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Hematopoietic progenitors express myelin basic protein and ensheath axons in *Shiverer* brain

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ABSTRACT

Oligodendroglia are cells of the central nervous system (CNS) that form myelin sheath, which insulates neuronal axons. Neuropathologies of the CNS include dysmyelination of axons in multiple sclerosis and CNS trauma. Cell replacement is a promising but largely untested therapy for dysmyelination. *Shiverer* mouse, a genetic mutant that does not synthesize full-length myelin basic protein (MBP), a critical prerequisite protein in CNS myelin sheath formation, provides an unequivocal model for determining the potential of stem cells to become oligodendroglia. We demonstrate that adult wild-type mouse bone marrow stem cells can express MBP and ensheath axons when transplanted into *Shiverer* brain.

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1. Introduction

Neuropathologies of the central nervous system include the involvement of dysmyelination of axons. Examples of these pathologies are multiple sclerosis, brain and spinal cord trauma and inherited leukodystrophies, where myelin is disrupted in lesion sites, accompanied by oligodendrocyte death, subsequent axonal damage and ultimately neuronal death and loss of function results. Myelin repair by endogenous oligodendroglia occurs but is limited in its extent. Therapeutic cell replacement is a promising but largely untested treatment for dysmyelination.

The myelin sheath is formed by multiple wrappings of an oligodendroglial cell plasma membrane around an axon between nodes of Ranvier. An individual oligodendrocyte may ensheath multiple, as many as fifty, individual axons. Myelin basic protein (MBP), which is obligatory for ensheathment, is associated with the major dense line of the myelin sheath, that is formed by condensation of the cytoplasmic faces of wrapped plasma membrane of oligodendrocytes. MBP constitutes 30–40% of the protein content of CNS myelin. CNS MBP is a family of alternatively spliced proteins that in mice have molecular masses of 21.5, 18.5, 17 and 14 kDa present in a ratio of 1:10:3.5:35, respectively. MBP expression is lost in dysmyelination lesion sites of CNS neuropathologies.

Shiverer mouse is a genetic mutant model of dysmyelination that does not synthesize classical myelin basic protein as a consequence of a deletion of exons 3–7 of the corresponding gene. *Shiverer shi/shi* homozygotic mice have oligodendrocytes but fail to make a compacted myelin sheath with the typical MBP major dense line. Hence, the mice have poor axonal insulation and neuroconduction and as a result they progressively exhibit persistent shivers, tremors and seizures leading to early death at 15 to 18 weeks of age. Oligodendroglial cell replacement therapies in *Shiverer* mice have been reported using embryonic stem cells (Brüstle et al., 1999), neural stem cells (Yandava et al., 1999; Mitome et al., 2001) and oligodendroglial precursor cells (Vignais et al., 1993) transplanted into neonatal *Shiverer* mice.

We discovered a subset of adult CD34+ bone marrow stem cells that naturally express novel alternatively spliced forms of myelin basic protein but do not express classical neural myelin basic protein that is crucial for oligodendroglial function (Grima et al., 1992; Zelenika et al., 1993; Marty et al., 2002; Goolsby et al., 2003). We developed a method to culture a pure population of these adult mouse bone marrow CD34+ stem cells (BMSCs) (Goolsby et al., 2003). These CD34+ cells exhibit not only the expected hematopoietic stem cell

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genes but surprisingly also express an array of embryonic stem cell genes — genes that have been used to induce pluripotency in adult fibroblasts by genetic manipulation (Takahashi and Yamanaka, 2006; Wernig et al., 2008). These CD34+ cells enigmatically also express lineage genes for each of the three embryonic germ layers: ectoderm, mesoderm and endoderm (Fig. 1A modified from Goolsby et al., 2003; Pessac et al., 2011, 2012). Furthermore, these CD34+ stem cells manifest differentiated neuronal major structural proteins like neurofilament and NeuN and they express genes that denote the oligodendrocyte phenotype like CNPase, NG2, GalC and MOSP, but they do not exhibit classical myelin basic protein, encoded by a major structural gene of oligodendrocytes, which is involved in their function of myelination of axons. These adult CD34+ stem cells are also multipotent in chimeric mice (Fig. 1B modified from Pessac et al., 2012). These are the rationale that led us to ascertain whether these adult bone marrow stem cells are also multipotent when transplanted into an adult animal model of neurodegeneration. For example, do they have the capacity to form oligodendroglia that express classical MBP and ensheath axons in *Shiverer* CNS. Therefore, cultured wildtype CD34+ BMSCs were injected into adult *Shiverer* mouse brain. They migrated throughout the brain and significantly some differentiated into morphologies reminiscent of oligodendroglia that expressed classical MBP and ensheathed axons.

2. Materials and methods

2.1. Pure cultures of CD34 + hematopoietic precursors from adult mouse bone marrow

Cells were cultured from adult wild-type C3H mice and from adult Cha.SWV(C3Fe)-*Mbp<shi>*/J *Shiverer* mice (Jackson Laboratories Bar

A)

Embryonic Stem Cell

Oct-4
Sox-2
Klf-4
Rex-1
LIN-28

Ectoderm		Mesoderm		Endoderm	
Neural	Hematopoietic	Cardiac	Pancreatic	Intestinal	
Pax-6	CD34	MEF-2C	Pdx-1	Villin	
HuC/HuD	Sca-1	Troponin C	Ptf-1a		
Neurofilament H	cKit	MLC-2	Ngn-3		
NeuN	AA4.1		FoxA2		
GAD65	CD45		Sox17		
	GATA-2		CXCR4		
GFAP(-)	LMO-2		Hnf1B		
CNPase	FLK-2 (-)		Hnf4A		
MOSP	Lin (-)		Hnf6		
MBP2			Nkx2.2		
Galactocerebroside			Pax-6		
NG2			Glucokinase		

B)

X-gal Staining of Chimeric Mice

	ROSA Chimeric Mouse Organs						Control Chimeric Mouse Organs			
Germ Layer / Tissue/Organ	**Mouse # (Sex: M=male, F=female)**									
Ectoderm	**#1 (M)**	**#2 (F)**	**#3 (F)**	**#4 (M)**	**#5 (F)**	**#6 (F)**	**#1 (M)**	**#2 (F)**	**#3 (F)**	**#4 (F)**
Brain	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)
Retina	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sp. Cord	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DRG	(ND)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Skin	(-)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
Mesoderm										
Bone marrow	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Heart	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Muscle	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Femur bone	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Endoderm										
Pancreas	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Lung	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Intestine	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Liver	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Bladder	(-)	(-)	(+)	(ND)	(ND)	(ND)	(-)	(-)	(-)	(-)

Fig. 1. A. Adult mouse CD34+ bone marrow stem cell gene expression. Embryonic stem cell gene and embryonic germ layer lineage gene expression in adult CD34+ cells were reported previously. B. X-gal staining of chimeric mice showed that adult ROSA mouse CD34+ bone marrow stem cells are multipotent when transplanted into wildtype mouse blastocysts. The ROSA CD34+ cells gave rise to X-gal+ brain, dorsal root ganglia, skin, bone marrow, bone, pancreas, intestine, liver and bladder.
Panel A modified from Goolsby et al. (2003) and Pessac et al. (2011, 2012). Panel B modified from Pessac et al. (2012).