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GENESIS MEDICINE INC.

Human FGF-1 as a Potential Treatment for Multiple Sclerosis (MS)

Zhittya Genesis Medicine, Inc.
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Introduction

Zhittya Genesis Medicine, Inc. (formerly Zhittya Regenerative Medicine, Inc.) is advancing a hypothesis that neurodegenerative diseases, such as Parkinson's disease, ALS and Alzheimer's disease, are initiated and progress due to a lack of blood flow or perfusion in the brains of individuals suffering from these disorders. This lack of adequate blood flow gradually starves neurons in different areas of the brain leading to dysfunctional neurons and eventually death of the neurons due to a lack of nourishment and oxygen, as well as improper removal of metabolic wastes from the neurons. There is no reason to doubt that similar mechanisms may also be occurring in multiple sclerosis (MS), a neurodegenerative disease characterized by the selective loss of neurons in the brain and spinal cord. A review of the medical literature supports the idea that a chronic lack of blood flow and "leaky" brain capillaries may also underly the development and progression of MS. A therapeutic agent such as FGF-1, that can regenerate and heal the damaged microvasculature could represent a novel disease modifying agent for the treatment of MS.

Multiple Sclerosis

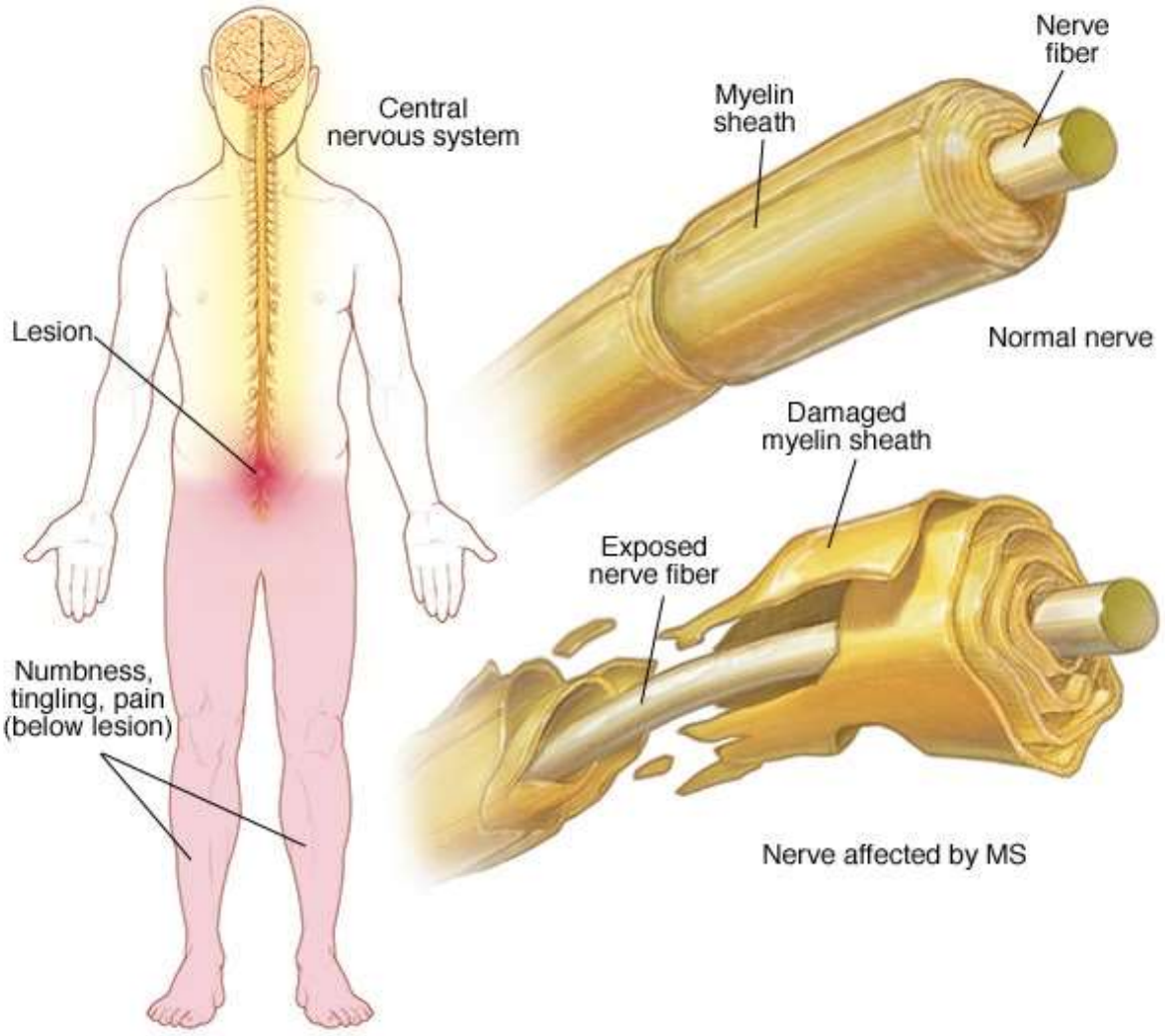
Since Charcot's first description of the disease towards the end of the 19th century, multiple sclerosis has been characterized by focal lesions throughout the central nervous system. These lesions result from the destruction of the myelin sheath which lines and protects the long axons which project out from neurons in the brain and spinal cord, and which results in the disruption of messages sent to downstream muscles and other tissues.

In some regions of the world, MS prevalence can exceed 100 per 100,000 people, and worldwide up to 2 million people are estimated to be affected by the disease. Figure 1 below shows the global distribution of MS and shows the distinctive distribution of the disease to areas with temperate climates.

Figure 1 Global Distribution of MS. *Distribution of multiple sclerosis by region and risk of developing MS by geographical location.*

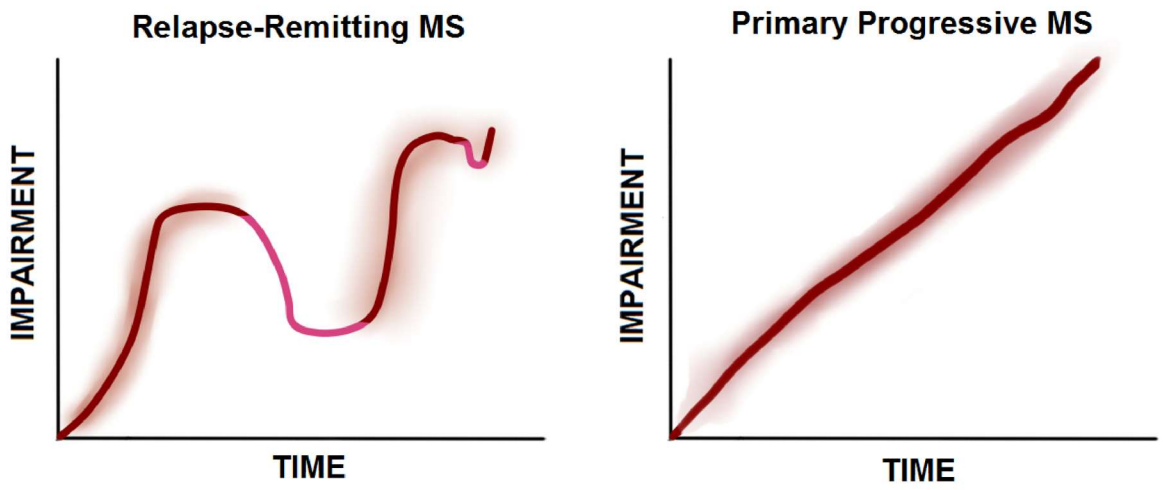
The peak age of onset is between 20 and 40 years, and disease progression often leads to severe neurological and physical disability. As shown in Figure 2 below, the disease affects the central nervous system and lesions that develop in the spinal cord lead to numbness, tingling, pain and muscle weakness below the lesion (left hand side of figure). On the right-hand side of the figure is depicted the main pathological finding in the disease, namely, damage to the fatty myelin sheath which surrounds and protects the nerve fiber. This leads to an exposed nerve fiber which itself becomes damaged and dysfunctional in relaying messages downstream.

Figure 2 MS Pathology. *Multiple sclerosis causes lesions in the central nervous system leading to symptoms below the lesion (left side of figure). The pathology of the disease results from damage to the nerve's myelin sheath (right side of figure).*



The exact cause and pathogenesis of MS are unknown. The most widely accepted hypothesis is that MS is an autoimmune disease that leads to destruction of the myelin sheath in the central nervous system neurons. Immune T-cells attack the myelin sheath and nerve fibers and the resulting inflammation and axon degeneration are believed to have a crucial role in the development of the focal lesions seen in this disease. However, the underlying mechanism that initiates this widespread nerve degeneration is not yet fully understood. It is also not known why some patients have a relapse-remitting form of the disease or a primary progressive form of MS disease as diagrammed in Figure 3 below.

Figure 3 Two Major Forms of MS. *The graphs show the two main forms of MS, the relapse-remitting form (left hand side of figure) and the primary progressive form (right hand side of figure) and how impairment varies over time.*



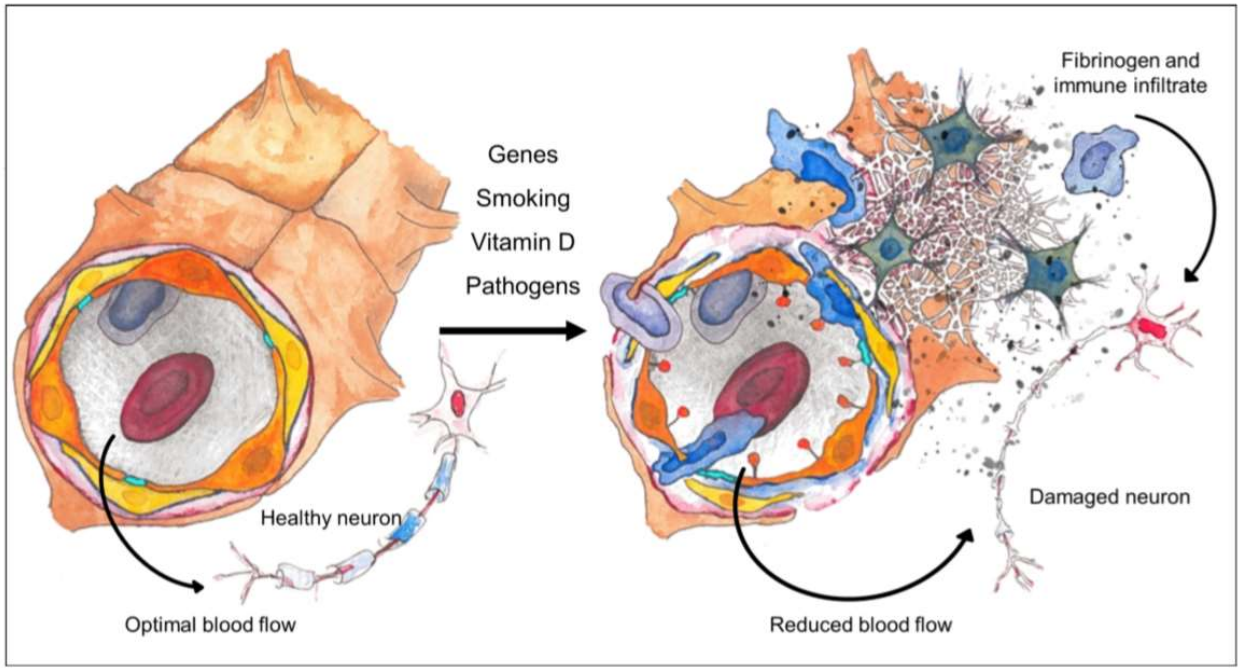
Vascular Dysfunction in Multiple Sclerosis

As stated above, although multiple sclerosis (MS) has traditionally been viewed and researched as an immune-mediated demyelinating and neurodegenerative disease of the human central nervous system (CNS), recent hypotheses and evidence suggest that vascular components may be initiating triggers for neuronal pathology and subsequent neurological manifestations of the disease. Much attention has been focused on disruption of the blood-brain barrier. The blood brain barrier is created by the very tight coupling of adjacent endothelial cells that line all of the small capillaries in the brain. The concept of a specialized boundary between the circulation and brain cells originated from the observation that dyes injected into the veins of animals stained all body tissues but the brain and spinal cord.

The blood brain barrier is now recognized as a tightly regulated cellular barrier which maintains healthy nerve cells by allowing the controlled exchange of oxygen and nutrients into the nerve cells and the removal of metabolic wastes from the neurons into the circulatory system. In multiple sclerosis, researchers now believe that a perfect storm of genetic and environmental

factors can act together on the brain's microvasculature and the blood brain barrier resulting in a lack of nourishment to the neurons, as well increased blood brain barrier permeability. This dysfunctional barrier leads to the leakage of attack immune cells into the central nervous system as shown in Figure 4 below.

Figure 4 MS is a Vascular Disease. *On the left, a healthy blood vessel leads to optimal blood flow and healthy neurons. On the right, multiple insults lead to disruption of the blood-brain barrier resulting in leakage of immune cells and toxic proteins (fibrinogen) which damage neurons in the vicinity*



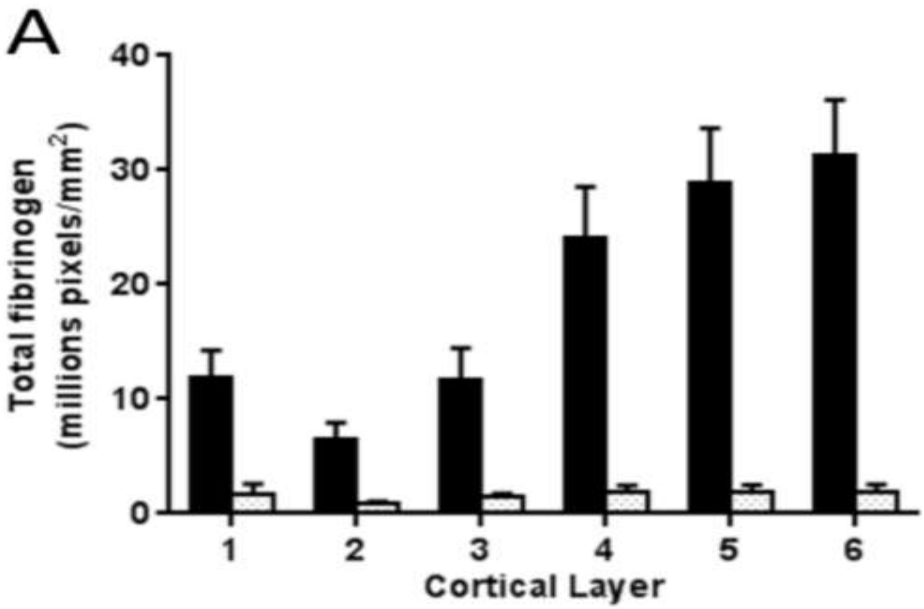
As shown in Figure 4, optimal blood flow and an intact blood brain barrier lead to healthy neurons in the central nervous system. Assault on the brain's microvasculature by defective genes, smoking, pathogens and aging leads to reduced cerebral/spinal cord blood flow and blood brain barrier disruption, allowing the escape of attacking immune cells and the deposition of large and toxic proteins onto the nerve fibers (one such toxic protein is fibrinogen as shown on the right side of Figure 4 above).

These two processes, reduced blood flow and disruption of the blood brain barrier, appear to interact in a vicious cycle, driving the destruction of neurons and the generation of focal lesions in the central nervous system, the hallmark of MS pathology. In figure 5 below, nuclear magnetic imaging displays the tell-tale focal lesions can be seen in the brain of a subject with MS.

Figure 5 Brain Lesions Seen on an MRI in a MS Patient. *Nuclear magnetic imaging of a subject with MS displaying the tell-tale focal lesions (yellow regions) in the brain of this individual.*

Medical research studies that support the hypothesis that MS is primarily a vascular disease come from a number of avenues and a few will be highlighted here. As stated above, disruption of the blood brain barrier results in the leakage of large proteins out of the blood's circulation into regions of the brain where they would not normally be located. One such protein, fibrinogen, is a large, "sticky" protein whose normal function within the blood vessel is to initiate the blood

Figure 6 Fibrinogen Leaks into the Brain in MS and is Toxic. *Fibrinogen, a molecule usually restricted to inside the blood vessel, is deposited in brain tissue in MS. Solid bars indicate fibrinogen levels in the cortex of MS patients, the white bars are normal individuals.*



clotting cascade. However, in MS where the blood brain barrier has broken down, fibrinogen can now escape from the blood vessels and coats the neurons and the long axons that branch from the cell body of the neuron. This causes a toxic reaction as cells in the brain that normally patrol the brain (microglial cells) see this fibrinogen coated neuron as abnormal and attack it. Figure 6 above shows the incredibly high amount of fibrinogen that is seen in cortical layers of the brain in MS patients (solid bars) when compared to normal individuals (white, dotted bars). This huge deposition of toxic fibrinogen on cortical neurons would certainly contribute to the pathology seen in MS.

Another clinical finding that could promote the development of ischemic brain lesions in individuals with MS is the observation that MS patients have globally decreased cerebral blood perfusion. Using sensitive MRI and PET imaging methodologies, it was demonstrated that

Figure 7 Cerebral Perfusion is Diminished in MS. *PET scans of normal individuals (top panel) and subjects with MS (bottom panel). Perfusion index is on the right side of the figure.*

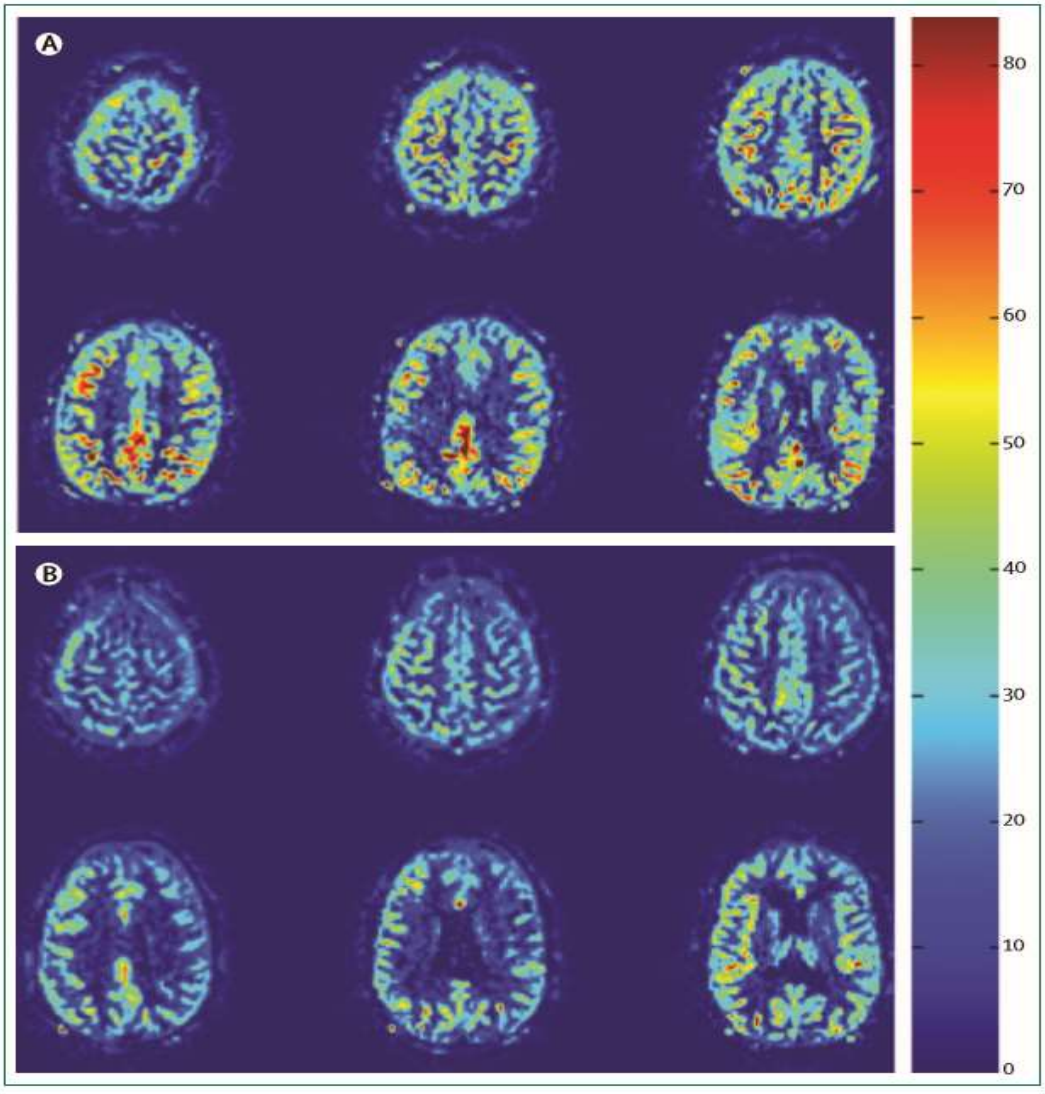


Figure 1: Cerebral perfusion in a patient with MS and a healthy individual

cerebral blood flow is decreased in both the grey and white matter of patients with MS. This can be clearly visualized in Figure 7 above which shows PET imaging of the brain of a healthy individual (panel A) and that of a patient with MS (panel B). On the right side of the figure is the PET intensity scale with red at the top of the scale indicating maximum blood perfusion. This type of "global" hypoperfusion in MS is thought to contribute to the demise of neurons in MS by further starving selected neuronal populations.

Finally, it is well established that patients with MS also have other cardiovascular issues that point to a general dysfunctional of the vascular system in this disease. Patients with MS have an increased risk for ischemic stroke and an increased risk of venous thrombosis and pulmonary embolism. Vascular comorbidities outside of the central nervous system are common in MS and can contribute to the disability seen in the disease. The debate on whether vascular events are the primary cause of neurological diseases or rather merely just a byproduct of a primary neuronal pathology is still open. However, evidence is mounting that vascular disease and endothelial cell dysfunction may be an important factor initiating and causing neuronal dysfunction and degeneration seen in multiple sclerosis.

Conclusions:

There is a large and accumulating body of evidence that vascular dysfunction and diminished blood flow in the brain is an initiating factor in the development of neurodegenerative diseases, including multiple sclerosis. There is no single unifying theory of what causes MS, but there is very good medical evidence that blood-brain barrier disruption and vascular changes are a prominent and early feature of MS pathogenesis, which may be influenced by genetic and environmental risk factors for MS.

These early vascular events could initiate a cascade of events which ultimately generates an immune response against the central nervous system and leads to MS. Focusing on the peripheral immune system alone, without taking in the contribution of the vascular system, may be limiting our understanding of the disease and the success of developing therapies for MS. A potent growth factor such as human FGF-1, which can stimulate the growth of new blood vessels and repair defective blood vessels by its direct action on the endothelial cells that line the blood vessel wall, makes FGF-1 a very attractive candidate to test in patients with MS.

Suggested additional reading

Multiple sclerosis as a vascular disease. Alireza Minagar, Wenche Jy, J. J. Jimenez & J. Steven Alexander. (2006) *Neurological Research* 28: 230-235.

Vascular aspects of multiple sclerosis. Miguel D'haeseleer, Melissa Cambron, Ludo Vanopdenbosch, Jacques De Keyser. (2011) *Lancet Neurology* 657-666.

Vascular pathology in multiple sclerosis: reframing pathogenesis around the blood-brain barrier. Jonathan Spencer, Jack Bell, Gabriele DeLuca. (2018) *J Neurol Neurosurg Psychiatry* 89:42–52.