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ORIGINAL ARTICLE

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The skin and hair as surrogate tissues for measuring the target effect of inhibitors of phosphoinositide-3-kinase signaling

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Abstract *Background*: The purpose of the study was to evaluate the use of phospho-Akt in mouse and human skin as a surrogate target for tumor phospho-Akt to measure the effect of antitumor inhibitors of phosphatidylinositol-3-kinase (PI-3-K)/Akt (protein kinase B) signaling. *Method*: The expression of phosphoSer^{473}-Akt was quantitatively assessed by Western blotting in human HT-29 colon, MCF-7 breast, A-549 non small cell lung tumor xenografts in mice, and by immunohistochemistry in mouse skin and human hair. *Results*: The pattern of PI-3-K isoforms in human hair keratinocytes was similar to that in tumor but mouse hair keratinocytes showed a different pattern. A high level of phospho-Akt staining was present in keratinocytes of the external root sheath of the hair and was inhibited by the PI-3-K inhibitor **PX-866** administered to mice, and in human hair exposed to **PX-866** in culture. The inhibition of phospho-Akt by **PX-866** in mouse hair keratinocytes was greater than inhibition of phospho-Akt in HT-29 and A-549 xenografts in the same mice. Phospho-Akt in mouse hair keratinocytes was inhibited by the Akt inhibitor **PX-316** to a lesser degree than in MCF-7 tumor xenografts. *Conclusions*: Hair offers a way of measuring the effects of PI-3-K signaling inhibitors and, in cancer patients, may provide a readily obtainable surrogate tissue for assessing PI-3-K and phospho-Akt inhibition in tumor.

Keywords Skin · Hair · Phospho-Akt · PtIns-3-kinase activity

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Introduction

The phosphoinositide-3-kinase (PtdIns-3-kinase)/Akt (protein kinase B) signaling pathway promotes cell survival in human tumors [3, 6]. PtdIns-3-kinases phosphorylate membrane PtdIns on the three position of the *myo*-inositol ring leading to the formation of PtdIns-3-phosphates and the recruitment of cytoplasmic Akt (protein kinase B) to the plasma membrane [10]. There are eight mammalian PtdIns-3-kinases that are divided into three main classes based on sequence homology and substrate preference [28]. Class I enzymes produce PtdIns(3,4,5)P_3 and are subdivided into class Ia, which includes p110α PtdIns-3-kinase and the closely related p110β and p110δ PtdIns-3-kinases. p110α and p110β are found in all adult human tissues, while p110δ is found in leukocytes. All class Ia kinases are associated with a p85 regulatory/adapter subunit. Three genes that can also generate splice variants encode at least 8 p85 adaptor subunits [24]. Different p85 subunits show differences in tissue distribution that may be of functional significance, but there does not appear to be a preferential association between the p85 subunits and any of the p110s. Class Ib PtdIns-3-kinase is represented by p110γ, which associates with a p110 adapter subunit and is confined largely to leukocytes [14]. Class II PtdIns-3-kinases are larger molecules of around 200 kDa without adapter subunits and give rise to PtdIns(3)P and PtdIns(3,4)P_2. Class III PtdIns-3-kinase has one member, Vps34p, which is constitutively active and is involved in lysosomal protein trafficking. The lipid product of Vps34p is PtdIns(3)P.

It is likely that all mammalian cells express representatives of each class of PtdIns-3-kinase [24]. Class 1a PtdIns-3-kinases are activated by transmembrane

receptors and oncogenic tyrosine kinases through the binding to Src homology 2 (SH2) domains found in all p85 isoforms [6] and by $p21^{Ras}$ [23]. Class 1b PtdIns-3-kinase is activated by $\beta\gamma$ subunits of heterotrimeric G proteins released upon the activation of 7-pass transmembrane receptors [14]. Class II PtdIns-3-kinases are activated by tyrosine kinase coupled receptors, although their mechanism of activation and function are largely unknown.

Akt binds through its pleckstrin homology (PH) domain to membrane PtdIns(3,4,5)P_3 and is activated by phosphorylation on Thr^{308} and Ser^{473} by membrane-associated phosphoinositide dependent kinases (PDKs) [2]. Activated Akt detaches from the plasma membrane [17] and phosphorylates a battery of targets including the forkhead transcription factor family members [20], the pro-apoptotic Bcl-2 family member Bad [8], apoptosis signaling kinase-1 (ASK-1), and procaspase-9, the initiator of the caspase cell death cascade [7]. The end result of PtdIns-3-kinase/Akt activation is the decreased expression of death genes and increased cell survival [20]. The dual specificity tyrosine-threonine/Ptdins-3-phosphatase tumor suppressor protein phosphatase and tensin homologue deleted on chromosome ten (PTEN) that is lost in many tumors prevents the accumulation of PtdIns(3,4,5)P_3 and, thus, attenuates Ptdins-3-kinase signaling [5].

Phosphoinositide-3-kinase/Akt signaling is activated in a large number of human cancers, either through increased PtdIns-3-kinase, increased Akt, deleted PTEN, or a combination of these mechanisms, and is a major factor in promoting cancer cell survival and resistance to many current cancer therapies [6]. Consequently, the pathway is of great interest for therapeutic intervention in cancer [4, 24, 30] and a number of inhibitors of PtdIns-3-kinase or Akt are under development as cancer drugs [10, 12, 19, 30]. An important aspect of the development of PtdIns-3-kinase/Akt signaling inhibitors as clinical agents, and indeed for any targeted cancer drug, is to have a way to evaluate the drug's ability to inhibit its target in patients [22]. This is most conveniently done in a surrogate normal tissue since it is difficult to obtain tumor biopsies before and after treatment from more than a few patients, particularly during the early phases of clinical drug development. We have investigated the use of skin and hair as surrogate normal tissues to measure the effects of inhibitors of PtdIns-3-kinase and Akt on PtdIns-3-kinase signaling and compared the effects to inhibition in human tumor xenografts.

Materials and methods

Compounds

Rabbit purified polyclonal anti-phosphoSer^{473}-Akt antibody (1:50 dilution), anti-Akt antibody (1:50 dilution), anti-human PtdIns-3-kinase 110α antibody (1:100 dilution), and anti-human PtdIns-3-kinase p110γ antibody (1:150 dilution) were obtained from Cell Signaling Technology (Beverly, MA, USA). Rabbit polyclonal anti-human PtdIns-3-kinase 110β antibody (0.75 μg/ml) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and mouse monoclonal anti-human PtdIns-3-kinase p110δ antibody (5 μg/ml) from Upstate (Chicago, IL, USA). Hydroxypropyl-β-cyclodextrin (Trappsol) ® was obtained from Cyclodextrin Technologies Development (High Springs, FL, USA). The PtdIns-3-kinase inhibitor **PX-866** (acetic acid (1*S*,4*E*,10*R*,11*R*,13*S*,14*R*)-[4-diallylaminomethylene-6-hydroxy-1-methoxymethyl-10,13-dimethyl-3,7,17-trioxo-1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxa-cyclopenta[a]phenanthren-11-yl ester) and the Akt inhibitor **PX-316** (D-3-deoxy-phosphatidyl-myo-inositol 1-[(R)-2-methoxy-3-octadecyloxypropyl hydrogen phosphate) were synthesized as previously described [18, 29]. Gefitinib (ZD 1839, Iressa™) was obtained from Astra Zeneca (Macclesfield, UK). For administration to mice, **PX-866** was dissolved at 1 mg/ml in 5% ethanol in 0.9% NaCl and **PX-316** at 10 mg/ml in 20% hydroxypropyl-β-cyclodextrin, 5% ethanol in 0.9% NaCl, and both were administered intravenously (i.v.) within 30 min. Gefitinib was administered orally (po) by gavage as a 7.5 mg/ml suspension in 0.1% Tween 20 in water.

Cells and animals

HT-29 human colon cancer cells, MCF-7 human breast cancer cells, and A-549 human non small cell lung cancer cells were obtained from the American Tissue Type Collection (Rockville, MD, USA) and grown in humidified 95% air, 5% CO_2 at 37°C in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cell lines were tested to be mycoplasma-free using a PCR ELISA kit (Roche Diagnostics Inc., Indianapolis, IN, USA). 10^7 HT-29, MCF-7, or A-549 cancer cells were injected subcutaneously into the flanks of female severe combined immunodeficient (*scid*) mice and allowed to grow to approximately 300 mm^3. Mice that received MCF-7 human breast cancer cells were implanted with a 60 day 17-β-estradiol slow release pellet (Innovative Research, Sarosota, FL, USA) 1 day before tumor implantation. Mice were administered **PX-866** as a single dose of 5–20 mg/kg i.v., 2.5 10 mg/kg po, 10 mg/kg i.v. and po, alone or 3 h before administration of the EGFR inhibitor gefitinib at 75 mg/kg po. The Akt inhibitor **PX-316** was administered as a single i.v. dose of 37.5–150 mg/kg.

Western blotting for tumor phospho-Akt

Tumors were rapidly excised from the mice and frozen as 5 mm^3 fragments in liquid N_2. For assay, the tumors were homogenized in 50 mM HEPES buffer, pH 7.5, 50 mM NaCl, 1% Nonidet® P40, and 0.25% sodium deoxycholate. Twenty microgram of total protein was

boiled for 5 min, loaded on a 12% acrylamide/bis-acrylamide gel, and separated by electrophoresis at 160 V for 40 min. Proteins were electrophoretically transferred to polyvinylidene fluoride membranes, pre-incubated with a blocking buffer of 137 mM NaCl, 2.7 mM KCl, 897 mM $CaCl_2$, 491 mM $MgCl_2$, 3.4 mM Na_2HPO_4, 593 mM KH_2PO4, 5% bovine serum albumin, and incubated overnight with anti-phospho-Ser^{473}-Akt polyclonal antibody or anti-Akt polyclonal antibody. Detection employed donkey anti-rabbit IgG peroxidase coupled secondary antibody and the Renaissance chemiluminescence system (Perkin Elmer Life Sciences, Boston, MA, USA) on Kodak X-Omat Blue XB films. Bands were quantified using Eagle Eye software (Stratagene Corp., La Jolla, CA, USA). Tumor Akt activity was expressed as the ratio of phospho-Ser^{473}-Akt to total Akt.

Immunohistochemistry for skin and hair phospho-Akt

Mouse skin was fixed in buffered 4% formaldehyde for 24 h followed by 70% ethanol before being embedded in paraffin, and 5 μm sections were cut. Hair from the temple area of six human volunteers was collected by plucking with tweezers and immediately immersed, root down, in McCoy's 5A cell culture medium containing 20% FBS, or the same medium with 100 nM **PX-866**. The study was approved by the Institutional Review Board and informed consent was obtained. After 10 min, the medium was removed and the hair follicles fixed in buffered 4% formaldehyde for 20 min. Excess hair was trimmed, and seven to ten intact roots were aligned in an agarose block, transferred to 70% ethanol and embedded in paraffin. Serial 5 μm sections were cut every 20 μm throughout the thickness of the block. Sections of mouse skin and human hair were baked and dewaxed automatically, and then stained for phospho-Akt and the four isoforms of PtdIns-3-kinase using a BOND-maX autostainer and Intense Polymer Detection System (Vision BioSystems, Norwell, MA, USA). Slides were lightly counter-stained with the onboard hematoxylin to visualize nuclei. Pictures of individual follicle sections were taken on a Nikon e90i fully motorized upright microscope with an RS Photometrics K4 (Roper Scientific, Tucson, AZ, USA) digital camera using a 10× plan-apo objective lens. Image analysis and reconstruction was performed using SimplePCI image analysis software (Compix Inc. Imaging Systems, Cranberry Township, PA, USA).

Results

Inhibition of phospho-Akt in mouse skin by a PtdIns-3-kinase inhibitor

Immunohistochemical staining for Ptdins-3-kinase in mouse skin using anti-human antibodies that cross-reacted with mouse protein showed weak staining for p110α and p110δ isoforms and strong staining for p110β throughout most of the mouse skin including the external root sheath keratinocyte layer of the hair follicles. Staining was cytoplasmic in all cases. Ptdins-3-kinase p110δ could not be detected in either mouse skin or hair. Staining for phosphoSer^{473}-Akt showed that its distribution in mouse skin was confined to the external root sheath keratinocyte layer of the hair follicles with no detectable staining in other areas of the skin, including the epidermal keratinocytes (Fig. 1a). The phospho-Ser^{473}-Akt staining in hair keratinocytes was cytoplasmic and lightly nuclear. Skin sections were cut along the length of the hair follicles and approximately 25% of the hairs stained for phosphoSer^{473}-Akt. When mice were administered the Ptdins-3-kinase inhibitor **PX-866** either po or i.v., there was a marked decrease in the number of hairs showing phosphoSer^{473}-Akt staining (Fig. 1a, b). The maximum inhibition of phosphoSer^{473}-Akt staining in hair was at 4 h after po and i.v. **PX-866** but was still more than 50% at 24 h (data not shown). Phospho-Ser^{473}-Akt in HT-29 tumor xenografts measured by Western blotting that has previously been reported to be maximal at 4–12 h [11] and in the present study at 4 h, was less sensitive than phosphoSer^{473}-Akt in hair to inhibition by **PX-866** (Fig. 1c).

The ability of the EGFR inhibitor gefitinib administered in combination with the PtdIns-3-kinase inhibitor **PX-866** to decrease phosphoSer^{473}-Akt in mouse hair and in A-549 non small cell lung tumor xenografts was studied. **PX-866** administered either i.v. or po caused a marked decrease in hair phosphoSer^{473}-Akt but only up to a 60% decrease in A-549 tumor xenograft phospho-Ser^{473}-Akt (Fig. 2). Gefitinib did not alter tumor phosphoSer^{473}-Akt but almost completely blocked mouse hair phosphoSer^{473}-Akt. The combination of **PX-866** and gefitnib gave almost complete phosphoSer^{473}-Akt inhibition in mouse hair, but only the same inhibition as produced by **PX-866** alone in A-549 tumor xenografts. At the same dose, gefitinib inhibited tumor phospho-EGFR greater than 70% (results not shown).

Inhibition of phospho-Akt in mouse skin by an Akt inhibitor

PhosphoSer^{473}-Akt in mouse hair and MCF-7 human breast cancer tumor xenografts was compared following administration of the Akt inhibitor **PX-316**. **PX-316** binds to the PH domain of Akt preventing Akt's translocation from the cytoplasm to the plasma membrane, thus blocking Akt activation [20]. **PX-316** decreased phosphoSer^{473}-Akt in MCF-7 breast cancer xenografts up to 85% at the highest dose, but only up to 50% in mouse hair (Fig. 3).

Human hair phosphoSer^{473}-Akt and PtdIns-3-kinase

Human hair was easily obtained from the temple and approximately 40 plucked hairs gave 7–10 hairs with

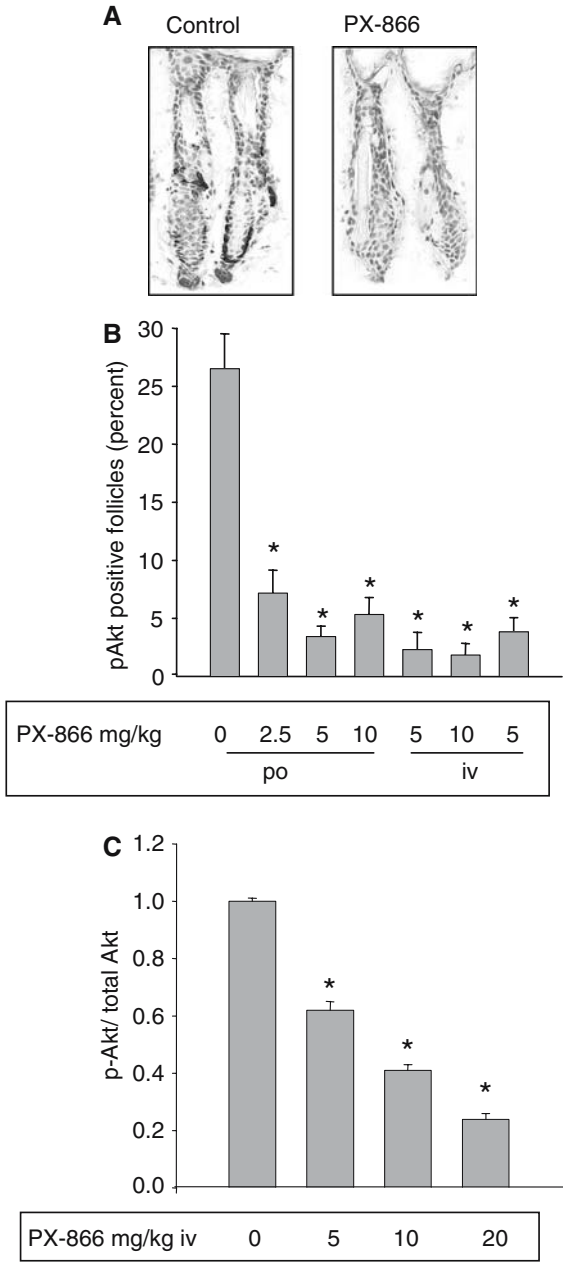

Fig. 1 Inhibition of phospho-Akt in mouse skin and HT-29 colon tumor xenografts by the PtdIns-3-kinase inhibitor PX-866. Scid mice with HT-29 human colon cancer xenografts were administered PX-866 i.v. or orally po at the doses shown. Four hours later, the animals were euthanized and skin and tumor removed. **a** Typical phosphoSer^{473}-Akt immunohistochemical staining of mouse skin. *Left panel* control mouse. *Right panel* a mouse treated with PX-866 at 10 mg/kg i.v. **b** The percent of hair follicles in mouse skin staining positive for phosphSer^{473}-Akt. Values are the mean of 10 fields on four mice and *bars* are SE. $*P < 0.05$ compared to control. **c** HT-29 tumor xenograft phosphoSer^{473}-Akt measured by Western blotting expressed relative to total Akt. Values are the mean of four tumors and bars are SE. $*P < 0.05$ compared to control

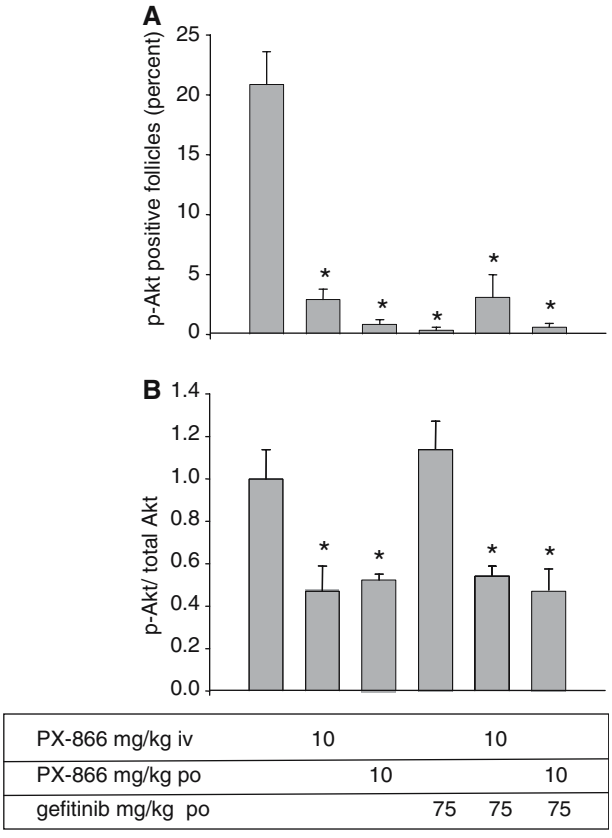

Fig. 2 Inhibition of phospho-Akt in mouse skin and A-549 non small cell lung cancer xenografts by PX-866 and gefitinib. Scid mice with A-549 human non small cell lung cancer xenografts were administered PX-866 i.v. or po, or gefitinib po at doses shown. When used in combination, the gefitinib was administered 3 h after the PX-866. Twenty four hours later, the animals were euthanized and skin and tumor removed. **a** The percent of hair follicles staining positive for phosphSer^{473}-Akt in mouse skin. Values are the mean of 10 fields on four mice and *bars* are SE. $*P < 0.05$ compared to control. **b** A-549 tumor xenograft phosphoSer^{473}-Akt measured by Western blotting expressed relative to total Akt. Values are the mean of four tumors and bars are SE. $*P < 0.05$ compared to control

intact roots after microscopic observation. The staining for phosphoSer^{473}-Akt in human hair was predominantly (74%) in the outer root sheath region of the hair shaft (Fig. 4a). Individual human hairs in short-term culture showed more than 80% decrease in phospho-Ser^{473}-Akt staining when incubated with 100 nM **PX-866** (Fig. 4b). Staining for PtdIns-3-kinase isoforms in the external root sheath of the hair shaft showed the presence of p110α, p110β, p110γ, and p110δ, and was similar to the pattern of PtdIns-3-kinase isoform staining in human A-549 non small cell lung cancer xenografts except for an increased staining of p110δ in the external root sheath keratinocytes (Fig. 5).

Discussion

The epidermis is contiguous with the external root sheath of the hair follicle, a complex structure responsible for creating the hair shaft [26]. The external root sheath of the human hair follicle has previously been reported to exhibit staining for EGFR, phospho-EGFR

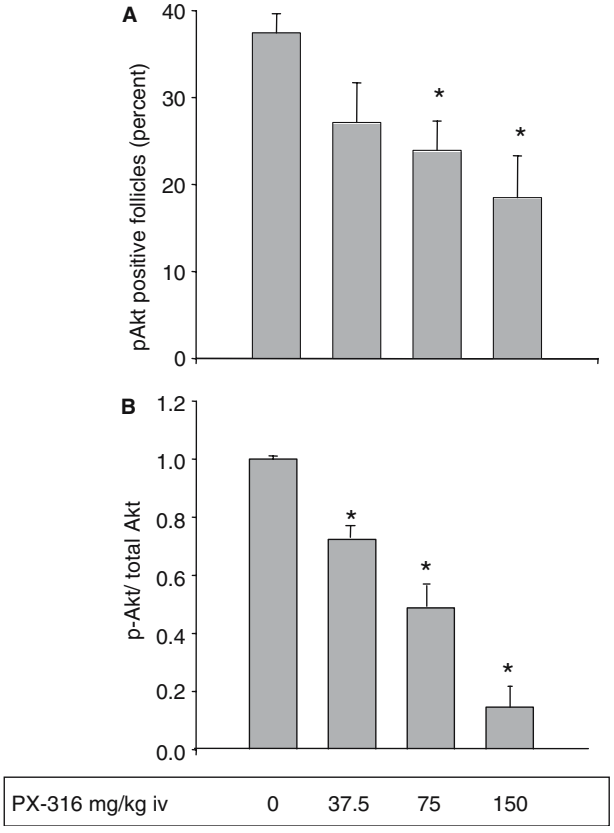

Fig. 3 Inhibition of phospho-Akt in mouse skin and MCF-7 breast tumor xenografts by the Akt inhibitor **PX-316**. Scid mice with MCF-7 human breast cancer xenografts were administered **PX-316** i.v. at the doses shown. Twenty four hours later, the animals were euthanized and skin and tumor removed. **a** The percent of hair follicles staining positive for phosphoSer^{473}-Akt in mouse skin. Values are the mean of 10 fields on four mice and *bars* are SE. *$P < 0.05$ compared to control. **b** MCF-7 tumor xenograft phosphoSer^{473}-Akt measured by Western blotting expressed relative to total Akt. Values are the mean of four tumors and bars are SE. *$P < 0.05$ compared to control

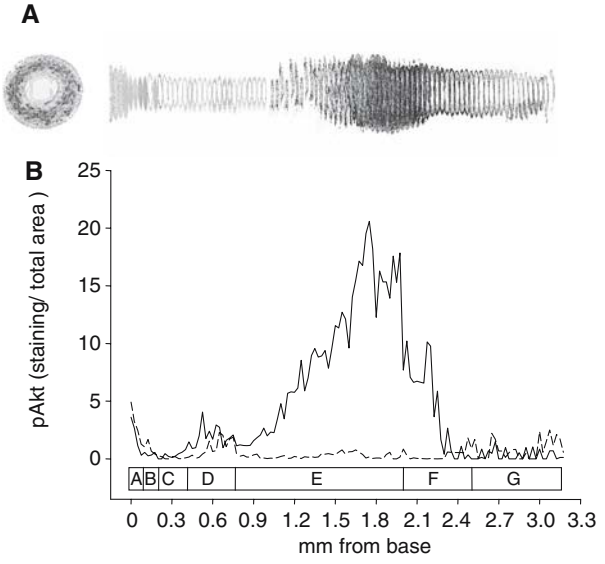

Fig. 4 Staining for phospho-Akt in human hair. **a** Axial and saggital views of a single human hair reconstructed from multiple 5 μm cross sections taken every 20 μm along the hair root showing staining for phospho-Akt. **b** Quantization of phospho-Akt staining along the length of the human hair root. The continuous line is human hair incubated for 10 min in culture media; *dashed line* is human hair incubated for 10 min in media with 100 nM **PX-866.** Values are the means of 7 hairs in both cases. The regions of the hair shaft are: *A* dermal papilla, *B* cell matrix, *C* medulla, *D* cortex and inner root sheath, *E* outer root sheath, *F* squamous epithelium, and *G* keratinized epithelium

[1], and phospho-MAPK [16]. We detected strong phospho-Akt staining in the external root sheath of the hair follicle in both mice and humans. It is noteworthy that double knockout mice lacking the two major Akt isoforms, Akt1 and Akt2, show impaired keratinocyte proliferation and severely impaired hair follicle development [21]. In the present study, we used phospho-Ser^{473}-Akt as a cellular indicator of the activity of the PtdIns-3-kinase signaling pathway to compare in mice the response to inhibition of PtdIns-3-kinase signaling in tumor xenografts with that in hair as a potential surrogate tissue for clinical trials of PtdIns-3-kinase signaling inhibitors. We found that mouse hair phosphoSer^{473}-Akt was inhibited by the experimental PtdIns-3-kinase inhibitor **PX-866** and, with the caveat that different techniques to measure phosphoSer^{473}-Akt were being compared, mouse hair phosphoSer^{473}-Akt was more sensitive to inhibition by **PX-866** than tumor xenograft phosphoSer^{473}-Akt. In contrast, the experimental Akt inhibitor **PX-316** gave greater inhibition of tumor xenograft phosphoSer^{473}-Akt than hair phosphoSer^{473}-Akt. The EGFR inhibitor gefitinib caused marked inhibition of phosphoSer^{473}-Akt in mouse hair but did not inhibit phospho-Akt in A-549 human tumor xenografts, despite causing marked inhibition of phospho-EGFR in the tumor. This has been previously reported for gefitinib in A-549 cells and was ascribed to the absence of ErbB-3 expression which is necessary to couple the EGFR to activation of PtdIns-3-kinase/Akt signaling [9].

There was no correlation between phospho-Akt staining and the relative staining for PtdIns-3-kinase isoforms. The major PtdIns-3-kinase isoform present in mouse skin and the external root sheath of the hair shaft was p110β; staining for p110α and p110δ was weak and p110δ was not detectable. Phospho-Akt staining was present only in the upper third of the external root sheath of both mouse and human hairs and was not seen in the cell matrix or dermal papilla of the hair follicle bulb where hair growth occurs. Thus, Akt signaling appears to play a role in hair development or maintenance unrelated to growth. In this regard, we have not observed inhibition of hair growth as a side effect in mice receiving long-term treatment with **PX-866** for up to 3 weeks [12]. All PtdIns-3-kinase isoforms were present in human hair and in the A-549 non small cell lung cancer xenograft. However, whether different patterns of PtdIns-3-kinase isoform expression could influence

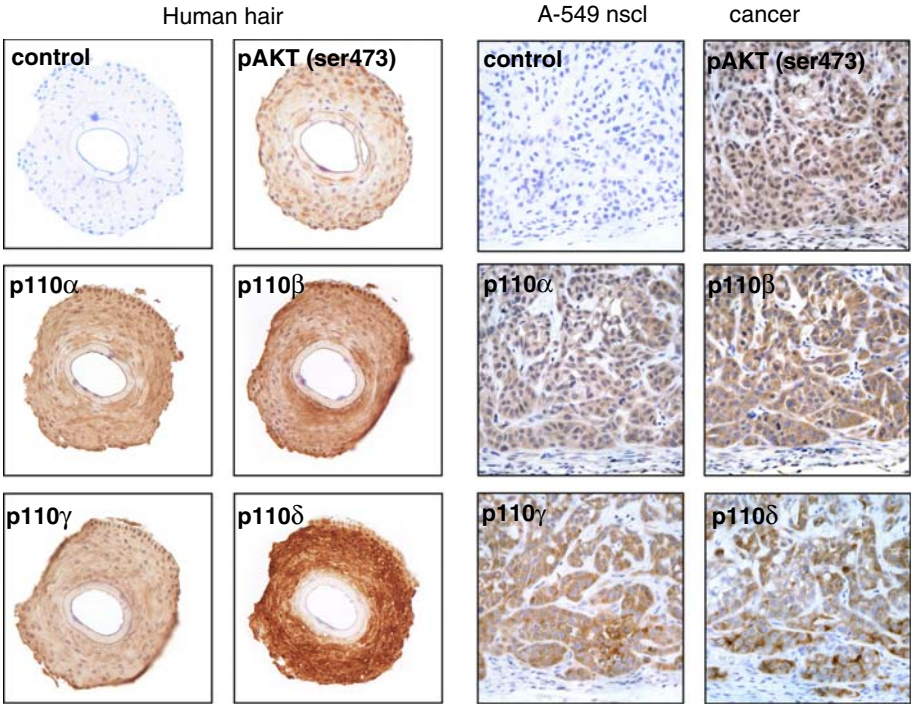

Fig. 5 Staining for PtdIns-3-kinase in the outer root sheath of human hair and in A-549 non small cell tumor xenografts. Typical photomicropraphs of sequential sections of the outer root sheath of freshly plucked human hair and A-549 human non small cell lung cancer xenografts stained for phospho-Akt, or for the PtdIns-3-kinase isoforms p110α, p110*β*, p110*γ* and p110*δ* with specific anti-human antibodies, and a counter stain of hematoxylin. The control shows counter stain with secondary antibody only

response between hair as a surrogate tissue and tumor in the clinical setting is not known and the response could depend upon the PtdIns-3-kinase inhibitor used. **PX-866** is an irreversible inhibitor of Ptdins-3-kinases p110α, p110*γ*, and p110*δ* but a weaker inhibitor of p110*β* [12]. Other reversible isoform-specific PtdIns-3-kinase inhibitors are being developed [10, 30] and they could conceivably cause a different response between hair and tumor. Tissue drug pharmacokinetics due to differences in skin and tumor perfusion may also play a role in determining the response to PtdIns-3-K and Akt inhibitors.

Skin samples obtained as punch biopsies have been previously been used as surrogate tissues to measure the effects of inhibitors of EGFR. EGFR inhibitors, as a class, cause acneiform eruptions and desquamation particularly on the face, and alterations in hair growth [15]. In normal adult skin, the EGFR is strongly expressed in keratinocytes in the basal layer of the epidermis and outer root sheath of follicles, as well as in cells of eccrine and sebaceous glands [13]. Albanell et al. [1] reported a decrease in epidermal keratinocyte phospho-EGFR staining in patients receiving the EGFR inhibitor gefitinib in a Phase I study. There was also a significant decrease in epidermal keratinocyte phospho-MAPK and in cell proliferation, and an increase in the cell cycle inhibitor p27. Malik et al. [16] observed a significant but non-dose related decrease in epidermal keratinocyte phospho-EGFR staining in up to 50% of patients receiving erlotinib in a Phase I study. There was no change in phospho-MAPK but a dose-related increase in p27. A study by Tan et al. [25] found no significant decrease of epidermal keratinocyte phospho-EGFR in patients with metastatic breast cancer following treatment with erlotinib. The study also reported no significant decrease in skin phospho-Akt following erlotinib treatment. Where inhibition of EGFR receptor activation was seen, it occurred at doses of inhibitor well below those that produce unacceptable toxicity, leading to the suggestion that skin EGFR activation might be used to select optimal doses of EGFR inhibitor rather than using maximum-tolerated doses [1]. In the above studies, it was not possible to make correlations of inhibition of skin EGFR with inhibition of tumor EGFR.

To our knowledge, there have been no reports of clinical studies using individual hair as a surrogate tissue for assessing the effects of cancer drugs. Hair is easier to obtain than a skin biopsy which requires local anesthesia, and hair has higher levels of phospho-Akt than skin. There is a report using individual hairs to measure EGFR, phospho-EGFR, ERK, and phospho-ERK in hair from normal volunteers as a prelude to clinical studies with EGFR inhibitors with the possibility of optimizing dose and treatment scheduling [27]. In this study, the proteins from each hair root were transferred to membranes before being stained with fluorescently labeled antibodies. In our study, we used direct immunohistochemical staining of plucked human hair from the temple. PhosphoSer^{473}-Akt staining was predominantly localized in the external root sheath of human hair. We were able to show in individual human hairs in a short-term culture that the phospho-Akt staining was susceptible to inhibition by **PX-866.**

In summary, we have shown that phosphoSer^{473}-Akt staining in the keratinocytes of the external sheath of

hair is inhibited by a PtdIns-3-kinase inhibitor administered to mice and to human hair in culture. The decrease in phosphoSer^{473}-Akt in mouse hair was greater than the decrease in phosphoSer^{473}-Akt in human tumor xenografts in the same mice. In contrast, inhibition of phospho-Akt in mouse hair by an Akt inhibitor was less than in human tumor xenografts. While in mouse hair, an EGFR inhibitor almost completely inhibited phosphoSer^{473}-Akt, there was no inhibition in human tumor xenograft, showing that signaling pathways in hair and tumor are not always identical. The results of the study suggest that individual human hairs could provide a minimally invasive way of measuring the effects of PtdIns-3-kinase signaling inhibitors in patients reflecting inhibition of tumor phospho-Akt.

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