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Neurorestoration after stroke

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Recent advancements in stem cell biology and neuromodulation have ushered in a battery of new neurorestorative therapies for ischemic stroke. While the understanding of stroke pathophysiology has matured, the ability to restore patients' quality of life remains inadequate. New therapeutic approaches, including cell transplantation and neurostimulation, focus on reestablishing the circuits disrupted by ischemia through multidimensional mechanisms to improve neuroplasticity and remodeling. The authors provide a broad overview of stroke pathophysiology and existing therapies to highlight the scientific and clinical implications of neurorestorative therapies for stroke.

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Stroke is a devastating neurological condition and a leading cause of morbidity and mortality worldwide. This severe disease is responsible for roughly 1 in every 18 deaths in the United States.[129] Nearly half of all stroke survivors require long-term care.[145] The functional and cognitive disabilities of stroke survivors result in significant long-term health care costs. It was estimated that direct and indirect stroke-related costs resulted in a health care expenditure of $73.7 billion in 2010.[116]

Currently, tissue plasminogen activator (tPA) is the only FDA-approved drug for acute ischemia.[46] While tPA has markedly improved stroke care, it must be administered within a narrow time frame, limiting its clinical utility. Less than 10% of stroke patients can benefit from such treatments due, in large part, to late referral to the hospital and an inability to meet other eligibility criteria.[2] Recently, endovascular therapies have also shown promise in treating acute stroke. After the acute period, stroke survivors face a myriad of challenges, including, but not limited to, hemiparesis and aphasia.[55] While evidence supports the utility of rehabilitation efforts after acute stroke,[150] complete neurological and physical recovery is rarely complete.

Thus, there is a distinct need for improved stroke recovery therapies. The profound vacuum in this field is particularly disappointing because evidence suggests that functional recovery is possible.[115] In this review, we discuss current stroke therapies and explore the burgeoning fields of cellular transplantation and neuromodulation as promising neurorestorative therapies for stroke (Fig. 1).

Stroke Pathophysiology

The cellular consequences of stroke include a complex and dynamic response of excitotoxicity, mitochondrial dysfunction, and oxidative stress.[53,112] While these pathways are well recognized in the promotion of neural and glial injury, stroke researchers have cultivated a more nuanced understanding of these mechanisms. Specifically, the pathways activated after ischemia also promote recovery; there exists duality in poststroke pathophysiology, which shifts depending on timing and the relative contribution of each constituent pathway.

Excitotoxicity and calcium (Ca^{2+}) overload are key contributors to the early stages of ischemic cell death. The lack of nutrients available to neurons after ischemia disrupts ionic gradients, resulting in excess release of excitatory amino acids—chiefly glutamate—driving an intracellular Ca^{2+} influx and setting in motion apoptotic and necrotic pathways.[102]

Mitochondria, reservoirs for proapoptotic and antiapoptotic proteins and cytochrome C, experience dysfunction secondary to Ca^{2+} accumulation.[103,114] Mitochondrial injury enables release of cytochrome C, activating cas-

ABBREVIATIONS BCI = brain-computer interface; G-CSF = granulocyte colony-stimulating factor; MSC = mesenchymal stem cell; NPC = neural progenitor cell; NSC = neural stem cell; NT2N = Ntera2/D1 neuron-like; PSD-95 = postsynaptic density-95; RCT = randomized controlled trial; tDCS = transcranial direct current stimulation; TMS = transcranial magnetic stimulation; tPA = tissue plasminogen activator.

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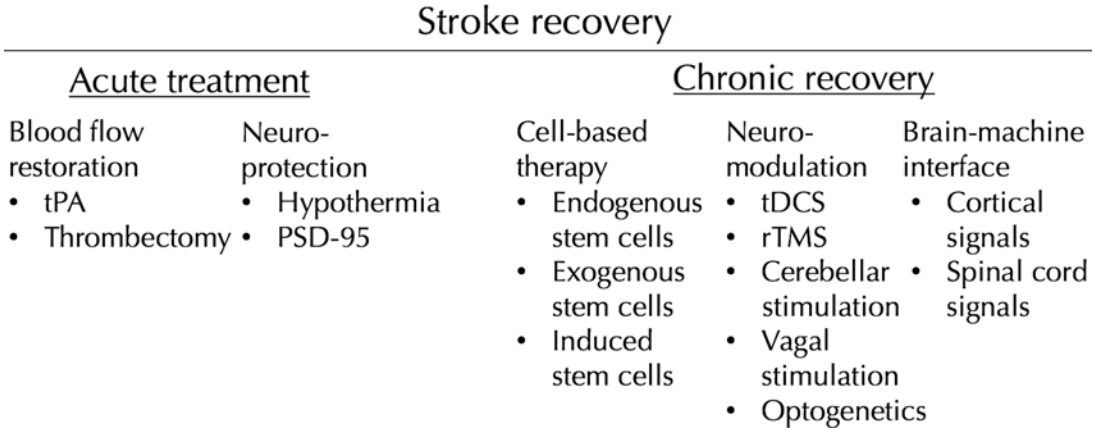

FIG. 1. Overview of neurorestorative modalities. rTMS = repetitive TMS; tDCS = transcranial direct current stimulation.

pase-dependent cellular death pathways. Reactive oxygen species, produced by mitochondria, have been implicated in reperfusion injury following ischemia.[83]

Oxidative and nitrosative stress, via free radicals, are also important mediators of ischemic injury and inhibitors of recovery. Ca^{2+} influx upregulates nitric oxide production, a byproduct of which is peroxynitrite, which can produce injury.[75] Other contributors to oxidative stress include mitochondrial dysfunction[83] and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase.[15] There are 2 general hypotheses regarding the mechanism of oxidative stress–related injury. In the first scenario, the redox environment of cells modulates signal transduction cascades that tip the balance between prodeath and prosurvival pathways.[34] In the second scenario, reactive oxygen species and perhaps reactive nitrogen species, including peroxynitrite, act directly as executioners of cell death.[24] Other contributors to stroke pathophysiology include protein misfolding, also a result of excess Ca^{2+},[130] glial injury,[108] and a broader proinflammatory response.[76]

In addition to the cellular insults incurred following stroke, neural circuits are also disrupted due to shifts in the excitation-inhibition balance in neural networks. In the setting of a long-term depression of inhibitory signals, cortical hyperexcitability peaks several weeks after stroke, though it can persist for months.[18,135] Sustained increase in glutamate transmission following stroke also contributes to greater excitatory signals.[23] Modulation of the tonic inhibition regulated by GABA(A) receptors has been shown to facilitate functional recovery in animal models.[29] The unaffected contralesional hemisphere can also influence the excitatory state of the damaged hemisphere.[113] Each component of the pathophysiological response following stroke, on both cellular and circuit levels, represents an opportunity for limiting initial injury and hastening recovery.

Existing Stroke Therapies

Blood Flow Restoration

Acute stroke therapy today largely consists of intravenous tPA, administered within a narrow time window.[16,64] This window initially broadened to administration within 3 hours of symptomatic onset and was extended to 4.5 hours after a large trial demonstrated continued benefit of tPA at this time point.[63] In 2015, endovascular therapies demonstrated a significant additive role in improving outcomes across 5 randomized controlled trials (RCTs) studying intraarterial thrombectomy.[8,21,58,81,133] Given the diversity of strokes and patient-specific characteristics (e.g., collaterals and vasculature), patient selection may be critical for the ultimate success of these therapies.[101] A key drawback of tPA and endovascular therapies is that the vast majority of stroke patients cannot get access to these treatments within the narrowly defined time limits. Beyond the acute time period, there is evidence that physical rehabilitation focused on the injured area is effective.[150] However, neurological recovery with physical rehabilitation is rarely complete—innovative approaches to enhance the body's endogenous regenerative abilities remain an opportunity for improvement.

Neuroprotection

The brain regions adjacent to the infarct, the ischemic penumbra, possess the greatest potential for poststroke recovery.[22] Thus, limiting periinfarct damage is the objective of many neuro-protective treatments. Promising preclinical studies have focused on single pathways to achieve neuroprotection; however, the failure of clinical trials investigating neuroprotective strategies suggests that multiple pathways must be disrupted in humans to achieve similar success.[42]

Mild neurological hypothermia (33°C), has demonstrated improved neurological outcomes for patients with global cerebral ischemia secondary to cardiac arrest and neonatal hypoxic-ischemic encephalopathy.[9,139] Mild hypothermia is currently being investigated as an acute stroke therapy, with trials to date proving the feasibility of this approach.[121]

Postsynaptic density-95 protein (PSD-95) represents an alternative neuroprotective target. This protein serves as an intermediary between NMDA receptors and the signaling pathways that produce the deleterious excitotoxic cascade. Inhibition of PSD-95 has been shown to reduce stroke volume in primates.[32] A double-blind RCT demonstrated safety and improved neurological outcome and

fewer acute infarcts in patients undergoing endovascular intracranial aneurysm repair who received a PSD-95 inhibitor.[69]

A third opportunity to enhance neuroprotection may exist in mediating the Ca^{2+} dysregulation observed after stroke. An acid-sensing ion channel, ASIC1a, is involved in the Ca^{2+} influx—inhibiting this channel may be neuroprotective.[154] In addition to the Ca^{2+} influx, failure of Ca^{2+} efflux contributes to the Ca^{2+} accumulation. Prostaglandin E2 EP1 receptors have been implicated in the failure of the Na^{+}/Ca^{2+} exchanger during ischemia. Inhibition of these receptors has been shown to be neuroprotective.[1,85] It remains unlikely that inhibition of any single stroke injury pathway will yield clinically meaningful neuroprotection. However, as researchers gain the ability to manipulate multiple recovery pathways, more effective neuroprotective therapies will emerge.

Cellular Replacement Therapies

Stem cells are undifferentiated cells that may specialize into multiple cell types and can self-renew. Stroke pathophysiology may be particularly amenable to stem cell therapy. After the initial injury and associated changes, there is no enduring neurodegenerative process inhibiting recovery. Two main lines of stem cell therapies for stroke have emerged: endogenous strategies focusing on facilitating mobilization, longevity, and production of existing neural stem cell and exogenous treatments in which cells are transplanted from another source into a patient.

Endogenous Stem Cells

The canonical niches for neural stem cells (NSCs) in the brain are the subventricular zone[57] and dentate gyrus.[3,43] Researchers have observed changes in migration patterns of neural progenitor cells (NPCs) following neurological injury,[56] a key finding underlying endogenous stem cell therapeutic strategies. Furthermore, ischemia induces NPC proliferation[118] and there is evidence of NPC differentiation into the predominantly injured cell type in a given region.[6]

Researchers have focused on neurogenesis-promoting pathways as potential methods to stimulate NPC proliferation.[41,91] This approach is characterized by the use of regulatory factors that have been implicated in neurogenesis, such as glial-derived neurotrophic factor, brain-derived neurotrophic factor, vascular endothelial growth factor, granulocyte colony-stimulating factor (G-CSF), basic fibroblast growth factor–2, insulin-like growth factor–1, bone morphogenetic protein–7, epidermal growth factor, and transforming growth factor–α.[27,41,60,80,91,97,127,131,136,149,152] Alternative strategies to increase NPC proliferation include antiinflammatory drugs, noncoding RNAs, and hormones such as erythropoietin and growth hormone.[71,137,151]

A complementary approach strives to limit NPC death through administration of G-CSF and insulin-like growth factor–1 to alter key survival pathways.[96] Inhibition of p53 and use of cyclosporine have also been studied as strategies to extend NPC survival.[47,104] Ongoing clinical trials are investigating the dual roles of G-CSF, activation of endogenous bone marrow cells and neuroprotection, to determine efficacy in stroke recovery.[44,84] A recent review of 10 studies comprising 711 patients reported that G-CSF is safe and well tolerated. Moreover, G-CSF may foster functional recovery, as measured by the National Institutes of Health Stroke Scale and modified Rankin Scale scores.[48]

Methods that drive NPC proliferation to a clinically meaningful degree remain elusive and carry with them an innate risk of tumorigenesis. As endogenous stem cell strategies are investigated in clinical trials, the propensity of these cells to give rise to malignancies must be closely monitored.

Exogenous Stem Cells

Exogenous stem cell therapies can be stratified as immortalized cell lines, NPCs or NSCs, and bone marrow–derived progenitors and stromal cells.[12] Immortalized cell lines are developed from tumor cells or from genetic manipulation. Ntera2/D1 neuron-like (NT2N) cells are derived from teratocarcinoma and differentiate into postmitotic neuron-like cells with addition of retinoic acid and mitotic inhibitors.[122] NT2N cell transplantation has been shown to improve outcome in several preclinical models.[66,132] ReNeuron's ReN001 cells, in which *myc* is genetically manipulated, have demonstrated dose-dependent recovery in stroke models in rodents[144] and have been engineered to be immortalized only in the presence of tamoxifen to minimize the risk of tumor formation.[143]

NPCs are derived from embryonic and fetal tissue and can differentiate into astrocytes, neurons, and oligodendrocytes.[52] Preclinical stroke models have revealed that NPCs can migrate to the injured brain regions and foster recovery.[86,128,155] There is also evidence that NPCs integrate into existing tissue and take on neuronal characteristics, including expression of synaptic proteins, synapse formation, and electrophysiological properties.[19,38,39]

Progenitor cells, derived from bone marrow, umbilical cord blood, and adipose tissue, have demonstrated improvement in recovery in preclinical models.[140] Several cell types are included in these strategies, and it appears that the mononuclear or marrow stromal cell component mediates recovery; however, it is not clear which specific subtype is responsible for improving functional outcomes.[105] Multiple trials have been performed or are ongoing that use these exogenous stem cells (Table 1).[98]

The advent of induced pluripotent stem cells created a paradigm shift in cell-based therapy. The ability to differentiate somatic cells such as fibroblasts into pluripotent stem cells bypassed many of the concerns of traditional stem cell therapy. Further development has led to vector- and transgene-free techniques to derive induced pluripotent stem cells that improve functional outcome after brain ischemia.[110] It is now possible to produce neural cells directly from mouse or human fibroblasts using transcription factors, bypassing pluripotent stages, a development that may have marked clinical significance.[117]

Delivery

Peripheral delivery techniques, whether intravenous or intraarterial, rely on inflammatory modulation or para-

TABLE 1. Current clinical trials of exogenous stem or progenitor cells for stroke recovery

Trial ID No.	Phase	Cell Type	Delivery Mechanism	Status
NCT00473057	I	BMMNC	IA or IV	Complete, no reported results
NCT02065778	I	BMMNC	IT	Complete, no reported results
NCT01501773	II	BMMNC	IV	Safe, feasible
NCT01849887	I	BMMNC	IV	Not currently recruiting
NCT00859014	I	BMMNC	IV	Safe, feasible
NCT02425670	II	BMMNC	IV	Safe, feasible, no efficacy benefit
NCT01832428	I/II	BMMNC	IT	Recruiting
NCT02245698	I	BMMNC	IT	Recruiting
NCT02290483	II	BMMNC	IA	Recruiting
India, 2011	I/II	BMMNC	IV	Safe, feasible, improved neurological outcomes
NCT01436487	II	Multistem	IV	Safe, feasible, no efficacy benefit
NCT02117635	II	CTX0E03, NSC	IC	Safe, improved neurological outcomes
NCT01151124	I	CTX0E03, NSC	IC	Not currently recruiting
NCT01453829	I/II	ASC	IA	Not currently recruiting
NCT01091701	I/II	MSC	IV	Not currently recruiting
South Korea, 2010	I/II	MSC	IV	Safe, feasible, improved neurological outcomes
NCT00875654	II	MSC	IV	Not currently recruiting
NCT01297413	I/II	MSC	IV	Recruiting
NCT01678534	I/II	MSC	IV	Not currently recruiting
Japan, 2011	I	MSC	IV	Safe, feasible, decreased infarct volume
NCT01714176	I	MSC	IC	Recruiting
NCT01716481	III	MSC	IV	Recruiting
NCT0146172	II	MSC	IV	Not currently recruiting
NCT01922908	I/II	MSC	IV	Not currently recruiting
NCT01468064	I/II	MSC, EPC	IV	Recruiting
NCT00761982	I/II	CD34+	IA	Safe, feasible, increased β-NGF
NCT00950521	II	CD34+	IC	Complete, no reported results
NCT00535197	I/II	CD34+	IA	Safe, feasible, reduced infarct volume
NCT01518231	I	CD34+	IA	Recruiting
NCT01438593	I	CD34+	IC	Not currently recruiting
NCT01310114	II	PDC	IV	Stopped by sponsor
NCT01327768	I	OEC	IC	Recruiting
NCT01287936	I/II	SB623	IC	Safe, improved neurological outcomes
BB-IND 7082	II	NT2N	IC	Safe, feasible, improved neurological outcomes in secondary end points
BB-IND 7082	I	NT2N	IC	Safe, improved neurological outcomes

ASC = adipose-derived stromal cells; BMMNC = bone marrow mononuclear cells; EPC = endothelial progenitor cells; IA = intra-arterial; IC = intracranial; IT = intrathecal; IV = intravenous; NGF = nerve growth factor; NT2N = tetracarcinoma cell-derived neurons; OEC = olfactory ensheathing cells; PDC = placenta-derived stem cells; SB623 = human mesenchymal stromal cells.

crine effects of the cells on the postischemic brain.[61,78] However, invasive transplantation, particularly intracerebral delivery, of stem cells provides for transplantation of large cell numbers at or near to the site and facilitates the trophic effects of stem cells. Advanced delivery methods are under development, including bioengineered polymers, to enhance stem cell survival and efficacy. Inert polymer matrices, such as hydrogels and particles, have been described for stem cell delivery.[148,157]

There are relative benefits and drawbacks for each delivery strategy. Intravenous administration is likely the safest and easy to perform but exposes cells to filtration by peripheral organs, such as the spleen and liver. Intraarterial transfusions enable improved targeting of cells with fewer cells being lost to other tissues, but these transfusions require arterial access. Intraventricular delivery increases safety risks inherent to accessing the ventricular system, but it provides closer proximity to the infarct. Intracerebral transplantation allows for direct delivery to perilesional tissue, but it is the most invasive technique.

Development of multimodal molecular imaging techniques will be invaluable to identify optimal stem cell

delivery methods and to monitor transplanted cells. One approach utilizes superparamagnetic iron oxide–based MRI of grafted cells to observe migration in experimental stroke models.[62,72,156] Our group combined this approach with reporter gene–based molecular imaging techniques. We were able to monitor the fate and function of grafted cells in real time using multimodal MRI and bioluminescent imaging.[39] Clinical translation of molecular imaging techniques will enable an improved understanding of which delivery strategies are best able to deliver healthy stem cells in clinically meaningful doses.

Clinical Trials

To date, clinical trials of cell transplantation for stroke have focused on assessing safety and efficacy. A significant concern for any stem cell therapy is risk of tumor formation—careful classification and understanding of the underlying biology are critical to avoid such adverse effects.[77] Immortalized NT2N cell lines were the first human cells used in a Phase I trial. Cells were implanted into the infarcts of 12 patients 0.5–6 years following a basal ganglia stroke.[93] No major adverse events occurred, and significant functional improvement was observed in this initial study. A subsequent Phase II trial with these NT2N cells recapitulated the safety of cellular transplantation though the primary outcome, motor function, was not met.[92] An open-label, single-blinded RCT using mesenchymal stem cells (MSCs) demonstrated significant functional improvement based on the modified Rankin Scale score with no difference in adverse events, and multiple other trials have shown safety and feasibility.[10,11,95] Furthermore, RCTs using bone marrow mononuclear cells have also shown safety and feasibility.[51,111,134] A phase 1/2A study of transplanted human modified bone marrow–derived stromal cells has demonstrated safety and feasibility with direct intracerebral transplantation 0.5–5 years poststroke with improvement in neurological outcomes.[142] Interim results from the first trial of NSCs for ischemic stroke, an open-label, dose-escalation study, have shown no adverse events and improved functional outcomes in 11 patients with follow-up between 9 and 24 months.[82] Demonstration of efficacy in double-blinded RCTs is needed, but numerous clinical trials are underway (Table 1) to determine whether cell-based therapy will become the next modality of restorative stroke therapy.

Neuromodulation

Neuromodulation is a well-established disruption of the excitatory-inhibitory balance in neural networks following ischemic stroke.[18,23,135] Modulation of the tonic inhibition regulated by receptors of inhibitory neurotransmitters has been shown to improve functional recovery in animal models.[29] Improved regulation and a well-developed understanding of how the excitatory-inhibitory balance is disrupted after stroke is critical to designing therapeutic approaches for stroke recovery.

Multiple neuromodulation techniques have been investigated to facilitate motor recovery after stroke. To date, however, both noninvasive transcranial magnetic stimulation (TMS) and invasive techniques have demonstrated limited clinical efficacy.[88,146] Epidural stimulation of the cortex showed promising initial clinical results,[17,99] but long-term benefits have not been supported when assessed in a large-scale clinical trial (Table 2).[100,124]

TABLE 2. Clinical studies of neuromodulatory techniques for stroke recovery

Authors & Year	No. of Patients	Intervention	Outcome
Levy et al., 2008	24	EECS	Safe & effective
Brown et al., 2008	10	EECS	Safe
Levy et al., 2016	164	EECS	No difference from control
Kim et al., 2006	15	rTMS	Improved motor function
Takeuchi et al., 2005	20	rTMS	Improved motor function
Khedr et al., 2005	26	rTMS	Improved motor function
Fregni et al., 2006	15	rTMS	Improved motor function
Malcolm et al., 2007	19	rTMS	No difference from control
Seniów et al., 2012	40	rTMS	No difference from control
Talelli et al., 2012	41	rTMS	No difference from control
Cunningham et al., 2015	12	tDCS	Proof of concept
Boggio et al., 2007	9	tDCS	Improved motor function
Hesse et al., 2011	96	tDCS	No difference from control

EECS = epidural electrical stimulation; rTMS = repetitive TMS.

Cortical Stimulation

Cortical stimulation represents a key strategy to restore the excitatory-inhibitory balance of the damaged brain and reorganize neural circuitry to enhance poststroke recovery. Noninvasive methods (e.g., TMS and transcranial direct current stimulation [tDCS]) and invasive methods (e.g., implantable epidural electrodes) have been explored.[141,153]

High-frequency TMS increases cortical excitability and low-frequency stimulation decreases excitability. These characteristics have been exploited to increase functional improvement of the affected extremity.[31,87,89] Stimulation of the contralesional hemisphere after stroke is an area of sustained interest; it can be recruited to improve recovery, but also imposes increased inhibition on the affected hemisphere.[50,113] Stimulation with tDCS has met similar outcomes, with improvement after stroke during therapy.[73] However, Cochrane reviews of TMS and tDCS concluded that further investigation is required to determine either technique's role in stroke recovery.[45,65] A double-blinded pilot RCT to evaluate the long-term efficacy of tDCS found evidence that stimulation of higher motor areas can help recruit adaptation of the contralesional hemisphere in patients with greater ipsilesional injury.[36,125] In sum, the

promise of noninvasive neuromodulation for stroke recovery, while demonstrating early promise,[13,74] has not borne out in larger clinical trials (Table 2).[68,107,123,126,138,147]

Invasive cortical stimulation offers the advantage of greater stimulus delivery duration at a more precise location. Upper-extremity recovery is a significant limitation following stroke, with only 20% of patients reaching full recovery at 6 months.[94] Preclinical and pilot human studies demonstrated improved recovery and safety with invasive stimulation techniques.[90,99] A clinical trial was initiated based on these findings,[67] evaluating invasive cortical stimulation in conjunction with rehabilitation, but was ultimately discontinued by the sponsoring company (Northstar Neuroscience). A better understanding of the proper stimulation sites and paradigms should facilitate translation of this technique to the clinical arena.

Cerebellar Stimulation

TMS has also been applied to the cerebellum. Recently, Bonnì et al. applied TMS over the lateral cerebellum of patients with ataxia due to chronic posterior circulation ischemic stroke. The authors observed both neurophysiological and clinical improvements.[14] Invasive cerebellar stimulation has also been studied. Deep brain stimulation of the cerebello-thalamo-cortical pathway, specifically of the lateral cerebellar nucleus,[119] has been shown in preclinical rodent models to modulate cerebral cortex excitability[7] and improve postischemia motor recovery.[106] More recently, chronic cerebellar DBS demonstrated promotion of long-term potentiation, neuroplasticity, and reparative reorganization.[33]

Vagal Stimulation

Given the observation that intensive training has been shown to facilitate a range of neuroplastic brain events,[20] researchers hypothesize that vagal nerve stimulation can enhance neuroplasticity and promote reorganization of neural networks.[35] A recent RCT of vagal nerve stimulation to augment upper-limb rehabilitation following stroke was shown to be safe and feasible.[40] Further prospective studies are necessary to evaluate the efficacy of this modality.

Optogenetics

After stroke, plasticity, both structural and functional, can occur in periinfarct regions. Neuronal activity in surviving cells can release activity-dependent factors that rewire neural connections and enhance recovery. Regulating the excitability in these neurons offers a path toward functional recovery.[25] Until recently, the ability to discretely stimulate precise neural circuitry remained beyond the grasp of neuroscientists.

The flourishing field of optogenetics may offer a solution to this roadblock because it modulates specific cell types with high precision and spatiotemporal resolution.[49] Optogenetics is a technique in which specific wavelengths of light are used to control living cells, particularly neurons, that have been genetically modified to express light-sensitive ion channels.[59,109] This technique has fundamentally extended the abilities of neuroscientists to manipulate neural circuits. In the context of stroke, optogenetic techniques revealed that even small ischemic injuries and depression in excitability could lead to relatively large effects on motor circuits.[4,25]

Optogenetics also has potential as a therapeutic and restorative modality. Optogenetics have been shown to mitigate seizures; similar strategies could be used to mediate neural excitability following stroke.[120] Given the ability to stimulate specific neural circuits, optogenetics could be used to precisely manipulate pathways, in particular, brain regions, to facilitate recovery. Our group has investigated whether optogenetics could be used to selectively improve functional outcomes following stroke.[26] The authors used transgenic mice expressing channelrhodopsin (ChR2) under a neuronal promoter to selectively increase neuronal activity in the ipsilesional primary motor cortex, after inducing stroke. They found that stimulated mice performed better on functional tests, gained weight more quickly, and demonstrated improved cerebral blood flow. Moreover, neurotrophin expression was observed in the contralesional motor cortex. This was the first study to demonstrate that optogenetics can be used to promote functional recovery after stroke.

Optogenetic techniques also present an opportunity to interrogate and augment cellular transplantation therapies. While NSC transplantation therapy has demonstrated promise, as discussed above, the mechanisms underlying functional recovery remain opaque. A recent preclinical study from our group sought to use optogenetics to better understand the mechanisms by which NSCs graft into the damaged brain and modulate local circuits. Stimulation of engrafted ChR2-expressing NSCs revealed upregulation of genes involved in neurotransmission, neuronal differentiation, axonal guidance, and synaptic plasticity. Furthermore, genes involved in the inflammatory response were downregulated. Most notably, optogenetic stimulation promoted stroke recovery.[37] A current drawback of optogenetics is that it requires genetic alteration, limiting its clinical applications. However, clinical gene therapy is making marked progress, and applying optogenetics to modulate recovery pathways may eventually be translated into clinical care.

Brain-Computer Interface

Brain-computer interface (BCI) research strives to restore motor control to individuals who have lost this ability. The applications of this burgeoning field to stroke recovery are evident. Because ischemia is usually an isolated event, and not a neurodegenerative process, many of the neural networks unaffected by the infarct remain whole, providing a basis for BCI therapy.

Movements are often controlled by deciphering cortical activity to produce movements in primates,[28] and, more recently, cortical signals recorded through high-density microelectrode arrays and electrocorticography grids allowed paralyzed patients the ability to control robotic limbs and computer cursors.[30,54,70,79]

Closed-loop systems have begun to explore the ability of primates to control limb function by utilizing cortical signals to stimulate spinal circuits to induce upper limb movements.[158] Noninvasive methods such as electroen-

cephalography-based systems have also been implemented in neurorehabilitation programs, and, as these technologies are developed further, they may replace implantable arrays.[5] These methods, however, are still limited by complications in long-term interfaces between tissue and electronics and the ability to accurately decipher integrated neural outputs of the cortex. As the ability to interpret cortical signals and robotics becomes more sophisticated, BCIs offer exciting potential to restore function to patients with hemiplegia or language impairment from stroke.

Conclusions

The burden of stroke is felt by patients and their families across the globe. While acute therapies exist, the complex pathophysiology of this disease has hindered efforts to augment functional recovery. Neurorestoration must remain a critical objective for stroke research; cell-based therapies and neuromodulation are the 2 fields that have demonstrated the greatest promise in promoting recovery. Through further focused study and aggressive translation efforts, neurorestoration will manifest a new frontier in stroke care.

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Disclosures

Dr. Steinberg serves on the Medtronic Neuroscience Strategic Advisory Board and is a consultant for Qool Therapeutics.

Author Contributions

Conception and design: all authors. Acquisition of data: Azad. Analysis and interpretation of data: Azad, Veeravagu. Drafting the article: Azad, Veeravagu. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Administrative/technical/material support: Steinberg, Azad. Study supervision: Steinberg.

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