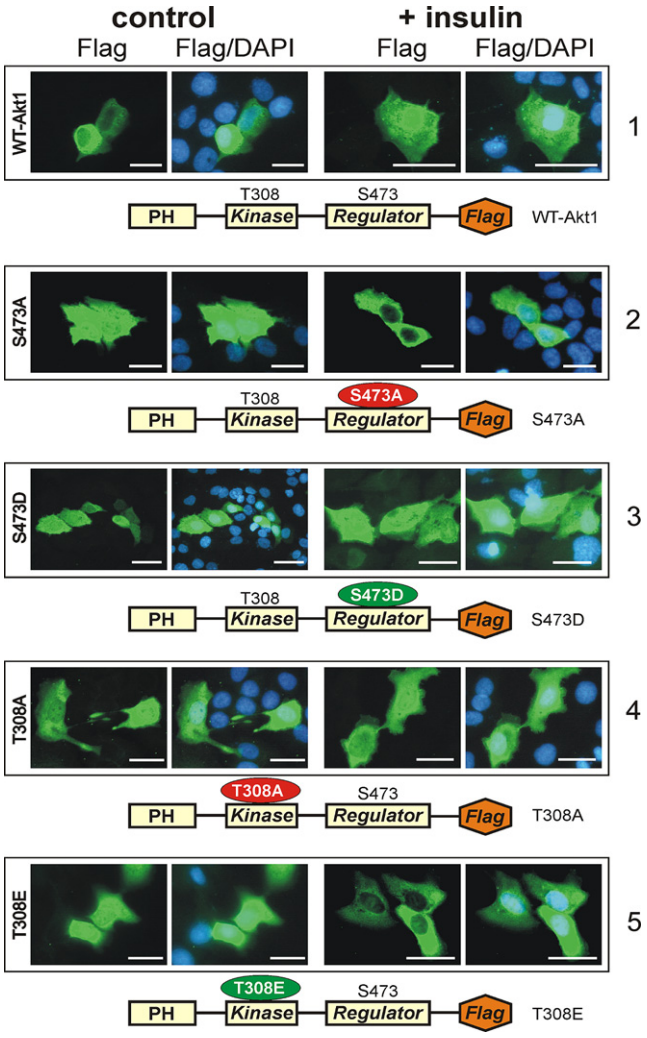

Fig. 4. Thr308 excludes activated Akt1 from nuclei in insulin-treated keratinocytes. HaCaT keratinocytes were transiently transfected with wild-type Akt1-Flag (*WT-Akt1*) and mutated Akt1-Flag constructs (Ser473 to Ala: *S473A*; Ser473 to Asp: *S473D*; Thr308 to Ala: *T308A*; Thr308 to Glu: *T308E*) as indicated. After 24 h of transfection, cells were left untreated (*control*) or stimulated with insulin (*+insulin*) for 10 min. Recombinant Akt1 protein was analyzed by immunofluorescence using an anti-Flag antibody. Nuclei were stained with DAPI. Bars, 30 μm.

accordance, the Ser473Asp mutation mimicking a constitutive phosphorylation of Akt1 at that respective residue could be observed again within the nuclei (panel 3). These data on Ser473 mutated Akt1 confirmed our results where we observed an increase of Ser473-phosphorylated Akt1 in nuclei of insulin-treated keratinocytes (Fig. 2C, left panels). However, and again in good accordance to the cell fractionation experiment, mimicking phosphorylation of Akt1 protein at residue Thr308 by a Glu replacement appeared to function as an essential prerequisite to drive an insulin-stimulated translocation of the kinase out of keratinocyte nuclei (panel 5), whereas the 'inactive' Thr308 to Ala mutant Akt1 protein was not excluded from keratinocyte nuclei (panel 4). These findings again suggested the importance of Thr308 phosphorylation in the control of a cytoplasmic localization of Akt1 in keratinocytes in close functional connection to insulin action. Taken together, data from transfection and cell fractionation experiments provide evidence that a fully activated Akt1 is preferentially located in the cytoplasm of insulin-stimulated keratinocytes—in place to drive the well-described Akt-dependent activation of the translation machinery [26] that is most likely to be important for wound keratinocyte protein expression. In addition, our study suggested differential roles of the activation-responsible Ser473 and Thr308 residues [2] with respect to Akt1 translocation processes in insulin-stimulated keratinocytes.

Acknowledgments

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References

[1] J.R. Testa, P.N. Tsichlis, Akt signaling in normal and malignant cells, Oncogene 24 (2005) 7391–7393.
[2] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor–mTOR complex, Science 307 (2005) 1098–1101.
[3] E. Calautti, J. Li, S. Saoncella, J.L. Brisette, P.F. Goetinck, Phosphoinositide 3-kinase signaling to Akt promotes keratinocyte differentiation versus death, J. Biol. Chem. 280 (2005) 32856–32865.
[4] B.R. Trash, C.W. Menges, R.H. Pierce, D.J. McCance, Akt1 provides an essential survival signal required for differentiation and stratification of primary human keratinocytes, J. Biol. Chem. 281 (2006) 12155–12162.
[5] S. Pankow, C. Bamberger, A. Klippel, S. Werner, Regulation of epidermal homeostasis and repair by phosphoinositide 3-kinase, J. Cell Sci. 119 (2006) 4033–4046.
[6] X.D. Peng, P.Z. Xu, M.L. Chen, A. Hahn-Windgassen, J. Skeen, J. Jacobs, D. Sundararajan, W.S. Chen, S.E. Crawford, K.G. Coleman, N. Hay, Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2, Genes Dev. 17 (2003) 1352–1365.
[7] S. Werner, R. Grose, Regulation of wound healing by growth factors and cytokines, Physiol. Rev. 83 (2003) 835–870.
[8] D.R. Plas, C.B. Thompson, Akt-dependent transformation: there is more to growth than just surviving, Oncogene 24 (2005) 7435–7442.
[9] C.M. Taniguchi, B. Emanuelli, R.C. Kahn, Critical nodes in signalling pathways: insights into insulin action, Nat. Rev. Mol. Cell Biol. 7 (2006) 85–96.
[10] I. Goren, E. Müller, J. Pfeilschifter, S. Frank, Severely impaired insulin signaling in chronic wounds of diabetic ob/ob mice. A potential role of tumor necrosis factor-α, Am. J. Pathol. 168 (2006) 765–777.
[11] B. Stallmeyer, H. Kämpfer, N. Kolb, J. Pfeilschifter, S. Frank, The function of nitric oxide in wound repair: inhibition of inducible nitric oxide synthase severely impairs wound reepithelialization, J. Invest. Dermatol. 113 (1999) 1090–1098.
[12] S. Frank, B. Stallmeyer, H. Kämpfer, N. Kolb, J. Pfeilschifter, Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes (HaCaT) and during cutaneous wound repair, FASEB J. 13 (1999) 2002–2014.
[13] P. Boukamp, R.T. Petrussevska, D. Breitkreuz, J. Hornung, A. Markham, N. Fusenig, Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line, J. Cell Biol. 106 (1988) 761–771.
[14] H. Kämpfer, U. Kalina, H. Mühl, J. Pfeilschifter, S. Frank, Counterregulation of interleukin-18 mRNA and protein expression during cutaneous wound repair, J. Invest. Dermatol. 113 (1999) 369–374.
[15] E. Schreiber, P. Matthias, M.M. Müller, W. Schaffner, Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells, Nucleic Acids Res. 17 (1989) 6419.
[16] M. Bachmann, H. Hennemann, P.X. Xing, I. Hoffmann, T. Möröy, The oncogenic serine/threonine kinase Pim-1 phosphorylates and inhibits the activity of Cdc25C-associated kinase 1 (C-TAK1), J. Biol. Chem. 279 (2004) 48319–48328.
[17] P. Martin, Wound healing—aiming for perfect tissue regeneration, Science 276 (1997) 75–81.
[18] A.J. Singer, R.A.F. Clark, Cutaneous wound healing, N. Engl. J. Med. 341 (1999) 728–746.
[19] T.F. Franke, S.I. Yang, T.O. Chan, K. Datta, A. Kazlauskas, D.K. Morrison, D.R. Kaplan, P.N. Tsichlis, The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase, Cell 81 (1995) 727–737.
[20] M. Andjelkovic, D.R. Alessi, R. Meier, A. Fernandez, N.J. Lamb, M. Frech, P. Cron, P. Cohen, J.M. Lucocq, B.A. Hemmings, Role of translocation in the activation and function of protein kinase B, J. Biol. Chem. 272 (1997) 31515–31524.
[21] N.A. Syed, K.N. Horner, V. Misra, R.L. Khandelwal, Different cellular localization, translocation, and insulin-induced phosphorylation of PKB alpha in HepG2 cells and hepatocytes, J. Cell. Biochem. 86 (2002) 118–127.
[22] D. Gupta, N.A. Syed, W.J. Roesler, R.L. Khandelwal, Effect of overexpression and nuclear translocation of constitutively active PKB-α on cellular survival and proliferation in HepG2 cells, J. Cell. Biochem. 93 (2004) 513–525.
[23] L.R. Potter, T. Hunter, A constitutively 'phosphorylated' guanylyl cyclase-linked atrial natriuretic peptide receptor mutant is resistant to desensitization, Mol. Biol. Cell 10 (1999) 1811–1820.
[24] L. Bertrand, D.R. Alessi, J. Deprez, M. Deak, E. Viaene, M.H. Rider, L. Hue, Heart 6-phosphofructo-2-kinase activation by insulin results from Ser-466 and Ser-483 phosphorylation and requires 3-phosphoinositide-dependent kinase-1, but not protein kinase B, J. Biol. Chem. 274 (1999) 30927–30933.
[25] M.J. Wick, K.R. Wick, H. Chen, H. He, L.Q. Dong, M.J. Quon, F. Liu, Substitution of the autophosphorylation site Thr516 with a negatively charged residue confers constitutive activity to mouse 3-phosphoinositide-dependent protein kinase-1 in cells, J. Biol. Chem. 277 (2002) 16632–16638.
[26] D. Ruggero, N. Sonenberg, The Akt of translational control, Oncogene 14 (2005) 7426–7434.