AKT1 Overexpression in Endothelial Cells Leads to the Development of Cutaneous Vascular Malformations In Vivo

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Background: Vascular malformations are clinical disorders in which endothelial cells fail to remodel and/or undergo programmed cell death, leading to abnormal persistence of blood vessels. The abnormal persistence of vessels makes therapy difficult because these lesions are resistant to interventions that are effective against hemangiomas. Akt1 is a serine-threonine protein kinase, which is a key mediator of resistance to programmed cell death. Our objective was to determine whether sustained activation of Akt1 could lead to vascular malformation in mice.

Observations: We examined the effect of constitutive activation of Akt1 in murine endothelial cells (MS1 cells). Overexpression of active AKT1 in MS1 cells led to the development of vascular malformations, characterized by wide endothelial lumens and minimal investment of smooth muscle surrounding the vessels. The histologic features of these vascular malformations are distinct from ras-transformed MS1 cells (angiosarcoma) and suggest that differing signal abnormalities give rise to human vascular malformations vs malignant vascular tumors.

Conclusions: Inhibition of Akt signaling may be useful in the treatment of vascular malformations. Examination of problematic hemangiomas and vascular malformations for the presence of activated Akt or downstream targets of Akt, such as mammalian target of rapamycin (mTOR), may predict response to treatment with Akt inhibitors or rapamycin. This study provides a potential rationale for the systemic and topical use of these inhibitors for vascular malformations and hemangiomas.

Arch Dermatol. 2007;143:504-506

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Methods

Cell Lines

MS1 cells are murine endothelial cells immortalized by infection with an ecotropic retrovirus encoding SV40 large T antigen. When injected subcutaneously into nude mice, these
cells form benign hemangiomas. Subsequent efforts to transduce MS1 cells with a myristoylated Akt retroviral supernatant were accomplished with 293T cells by cotransfection with pbabepuroHA-myrAkt (or control pbabepuru) and pCLEco, which encodes gag, pol, and env proteins specifying the ecotropic receptor, using a FuGene 6 transfection reagent (Roche Applied Science, Indianapolis, Ind). Thirty-six hours after transfection, the 293T medium was collected and filtered through a 0.45-µm syringe filter onto the MS1 cells, and protamine sulfate was added to a concentration of 0.6 mg/mL. After 3 hours, the medium was replaced with Dulbecco modified Eagle medium plus 5% fetal calf serum. Thirty-six hours after infection, the cells were selected with 1-µg/mL puromycin for 3 days, at which time 100% of the noninfected cells were dead.

WESTERN BLOT ANALYSIS

Cells were grown on 10-cm dishes and allowed to reach approximately 80% confluence before protein isolation. Sample aliquots normalized for protein content were size fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were transferred to a polyvinylidene fluoride microporous membrane. The blots were incubated in blocking solution (phosphate-buffered saline with 2% [weight-volume ratio] bovine serum albumin) for 1 hour at room temperature. The blots were then incubated overnight in sheep polyclonal anti-Akt (Upstate Biotechnology, Lake Placid, NY), rabbit polyclonal antiphosphorylated Akt (Ser 473; Cell Signaling Technology, Beverly, Mass), monoclonal antiras antibody (Transduction Laboratories, Lexington, Ky), or monoclonal antiantiactin (Chemicon International Inc, Temecula, Calif). Blots were washed 3 times with phosphate-buffered saline with 0.05% Tween 20 and were then incubated 1 hour at room temperature in secondary antibody solution consisting of a 1:16 000 dilution of horseradish peroxidase–conjugated anti–rabbit IgG (Amersham Pharmacia Biotech, Piscataway, NJ) or 1:10 000 dilution of horseradish peroxidase–conjugated anti–mouse IgG (Amersham Pharmacia Biotech) and washed 3 times with phosphate-buffered saline with 0.05% Tween 20. Finally, the blots were developed using enhanced chemiluminescence.

IN VIVO TUMORIGENESIS STUDIES

One million MS1 or MS1 m-Akt cells were injected subcutaneously into nude mice as described in our previous studies. MS1-derived cells were allowed to remain in nude mice up to 2 months prior to killing. Tumor tissue was fixed in formalin and stained with hematoxylin-eosin.

RESULTS

CONSTITUTIVELY ACTIVE Akt EXPRESSION LEADS TO THE DEVELOPMENT OF VASCULAR MALFORMATIONS

Introduction of AKT1 into endothelial cells leads to a high-level expression of activated Akt (Figure 1). Akt is also known to cause phosphorylation of another transcription factor, FKHR-1, which is accomplished in our cells by Akt introduction (Figure 1). To determine the effect of Akt activation on in vivo transformation, 1 million cells were injected subcutaneously into the right flank of 3 male nude mice. Long-term implantation (1 month) of MS1 m-Akt and SVR angiosarcoma (hematoxylin-eosin, original magnification ×40). Note the contrasting histologic features of wide vascular lumens with minimal smooth muscle investment in Akt overexpression cells compared with the highly cellular morphologic features of the SVR angiosarcoma.

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We have previously shown that ras transformation increased angiogenic activity in immortalized endothelial cells and induces Akt as well as other signaling pathways. Akt is a well-studied downstream mediator of ras activity, and Akt has been implicated in transformation and protection from apoptosis.7,8 We studied the effect of Akt overexpression alone to determine whether Akt could confer the ability of endothelial cells to persist in vivo. Surprisingly, we found that constitutive overexpression of Akt led to the development of lesions that closely resemble vascular malformations in that they have wide lumens with small amounts of smooth muscle investment.

Recent data have shown that too much Akt can perturb angiogenesis. Targeting Akt to endothelial cells of transgenic mice leads to perinatal lethality owing to the development of vascular malformations.9 Interestingly, in the mice that have the induced akt transgene, total Akt levels remain the same, even though induction of Akt by the withdrawal of doxycycline leads to increased phosphorylation of Akt.9 This may be due to a feedback suppression of endogenous Akt expression by Akt induction. Our model has the advantage that it can be transplanted to nude mice and thus be used to evaluate potential therapies for vascular malformations.

Our findings provide a rationale for the use of Akt inhibitors and drugs that work downstream of Akt, such as rapamycin,11 for the treatment of vascular malformations. Akt inhibitors in clinical trial include perifosine and miltefosine, drugs originally developed for the treatment of leishmaniasis.12-13 Another protein, mammalian target of rapamycin (mTOR), and downstream activators of mTOR, such as S6 kinase, can be detected in paraffin sections by immunohistochemical analysis.14 Rapamycin has already been used for the treatment of other vascular neoplasms, including Kaposi sarcoma, as well as dermatomyositis, and is well tolerated by patients.15,16 Our findings suggest that vascular malformations that demonstrate activation of the Akt pathway, including mTOR and S6 kinase, may be treated with currently available drugs such as rapamycin and novel drugs such as Akt inhibitors.

Accepted for Publication: August 14, 2006.
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Financial Disclosure: None reported.
Funding/Support: Dr Arbiser was supported by grants R01 AR47901, P30 AR42687, and Emory Skin Disease Research Core Center Grant P30 AR42687 from the National Institutes of Health, as well as by a VA Merit Award.

Acknowledgment: We thank J. Ann Le Good, PhD, Mary Hendrix, PhD, and Elisabeth Seftor, PhD, for their critical evaluation of this manuscript and J. Dewald, BS, for performing Western blot analysis.

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