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HuGE, a novel GFP-actin-expressing mouse line for studying cytoskeletal dynamics

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Abstract

Analysis of actin remodeling in live cells and tissues has become an increasingly important tool to study actin-dependent cellular processes. To facilitate these experiments in the mouse we have generated a GFP-actin-expressing line (huGE) by knock-in of the GFP-actin gene into the profilin 1 locus. Here we show that GFP-actin is expressed throughout embryonic development and in all tissues except skeletal muscle, in a pattern similar to profilin 1. Particularly high expression of GFP-actin was observed in bone marrow and all blood cells. The GFP-actin fusion protein is functional as shown by its co-localization with endogenous actin in F-actin-rich structures. Therefore, the huGE mouse line provides a novel tool to monitor actin dynamics in mouse embryos and a wide range of organs.

Keywords: GFP-actin; Actin dynamics; Lymphocytes; Profilin 1

Introduction

The actin cytoskeleton is a highly dynamic structure employed by the cell to regulate shape changes. Apart from cell crawling actin dynamics is important in endocytosis (Yarar et al., 2005), cell division (Gerisch and Weber, 2000), and secretion (Trifaro et al., 2002). Even chromosome segregation (Lenart et al., 2005), chromatin remodeling, transcription (Bettinger et al., 2004) and nuclear stability (Bohnsack et al., 2006) depend on actin.

These different processes depend on changes in length and alignment of actin filaments, parameters which are regulated by a plethora of actin-binding proteins. Hence, observing actin dynamics in live cells and tissues has become an essential technique in cell biology. GFP-tagged versions of actin are widely used to visualize the actin cytoskeleton in live cells (Choidas et al., 1998; Westphal et al., 1997). Normally, expression vectors are transfected or the respective mRNA is injected into the cell of interest. However, in tissues or whole animals this approach is limited. An alternative method is the use of transgenic mouse models expressing the GFP-actin gene under the control of a tissue-specific promoter. Such mouse lines have been described expressing GFP-actin in keratinocytes (Vaezi et al., 2002) and neurons (Fischer et al., 2000). However, a "universal GFP-actin mouse" expressing GFP-actin in a broad range of cell types and tissues has not been available yet. Our aim was to generate a mouse line by a knock-in of the GFP-actin fusion into the profilin 1 locus, placing it under the control of the authentic profilin 1 promoter. Here we demonstrate that this mouse line is suitable for studying the actin cytoskeleton in embryonic development, as well as in a wide range of organs and primary cell lines.

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Materials and methods

Cloning of the targeting construct and generation of huGE mice

The GFP cDNA was fused to the 5′ end of the human β-actin cDNA (Ponte et al., 1984) and the bovine growth hormone polyA signal was added at the 3′ end. The GFP-β-actin-polyA fusion gene was then cloned into the NcoI site comprising the start codon of the mouse profilin 1 gene. Downstream of the GFP-actin gene a loxP site-flanked neo^r cassette was introduced to allow selection of transfected embryonic stem (ES) cells. ES clones carrying a targeted knock-in allele were identified by Southern blot. Injection of ES cells, generation of chimeras and germline breeding of the knock-in allele were performed using standard procedures. Mice with a targeted profilin 1 locus were crossed to cre-deleter mice (Schwenk et al., 1995) in order to delete the selection marker. Removal of the neo^r cassette was confirmed by Southern blot and PCR. The resulting mouse line was named huGE (for human GFP-actin expressing). All huGE animals used in the experiments carried one GFP-actin knock-in allele and one profilin 1 allele. Mice were genotyped by PCR using specific primers for GFP and β-actin.

Tissue lysates and Western blot analysis

Organs were placed in PEB buffer (20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 5 mM EGTA, 2 mM EDTA, 0.5% TritonX-100, protease inhibitors) and dissociated using 20–30 strokes with a tight-fitting Dounce homogenizer. After centrifugation the cleared cytoplasmic supernatant was boiled in SDS sample buffer (22 mM Tris–HCl, pH 6.8, 4% glycerol, 0.8% SDS, 1.6% β-mercaptoethanol, bromophenolblue) and separated by SDS-PAGE. Equal loading was monitored by Coomassie staining. After transfer to Immobilon-P (Millipore) the membrane was blocked in 5% non-fat milk in NCP buffer (150 mM NaCl, 20 mM Tris, 0.05% Tween-20, 0.02% Na-azide) overnight. Primary and secondary antibodies were diluted in blocking solution. The profilin 1-specific antibody was raised in rabbit against recombinant mouse profilin 1 (Di Nardo et al., 2000). The polyclonal anti-GFP antibody was generously provided by Dr. Jan Faix (University Hannover, Germany). Bound antibodies were detected with appropriate secondary antibodies and enhanced chemiluminescence.

Immunofluorescence microscopy

Cells were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) for 20 min at room temperature, permeabilized with 0.1% Triton/PBS for 10 min and stained with Alexa Fluor-594-conjugated phalloidin (Molecular Probes). Cells were mounted in gelvatol and analyzed using a Leica DMR microscope.

Culture of primary cell lines

Bone marrow-derived macrophages: Bone marrow was flushed from the thigh of huGE mice using Dulbeccos's modified Eagle's medium (DMEM) (supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM non-essential amino acids, 0.1 mM β-mercaptoethanol; all reagents from GIBCO), cells were plated in DMEM plus 10 ng/ml GM-CSF (BioCarta Europe). Within 8 days the cultured cells differentiated into macrophages. For Western blotting cells were lysed directly on the plate, for immunofluorescence cells were fixed and stained as described before.

Skin fibroblasts: A piece of skin from young animals was cut into small pieces and placed into a culture dish with DMEM. After 10 days in culture fibroblasts had grown out from the explant which were used subsequently for Western blotting or fixed for immunofluorescence.

Preparation of embryonic fibroblast lines and *Listeria* infection

E13 embryos were prepared and the head, liver and other intestinal organs were removed. The carcasses were washed in PBS and trypsinized for 30 min. After addition of DMEM and vigorous pipetting a single cell suspension was obtained. Cells were plated in DMEM and split before complete confluency was reached. Cells were passaged for about 3 months after which they became spontaneously immortalized. These permanent embryonic fibroblasts were infected for 2 h with *Listeria monocytogenes* strain 1040 (generous gift by Dr. Fred Southwick, University of Florida/Gainesville) in DMEM medium without antibiotics. After several washes cells were incubated for another 6 h in DMEM medium with penicillin/streptomycin to restrict bacterial growth in the medium. Cells were then fixed and mounted for further analysis.

FACS analysis of bone marrow and blood cells

Bone marrow cells were flushed as described before. Blood was collected directly into FACS buffer (0.5% FCS, 2 mM EDTA in PBS) from the vena cava of huGE mice. White blood cells were purified by a single centrifugation on a Ficoll cushion, washed and stained