Akt/PKB regulates laminin and collagen IV isotypes of the basement membrane

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Basement membranes are important for epithelial differentiation, cell survival, and normal and metastatic cell migration. Much is known about their breakdown and remodeling, yet their positive regulation is poorly understood. Our previous analysis of a fibroblast growth factor (FGF) receptor mutation raised the possibility that protein kinase B (Akt/PKB) activated by FGF is connected to the expression of certain laminin and type IV collagen isotypes. Here we test this hypothesis and demonstrate that constitutively active Akt/PKB, an important downstream element of phosphoinositide 3′-kinase signaling, induces the synthesis of laminin-1 and collagen IV isotypes and causes their translocation to the basement membrane. By using promoter–reporter constructs, we show that constitutively active phosphoinositide 3′-kinase-p110 or Akt/PKB activates, whereas dominant negative Akt/PKB inhibits, transcription of laminin β1 and collagen IV α1 in differentiating C2 myoblast- and insulin-induced Chinese hamster ovary–T cell cultures. These results suggest that Akt/PKB activated by receptor tyrosine kinases is involved in the positive regulation of basement membrane formation. The possible role of Akt/PKB-induced laminin and collagen IV synthesis in cell survival and differentiation will be discussed.

transcription | extracellular matrix | receptor kinases | P13K | signal transduction

Secreted laminin and type IV collagen chains form heterotrimers because of their mutual affinity and assemble into the basement membrane's (BM's) protein network, where they associate with additional proteins such as nidogens and heparan sulfate proteoglycans (HSPG) (1). Collagen IV and laminin isotypes bind to the cell membrane through integrin or dystroglycan receptors and induce cytoskeletal rearrangements, which contribute to the BM's final mat-like structure (2). They initiate signaling pathways through both their receptors (3) and HSPGs associated with them. HSPGs bind and present a number of polypeptide growth factors to cellular receptors (3, 4). Thus, the BM provides a platform for multiple signaling mechanisms, which may explain its importance in epithelial differentiation, cell migration, and morphogenesis. BMs are also involved in cell survival and metastasis formation, and their thickening is typical of late-stage diabetes. Therefore, the assembly (1, 5) and distribution of multiple BM components (6) and their breakdown by metalloproteinases (7) attracted extensive interest. Yet the positive regulation of BM components is poorly understood, although there is evidence for their *de novo* synthesis during development. Laminins (8) and their receptors (9, 10) are required in early embryogenesis and branching organogenesis (11–13). Because receptor tyrosine kinases are major inducers of cell differentiation and morphogenesis, we were interested whether they are involved in the positive regulation of BM components.

We were led to the analysis of these questions by recent results with embryonic stem (ES) cell-derived embryoid bodies. Dominant negative fibroblast growth factor (FGF) receptor (dnFGFR) cDNA inhibited BM formation and cell differentiation in this model. This mutation had little effect on E-cadherin or on the activation of the integrin-dependent kinase focal adhesion kinase, but abrogated the phosphorylation of protein kinase B (Akt/PKB) (14). While investigating the mechanism of this mutation, we found that FGF signaling is required for the synthesis of laminin and collagen IV isotypes characteristic of the early embryo. Their importance in FGF-induced embryoid body differentiation was demonstrated by partial rescue of the dnFGFR phenotype by Matrigel and purified laminin-1 (15). This finding raised the intriguing possibility that Akt/PKB may be involved in the activation and synthesis of laminin and collagen IV isotypes.

Akt/PKB is an antiapoptotic cytoplasmic serine–threonine kinase activated by phosphoinositide 3′-kinase (PI3K), which is controlled by cell surface receptors. Among the downstream targets of Akt/PKB are important transcriptional regulators, such as the *forkhead* multigene family and NFκB (16), but the multiple cellular mechanisms controlled by Akt/PKB are still subject to extensive research. In this study, we use constitutively active and dominant negative mutations of PI3K-p110 and Akt/PKB to test our hypothesis for its role in the positive control of the BM matrix.

Materials and Methods

Cell Culture and Cytology. ROSA 11 or AB-2.2 ES cells were cultured, and embryoid bodies were grown as before (14). Chinese hamster ovary (CHO) cells and the CHO-T clone, which expresses the insulin receptor (17) as well as C2 mouse myoblasts (18), were grown and differentiated as described. Embryoid bodies were observed by light microscopy after semithin sectioning (15) and electron microscopy after methacrylate embedding. PHOTOSHOP-5 (Adobe Systems, Mountain View, CA) and FREEHAND (Macro Media, Edinburg, U.K.) were used for digital images.

Constructs. To test laminin or collagen IV expression, constitutively active Akt1/PKBα (myrAkt$^{\Delta 4-129}$) (19) and p110α (p110$\alpha^{K227E\text{-}5'myr}$) (20) or a "kinase dead" dominant negative mutant of Akt/PKB (21) Akt^{AAA} or dnAkt) were used. To analyze collagen IV transcription, a fragment containing the 0.78-kb common promoter and the 5.0-kb enhancer of Col4A1 (22) was ligated into the pGL2 luciferase vector (Promega). To assay laminin transcription, a 2.3-kb genomic fragment upstream of the initiation site of LamB1 was isolated "ex silico" and ligated into pGL2. As control, enhanced green fluorescent protein (EGFP) was used in the same vector.

Abbreviations: BM, basement membrane; FGF, fibroblast growth factor; dnFGFR, dominant negative FGF receptor; ES, embryonic stem; PI3K, phosphoinositide 3′-kinase; Akt/PKB, protein kinase B; dnAkt/PKB, dominant negative Akt/PKB; WT, wild type; CHO, Chinese hamster ovary; EGFP, enhanced green fluorescent protein.

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DNA Transfer: Stable Expression. The pBabePuro retroviral expression vector (23), carrying myrAkt$^{\Delta 4-129}$ or p110$\alpha^{K227E\text{-}5'myr}$, or LacZ (as control) controlled by the PGK1 promoter was used. Ecotropic packaging cells (24) were used to infect ES cells. Puromycin-resistant stable clones expressing myrAkt$^{\Delta 4-129}$, or p110$^{K227E\text{-}5'myc}$ were collected. Transient transfection into CHO-T and C2 cells was with Lipofectamine, used according to the manufacturer's suggestions (GIBCO/BRL). As control, EGFP in the same expression vector was used.

RNA isolation and blot hybridization were as described (15).

Quantitative real time PCR (TIB Molbiol, Berlin) was performed on a LIGHTCYCLER (Roche Diagnostics), with quantitation of GAPDH mRNA synthesized by the cells as control. Total RNA was transcribed into cDNA by using oligo-dT$_{12-18}$ primers and Superscript II Reverse Transcriptase (GIBCO). Primer pairs for laminin α1: Fw-TGTAGATGGCAAGGTCTTATTTCA and Rev-CTCAGGCAGTTCTGTTTGATGT; for α2: Fw-TGTCGTGGGGATTCTGTATGTC and Rev-CAAGAAGGTCCAATCCAACTTT; for α4: Fw-GAAATGCGAATGGCTACCTA and Rev-TCATGCACCCCCTAAAGTTGAT; for α5: Fw-GACACCACGGGCATCAGT and Rev-CCATACCCCTGTGACATTGCAT; for GAPDH: Fw-TTGTCAGCAATGCATCCTGC and Rev-CCGTTCAGCTCTGGGATGAC. Probes for detection: for α1: fluorescein-CTGAAAGCCCCCACACCCATTCCA and LIGHTCYCLER red (LCR) 640- TCGGCAGACACCAACGATCCCATTTA; for α2: fluorescein-ACCTCCAGCTTTCACGTTGGGACAT and LCR 640-CTTTGCGAATGCAGAGAGTGGGACTT; for α4: fluorescein-GCGCCCCAAGAAGTCTTACAGTCC and LCR 640-GACCCTAAGAGCACACCTTCCCCT; for α5: fluorescein-GTGGCCGTGAAAAGTCATGGTGC and LCR 640-TCCCACTAGCAGGTCAGCGGTGCGA-PDH; fluorescein-CACCCAGAAGACTGTGGATGGCCCCT and LCR-640 TGGAAAGCTGTGGCGTGATGGCCG. Standard curves were obtained for each set with serial dilutions of RNA. Amplification curves were generated by LIGHTCYCLER software. The number of cycles (n) corresponding to the start of the logarithmic phase of the fluorescence signals was used to calculate the amount of mRNA by the formula $2^{(\Delta n)}$, where $\Delta n = n_{GAPDH} - n_{laminin}$ Each increase in Δn by 1 equals a 2-fold increase of specifically amplified mRNA.

For luciferase assays, 1.4×10^5 CHO cells or 3×10^4 C2 cells were plated into 24-well plates. Transfection of CHO-T cells was followed by incubation for 16 h in 10% serum or for 24 h in 0.5% serum and, for insulin treatment, for an additional 24 h in 0.5% serum plus 0.4 units insulin/ml. C2 myoblasts were grown in Dulbecco's high glucose medium with 20% FCS. Differentiation was induced by changing to 10% horse serum and 0.04 units insulin/ml after transfection for 24 h.

Immunoprecipitation. Embryoid bodies were labeled with [^{35}S]methionine (1 mCi/ml) and extracted as described (25). To analyze incorporation of single laminin chains into heterotrimers, aliquots of radioactivity were precipitated from each sample under nonreducing conditions by using affinity-purified anti-laminin α1, α2, or α4 antisera (25–27) or mAb 4C7 against laminin α5 (28). To detect these and the associated chains, precipitates were boiled in the presence of 2-mercaptoethanol and subjected to SDS/PAGE under reducing conditions. Polyclonal antibodies detecting collagen α1 (IV) and α2 (IV) (gift from R. Timpl) were used similarly. Gels were fixed, enhanced with Amplify (Amersham Pharmacia), dried, exposed, and quantified in a PhosphorImager (Fuji). For immunoblotting, cell lysates from embryoid bodies expressing constitutively active Akt/PKB were separated by SDS/PAGE under reducing conditions, and the blot was reacted with the laminin α1-specific antibody.

Results

Activated Akt/PKB Induces Increased Synthesis of Laminin and Collagen IV Isotypes. First, we investigated the effect of increased PI3K and Akt/PKB signaling on embryoid body differentiation. Constitutively active PI3Kαp110 (p110$^{\alpha K227E-5'myr}$) or constitutively active Akt1/PKBα (myrAkt$^{\Delta 4-129}$) was introduced into wild-type (WT) ES cells by retroviral infection. Clones that stably expressed these constructs (p110$^{\alpha K227E\text{-}5'myr}$-expressing clones were less stable than those with myrAkt$^{\Delta 4-129}$) differentiated faster than WT (Fig. 1*A*), and the resulting embryoid bodies displayed greatly expanded BMs (Fig. 1 *B* and *C*). By electron microscopy, massive accumulation of a extracellular matrix was apparent between the visceral endoderm and the columnar ectoderm (Fig. 1 *D* and *E*).

To investigate whether accumulation of BM-like structures is connected to increased protein synthesis, antibodies to various laminin-α chains or to type IV collagen were used to precipitate [^{35}S]methionine-labeled polypeptides. Selective increase of proteins reacting with antibodies to laminin-α1 and collagen IV was observed in embryoid bodies expressing myrAkt$^{\Delta 4-129}$ (Fig. 2*A*). The increase was 55-fold, as compared with the WT, in bands isolated by the laminin-α1 chain-specific antibody (Fig. 2*A* and Table 1). A slight increase of α2 containing laminins was also noted, whereas the amount of α4- and α5-specific laminins did not increase, suggesting that in embryoid bodies, Akt/PKB preferentially affect the laminin-1 isotype. This finding corresponded well with the embryonic expression pattern of this laminin isotype (6).

The electrophoretic pattern of polypeptides precipitated by anti-laminin-α1 revealed a 400- and a 200-kDa band under reducing conditions (Fig. 2*A*). The 400-kDa polypeptide represents the α1 chain, whereas the 200-kDa band represents the β1 and γ1 chains. Thus, the antibody precipitated the entire heterotrimer, which on reduction separated to its component chains. Further support derived from lysates immunoblotted with anti-laminin α1. Here, a single 400-kDa band of laminin α1 was detected in cultures expressing myrAkt$^{\Delta 4-129}$ but not in the control (Fig. 2*B*). The collagen α1- (IV) and α2- (IV) specific antibody detected a commensurate 62-fold increase due to Akt/PKB. Type IV collagen heterotrimers migrated as 170- and 330-kDa bands (Table 1 and Fig. 2*A*). Shorter exposure resolved the smaller polypeptide to a 160- and a 167-kDa band, corresponding to the α1 and α2 chains of type IV collagen, whereas the 330-kDa band separated into two homo- and heterodimer bands (Fig. 2*C*). These results collectively suggest that the BM thickening in embryoid bodies, which express constitutively active Akt/PKB, is because of a massive increase in laminin-1 and collagen IV synthesis. They also show that the overexpressed protein chains assemble into their native heterotrimeric form and translocate to the BM.

Increased amino acid incorporation suggested that myristoylated Akt/PKB induced active polypeptide synthesis. To obtain further evidence, RNA blot hybridization was performed. Significant increase of LamA1, LamB1, LamC1, and Col4A1 transcripts followed the kinetics of embryoid body differentiation from two different ES clones expressing myrAkt$^{\Delta 4-129}$. Increased steady-state mRNA levels were detected at day 2. They reached maximum at day 4 (Fig. 3*A*), when BM formation is at its highest (15). Significant activation was detected with probes specific to the genes encoding the α1, β1, and γ1 chains of laminin-1 as well as with the probe to collagen α1 (IV) (Fig. 3*B*). Quantitative real-time reverse transcription–PCR clarified the amount and type of various laminin α chain mRNAs and revealed selective activation of LamA1, the gene specific for isotype 1. The degree of increase (57-fold) fitted well with enhanced protein synthesis (Table 1).

These results demonstrate the activation of laminin and

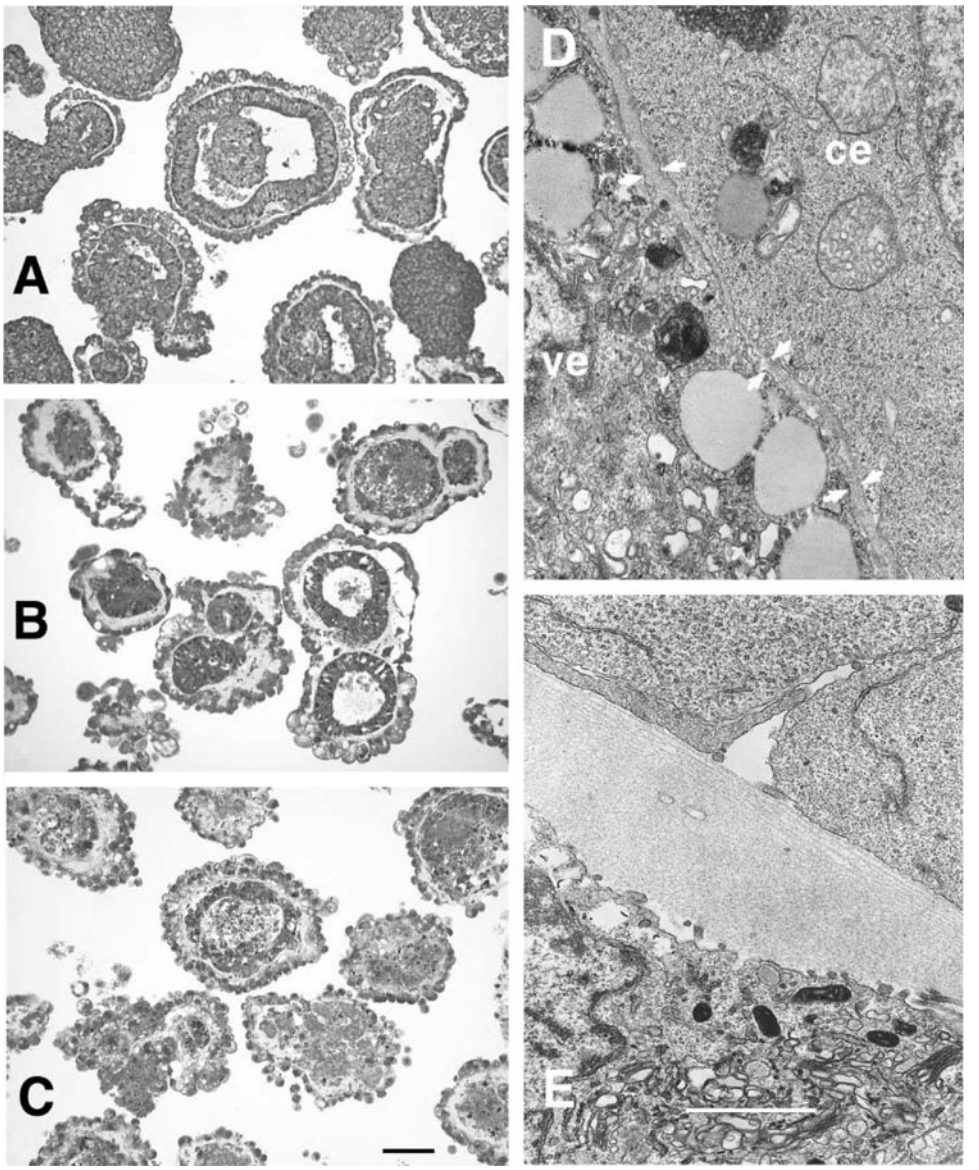

Fig. 1. Greatly extended BMs in embryoid bodies expressing constitutively active Akt/PKB or PI3K-p110. (*A*) Control, AB2.2-derived embryoid body; (*B*) embryoid body expressing PI3K-p110$^{K227E\text{-}5'myr}$ (these clones proved to be unstable); (*C*) embryoid body expressing myrAkt$^{\Delta4-129}$. (Bar = 35 μm.) (*D*) Electron microscopy of control (arrows indicate the BM) and (*E*) myrAkt$^{\Delta4-129}$-expressing embryoid body. All cultures at day 6. ce, columnar ectoderm or epiblast; ve, visceral endoderm. (Bar = 0.5 μm.)

collagen IV synthesis by Akt/PKB and show their translocation to the BM. They confirm our suggestion of Akt/PKB-mediated control of genes encoding the network forming proteins of the BM, which was based on the abrogation of laminin and collagen IV synthesis by a dnFGFR mutation coupled with reduced Akt/PKB phosphorylation (14, 15).

Next, it was important to clarify the mechanism of laminin and collagen IV induction by Akt/PKB. We chose an approach that does not overactivate multiple mediators connected with the PI3K pathway. To this end, we used systems where BM formation is activated endogenously. Induction of laminin and collagen IV synthesis was assayed by specific inhibition with a dominant negative mutation of Akt/PKB (19). As readout, luminescence from a luciferase reporter, ligated to the regulatory region of laminin β1 or collagen α1 (IV), was measured. This arrangement enabled testing of whether endogenously activated Akt/PKB induces laminin and collagen IV synthesis at the level of transcription.

Dominant Negative Akt/PKB Inhibits Laminin and Collagen IV Transcription in Differentiating Myoblasts. Muscle fibers are enveloped in BMs that also contribute to the neuromuscular synapse. During myogenesis, individual myoblasts aggregate and form syncitia covered by BM. Thus, BM formation is an essential part of myogenesis. We studied laminin and collagen IV transcription as a function of primary muscle fiber formation in C2 myoblasts (18). Undifferentiated myoblasts were transfected with LamB1-luciferase or Col4A1-luciferase together with dnAkt/PKB. As control, EGFP was used in the same vector. Luminescence was measured 24–36 h later at an early stage of myoblast fusion. Considerable activation was observed from both promoters because of their activation during myogenesis (Fig. 4*A*). Complete abrogation of this reaction by dnAkt/PKB demonstrated that endogenous Akt/PKB signaling is required for the transcriptional activation of laminin β1 and collagen α1 (IV) regulatory regions during myoblast differentiation (Fig. 4*A*). The

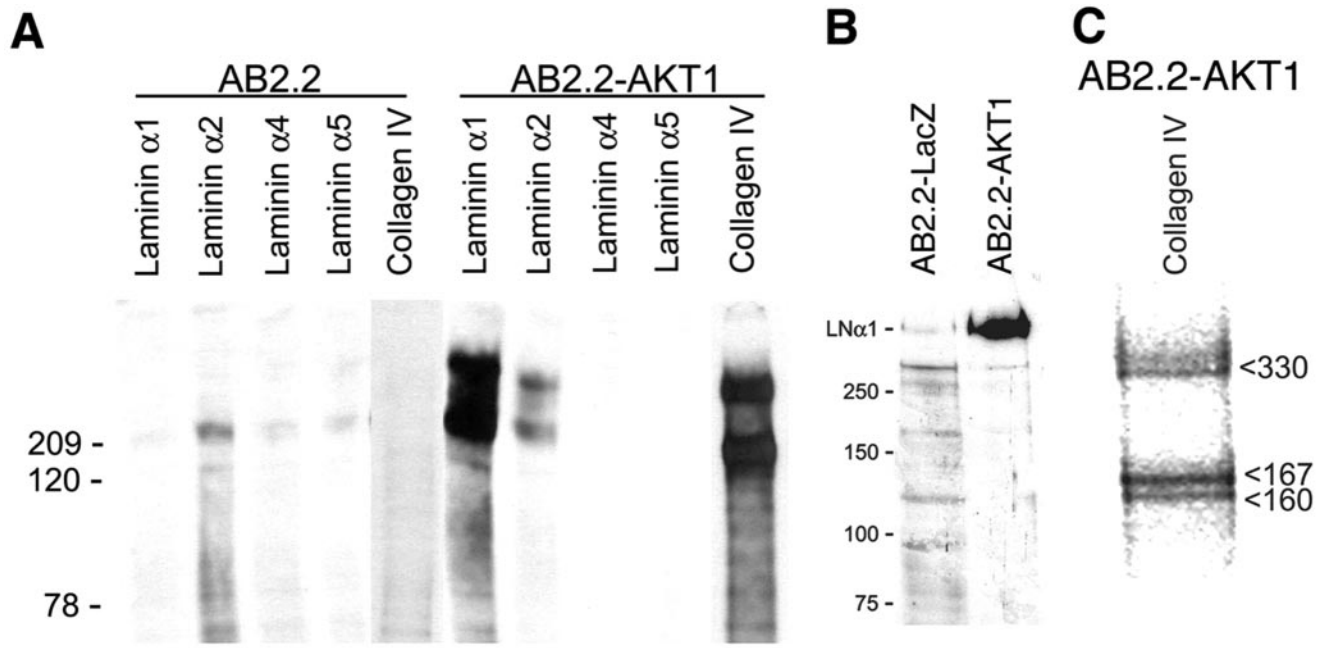

Fig. 2. Constitutively active Akt/PKB induces increased expression of laminin and collagen isotypes. (*A*) Immunoprecipitation of cell lysates from sixth-day embryoid body cultures labeled with [^{35}S]methionine (1 mCi/ml). Precipitation with antibodies to laminin α chains or collagen IV. Protein complexes were dissociated by reduction and resolved by SDS/PAGE. The control bands become visible by overexposure of the film. (*B*) Immunoblot of cell lysates from AB2.2-derived control embryoid bodies and from those expressing constitutively active Akt/PKB. The blot was reacted with antibody to laminin α1. Three-fold excess was applied from the control lysate. (*C*) Shorter exposure of biosynthetically labeled collagen IV immunoprecipitates from embryoid bodies expressing myrAkt$^{\Delta 4-129}$ resolved the 160-kDa band into a 160- and a 167-kDa band and separated the ≈330-kDa band into hetero- and homodimers. AB2.2 untreated control, AB2.2-derived embryoid bodies; AB2.2-LacZ, embryoid body from ES cells carrying the control vector with LacZ; AB2.2-Akt1, a clone expressing constitutively active Akt/PKB.

upstream elements inducing myogenesis after partial growth factor withdrawal are yet to be defined. Nevertheless, whereas Fgf signaling inhibits myogenesis, insulin, which was added to the differentiation medium, promotes myogenesis by activating NFκB (29), which is a target of Akt/PKB (16).

It follows that the synthesis of collagen and laminin IV isotypes is regulated by transcriptional machinery induced by Akt/PKB. Additional conclusions are relative to the laminin isotypes expressed in muscle fibers. The BM of muscle fibers contains the laminin-2 isotype made of α2, β1, and γ1 chains, whereas neuromuscular synapses that are partially formed in these cultures contain laminin α4 and α5 chains (30). Because our construct carried regulatory sequences of the rather widely distributed β1 chain, it had the potential to detect more than one laminin isotype. It is therefore possible that the PI3K–Akt/PKB pathway activates multiple loci of various laminin and collagen IV chains. Coordinate activation of laminin and collagen IV has been observed previously in the retinoic acid induced differentiation of F9 teratocarcinoma cells (31).

Akt/PKB Activated by the Insulin Pathway Induces LamB1 and Col4A1 Transcription. The PI3K pathway can be activated by numerous signaling receptors. We were therefore interested in whether Akt/PKB-activated laminin and collagen IV synthesis is controlled by one or more receptor. Whereas muscle differentiation may not be connected to a single receptor, our previous results with embryoid bodies implicated an FGFR mutation in the abrogation of laminin-1 and collagen IV synthesis (15). Here, as a third system, we used CHO cells, which naturally express laminin isotypes (32). Their CHO-T clone, which carries an insulin receptor (17), provided the opportunity to test whether insulin signaling might also activate laminin and collagen IV expression.

Table 1. Relative amounts of mRNA and protein in embryoid bodies

	Ab2.2 mRNA*	AKT mRNA*	Times increase	Ab2.2 protein†	AKT protein†	Times increase
Laminin α 1	11.4 ± 1.0	652 ± 58	57	70	3,873	55
α2	0.21 ± 0.06	0.54 ± 0.11	2.6	82	773	9.4
α4	3.63 ± 0.82	3.54 ± 0.88	0.98	40	17	0.42
α5	0.81 ± 0.08	0.04 ± 0	0.05	296	11	0.04
Collagen IV	ND	ND		14	867	62

*Quantitative reverse transcription–PCR with sequence-specific hybridization probes. Expression of laminin was normalized to GAPDH expression levels and expressed as relative amounts of mRNA. Mean ± SD (n = 3). No laminin α3 chain mRNA was detected in either sample, although the probes did detect this mRNA in cells known to express laminin α3. ND, not done.

†Relative amounts of protein. Immunoprecipitation of proteins from ^{35}S-methionine labeled cells. Aliquots of equal radioactivity of lysates were precipitated with chain-specific antibodies. Polypeptide chains were resolved by SDS/PAGE, and the results were quantified in a PhosphoImager (Fuji).

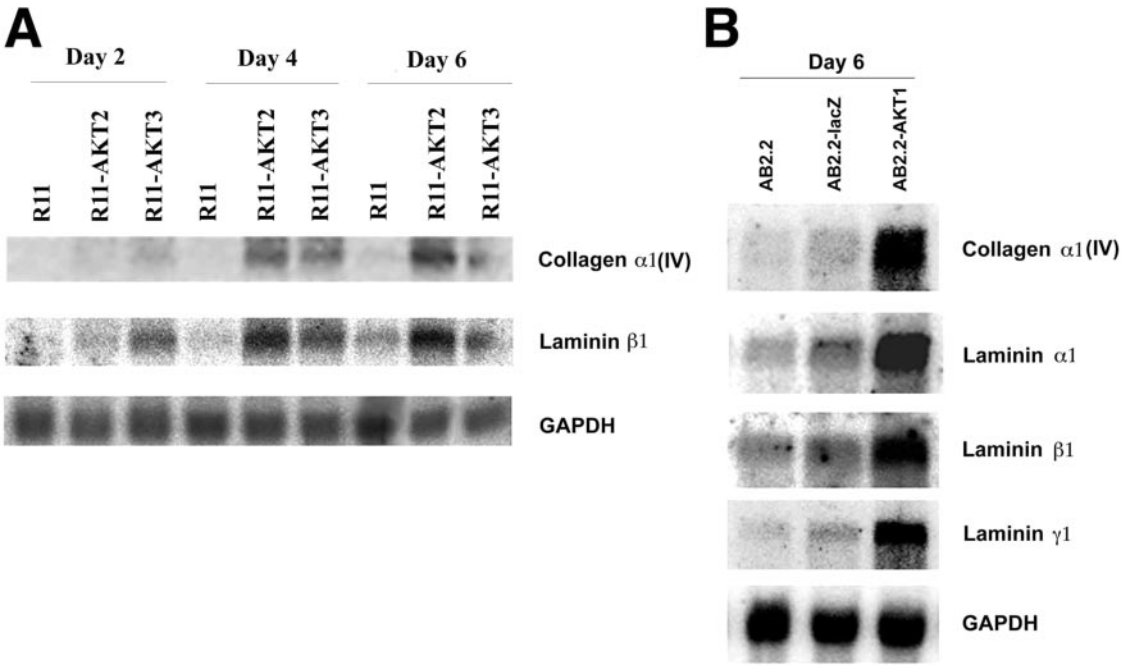

Fig. 3. Increased synthesis of laminin and type IV collagen mRNA. RNA blot hybridization of ROSA 11 (R11), AB2.2 or AB2.2-LacZ derived control embryoid bodies and R11- (Akt2 and -3) or AB2.2- (AB2.2–1) derived embryoid bodies deriving from clones expressing constitutively active Akt/PKB. (*A*) The kinetics of increased laminin-α1 and type IV collagen expression follows the kinetics of embryoid body differentiation. (*B*) Expression of laminin α1, β1, and γ1 and collagen α1 (IV) chain RNA in control (AB2.2) embryoid bodies in embryoid bodies expressing ''empty'' (LacZ-containing) vector and in a AB2.2-derived embryoid body clone (AB2.2-Akt1) expressing constitutively active Akt/PKB.

First, constitutively active Akt/PKB or PI3K-p110 was tested. We observed a 10- to 20-fold increase of Col4A1 transcription as the result of this activation (Fig. 4*B*). Activation by constitutively active PI3K was inhibited by 75%, whereas that by myristoylated Akt/PKB was inhibited by 50%, as a result of treatment with Ly294002. In the next experiment, insulin was added to CHO-T cells. A high level of hormonally induced transcription was obtained, and it was almost completely inhibited by dnAkt/PKB. This construct also inhibited basal level transcription, lending further support for the involvement of Akt/PKB in the regulation of laminin and collagen IV isotypes (Fig. 4*C*). It follows that the insulin pathway can induce LamB1 and Col4A1 transcription through Akt/PKB. We conclude, therefore, that more than one receptor tyrosine kinase maybe involved in the positive regulation of the BM matrix.

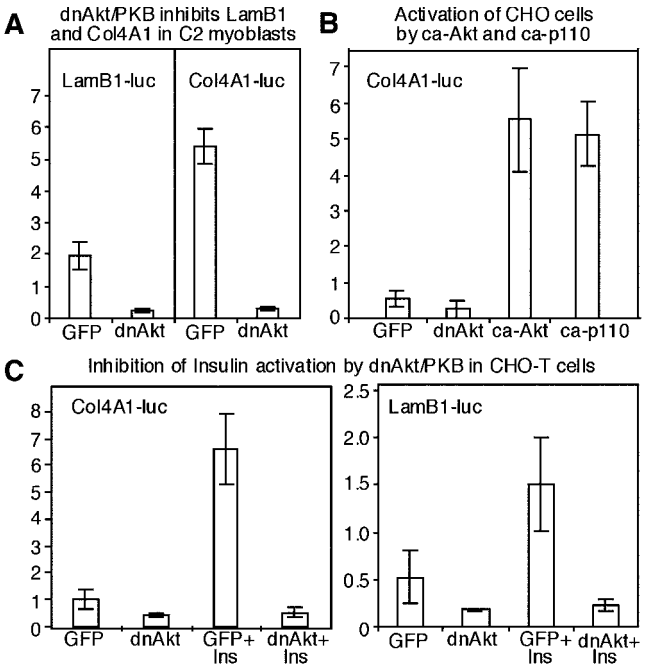

Fig. 4. PI3K-p110 and Akt/PKB are positive regulators of laminin and collagen IV transcription. ca-Akt, constitutively active Akt/PKB; ca-p110, constitutively active PI3Kp110; dnAkt, dominant negative Akt/PKB; GFP, empty or control vector incorporating EGFP; Ins, insulin treatment; luc, luciferase. Each experiment was repeated at least three times. Construct, transfection, and luciferase assays as in *Materials and Methods.*

It should be mentioned that Akt/PKB is a well known target of insulin signaling (for review, see ref. 16). Interestingly, late-stage diabetes is characterized by widespread BM hypertrophy, similar to that observed in our embryoid body cultures that express myrAkt$^{\Delta 4-129}$. Our findings may help to analyze the causes of this pathology.

Discussion

We demonstrate that Akt/PKB activates transcription and synthesis of laminin and collagen IV isotypes. Results with embryoid bodies shown here demonstrate that laminin and collagen IV chains assemble into their native heterotrimeric structure and translocate to the BM on activation by Akt/PKB. In differentiating myoblasts, dnAkt/PKB inhibited physiologically induced laminin and collagen synthesis by competing with the endogenously activated WT molecule. It appears, therefore, that receptor tyrosine kinases beyond FGF, including the insulin receptor, activate this pathway. Similar observations were made with 293T human kidney cells (unpublished work). Evidence for Akt/PKB activation by the integrin activated cytoplasmic kinase, focal adhesion kinase (33), raises the question whether integrins may also activate laminin and collagen IV isotypes.

We demonstrate that Akt/PKB activates laminin and collagen IV at the level of transcription. Akt/PKB controls numerous

transcription factors (16). Whether the *forkhead* family, the NFκB system, or other mechanisms connect Akt/PKB activation with the transcription of laminin and collagen IV chains remains to be determined. It is tempting, nevertheless, to speculate that this regulation represents the positive side of BM remodeling, whereas metalloproteinases represent its negative side. Such positive regulation of BM formation could result in the local amplification of cell signaling mediated by the various signaling molecules associated with the BM.

Our hypothesis stems from *in vitro* experiments and therefore requires support at the organism level. Targeted mutagenesis of PI3K-p110α causes early midgestation lethality of the mouse embryo (34), and its dominant negative and constitutively active forms affect angiogenesis in the chorioallantoic membrane of the chicken embryo (35). According to recent gene targeting experiments, loss of Akt2 function results in type II diabetes (36), whereas loss of Akt1 causes growth retardation and increased apoptosis (37). Failure of these models to cause a more severe phenotype may indicate substrate specificity, and mutant crosses may clarify which isotype or isotypes are involved in cell signaling, laminin, and collagen IV synthesis.

The analysis discussed here stems from results with a dnFgfr mutation of embryoid bodies, characterized by defective epithelial differentiation and deficient Akt/PKB activation (14). This mutation was non-cell-autonomous, that is, mutant cells could not produce but could respond to an extracellular differentiation factor of the WT. This factor acted at a short radius or by contact, and it was identified with matrix forming elements of the BM at two counts. Mutant embryoid bodies synthesized no laminin and collagen IV chains, and the defect was partially restored by externally added Matrigel or purified laminin-1 (15). We therefore predicted that the PI3K–Akt/PKB pathway regulates laminin and collagen synthesis. The present data validate and expand this hypothesis and extend it to additional receptors and pathways.

Another FGF-mediated developmental mechanism relates to gastrulation. Loss of Fgfr1 causes retention of mesoderm precursors in the primitive streak. These cells, in contrast to the WT, fail to down-regulate E-cadherin, an adhesion molecule that interacts with the β-catenin-wnt pathway. Ciruna and Rossant conclude that retention of E-cadherin by Fgfr1$-/-$ mesoderm precursors results in impaired adhesion and differentiation (38). This FGF-controlled cell-autonomous mechanism of gastrulation complements our results, which are relevant for the earlier pregastrulation egg-cylinder stage of mammalian development. We assume that the two mechanisms are not exclusive; they may occur either in succession or in synchrony.

We describe the activation of laminin and collagen IV in four different cellular systems, emphasizing that this positive control mechanism may have broader relevance. Akt/PKB is best known for its antiapoptotic effect. Its *in vivo* manifestation is associated with mutations that inactivate PTEN, its negative regulator, and occur frequently in advanced human cancer (39). The apoptotic process most closely associated with the BM is anoikis, the programmed death of normal cells that detach from the BM. Significantly, most tumor cells are free from this restriction (40). BM attachment and Akt/PKB expression both rescue detached cells by a mechanism that is active before the caspase-mediated cell death cascade becomes effective (41). This mechanism could be related to the activation of BM assembly via the PI3K pathway.

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