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Migration of transplanted neural progenitor cells in a ferret model of cortical dysplasia

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Abstract

Although altered gene expression clearly causes failure of the neocortex to form properly, many causes of neocortical dysplasia arise from environmental or unknown factors. Our lab studies a model of cortical dysplasia induced by injection of methylazoxymethanol (MAM) into pregnant ferrets on embryonic day 33 (E33), which shares many features of neocortical dysplasia in humans. E33 MAM treatment results in characteristic deficits that include dramatic reduction of layer 4 in somatosensory cortex, widespread termination of thalamic afferents, and altered distribution of GABAergic elements. We determined the ability of immature cells to migrate into MAM-treated cortex using ferret neural progenitor cells obtained at E27 and E33 and mouse neural progenitor cells obtained at E14. When these cells were transplanted into organotypic cultures obtained from normal and E33 MAM-treated ferret cortex prepared on postnatal day 0 (P0), all progenitor cells migrated similarly in both hosts, preferentially residing in the upper cortical plate. The site of transplantation was significant, however, so that injections into the ventricular zone were more likely to reach the cortical plate than transplants into the intermediate zone. When similar cells were transplanted into ferret kits, ~P7–P9, and allowed to survive for 2–4 weeks, the donor cells migrated differently and also reached distinct destinations in normal and MAM-treated hosts. MAM-treated cortex was more permissive to invasion by donor cells as they migrated to widespread aspects of the cortex, whereas transplants in normal host cortex were more restricted. E27 neural progenitor cells populated more cortical layers than later born E33 neural progenitor cells, suggesting that the fate of transplanted cells is governed by a combination of extrinsic and intrinsic factors.
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Keywords: Stem cells; MAM; Neuronal migration; Neocortical development; Transplantation; Organotypic culture; Mouse; Ventricular zone

Introduction

During development of the cerebral cortex, progenitor cells line the lateral ventricles, in a region known as the ventricular zone (VZ). Pyramidal cells arise from the dorsal telencephalon and migrate radially along a glial scaffold towards the pia. Interneurons on the other hand are born in the VZ of the ganglionic eminences in the ventral telencephalon. These cells migrate tangentially from the ventral telencephalon and into the cortical plate (Anderson et al., 2001; Marin and Rubenstein, 2001). The migrating cells populate the cerebral cortex in an "inside–out" manner so that the later born cells bypass earlier-born cells to populate more superficial layers (Rakic, 1990; Parnavelas, 2000; Kriegstein and Noctor, 2004). In the ferret, which has a gestation period of 41 days, corticogenesis is relatively protracted and continues until approximately 2 weeks after birth, depending on the cortical region (Jackson et al., 1989; Noctor et al., 1997).

The complex process of cortical development can be disrupted due to genetic and environmental factors. Defects in genes such as *LIS1*, *DCX*, and *FLN* identified in humans result in cortical malformations including lissencephaly, doublecortex, and ectopic neuronal clusters (Gleeson et al., 1999). Epigenetic factors such as alcohol, drugs of abuse, methyl mercury, cigarette smoke, and radiation can produce a constellation of cortical defects consisting of abnormal proliferation and migration, cell death, and other central nervous system (CNS) changes. For example, fetal alcohol syndrome can result in altered neuronal distribution and migration, as well as neuronal heterotopias

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(Miller, 1993, 1996, 2006; Ikonomidou et al., 2000; Rice and Barone, 2000; Mendola et al., 2002; Bailey et al., 2004).

Often, cortical dysplasias in humans are not associated with specific genes and result from environmental causes or the combination of genetic and environmental effects (Montenegro et al., 2002; Sisodiya, 2004). Additional evidence points to trauma and infectious diseases during pregnancy as contributing to human cortical dysplasias (Marin-Padilla, 1999; Deukmedjian et al., 2004). As a result, we developed a model of cortical dysgenesis that mimics many features of human disorders (Noctor et al., 2001; Palmer et al., 2001; McLaughlin and Juliano, 2005).

The research reported here focuses on congenital neural migration deficits during the neonatal period. To do this, we used an animal with a highly convoluted cortex, the ferret. The ferret is an ideal model to study cortical migration and transplantation partly because it has a protracted period of corticogenesis; cells continue to divide and migrate until several weeks after birth. In addition, radial glia guide migrating cells from the VZ to the cortical plate (CP) and persist until 2–3 weeks after birth (Voigt, 1989; Juliano et al., 1996). The presence of these glial guides may facilitate repair if transplanted cells are delivered during the window of time when the radial glia are still guiding endogenous neurons to the CP. In our model, an antimitotic (methylazoxymethanol; MAM) is delivered to pregnant ferrets, which temporarily disrupts the birth of cortical neurons (Cattaneo et al., 1995; Cattabeni and Di Luca, 1997; Noctor et al., 2001; Jablonska et al., 2004; McLaughlin and Juliano, 2005). When MAM is injected on embryonic day 33 (E33), the birth of cells populating layer 4 of the somatosensory cortex is interrupted, leading to a set of characteristic effects. While many features of the E33 MAM-treated ferret appear normal, including the topographic maps of somatosensory and visual cortex, the general architecture of the cortex outside of layer 4 in somatosensory cortex, and the distribution of excitatory amino acid receptors, other features are abnormal (Noctor et al., 1997, 2001). These include alteration of thalamocortical projections, which normally terminate in layer 4, the distribution of $GABA_{A\alpha}$ receptors and GABAergic neurons, and the neuronal responses recorded through the cortical layers (Noctor et al., 2001; Palmer et al., 2001; Jablonska et al., 2004; McLaughlin and Juliano, 2005). The E33 MAM-treated ferret model makes an excellent host to accept transplanted neural progenitor cells from embryonic ferrets and mice. The presence of characteristic migratory deficits allows us to assess differences between normal and MAM-treated cortex after receiving donor transplants. In the current study, we used donor cells from 3 sources to determine the potential of distinctive contributors to migrate into damaged cortex *in vivo* and *in vitro*. We included donor cells from (i) embryonic day 27 (E27) ferrets, which are young, theoretically multipotential, and able to migrate to different cortical layers (Bohner et al., 1997), (ii) ferret embryos at E33, which correspond to the population of cells missing from the treated ferrets, and (iii) E14 mouse neural progenitor cells to demonstrate the capability of cells from another species to survive, migrate, and respond to cues after transplantation.

Methods

MAM injection

On the 33rd day of gestation, pregnant ferrets (Marshall Farms, New Rose, NY) were injected IP with methylazoxymethanol (14–17 mg/kg; Midwest Research Institute dissolved in 5 ml of 0.9% sodium chloride). MAM inhibits mitosis for approximately 8 h (Cattaneo et al., 1995; Noctor et al., 2001; Palmer et al., 2001; Jablonska et al., 2004; McLaughlin and Juliano, 2005).

Organotypic cultures

P0 ferret kits were anesthetized with either 50 mg/kg euthasol or sodium pentobarbital. When the animal was unresponsive to painful stimuli, the brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF (containing H_2O, and in mM amounts, NaCl, 124; $NaHCO_3$, 26; glucose, 10; NaH_2PO, 1.2; KCl, 3.2; $MgSO_4$, 1.2; $CaCl_2$, 2.4)). Coronal slices (500 μm) were cut using a tissue chopper and placed on a 0.4-μm culture plate insert (Millicell-CM, Bedford, MA) in a 6-well plate with enough media to form a meniscus above the slice. We used minimum essential medium (MEM) with Earle's salts without L-glutamine (Gibco) supplemented with 10% normal horse serum (Gibco), 0.001% gentamicin, L-glutamine-200 mM 100× (Gibco), and 0.6% glucose (Palmer et al., 2001). Ferret neural progenitor or mouse cells were injected onto the surface of the slice 24 h later. The slices with transplanted cells were placed back into the incubator for an additional 3–5 days to allow enough time for the transplanted cells to migrate.

Preparation of ferret neural progenitor cells

Under sterile conditions, the uterine horns containing either E27 or E33 embryos were removed from a pregnant ferret under 1–2% isoflurane anesthesia. The uterine horns were immediately placed in ice-cold aCSF the embryos removed, and the brains isolated. The meninges and the rostral and caudal poles were removed from the telencephalon, the cerebral cortices were dissected from the brains, and these were placed in ice-cold oxygenated aCSF. The tissue was coarsely chopped using microdissection scissors and then transferred to a 15-ml centrifuge tube containing PBS without calcium or magnesium (Gibco) and 0.6% glucose (Sigma). The solution was then mechanically triturated to form a single cell suspension using a series of 9-in. fire polished Pasteur pipettes. Cell density was determined and the cells prepared for labeling and acute transplantation either into organotypic slices or into host animals. Preparation of cells for transplantation was completed within 2 h. The cells were tested for viability using Trypan blue exclusion.

Cells were also proliferated, passaged, and differentiated to determine their potential fates both *in vivo* and *in vitro*. We refer to these cells as ferret neural progenitor cells (fNPs) with the *caveat* that they may contain post-mitotic cells. Our experiments showed that single cells placed in a flask with media or at low