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Generation of a Reporter Mouse Line Expressing Akt and EGFP Upon Cre-Mediated Recombination

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Summary

The serine-threonine kinase Akt regulates multiple biological processes. An important strategy to study Akt signaling in different tissues is targeted activation of this pathway *in vivo*. The current studies describe the generation of a mouse model that combines a double reporter system with activation of a constitutively active form of Akt1 (caAkt) in a Cre-dependent manner. Before Cre recombination, these mice express *LacZ* during development as well as in most adult tissues. After Cre-mediated excision of the *LacZ* reporter, functionality of the transgene was demonstrated by expression of the caAkt mutant along with the second reporter, EGFP in different pancreatic compartments and in the nervous system. This animal model provides a critical reagent for assessing the effects of Akt activation in specific tissues. The lineage-tracing properties provide a useful tool to study the role of Akt signaling in regulation of differentiation programs during development and plasticity of mature tissues.

Keywords

Akt1 overexpression; Cre-mediated expression; double lineage tracing; mouse model; PI3-kinase signaling

INTRODUCTION

The serine-threonine kinase Akt has been identified as a major effector of the phosphatidylinositol 3-kinase (PI3K) signaling pathway. In mammals, three separate genes encode three Akt isoforms that share 85% sequence similarity. Upon various mitogenic signals, full activation of Akt leads to its nuclear translocation and phosphorylation of its downstream targets (Elghazi *et al.*, 2006). This kinase plays a critical role in the modulation of several biological processes including cell development, proliferation, survival and metabolism in various organs and has also been involved in tumorigenesis (Zdychova and Komers, 2005).

The overall goal of this study was to generate a mouse model with lineage-tracing that conditionally over-expresses a constitutively active form of Akt1 (caAkt) upon Cre-mediated recombination. Before Cre-mediated recombination, *LacZ* is expressed in the majority of embryonic and adult tissues. Upon Cre excision in different pancreatic compartments and neuroglial progenitors, *LacZ* expression is replaced by expression of the caAkt mutant and

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EGFP. This unique mouse strain provides a new reagent for the study of Akt signaling *in vivo*.

The expression vector used to generate this mouse model (pCALL2-caAkt) was previously shown to provide a strong and widespread expression (Lobe *et al.*, 1999; Niwa *et al.*, 1991; Novak *et al.*, 2000). An HA-tagged constitutively active form of Akt1 (caAkt) and enhanced green fluorescent protein (IRES-EGFP) were subcloned downstream of the *loxP*-flanked *LacZ*/neoR (Fig. 1a). In the absence of Cre recombinase, this promoter regulates the expression of a *loxP*-flanked *LacZ*/neoR. Upon Cre-mediated excision, *LacZ* expression is switched to expression of the caAkt mutant and EGFP (Fig. 1a).

The pCALL2-caAkt construct was used to generate transgenic mice using ES-based transgenesis. Four clones containing a single inserted copy of the transgene were selected for generation of chimeric mice by ES cell/embryo aggregation. Figure 1d shows the number of founders and the degrees of chimerism obtained. We successfully obtained germ line transmission from chimeric mice in three out of four lines (10, 42, and 56). For subsequent discussion, these transgenic mice will be called pCALL2-caAkt. The mouse colony was expanded by crossing to C57Bl/6 mice. The progeny followed the expected Mendelian ratio, were fertile, and did not exhibit any gross abnormalities.

Expression of the transgene was assessed by X-gal staining in transgenic mice from the three different lines generated. Line 10 exhibited weak *LacZ* expression and was not further characterized. Lines 56 and 42 showed similar strong homogeneous *LacZ* expression in most of the organs (Figs. 2 and 3). Sagittal sections obtained from the central nervous system demonstrated high transgene expression in the cortex, hippocampus, dentate gyrus, olfactory bulb, corpus callosum, and cerebellum (Fig. 2a,b). Higher magnification of the hippocampus and cerebellum are shown in Figure 2c,d. Interestingly, very few *LacZ* positive cells were found in the midbrain and the thalamus region, whereas, the hypothalamus was negative (Fig. 2b). Expression of the transgene was detected in the myocardium, endocardium (Fig. 2f,g), and skeletal muscle (Fig. 2i,j). In the respiratory system, the terminal bronchioles and alveoli exhibited *LacZ* expression (Fig. 2l,m). Analysis of the kidney showed higher levels of expression in the cortex when compared with the medulla region (Fig. 2o-q). In the gastrointestinal tract, the transgene was detected in the smooth muscle area of the gastric wall (Fig. 3a-c). Higher magnification revealed mosaic expression of the transgene in the columnar epithelium of the stomach (Fig. 3a-c), the small and large intestine (Fig. 3d-i and data not shown for large intestine). High and diffuse levels of *LacZ* staining were observed in all the pancreatic compartments (Fig. 3j-l). Only two organs, the liver and the spleen displayed very few *LacZ* positive cells (Fig. 3m-r).

Whole mount analysis in embryos at embryonic day 10.5 showed widespread *LacZ* expression throughout the body compared with their control littermates (Fig. 4a-c). One-hour staining revealed *LacZ* expression in the otic vesicles, somites, limb buds, and gastric tract (Fig. 4b). Longer exposure to the substrate allowed visualization in the eye, forebrain, midbrain, branchial arch, spinal cord, tail bud, and the stomodeum (Fig. 4c). Sagittal sections from these embryos showed *LacZ* expression in several developing organs as indicated in Figure 4.

Functionality of the transgene was assessed by crossing pCALL2-caAkt transgenic mice with Pdx1-Cre (Gu *et al.*, 2002), RIP-Cre (Herrera, 2000), or Elastase-Cre (Grippio *et al.*, 2002). In Pdx1-Cre mice, Cre recombinase is expressed in pancreatic progenitors and the duodenum during early stages of development and becomes restricted to differentiated β -cells in the adult pancreas. Four-week-old pCALL2-caAkt pancreata exhibited expression of β -galactosidase in about 90% of the acinar, ductal, and β -cell compartments while lacking EGFP expression (Fig. 5a). In pCALL2-caAkt^{Pdx1-Cre} mice, EGFP fluorescence was expressed in ducts, β - and acinar

cells (Fig. 5a and data not shown). β -galactosidase positive cells remained in some areas of the pancreas indicating some degree of mosaicism of the Cre recombinase (Fig. 5a). Transgene expression assessed by HA staining with the acinar marker amylase showed that pCALL2-caAkt^{Pdx1-Cre} pancreata exhibited homogenous HA staining in the acinar tissue (Fig. 5b). Phosphorylation of GSK3- β , an Akt target, demonstrated higher levels of phospho-GSK3- β in islets from pCALL2-caAkt^{Pdx1-Cre} animals indicating increased Akt signaling (Fig. 5c). Activation of Akt in mature β -cells was assessed by crossing pCALL2-caAkt mice to RIP-Cre animals (pCALL2-caAkt^{RIP-Cre}). In these mice, Cre recombinase is restricted to differentiated pancreatic β -cells. Pancreata from 5-week-old pCALL2-caAkt^{RIP-Cre} showed EGFP and HA staining in pancreatic β -cells (Fig. 6a). Of note is the abnormal appearance of islets from pCALL2-caAkt^{RIP-Cre} mice, a finding that is currently being characterized. Finally, to evaluate recombination of the transgene in the acinar tissue, we used Elastase-Cre animals. Pancreata from pCALL2-caAkt showed homogenous staining for amylase without EGFP fluorescence (Fig. 6b). pCALL2-caAkt^{Ela-Cre} mice exhibited EGFP fluorescence within amylase expressing cells (Fig. 6b). To assess the recombination potential in a different organ, we crossed pCALL2-caAkt mice to mice expressing Cre recombinase under the control of the brain lipid binding protein promoter (BLBP-Cre). These mice express Cre recombinase in embryonic neuroglial progenitor cells (Hagedus *et al.*, 2007). Upon Cre-mediated excision, EGFP fluorescence was detected both in the glial cells of the cerebellar white matter and in the neuronal cells of the molecular and granule cell layer (Fig. 6c). Interestingly, there was no EGFP expression in Purkinje cells.

In the current studies we generated a mouse model with reliable and reproducible activation of Akt signaling by Cre-mediated recombination. These mice were born in the expected Mendelian ratio with no abnormalities in glucose metabolism (data not shown). Widespread expression of the transgene both during development and in adulthood was observed. These animals did not show an increased susceptibility to malignant transformation for up to 12 months of age. Functionality of the transgene assessed by expression of EGFP and activation of downstream targets of Akt was demonstrated using several Cre lines for different pancreatic and neuronal compartments. These studies suggest that this animal model represents a suitable system to study Akt signaling *in vivo*.

The strategy used to conduct this study had been previously employed to generate different transgenic models (Ding *et al.*, 2002; Lobe *et al.*, 1999; Novak *et al.*, 2000). Consistent with previous reports, the chicken β -actin promoter with upstream CMV enhancer was expressed during developmental stages and in most adult tissues. Expression in the gastrointestinal track showed a mosaic expression of the transgene. While it is difficult to prove, expression in the intestinal crypt and villi suggest that the cryptal pluripotent cells expressed the transgene. Interestingly, the liver and spleen exhibited very few LacZ positive cells. Absence of LacZ expression in the liver can be accompanied by strong EGFP expression in this tissue after Cre excision as reported in another transgenic mouse model using the same strategy and promoter (Z/EG mice) (Novak *et al.*, 2000).

The cross to various specific Cre-lines for different pancreatic compartments demonstrated that recombination of the LacZ stop codon was functional and that the distal component of the transgene was intact. Expression of the HA tag from the Akt mutant and phosphorylation of downstream targets of Akt signaling (e.g., GSK-3 β) suggested that the caAkt mutant was operational. Recombination in pancreatic progenitors (pCALL2-caAkt^{Pdx1-Cre} mice) resulted in endogenous fluorescence and activation of Akt signaling in the three pancreatic compartments. LacZ expression was still observed in some areas of the pCALL2-caAkt^{Pdx1-Cre} suggesting incomplete recombination and a certain degree of mosaicism. In pCALL2-caAkt^{Ela-Cre} mice, acinar cells displayed diffuse EGFP. Similar efficiency of recombination and activation of Akt signaling was obtained in pancreatic islets using the RIP-

Cre mice. Interestingly, these mice exhibited endogenous EGFP in β -cells and in some acinar cells implying potential β -cell to acinar transdifferentiation. To further demonstrate the utility of this mouse strain in the study of another organ, we examined Akt activation in the developing central nervous system. Cre expression in the BLBP1 neuroglial progenitor cells resulted in EGFP expression in both glial and neuronal lineage cells as well as increased Akt activity in the fore-brain (Hagedus *et al.*, 2007).

In summary, we believe that these double reporter transgenic mice represent a valuable tool for further characterization of the Akt pathway in different organs and at different developmental stages. The lineage tracing capabilities of this model are powerful tools to elucidate the importance of Akt signaling in cell fate allocation during development as well as in the plasticity of mature cells.

MATERIALS AND METHODS

DNA Construct

To generate a mouse model with expression of a constitutively active Akt in a temporal and cell-specific manner, we used the pCALL2 vector (Lobe *et al.*, 1999; Novak *et al.*, 2000). This construct contains a chicken β -actin promoter with upstream cytomegalovirus (CMV) enhancer (pCAGGS) (Niwa *et al.*, 1991). This promoter is followed by a *loxP*-flanked *LacZ*/neoR fusion with three SV40 polyadenylation signals (pA), a constitutively active form of Akt (caAkt) that contains an HA epitope, and an internal ribosomal entry site (IRES) that allows joint but independent translation of enhanced green fluorescent protein (EGFP). After the EGFP, the construct contains a rabbit β -globin polyadenylation sequence. This allows visualization of caAkt expressing cells and lineage tracing experiments (Fig. 1a). For subsequent discussion, the expression vector was called pCALL2-caAkt.

ES Cell Culture

To generate these mice we used ES-based transgenesis. This strategy requires the production of a ubiquitously expressed conditional transgenic line with a single copy transgene. The pCALL2-caAkt vector was electroporated by standard techniques into R1 embryonic stem (ES) cells (Nagy, 1997; Nagy *et al.*, 1993) by the Washington University Mouse Genetics Core. After 7 days of culture, ES cell clones that carried the transgene were selected through neomycin resistance. After selection, colonies were picked and grown in duplicate 96-well plates. One duplicate was used for *LacZ* staining (Lobe *et al.*, 1999; Novak *et al.*, 2000). ES clones with strong, uniform expression of *LacZ* reporter (>90%) were expanded and frozen (Fig. 1b).

Generation of Transgenic Mice Expressing caAkt in a Cre-Excision Manner

After electroporation of the transgene into R1 cells and neomycin selection, 240 ES clone colonies were picked and grown in triplicate 96-well plates. Eighteen ES clones with strong and uniform *LacZ* expression in more than 90% of the cells were selected by *LacZ* staining (Fig. 1b). The genomic DNA from these clones was digested with multiple restriction enzymes recognizing unique sites in the transgenic vector (not in the probe region) (BglII, ScaI, StuI, and XbaI) and were subjected to Southern blot analysis by hybridization with labeled *LacZ* cDNA or *LacZ* or β -globin probes. To avoid any possible recombination between multiple *loxP* sites, clones with a single copy of the transgene that expressed high level of *LacZ* were thawed and used to generate chimeric mice by conventional techniques (Nagy, 1997). The Washington University Mouse Genetics Core performed these procedures. Chimeric mice were mated with C57/B6 mice to produce hemizygous transgenic offspring and to identify germline transmitters (Fig. 1c,d). The pCALL2-caAkt transgene was geno-typed by PCR using genomic DNA isolated from the toe. We used the following primers: pCALL2 5':

GTTGCAGTGCACGGCAGATACACTTGCTGA; pCALL2 3':
GCCACTGGTGTGGGCCATAATTCAATTCGC.

Tissue Preparation and *LacZ* Staining

Various tissues from 4-week-old mice were dissected and fixed for 6 h in *LacZ* fixing solution (0.2% glutaraldehyde, 5 mM EGTA, pH7.3, 0.1 M MgCl₂ in PBS, pH7.3). Tissues were washed in PBS and cryoprotected in 30% sucrose overnight. Tissues were then embedded in OCT over dry ice. Sixteen-micrometer frozen sections were collected onto polylysine-coated slides and stored at -20°C. Prior to staining, slides were fixed in PBS containing 0.2% glutaraldehyde for 10 min, washed in *LacZ* wash buffer (2 mM MgCl₂, 0.01% sodium deoxycholate, 0.02% Nonidet-P40 in PBS, pH7.3) and then stained in *LacZ* wash buffer containing 1 mg/mL X-Gal (Amresco), 5 mM potassium ferrocyanide, and 5 mM ferricyanide at 37°C. After staining, slides were rinsed in *LacZ* wash buffer and then PBS.

Whole Mount *LacZ* Staining

Embryos were harvested and rinsed in ice-cold PBS. Fixation was carried out in 2% PFA; 0.2% glutaraldehyde in PBS for 30 min on ice. Embryos were fixed for an additional 60 min on ice in *LacZ* fixing solution. Samples were placed in *LacZ* wash buffer and then into *LacZ* staining solution at 37°C. After staining, embryos were extensively rinsed in PBS, stored in *LacZ* wash buffer at 4°C and observed under a Leica dissecting microscope hooked to a digital camera. Embryos were then postfixed for 1 h in *LacZ* fix solution, followed by 2 h in 4% PFA and prepared for frozen section as described above. Ten-micrometer sagittal sections were restained with *LacZ* stain buffer.

Assessment of EGFP Expression in Transgenic Lines and Immunostaining

Frozen pancreatic sections from single (pCALL2-caAkt) and double (pCALL2-caAkt^{Pdx1-Cre}; pCALL2-caAkt^{RIP-Cre}; pCALL2-caAkt^{Ela-Cre}) mice were incubated overnight with anti insulin (1:800; Dako), anti β-galactosidase (1:1,000; Capell), anti amylase (1:400; Sigma) anti HA (1:200; Covance) antibodies. Sections were then incubated for 4 h with the appropriate secondary antibodies (Jackson ImmunoResearch). Endogenous EGFP was visible under a Leica fluorescent microscope using the FITC filter. Fluorescent images were obtained using a Leica microscope DM4000B with a Leica DFC 350FX camera and subsequently processed with Leica Application Suite (LAS) software v.2.5.0.

EGFP recombination in the brain was assessed in mice transcardially-perfused with PBS and 4% PFA. Following overnight post-fixation, coronal slices were cryoprotected in 30% sucrose and then embedded in OCT. Sixteen-micrometer frozen sections were permeabilized with 0.1% Triton-X100 prior to blocking in 10% goat serum. Anti-GFP (1:200; Molecular Probes) was applied overnight at 4°C. Sections were then incubated with Alexa-labeled secondary antibody (molecular probes). Stained sections were coverslipped with DAPI mounting medium.

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LITERATURE CITED

- Ding H, Wu X, Nagy A. Mice with Cre recombinase activatable PDGF-C expression. *Genesis* 2002;32:181–183. [PubMed: 11857815]
- Elghazi L, Balcazar N, Bernal-Mizrachi E. Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. *Int J Biochem Cell Biol* 2006;38:157–163. [PubMed: 16213777]
- Grippio PJ, Nowlin PS, Cassaday RD, Sandgren EP. Cell-specific transgene expression from a widely transcribed promoter using Cre/lox in mice. *Genesis* 2002;32:277–286. [PubMed: 11948915]
- Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN31 cells are islet progenitors and are distinct from duct progenitors. *Development* 2002;129:2447–2457. [PubMed: 11973276]
- Hagedus B, Dasgupta B, Shin J, Emmett RJ, Hart-Mahon EK, Elghazi L, Bernal-Mizrachi E, Gutmann DH. Neurofibromatosis-1 regulates neuronal and glial cell differentiation from neuroglial progenitors in vivo by both cAMP- and Ras-dependent mechanisms. *Cell Stem Cell* 2007;1:443–457. [PubMed: 18371380]
- Herrera PL. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 2000;127:2317–2322. [PubMed: 10804174]
- Lobe CG, Koop KE, Kreppner W, Lomeli H, Gertsenstein M, Nagy A. Z/AP, a double reporter for cre-mediated recombination. *Dev Biol* 1999;208:281–292. [PubMed: 10191045]
- Nagy, A. Formation of mouse chimeric embryos from ES cells. Taylor & Francis; Amsterdam: 1997. p. 4
- Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc Natl Acad Sci USA* 1993;90:8424–8428. [PubMed: 8378314]
- Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 1991;108:193–199. [PubMed: 1660837]
- Novak A, Guo C, Yang W, Nagy A, Lobe CG. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis* 2000;28:147–155. [PubMed: 11105057]
- Zdychova J, Komers R. Emerging role of Akt kinase/protein kinase B signaling in pathophysiology of diabetes and its complications. *Physiol Res* 2005;54:1–16. [PubMed: 15717836]

FIG. 1. Generation of conditional transgenic mice over expressing a constitutively active mutant of Akt (caAkt) in a cell specific manner. **(a)** Diagram of the pCALL2-caAkt transgene construct before and after Cre-mediated excision of the floxed *LacZ*-neoR sequence. **(b)** Low (upper panel) and high power (lower panel) magnification of *LacZ* staining for some of the ES clones used to generate chimeric mice. **(c)** Southern-blotting analysis from ES clones used to generate chimeric mice. Southern blotting was performed using β -globin (left panel) and *LacZ* (right panel) probes (position of the probes is depicted with a black bar on Fig. 1a). **(d)** Degree of chimerism and number of founders obtained.

FIG. 2.

Transgene expression assessed by *LacZ* expression in organs from control and pCALL2-caAkt transgenic lines. Tissue sections from various organs were stained for *LacZ*. (a,b) sagittal section of the brain, (c,d) magnification of the hippocampus region (c) and the cerebellum (d). Cbx, cerebellum; Ctx, cortex; Mb, midbrain; Mob, main olfactory bulb; Cc, corpus callosum; Hip, hippocampal region; Th, thalamus; Hy, hypothalamus; My, medulla; Dg, dentate gyrus. (e-g) *LacZ* staining in sections obtained from the heart, (h-j) skeletal muscle, (k-m) lung, (n-q) and kidney. Scale bar, 500 μ m (a,b,e,h,i,k,l,n,o); 100 μ m (f,g,j,m,p,q).

FIG. 3.

Transgene expression assessed by *LacZ* expression in organs from control and pCALL2-caAkt transgenic lines. (a–c) *LacZ* staining in sections from the stomach, (d–f) small intestine, (g–i) pancreas, (j–l) liver, (m–o) and spleen. Sp, spleen. Scale bar, 500 μ m (h,j,k,m,n); 100 μ m (a,d,g,i,l,o); 50 μ m (b,c,e,f).

FIG. 4.

Transgene expression assessed by *LacZ* expression during early development. Whole mount *LacZ* expression pattern in E10.5 wild-type (**a**) and pCALL2-caAkt (**b,c**) transgenic embryos. (1) eye, (2) prosencephalon, (3) mesencephalon, (4) isthmus, (5) metencephalon, (6) myelencephalon, (7) otic vesicle, (8) infundibulum, (9) spinal cord, (10) somites, (11) tail bud, (12) limb bud, (13) branchial arch, (14) digestive tract, (15) stomodeum. Sagittal section of E10.5 wild-type (**d**) and pCALL2-caAkt (**e-h**) transgenic embryos. 0 forebrain, (1) hindbrain, (2) mandibular component of the first branchial arch, (3) endocardial cushion tissue associated with the wall of the atrio-ventricular canal, (4) common atrial chamber (5) elements of the

hepatic/biliary primordia within septum transversum, (6) mesonephric tubules and ducts, (7) lumen of the stomach, (8) midgut and duodenum, (9) somites. Scale bar, 500 μm (d–h).

FIG. 5.

Assessment of Cre-mediated recombination in pancreatic progenitors (pCALL2-caAkt^{Pdx-Cre}). (a) Immunofluorescence staining for insulin (blue), β -galactosidase (red) and endogenous EGFP (green) in 5-week-old pCALL2-caAkt (top panel) and pCALL2-caAkt^{Pdx-Cre} (lower panel). (b) Immunofluorescence staining for amylase (blue), HA (red) and endogenous EGFP (green) in 5-week-old pCALL2-caAkt and pCALL2-caAkt^{Pdx-Cre}. (c) Activity of the transgene observed by immunofluorescence staining for insulin (green) and phospho GSK3- β (red) in 5-week-old pCALL2-caAkt and pCALL2-caAkt^{Pdx-Cre}. Nuclei are stained with DAPI. Scale bar, 50 μ m.

FIG. 6.

Assessment of Cre-mediated recombination in β -cells, acinar cells and embryonic neuroglial progenitor cells. (a) Immunofluorescence staining for insulin (blue), HA (red), and endogenous EGFP (green) in pCALL2-caAkt (a, top panel) and pCALL2-caAkt^{RIP-Cre} (a, lower panel). (b) Immunofluorescence staining for insulin (blue), amylase (red) and endogenous EGFP (green) in pCALL2-caAkt (top panel) and pCALL2-caAkt^{ELA-Cre} (lower panel) (c) Immunofluorescence staining EGFP (red) in pCALL2-caAkt^{BLBP-Cre}. Nuclei are stained with DAPI. WM, white matter; GCL, granule cell layer; PC, Purkinje cell; ML, molecular layer. Scale bar, 50 μ m.