

Implantation of Adult Human Neural Progenitors into an Animal Model of Cortical Dysplasia

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Background:

Adult human neural progenitor cells (AHNPs) provide a potential method to treat certain brain disorders by restoring a population of neurons that has been lost or whose function is otherwise reduced. For treatment in the post-natal setting, it is logical that the most promising scenarios for neural restoration would involve abnormalities of local circuitry; i.e., restoration of a neural population that typically makes contacts with nearby neurons rather than distant targets that require accurate axonal path-finding that extends over long distances. Epilepsy would be a promising candidate disease for such therapies because, in some cases, there is strong evidence that the primary pathology in the epileptic “focus” is an aberration in local neuronal circuitry.

Cortical dysplasia (CD) often produces severe epilepsy in children and adults. This is a spectrum of disorders that share in common a deviation from normal development during the formation of the neocortex. There is evidence from studies of human focal CD that a reduced number and function of inhibitory interneurons is a common finding and may be responsible for the epileptogenicity of this tissue. My laboratory has studied an animal model of CD, in utero irradiation, for many years. The principle findings of this work demonstrate a reduction in the number and function of inhibitory interneurons in the neocortex. The current project will attempt to restore normal function in the neocortex of irradiated rats using transplanted AHNPs. The ultimate goal is to use AHNPs to treat CD-related epilepsy in humans.

Aims:

Aim 1:

Determine the optimal methods for transplantation of AHNPs into the neocortex of irradiated rats.

Aim 2:

Describe the neuronal phenotypes of the differentiated AHNPs in the host neocortex. Determine the degree to which AHNPs are integrated into the local circuitry using histology and electrophysiology.

Aim 3:

Determine if AHNPs restore normal inhibitory activity to the local circuitry of irradiated rats.

Methods:

General methods:

Animal preparation:

Pregnant Sprague Dawley rats will be exposed to 225 cGy external gamma-radiation using a linear accelerator on E17. Offspring will be born normally and weaned at P21.

Culture of adult human neural progenitors:

AHNPs are obtained from surgery for treatment of intractable epilepsy from temporal cortex, hippocampus and periventricular white matter as previously described (Walton et al. 2006). AHNPs are expanded as described (Walton et al., 2006). Briefly, cells are grown as monolayers on uncoated plastic culture dishes in N2 medium supplemented with 5 % FCS, EGF and FGF2 (20 ng/ml each). Growth factors are added every other day. Upon confluence, cells are passaged using trypsin and split 1:2. GFP-AHNPs are transduced using a Lenti-GFP construct under the SV40 promoter and purified by cell sorting on a FACSVantage (BD, Franklin Lakes, NJ).

Cortical transplantation of AHNPs:

Grafting of GFP-AHNPs into focal cortical dysplasia animals is performed as described (Zheng et al., 2006). Briefly, rat pups at pn1 are anesthetized by hypothermia and placed under a dissecting microscope. Their heads are transilluminated and a Hamilton syringe is lowered through the scalp and skull into the cortex. Approx. 50,000 GFP-AHNPs are injected in a volume of 1 μ l into the right neocortex. Animals are allowed to recover and returned to their dam post-grafting. At 7, 14 and 28 days after transplantation, animals are deeply anesthetized with Avertin and perfused with 4 % paraformaldehyde in 0.1 M PBS.

Histology:

At age 2 wk and 4 wk, animals are sacrificed and processed for immunohistochemistry. Coronal sections (30 μ m) are obtained using a cryostat thorough the motor cortex. Labeled antibodies for GAD67, parvalbumin, somatostatin and calretinen are used to identify subtypes of interneurons. Antibodies for VGlut1, VGlut2 and VGAT are used to identify glutamatergic and GABAergic pre-synaptic terminals.

Slice physiology:

At age 2 wk and 4 wk, animals will be sacrificed for in vitro slice experiments. Slices are obtained and whole cell patch clamp recordings are performed as previously described (Xiang et al. 2006). Transplanted cells are identified using fluorescent microscopy and neurons are imaged for patch clamping using infrared differential interference microscopy.

Specific experiments:

Aim 1:

We will use fluorescent microscopy to quantify the number of surviving transplanted neurons in each animal. We will also measure the maximum distance of migration from the injection site in the coronal plane. These experiments will be performed at 2 wk and 4 wk.

Aim 2:

We will use fluorescent microscopy to determine the identity of transplanted cells by looking for expression on GAD 67, parvalbumin, somatostatin and calretinen. Pyramidal cells will be identified based on morphology and absence of labeling with interneurons-specific antibodies.

We will analyze synaptic connectivity using VGlut1, VGlut2, and VGAT to label glutamatergic and GABAergic pre-synaptic terminals. Confocal microscopy will be used to determine the number of each type of terminal on the dendrites of transplanted neurons.

Synaptic connectivity will be analyzed physiologically using whole cell recordings from transplanted cells. They will first be characterized according to basic membrane and firing properties. Then spontaneous and miniature EPSCs and IPSCs will be recorded. These will be compared to nearby host neurons of a similar type. They will also be compared between controls and irradiated animals to see if the prior radiation treatment affects the degree of connectivity in the transplanted cells.

Aim 3:

We will use whole cell and field potential recordings to determine if the transplanted neurons help to restore the normal balance of excitation and inhibition in the dysplastic cortex of irradiated rats. We will record from layer V pyramidal cells and quantify spontaneous and miniature EPSCs and IPSCs in these cells. Prior studies show that IPSCs will be reduced in frequency in the irradiated rat cortex. We will compare controls, irradiated rats without transplantation, and irradiated with transplantation to see if the normal level of inhibitory synaptic activity is achieved in the irradiated rats after transplantation. We will also examine bursting behavior in slices in the presence of the convulsant, 4-aminopyridine (4-AP). We hypothesize that transplantation will restore the level of bursting to that of controls.

Future Plans:

Successful completion of the proposed experiments will establish a solid foundation for continuing this project as an NIH-funded ROI grant. The ultimate goal of this line of research is to apply similar transplantation techniques to human epilepsies with a documented lack of inhibitory neurons and inhibitory synaptic activity; this includes human focal CD (Calcagnotto et al. 2005).