VIB
Rijvisschestraat 120
B-9052 Ghent, Belgium

VIB research in focus

01
2005

VIB invests strongly in deciphering the molecular mechanisms of life. And we would like to keep you informed about our most significant scientific breakthroughs. Several times a year, we'll be sending you brief articles focusing on the best research results from VIB's scientists.

This time, we focus on one of the many successful topics addressed by Peter Carmeliet and his team: the role of VEGF in neurodegeneration.

Wishing you much reading pleasure!

VIB Communications
info@vib.be

Peter Carmeliet

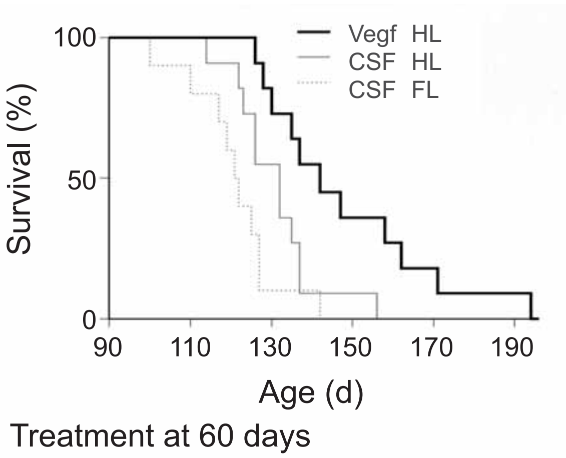
The unexpected role of VEGF in treating Amyotrophic Lateral Sclerosis (ALS)

VEGF (Vascular Endothelial Growth Factor) has been shown to be an essential regulator of blood vessel growth. Recent evidence shows that this molecule also protects against motoneuron loss, confirming the link between blood vessels and neurons. Now, VIB researchers Peter Carmeliet and his team have shown that VEGF plays a major role in the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS, or Lou Gehrig's Disease).

Angiogenesis – the generation of new blood vessels – is an essential process during the development of the embryo and in tissue remodeling during pregnancy or wound healing. Abnormal angiogenesis is associated with numerous diseases: for example, excessive angiogenesis promotes cancer and inflammatory disorders, while insufficient angiogenesis leads to ischemic tissue disease.

Peter Carmeliet and his team in the VIB Department of Transgene Technology and Gene Therapy (Catholic University of Leuven) have a long-standing interest in how VEGF controls angiogenesis during both development and disease.

In the mid-1990s, Carmeliet's group investigated the functions of VEGF *in vivo* by knocking out the VEGF gene in mice. To their surprise, loss of even a single VEGF allele resulted in early embryonic death due to severe vascular defects at a time when angiogenesis is critical for embryonic growth (Carmeliet *et al., Nature,* 1996). 'This was entirely unexpected,' Carmeliet says, 'and it was the first example of such a severe haploinsufficient phenotype, but it established the key role of VEGF in vascular development.'



VEGF treatment prolongs the survival of ALS rat models. The control animals are injected with cerebrospinal fluid (CSF). They show onset of ALS in forelimbs (FL) and hindlimbs (HL), whereas those treated with VEGF show only hindlimb onset and live longer.

'Recombinant VEGF protein delivery shows unsuspected therapeutic efficacy' *(Peter Carmeliet)*

It's good to express your VEGF

The team's next area of investigation was the up-regulation of VEGF expression by hypoxia, or a shortage of oxygen. Hypoxia induces cells to express VEGF – thus enhancing angiogenesis and tissue perfusion and thereby restoring the delivery of oxygen to the cells.

To evaluate the physiological relevance of hypoxia-induced VEGF expression, Carmeliet's group generated $VEGF^{\partial/\partial}$ knock-in mice by deleting the hypoxia response element in the VEGF promoter. This element is a 28-base pair sequence known to bind hypoxia inducible transcription factors. The $VEGF^{\partial/\partial}$ mice were viable but, unexpectedly again, they became paralyzed when they reached adulthood. Carmeliet recalls: 'We were all puzzled by the fact that adult $VEGF^{\partial/\partial}$ mice were unable to move around in their cages and developed signs of paralysis. So, we had to find out what was going on in these mice.'

What they discovered was that the $VEGF^{\partial/\partial}$ mice had developed profound muscle weakness, which was caused by a progressive degeneration of motoneurons in the spinal cord and brain stem. At this point, the researchers noticed several parallels between the $VEGF^{\partial/\partial}$ phenotype and the neuropathological and clinical features found in mSOD1G93A transgenic mice and human patients with ALS (Oosthuyse *et al., Nature Genetics,* 2001).

An incurable, paralyzing, neurodegenerative disorder, ALS commonly affects healthy people in the most active period of their lives – often striking without warning signs or previous family history. In ALS patients, motoneurons progressively degenerate, leading to a progressive debilitation of the muscles that control speech, swallowing, locomotion, and breathing. Affected individuals typically become paralyzed and die of respiratory failure within 3-5 years of initial diagnosis. A causative gene (superoxide dismutase, SOD) has been identified for a small fraction of familial ALS cases, but for the majority of the more common sporadic form of the disease the etiology remains enigmatic.

VEGF and motoneuron degeneration

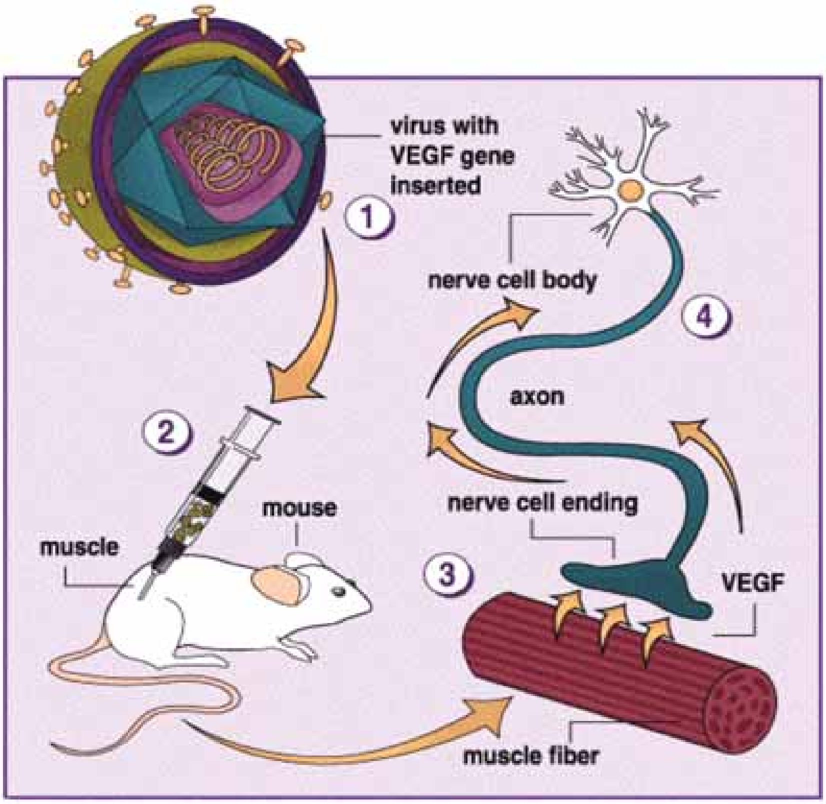
The finding that VEGF could play a role in motoneuron degeneration in mice was so unexpected that the team set about to provide more evidence that VEGF had a similar role in humans. It turned out that individuals with ALS had significantly lower circulating levels of VEGF. The researchers then established a molecular link with VEGF regulation when genotyping of three common variations in the promoter and 5' untranslated region of the VEGF gene in the DNA of ALS patients revealed the existence of two at-risk genotypes, which increased the risk for ALS (Lambrechts *et al., Nature Genetics,* 2003).

When the influence of these at-risk genotypes was assessed *in vitro*, it was found that they reduced VEGF expression by almost half. Genotyping of over 700 ALS patients and appropriate controls in the largest ALS association study performed at the time revealed that persons with the at-risk genotype had nearly twice the risk of developing the disease – clear evidence that VEGF is a modifier gene of motoneuron degeneration in human ALS. The researchers also found that crossing a standard mouse model for ALS (mSOD1G93A mutant transgenic mice) with the $VEGF^{\partial/\partial}$ mutant mice enhanced the ALS symptoms in the double-transgenic mice, resulting in an earlier onset of muscle weakness due to motoneuron loss, and a shorter lifespan.

VEGF's therapeutic potential

Knowing that VEGF and ALS were interconnected made it possible to design experiments to examine the therapeutic potential of VEGF as a novel ALS treatment. A first positive result was the discovery that administration of VEGF could protect wild-type mice from motoneuron loss in stressed conditions.

In a collaborative effort with the British biotechnology company Oxford Biomedica, Carmeliet's team also found that a single injection of a lentiviral vector expressing VEGF delayed the onset of the disease and slowed down the progression of motoneuron degeneration in the mSOD1G93A mutant mouse model.
Most notably, the VEGF gene treatment increased the life expectancy of the ALS mice by 30%, without causing toxic side effects (Azzouz *et al., Nature,* 2004). According to Carmeliet, 'This experiment provided the first promising evidence that delivery of VEGF was capable of significantly prolonging the survival of pre-clinical ALS animal models.'



A single injection in multiple muscles of a lentiviral vector expressing VEGF delays the onset of the disease and slows down the progression of motoneuron degeneration in the mutant mouse model. The particles (1) injected into the muscles of mice (2), are transported from the muscle cells (3) up into nerve cells (4), where they improve cell survival.

(Graphic courtesy of the Muscular Dystrophy Association, www.mdausa.org)

Even though these encouraging gene transfer results have provided proof-of-concept for the beneficial role of VEGF in ALS, the clinical utility of VEGF gene therapy for ALS remains to be established. An alternative approach is to deliver recombinant VEGF protein, with the important advantage that the duration and dosage of VEGF administration can be controlled.

'VEGF apparently exerts its beneficial effects by delaying motoneuron degeneration in the brain stem and spinal cord.'

Undaunted by the fact that previous clinical trials of protein delivery of neurotrophins had all failed, Carmeliet and colleagues set out to succeed by optimizing the route, dosage and duration of administration of recombinant VEGF. They found that continuous intracerebroventricular administration via osmotic mini-pumps delayed disease onset and improved motor performance in two rat models, one with a severe form of ALS and one with a milder form. Importantly, the researchers could show that VEGF prolonged the rats' survival when treatment was initiated both before and at the onset of the disease – a finding relevant to the potential clinical use of VEGF in human patients (Storkebaum *et al., Nature Neuroscience,* 2005).

'This is the first example that delivery of a neurotrophic protein prolongs survival of ALS rodent models,' Carmeliet comments. 'The therapeutic effect of VEGF protein delivery is the most promising in the field.'

With their latest publication – reporting their exciting pre-clinical results – the work of Carmeliet and colleagues paves the way for the start of clinical trials using VEGF in the treatment of human patients with ALS.

References

Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A
Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele.
Nature 380, 435-439, 1996.

Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, J. Hillan K, W. Moore M
Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene.
Nature 380, 439-442, 1996.

Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, Van Dorpe J, Hellings P, Gorselink M, Heymans S, Theilmeier G, Dewerchin M, Laudenbach V, Vermylen P, Raat H, Acker T, Vleminckx V, Van Den Bosch L, Cashman N, Fujisawa H, Drost MR, Sciot R, Bruyninckx F, Hicklin DJ, Ince C, Gressens P, Lupu F, Plate KH, Robberecht W, Herbert JM, Collen D, Carmeliet P
Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration.
Nat Genet. 28, 131-138, 2001.

Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, Wyns S, Thijs V, Andersson J, van Marion I, Al-Chalabi A, Bornes S, Musson R, Hansen V, Beckman L, Adolfsson R, Pall HS, Prats H, Vermeire S, Rutgeerts P, Katayama S, Awata T, Leigh N, Lang-Lazdunski L, Dewerchin M, Shaw C, Moons L, Vlietinck R, Morrison KE, Robberecht W, Van Broeckhoven C, Collen D, Andersen PM, Carmeliet P
VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death.
Nat Genet. 34, 383-394, 2003.

Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, Carmeliet P, Mazarakis ND
VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model.
Nature 429, 413-417, 2004.

Storkebaum E, Lambrechts D, Dewerchin M, Moreno-Murciano MP, Appelmans S, Oh H, Van Damme P, Rutten B, Man WY, De Mol M, Wyns S, Manka D, Vermeulen K, Van Den Bosch L, Mertens N, Schmitz C, Robberecht W, Conway EM, Collen D, Moons L, Carmeliet P
Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS.
Nat Neurosci. 8, 85-92, 2005.