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Sensors of Succinate: Neural Stem Cell Grafts Fight Neuroinflammation

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https://doi.org/10.1016/j.stem.2018.01.019

In this issue of *Cell Stem Cell*, Peruzzotti-Jametti et al. (2018) demonstrate how neural stem cells, transplanted in a mouse model of multiple sclerosis, respond to extracellular succinate and modulate neuroinflammation by releasing anti-inflammatory prostaglandin E2 and scavenging succinate. This mechanism reduces CNS damage and ameliorates motor impairment.

Neural stem cell (NSC) transplantation has the potential to become a novel therapeutic approach for a variety of CNS disorders. Differentiation of grafted NSCs for cell replacement and reconstruction of circuitry can achieve functional recovery in the host brain. Transplanted NSCs have also led to improvements in animal models of human diseases through other modes of action such as immunomodulation. Emerging experimental evidence indicates that cross-talk between grafted NSCs and immune cells, e.g., microglia and infiltrating monocyte-derived macrophages (MDMs), plays an important role for regenerative responses in the adult brain (Kokaia et al., 2012; Martino et al., 2011). Currently, the molecular mechanisms underlying this interaction are poorly understood. In this issue of *Cell Stem Cell*, Peruzzotti-Jametti et al. (2018) identify a novel regulatory mechanism involved in the dialog between NSCs and immune cells in a model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE) (Figure 1).

Peruzzotti-Jametti and co-workers (Peruzzotti-Jametti et al., 2018) first demonstrated that intracerebroventricular injection of NSCs or induced NSCs (iNSCs) in mice with chronic EAE led to long-lasting improvement of behavioral scores and reduced demyelination and axonal loss in the spinal cord. In the transplanted animals, microglia and MDMs with pro-inflammatory phenotype decreased whereas those that were anti-inflammatory increased. Similarly, brains of NSC- and iNSC-transplanted EAE mice exhibited reduced expression of pro-inflammatory genes. The chronic EAE caused several cerebrospinal fluid (CSF) metabolites to significantly increase, including succinate, and, interestingly, transplantation of NSCs and iNSCs resulted in a drop in succinate CSF levels. This metabolite, released from inflammatory macrophages, has previously been identified as a pro-inflammatory signal enhancing IL-1β production (Littlewood-Evans et al., 2016; Tannahill et al., 2013). Taken together, the findings of Peruzzotti-Jametti and co-workers (Peruzzotti-Jametti et al., 2018) suggested a previously unknown molecular mechanism involving succinate underlying the anti-inflammatory action of grafted NSCs and iNSCs.

In order to clarify this mechanism in detail, a series of *in vitro* experiments were carried out. When culturing bone-marrow-derived, lipopolysaccharide (LPS)-activated macrophages with or without NSCs/iNSCs in a trans-well system, the authors found that the NSCs/iNSCs downregulated several pro-inflammatory genes while concomitantly upregulating anti-inflammatory genes in the activated macrophages. Importantly, co-culturing LPS-activated macrophages with NSCs/iNSCs caused a substantial reduction of both extracellular and intracellular succinate. These *in vitro* experiments raised the possibility that NSCs/iNSCs could influence the phenotype of activated macrophages by altering the levels of succinate. The authors then analyzed whether succinate released by activated pro-inflammatory macrophages could affect NSCs/iNSCs. In support of this idea, both NSCs and iNSCs indeed expressed the succinate receptor (SUCNR1/GPR91) and, indicative of its activation, the cells released intracellular calcium stores and upregulated phospho-p38 mitogen-activated protein kinase when exposed to succinate.

The authors then sought to determine how grafted NSCs/iNSCs exerted their anti-inflammatory actions. First, they showed that SUCNR1 stimulation in NSCs induced secretion of prostaglandin E2 (PGE2). However, experiments comparing the effects of blocking SUCNR1 with those of inhibiting prostaglandin signaling provided evidence of additional mechanisms. To explore this possibility, the authors used NSCs isolated from *SUCNR1*$^{-/-}$ mice and controls and found that only in wild-type mice, succinate stimulation induced expression of the dicarboxylate co-transporter SLC13A5, known to be involved in succinate transport. In addition, transplantation of NSCs/iNSCs in EAE mice caused depletion of succinate in CSF. Based on these findings, the authors hypothesized that succinate could activate SLC13A5 in NSCs/iNSCs to scavenge extracellular succinate. In support, they found that SUCNR1 signaling in NSCs induced uptake of succinate *in vitro*, decreasing its extracellular pool and preventing activation of pro-inflammatory macrophages.

Finally, *in vivo* experiments confirmed that transplanted NSCs, responding to SUCNR1 signaling, exhibit their anti-inflammatory effect by scavenging succinate. Transplantation of *SUCNR1*$^{-/-}$ NSCs in EAE mice induced only slight behavioral recovery and failed to shift pro-inflammatory microglia and MDMs toward an anti-inflammatory phenotype. Moreover, grafted *SUCNR1*$^{-/-}$ NSCs did not affect CSF levels of succinate.

It now seems highly warranted to explore the extent to which the

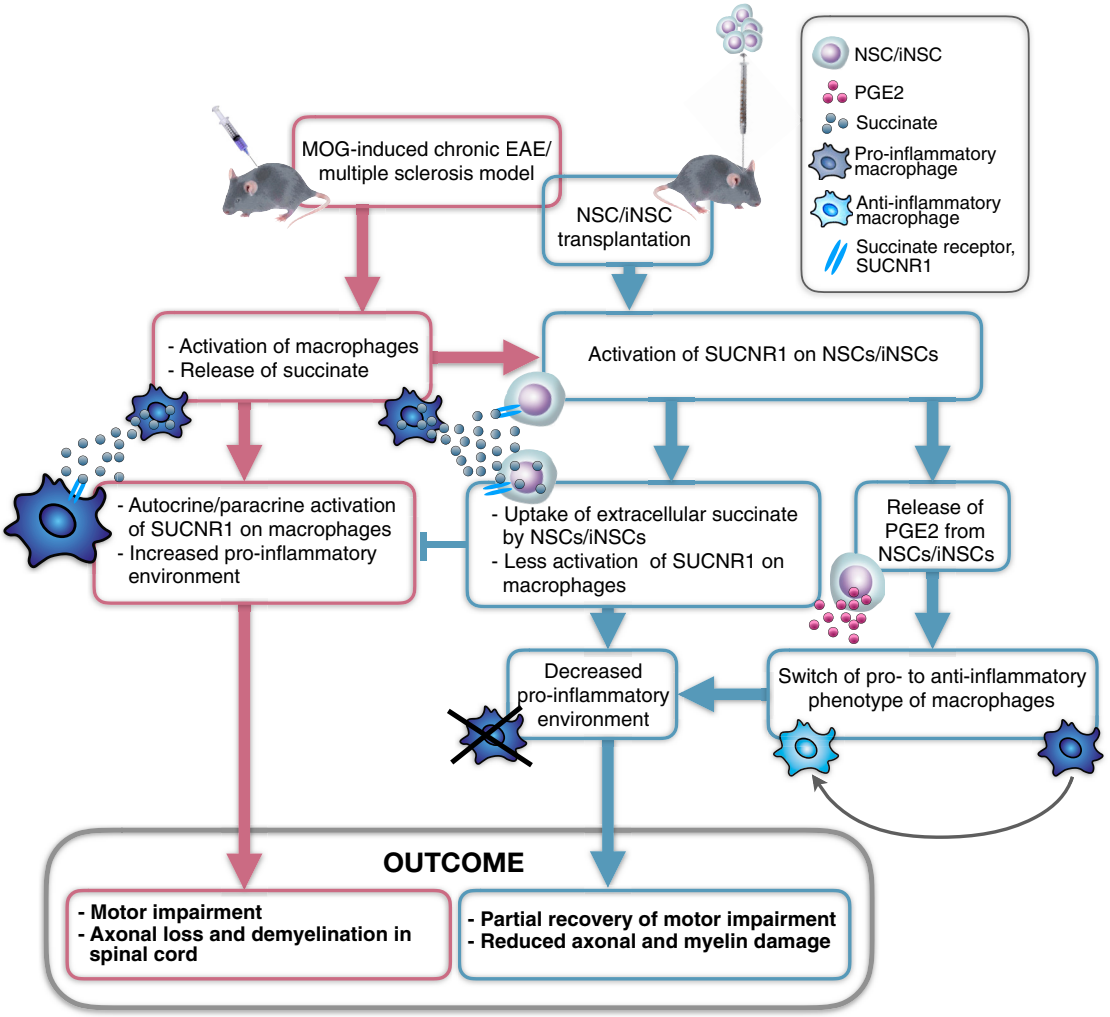

Figure 1. Transplantation of NSCs or iNSCs in an Animal Model of Multiple Sclerosis Leads to Reduced Pathology and Functional Improvements

Red color depicts a multiple sclerosis (MS) model with activation of immune cells (microglia and MDMs), release of succinate, and detrimental outcome of inflammation. Blue color illustrates events induced by transplantation of NSC or iNSCs: decreased levels of macrophage-derived succinate, release of anti-inflammatory PGE2 from NSC/iNSCs, switch of pro- to anti-inflammatory activity of macrophages, and overall suppression of the pro-inflammatory environment.

immunomodulatory mechanisms reported in NSC grafts in EAE (Peruzzotti-Jametti et al., 2018) are involved in other brain disorders associated with inflammation. For example, grafted NSCs have been reported to suppress inflammation after ischemic stroke (Bacigaluppi et al., 2009; Horie et al., 2011; Kelly et al., 2004). We recently showed (Wattananit et al., 2016) that during the first week after stroke in mice, microglia in the ischemic hemisphere exhibited predominantly pro-inflammatory activity, whereas MDMs were largely pro-inflammatory at day 3, 50% were pro-inflammatory at day 7, and the anti-inflammatory phenotype dominated thereafter. Succinate already accumulates in the brain during ischemia but is then rapidly metabolized (Chouchani et al., 2014). Therefore, it seems more likely that succinate derived from activated microglia and MDMs in the post-ischemic phase could interact with the grafted NSCs, leading to release of PGE2 and extracellular succinate scavenging. Whether this mechanism, switching to a more anti-inflammatory tissue environment, occurs after NSC transplantation in stroke and contributes to repair and improved recovery needs to be determined.

From a clinical perspective, it is particularly important that the new mechanism also operates in human-derived NSCs. Thus, the authors found that both human fetal NSCs and iNSCs expressed functional SUCNR1, and activation of the SUCNR1 signaling pathway triggered release of PGE2 leading to anti-inflammatory effects. Human NSCs also upregulated expression of succinate transporters in response to succinate. Taken together, these new findings support the idea of delivering human NSCs as a potential immunomodulatory therapeutic strategy in human multiple sclerosis. In fact, phase 1 studies with intraventricularly or intrathecally administered human NSCs have already been initiated in MS patients, e.g., by IRCCS Ospedale San Raffaele, Milan, Italy (ClincalTrials.gov

NCT03269071). The experimental basis for clinical trials has been strengthened by the present identification of one major molecular mechanism underlying a possible clinical improvement following NSC transplantation. In the future, it will be important to compare the therapeutic efficacy of NSC transplantation with that of pharmacological interventions targeting the same mechanism. However, a major advantage of grafted NSCs, illustrated by the present findings, is their unique properties with multiple modes of action, not only as sources of cells for replacement but also as sensors of the microenvironment, aiming to maintain homeostasis and optimize the conditions for repair and recovery.

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Reversing Time: Ezh1 Deficiency Hastens Definitive Hematopoiesis

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https://doi.org/10.1016/j.stem.2018.02.006

The inability to derive multipotent hematopoietic stem cells *in vitro* stems in part from a limited understanding of how multipotency is acquired during development. Recently in *Nature*, Vo et al. (2018) reveal the epigenetic enzyme Ezh1 as a master regulator of multipotency during hematopoietic stem cell development.

The generation of definitive, adult hematopoietic stem cells (HSCs) from autologous pluripotent cells is one of the highest research priorities in the field of hematology. Though allogeneic HSC transplantation from cord blood or HLA-matched mobilized donors is the standard of care for both malignant and hereditary hematological disorders, availability of donors is the biggest obstacle to treatment. The generation of HSCs from patient-derived induced pluripotent stem cells (iPSCs) represents a potentially unlimited source of autologous HSCs. However, despite decades of work, the derivation of definitive HSCs from iPSCs remains elusive; absent transcriptional reprogramming, hematopoietic differentiation of pluripotent cells generates embryonic-like, lineage-restricted progenitors with limited self-renewal capability. A critical limitation of this work is a fundamental lack of understanding of the mechanisms that regulate multipotency across hematopoietic development.

Recently in *Nature*, elegant work by George Daley and colleagues has shed a new light on definitive HSC establishment by identifying the chromatin remodeling factor Ezh1 as a critical regulator of hematopoietic multipotency during development (Vo et al., 2018) (Figure 1). Ezh1 and Ezh2 are homologs that can interchangeably associate with two other components, Eed and Suz12, to form the Polycomb repressive complex 2 (PRC2) (Margueron and Reinberg, 2011). Canonical PRC2 facilitates transcriptional repression by mediating reduced chromatin accessibility catalyzed by methylation of histone H3 at Lysine 27 (H3K27). Examination of the function of PRC2 in