Investigating muscle regeneration

Dr Ryuichi Tatsumi is investigating the role of Semaphorin3A in muscle regeneration. Here, he describes how he developed his interest in the neural guidance factor developed and the next steps for his research.

Can you outline the main objectives of your project investigating semaphorin3A (Sema3A) in muscle regeneration?

Despite advances in understanding of the molecules and mechanisms involved in regulating myogenesis (hypertrophy/hyperplasia and the regeneration of muscle fibres), which have been centred on resident myogenic satellite cells, it was only very recently that regenerative motoneuritogenesis was addressed. This includes the coordination and remodelling of the intramuscular motor neuron (motoneuron) network, critical for restoring the functional properties of muscle following injury.

While the spatiotemporal regulatory mechanisms coordinating these processes with myogenesis remain unclear, we hypothesise that various neural factors – including attractive and repulsive axon-guidance cue ligands – may be involved in the signalling pathway. The purpose of my study is to clarify the role of the secretion of the neural chemorepellent Sema3A from early differentiated satellite cells (myoblasts) in regenerative motoneuritogenesis.

What led to your interest in Sema3A?

We were looking for an unknown membrane receptor with a low affinity for hepatocyte growth factor (HGF) that may induce myostatin expression in cultured satellite cells. Several studies showed that the Plexin A family receptor protein has a Sema domain (a Met-related sequence module) that provides a binding site for extracellular ligands. Met is the sole high affinity membrane receptor for HGF.

Sema3A, originally identified as a neural chemorepellent, is an important focus for research on neural cells, as well as other cells, tissues and organs. When I searched for papers on Sema3A there were no results in the field of muscle, which indicated that its function in muscle was completely unknown. Therefore, we investigated whether satellite cells could produce Sema3A in cultures, which they could. Subsequent experiments demonstrated that Sema3A production takes place at the early myogenin differentiation phase in response to HGF. This reminded me of a scanning electron micrograph I had seen in an old textbook. The micrograph clearly illustrates the 3D relationship between muscle fibres, motoneuron terminals, satellite cells and blood capillaries, and led me to hypothesise that satellite cell secreted Sema3A may mediate regenerative motoneuritogenesis.

Why is the Sema3A gene upregulated after muscle injury?

Satellite cell derived Sema3A regulates regenerative motoneuritogenesis, including attachment of motoneuron terminals onto regenerating or generating muscle fibres/myotubes. In other words, Sema3A may determine when, where and how motoneurons innervate fibres.

Can you discuss HGF and how it effects secretion of Sema3A?

HGF is a multifunctional growth factor in a variety of cells, tissues and organs. In muscle tissue, HGF is the sole factor that can activate quiescent satellite cells to enter the cell cycle. The specific membrane receptor, Met, generates a signal, as revealed by our studies. Activated and proliferating satellite cells produce and secrete HGF, which upregulates cell proliferation and inhibits differentiation.

We recently demonstrated that Sema3A production and secretion turn on during the early differentiation period in response to HGF. Met may not be involved in this signalling and unknown receptors may be. Our preliminary results indicate that transmembrane type proteoglycans (Syndecans 2, 4) may be receptors for HGF ligands to impact Sema3A expression.

Could you provide some insight into the methodology behind your research on the role of Sema3A in muscle regeneration?

We found that the Sema3A expression pathway is activated by the association of HGF ligands with membrane receptors. Thus, it might be possible that ligand molecules (agonists) other than HGF can bind to the receptor to generate a similar signal. If we could find the agonist in food ingredients, it would help develop a safe methodology for muscle regeneration by the supplementation of functional food ingredients.

In terms of fibre type regulation during muscle regeneration, the signalling pathway is thought to be activated by the association of Sema3A ligands with the binding receptor neuropilin-1 (Nrp1). It is possible that other ligands could bind Nrp1 to generate the signal and we have already found a promising candidate in food ingredients. Primary differentiation cultures of rat satellite cells and in vivo feeding experiments in rats have demonstrated that the Sema3A agonist can mimic the Sema3A response, significantly improving the anti-fatigue performance of calf muscles due to increased slow fibre composition.

Do you intend to develop this research further?

Yes. I wish to evaluate Sema3A in vivo using satellite cell specific Sema3A-knockout mice. These models are ready for muscle injury experiments and will hopefully provide direct evidence for Sema3A in muscle regeneration.
The secret of Sema3A

Sema3A is a multifunctional molecule involved in several significant processes, but studies have so far neglected its potential impact in muscle biology. Research from Kyushu University in Japan is beginning to unravel its important role and results to date are promising.

**AS ANIMALS GROW** and move, muscles grow and regenerate. The ability to regenerate becomes even more important following injury. It is well established that this involves the attachment and re-attachment of motor neurons (motoneurons) to muscle fibres, but the underlying molecular mechanism remains unclear. Elucidation of this pathway would signal a real breakthrough and could lead to the development of techniques to rebuild motoneurons, promote muscle growth and regeneration, and inhibit muscle loss.

Skeletal muscle satellite cells are crucial to these processes. These resident myogenic stem cells are found at junctions between interfaces of motoneurons and muscle fibres in frequencies 20 times greater than non-junctional regions. They are present both in activated and quiescent forms. Mechanical damage or other perturbations initiate a process beginning with their activation. Accordingly they migrate and enter the cell proliferation cycle, where they differentiate and fuse to form primary myotubes – new muscle fibres – or build on the remainder of existing fibres in a process called myogenesis.

Dr Ryuichi Tatsumi of the Graduate School of Agriculture at Kyushu University, Japan, is working to better understand the intricacies of this mechanism. Since joining the University of Arizona in 1996, Tatsumi has studied postnatal muscle growth and regeneration, particularly in terms of the hepatocyte growth factor (HGF)-induced activation and quiescence of satellite cells. From 2006 he has focused specifically on semaphorin 3A (Sema3A) and the mechanisms that induce its expression. Although originally identified as an axon guidance molecule (a class 3 vertebrate-secreted semaphorin and a potent neural chemorepellent), Sema3A also has significant roles in angiogenesis, organogenesis, immunity and tumour progression, and is now recognised as a multifunctional modulator.

**HEPATOCYTE GROWTH FACTOR**

Tatsumi’s team has revealed the essential role of HGF in the satellite cell activation cascade. Even at very low levels, the rapid release of HGF from extracellular tethering (pool) initiates a series of events that culminates in the activation of the satellite cell. However, it also plays a role at high levels. Tatsumi elucidated that HGF, when present in concentrations over 10-50 ng ml⁻¹, re-establishes quiescence in satellite cells. Following satellite cell activation, HGF stimulates the expression and secretion of myostatin, an important growth differentiation factor. In doing so, HGF is able to return the satellite cell to its quiescent state.

Tatsumi thus proposed a negative feedback loop, in which the time coordinated increase in HGF concentration modulates these two opposing pathways. Low levels of HGF are sufficient to initiate the activation of satellite cells, which then release HGF, increasing its extracellular concentration and triggering return to the quiescent state.

HGF is produced in response to muscle damage not only by proliferating satellite cells, but also by spleen and liver cells, and anti-inflammatory macrophages critical for tissue repair. Because HGF is produced from many different sources, local concentrations around satellite cells are able to reach the threshold required to stimulate myostatin expression. The time lag in reaching these concentrations delays quiescence during the early stages of muscle regeneration, enabling more satellite cells to be active in order to maximise repair.

**COORDINATING REGENERATION**

Recent years have seen great progress in the understanding of myogenesis regulation, however, comparatively little attention has been paid to its coordination with muscle regeneration. Although the mechanisms which coordinate these processes lack clarity, it is likely that various neural factors – like Sema3A – are involved.

Tatsumi’s investigations have shown that Sema3A is upregulated and secreted by satellite cells exclusively in the early differentiation phase of muscle regeneration, when proliferating satellite cell myoblasts differentiate and fuse to each other to form immature muscle fibres. He demonstrated this in response to muscle injury in vivo, as well as in response to HGF treatment in primary cultures. Tatsumi consequently hypothesised that satellite cells may mediate regenerative motoneuritogenesis. This is a vital step for properly functioning muscle and includes the attachment and re-attachment of motoneuron terminals onto generating and regenerating muscle fibres.

To prove this, Tatsumi designed a study to identify a paracrine source of HGF release. A series of in vivo

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**Key findings**

Tatsumi’s work has led to a number of important discoveries about the role of Sema3A in muscle regeneration:

- Satellite cells up-regulate the expression and secretion of a chemo-repulsive axon-guidance cue Sema3A. This occurs exclusively during the early-differentiation phase in response to muscle injury and therefore may actively promote regenerative motoneuritogenesis that includes a delay in motoneurite sprouting and the attachment of motoneuron terminals onto damaged and generated muscle fibres in synchrony with the recovery of muscle fibre structures and functions.

- Anti-inflammatory macrophages (M2) are a paracrine source of HGF that impacts Sema3A upregulation, therefore highlighting a heretofore unexplored regulatory axis of M2 macrophages-satellite cells (myoblasts)-intramuscular motoneuron terminals, as an essential element of paracrine intramuscular-communications in regenerating muscle.

- Satellite cell cultures produced from slow-fibre muscle secrete more Sema3A than those from fast-fibre muscle. Sema3A knockdown cultures also reduce slow-type myosin expression, conceiving a new hypothesis that Sema3A ligands impact slow-fibre generation in injured muscle and again highlighting the essential role that satellite cell-derived Sema3A plays in muscle regeneration.
vitro/ex vivo experiments showed that activated anti-inflammatory macrophages (M2) produce and secrete HGF, thereby promoting the secretion of Sema3A and chemoattraction of proliferated myoblasts. M2 cells were most active at the early differentiation phase, three to five days post injury. During this stage, M2 cells infiltrate and recruit myoblasts (a preliminary form of muscle cell) to the injury site, with a delay following the invasion of phagocytic pro-inflammatory macrophages (M1).

Therefore, the activation of Sema3A expression signalling is specific to the early differentiation stage of muscle regeneration. Moreover, Tatsumi proposed a clear model for Sema3A signalling: M2 cells produce HGF, promoting secretion of Sema3A by satellite cells, which in turn acts as a chemoattractant to guide cells to the site of injury. This may ensure a coordinated delay in the re-attachment of motoneuron terminals onto damaged fibres early in muscle regeneration, acting to synchronise the recovery of muscle fibres with reduced inflammation soon after injury.

These findings reveal a hitherto unknown role for satellite cells as the source of Sema3A during muscle regeneration. Satellite cells appear to have important, but to date unexplored, regenerative activity and could be crucial to muscle growth in the earliest stages of life.

DETERMINING MUSCLE FIBRE TYPE

Sema3A also has potential implications in the regulation of fibre type: “Fast/slow muscle fibre types are responsible for the sensory, contractile and metabolic properties of skeletal muscle,” Tatsumi elucidates. It seems Sema3A may mediate the early-determination of this fibre type during regeneration.

To further investigate this, Tatsumi has conducted experiments using Sema3A knockdown cultures (ie. reduced expression of Sema3A). These cultures have been shown to reduce the expression of slow type myosin. This strongly suggests that Sema3A ligand is essential for slow fibre formation during the early period of muscle regeneration.

Sema3A mediated fibre type regulation could contribute to the maintenance, survival and remodelling of mature muscle: “My recent results demonstrate that Sema3A acts as a ‘survival factor’ during muscle regeneration and restores the original fibre type composition of individual muscles,” Tatsumi explains. These findings will not only facilitate progress in existing research, but may also open new fields of myogenic stem cell research.

POTENTIAL FOR NOVEL THERAPEUTICS

Given the important functions of Sema3A, the expression mechanisms revealed by Tatsumi have caused great excitement in the muscle biology community, as he describes: “Our study may contribute to novel strategies that promote an increase in preferable slow/fast fibre composition in meat production technologies. This could also apply to human sports and health sciences”.

In order to build on his success so far, Tatsumi plans to conduct further research to improve understanding of the signalling pathways described. The team has already completed a number of in vitro experiments and is in the process of conducting others using satellite cell specific Sema3A knockout mice, with a publication in preparation for Nature.

In the longer term, this knowledge could be used to identify new therapeutics that promote muscle injury repair. This includes traumatic lesions that damage motoneuron processes, as well as devastating neuromuscular disorders. It could also lead to a strategy to overcome a problem that will affect everyone – age-related muscle loss.

To clarify the essential roles of neural chemorepellent Sema3A secretion burst from early differentiated satellite cells (myoblasts) in regenerative motoneiritogenesis and myogenesis.

KEY COLLABORATORS

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