Available online at www.sciencedirect.com
ScienceDirect
Experimental Neurology 211 (2008) 107–114
Experimental Neurology
www.elsevier.com/locate/yexnr

Mice lacking the transcription factor Ikaros display behavioral alterations of an anti-depressive phenotype

Tim-Rasmus Kiehl [a,*], Sandra E. Fischer [a], Shereen Ezzat [b,c], Sylvia L. Asa [a]

[a] Department of Pathology, University Health Network, 610 University Avenue, Toronto, ON, Canada M5G 2M9
[b] Department of Medicine, Mount Sinai Hospital, 610 University Avenue, Toronto, ON, Canada M5G 2M9
[c] The Freeman Centre for Endocrine Oncology and Ontario Cancer Institute, University of Toronto, 610 University Avenue, Toronto, ON, Canada M5G 2M9

Received 16 October 2007; accepted 11 January 2008
Available online 5 February 2008

Abstract

The Ikaros (Ik) family of transcription factors has critical functions in immune regulation, lymphohematopoiesis and the hypothalamic-pituitary axis. Ik influences cell fate decisions through transcriptional activation of target genes and its interaction with chromatin remodeling complexes. While Ik is well-described in the lymphoid system and pituitary, its presence and function in the brain has received limited attention to date. This study describes the transient spatio-temporal expression of Ik in striatal medium spiny neurons of the developing murine CNS. To determine the impact of Ik deficiency, standardized behavioral tests were performed. In the elevated plus-maze and contextual fear conditioning tests, homozygous Ik-deficient mice performed similarly to wild-type or heterozygote mice. However, significant differences were observed in Ik-null mice in several behavioral tests. Pinch-induced catalepsy was markedly extended. In the Porsolt forced swim test, Ik-null mice showed reduced immobility, consistent with an anti-depressive effect. The acoustic startle response of Ik-null mice was also markedly diminished. Our findings extend the role of the Ikaros zinc-finger protein to the maturation and differentiation of striatal medium spiny neurons and indicate important actions for Ik in the development of neurocognitive functions and affecting depressive behaviors.

Keywords: Striatum; Brain; Ikaros; Depression; Medium spiny neuron

Introduction

The striatum is a central component of the basal ganglia and performs critical functions in the integration of information, including motor control, cognition, and emotion (Gerfen, 1992; Graybiel 1995). Its importance is also reflected by a number of neurological disorders, including Parkinson's disease, Huntington's disease, schizophrenia and bipolar disorder. Despite major advances in the understanding of basal ganglia circuitry, relatively little is known about the developmental programs that specify striatal neurochemistry and connectivity.

Over 90% of striatal neurons are the GABAergic medium spiny neurons (MSN). During development, the ventricular zone (VZ) and subventricular zone (SVZ) give rise to all neurons and glia that will populate the cerebral hemispheres. After passing through the cell cycle in the VZ or SVZ, cells migrate outward, where they undergo terminal differentiation. In contrast to the cerebral cortex, the mechanisms governing cell migration and maturation in the basal ganglia are still poorly understood.

A transcription factor that has previously been observed in the striatum during development is Ikaros (Georgopoulos et al., 1992; Agoston et al., 2007). The Ikaros (Ik) family of zinc-finger transcription factors is essential in the development and function of leukocytes, including all classes of lymphocytes (NK, T, and B cells), monocytes/macrophages, and dendritic cells (Georgopoulos et al., 1994). This has been confirmed in studies of mice homozygous for a targeted deletion in the Ik gene, which develop a variety of defects in their lymphoid compartments (Wang et al., 1996). After a period of differentiation, Ik expression is down-regulated in most of the cell types involved (Klug et al.,

* Corresponding author. University Health Network, 200 Elizabeth St., E11-444, Toronto, Ontario, Canada M5G 2C4. Fax: +1 416 340 4626.
E-mail address: rasmus.kiehl@uhn.on.ca (T.-R. Kiehl).

0014-4886/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2008.01.014

1998). It was previously shown that Ik is a component of a transcriptional complex that is capable of recruiting multiple co-repressors with histone deacetylase complex (HDAC) activity (Yu et al., 2002). The transient expression, which disappears once a mature cellular phenotype is reached, as well as the chromatin remodeling activity of Ik, point to a role in cell fate specification.

Recently, we showed that corticomelanotrophs in the anterior pituitary also express Ik (Ezzat et al., 2005), and that Ik plays an essential role in hypothalamic-mediated somatic growth (Ezzat et al., 2006). These findings suggest that Ik facilitates the differentiation and maturation of cells required for immune and endocrine homeostasis. However, neurobehavioral assessment of these mice has not been previously reported. In the current study, Ik-deficient mice were evaluated with detailed neurobehavioral tests. We also describe the spatio-temporal pattern of Ik expression during development in the wild-type mouse and examine the impact of Ik deficiency on striatal neurotransmitter systems.

Materials and methods

Animals and genotyping

Ikaros-null mice were derived as described (Ezzat et al., 2006) from a strain generated by Georgopoulos and colleagues (Wang et al., 1996). Mice were propagated in the original C57BL/6 background. Germline allelic transmission was verified by PCR analysis using tail DNA as described (Wang et al., 1996). Mice were initially housed at the Animal Care Facility at the Ontario Cancer Institute until 1 month of age. They were subsequently transferred to the Centre for Phenogenomics, Toronto, where neurobehavioral testing was performed starting at 2 months of age and was completed at age 3 months. Three batches of mice were tested in order to arrive at sufficient numbers, with a total of 9 homozygous Ik-null mice ($Ik^{-/-}$; 3 males, 6 females), 12 heterozygotes ($Ik^{+/-}$; 6 males, 6 females), and 12 wild-type animals ($Ik^{+/+}$; 6 males, 6 females). After the completion of testing, animals were sacrificed and from each animal, one brain hemisphere was fixed in 10% buffered formalin and embedded in paraffin; the other hemisphere was snap-frozen in isopentane. All experiments were carried out in accordance with the rules and regulations of the Animal Care and Use Committee at the University of Toronto.

Components of the study that used wild-type mice for the evaluation of normal expression of Ik were performed in the outbred ICR strain (Institute for Cancer Research). This additional strain was chosen to assess the validity of findings across strains. Tissue was harvested at time points E14, E16, E18, as well as P1 through P20, 6 weeks and 3 months, from at least 3 animals per time point. Brains were fixed in 10% neutral buffered formalin and embedded in paraffin. Hemi-brains from postnatal days 1 through 20 were oriented in the coronal plane and combined into a single array block containing all time points. The care of animals was approved by the Institutional Animal Care Facilities at the Ontario Cancer Institute, where animals were housed.

Morphologic and immunohistochemical studies

Fetal, neonatal and adult mouse brains were fixed in neutral buffered formalin and embedded in paraffin and sectioned in the coronal plane. Sections were stained with hematoxylin and eosin, and serial sections were used for immunolocalization studies. To examine Ik-null and Ik heterozygote mutant mice for potential neuroanatomical defects, serial sections were taken at 5 μm in the entire rostro-caudal extent (three mice analyzed per genotype group, four age points examined). Every tenth section was stained with hematoxylin and eosin. Immunolocalization of Ik was performed with the 4E9 mouse monoclonal antibody that recognizes the C-terminal tail of Ik, as previously described (Ezzat et al., 2003). The following antibodies were used for the immunohistochemical assessment of the striatal neurochemical architecture: DARPP-32 (Cell Signaling Technologies 2302; dilution 1:100), Glutamic acid decarboxylase/GAD 65 and 67 (Chemicon AB1511; dilution 1:500), Substance P (Chemicon AB1566; dilution 1:3000), Met-enkephalin (Abcam ab22620; dilution 1:500), and Choline acetyl transferase/ChAT (Chemicon AB143; dilution 1:500), Dopamine D1 receptor (Sigma D6692; dilution 1:100), Dopamine D2 receptor (GeneTex GTX71748; dilution 1:100).

Elevated plus-maze (anxiety-like behavior)

The elevated plus-maze is a commonly used test for measuring anxiety-like behavior and innate fear in rodents (Crawley, 2000; Rogers and Cole, 1994). The maze consists of two open (25 × 5 cm) and two enclosed arms (25 × 5 × 30 cm), arranged such that the two arms of each type are opposite each other and extend from a central platform (5 × 5 cm). The floor and side walls of the maze consist of opaque Plexiglass material. The maze is elevated to a height of 50 cm. Testing was performed in a dimly lit experimental room. Mice were individually introduced to the center, the head facing the open arm. Behavioral parameters were recorded using a digital camera and were analyzed using Observer 50 software (Noldus Information Technology, Netherlands). The percentage of each of the following parameters was measured: 1.) open arm time, enclosed arm time, and central platform time as a percentage of total testing time; 2.) open arm entries, enclosed arm entries, and central platform number of entries as a percentage of total number of entries; 3) total entries; 4) head-dips (exploratory behavior of head and shoulders over the open sides of maze); 5) number of passages from one enclosed arm to another, and 6) risk assessment (posture of body stretched forward and followed by retraction to the original position). The maze was cleaned between sessions using 70% ethanol.

Acoustic Startle Response (ASR) and Pre-pulse Inhibition (PPI; motor and autonomic reaction)

Testing was performed in four standard startle chambers (Med Associates Inc Startle Reflex System, Georgia, Vermont), as previously described (Lipina et al., 2007). During the test, the animal was confined to a holder, resting on a platform in a