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BMP4 is a peripheral-derived factor for motor neurons and attenuates glutamate-induced exictotoxicity *in vitro*

INTRODUCTION

In the neuromuscular system, a dynamic interaction occurs among motor neurons, Schwann cells and muscle fibers. Motor neuron-derived agrin, for instance, can induce the formation of the neuromuscular junction (NMJ) (Cohen and Godfrey, 1992, Reist et al., 1992), while signals from skeletal muscle fibers and Schwann cells are able to regulate the survival of motor neurons (Riethmacher et al., 1997, Grieshammer et al., 1998). The large variety of neurotrophic factors that can support motor neuron survival in culture and in animal model of nerve injury indicates that developing and postnatal motor neurons depend on a cooperation of these molecules (Henderson et al., 1993, Hughes et al., 1993, Li et al., 1994, Sakamoto et al., 2003). Recent studies using genetic deletion of a single, or even multiple, growth factors only lead to a partial loss of motor neurons (Liu and Jaenisch, 2000, Oppenheim et al., 2000, Oppenheim et al., 2001). This may suggest that motor neurons may be affected by numerous muscle fiber- and Schwann cell-derived survival factors. Equally, this may also indicate that there are distinct subpopulations of motor neurons that rely on different survival factors (Oppenheim et al., 2000).

Bone morphogenetic proteins (BMPs), the largest subgroup within TGF-β superfamily, were originally identified by their ability to induce bone differentiation (Wozney et al., 1988). To date, more than 20 BMPs have been identified with diverse biological functions, such as cell proliferation, differentiation, morphogenesis and apoptosis (Hogan, 1996, Ten Dijke et al., 2002). Like other TGF-β superfamily members, BMPs signal through a complex involving both a type I and a type II receptor. In general, the type II receptors control the ligand-binding specificity, while the type I receptors determine which downstream signaling pathway is activated. There are five type II receptors and seven type I receptors (Shi and Massague, 2003). The type II receptors associate with specific subfamilies of the superfamily and are referred to as the TGF-β, BMP, Mullerian inhibiting substance and activin A/B type II receptors. The seven type I receptors are ALK1 to ALK7.

It has been shown that BMP signaling is required for the normal development of the *Drosophila* neuromuscular system. Mutations of *Glass bottom boat* (BMP homolog) and its receptors (*Wishful Thinking* for the type II receptor and *Thickveins* for the type I receptor) lead to profound defects at the *Drosophila* NMJ, such as decreased neurotransmitter release, reduced synaptic size and aberrant presynaptic ultrastructure (Marques et al., 2002, McCabe et

al., 2003, Eaton and Davis, 2005). However, the function of BMPs in the mammalian neuromuscular system remains unclear. In this study, we report BMP4 as a peripheral-derived factor that may regulate the survival of motor neurons.

MATERIALS AND METHODS

Animals. The Nation Cheng-Chi University's Animal Ethics Committee approved all experiments. C57Bl6 mice were bred and maintained in Modular Animal Caging System® (Alternative Design) and their food sterilized by gamma irradiation. The room had a 12h light / 12h dark phase, with the dark phase beginning at 8 pm.

Isolation of motor neuron RNA. Motor neuron mRNA was isolated using laser capture microdissection as previously described (Wang et al., 2005). Briefly, lumbar spinal cords were sectioned in a cryostat, stained with cresyl violet and their motor neurons harvested using the PixCell 2 LCM System and CapSure HS LCM Caps (Arcturus Engineering, Mountain View, CA). Typically, 4-500 motor neurons were collected from each mouse. The purity of the mRNA was confirmed by measuring the abundance of a glial marker, glial fibrillary acidic protein.

RNA preparation, cDNA synthesis, and real-time PCR. Total RNA was prepared using the RNeasy Mini Kit (Qiagen). The RNA was treated with DNase and converted to cDNA using oligo-d$(T)_{15}$ (Invitrogen) and SuperScript III reverse transcriptase (Invitrogen) as described before (Wang et al., 2009). Real-time PCR reactions were performed using a 7300 Real-Time PCR System (Applied Biosystems), SYBR Green Master Mix (Applied Biosystems) and gene specific primers were TACATGGCCTCCAAGGAGTAAGAA and GGATGGAAATTGTGAGGGAGATG for GAPDH; GTGAGGAGTTTCCATCACGAAGA and TGCCGAGGAGATCACCTCATT for BMP4; AGGATCAGGTGAAAAGATCAAGAGA and GCAAGGTACACAGCAGTGCTAGATT for BMPRII. A two-step PCR reaction was carried out with denaturation at 95°C for 15 seconds, annealing and extension combined at 60°C for one minute in a total of 40 cycles. The uniqueness of amplicons was analyzed using dissociation curves.

Operations. Adult mice were anaesthetized with isoflurane gas (2–3% by volume, 0.4 L/min). The left hypoglossal or sciatic nerve was double ligated as previously described (Jiang et al., 2000). A midline incision was made in the ventral neck and the tendon of the digastric muscle sectioned to expose the hypoglossal nerve. The nerve was then ligated in two places, 1-2 mm apart, using fine surgical thread. The sciatic nerve was exposed by separating the anterior border of the biceps femoris from other structures and ligated as described for the hypoglossal nerve. Eighteen to 20 hours after ligation, the animals were killed in a CO_2 chamber and their

nerves dissected. In some experiments, the extensor digitorum longus (EDL), soleus, and tibialis anterior muscles were denervated by sectioning the sciatic nerve in one of the hind limbs, as previously described (McGeachie et al., 2001). The mice were killed by cervical dislocation 7 days after the operation. The muscles were removed and either processed for RNA analysis or snap frozen in melting isopentane for immunohistochemical analysis.

Immunostaining. The whole-mount tissues, cryosections or culture cells were stained by immunohistochemistry and immunocytochemistry, as previously described (Russell et al., 2000, Wang et al., 2007). Antibodies included anti-BMP4 (Santa Cruz Biotechnology), anti-BMPRII (R&D System), anti-synaptophysin (Abcam), anti-neurofilament 200 (Sigma), anti-S100 beta (Abcam). The immunoreactivity was visualized using fluorescent Alexa Fluor 350, Alexa Fluor 488 and BODIPY-fl conjugated secondary antibodies (Invitrogen) or using 3-amino-9-ethylcarbamide (AEC) (Sigma) as the chromogen. NMJs were labeled with rhodamine-conjugated-α-bungarotoxin (Molecular Probes). Non-specific binding was controlled for by replacing the primary antibody with non-immune IgG (Sigma). The fluorescent intensity of BMP4 in culture cells was quantified using Image J software.

Cell cultures. Mouse muscle cells, C2C12 and neuroblastoma×glioma hybrid cells, NG108-15 were purchased from Taiwan National Health Research Institute's Cell Bank. C2C12 cells were grown as myoblasts in medium containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS), 100 U/ml penicillin G, and 100μg/ml streptomycin. C2C12 myoblasts were induced to differentiate and fuse into myotubes by replacing the culture medium with DMEM and 2% HS. Undifferentiated NG108-15 cells were maintained in medium containing DMEM supplemented with 10% FBS, 100 μM hypoxanthine, 15 μM thymidine and 1μM aminopterin. NG108-15 cells were induced to differentiate by adding 1mM of dibutyryl cAMP (Sigma) into the culture medium. To test the neurotrophic effects of BMP4, differentiated NG108-15 cells were treated with 50 μM glutamate (in DMEM supplemented with 10 μM glycine) and BMP4 for 12 hours. Afterwards, cells were returned to original culture medium with or without BMP4 for 8 hours. Cell viability was then examined under a microscope (10x eyepiece and objective lens) using trypan blue exclusion assay.

RESULT

The type II BMP receptor is associated with the NMJ

We have previously shown that BMPRII mRNA and protein were detected in the cell bodies of motor neurons (Fig. 2A and Wang et al., 2007). We further examined the expression BMPRII at other parts of the neuromuscular system. In the EDL muscle and soleus muscle (not

illustrated), strong BMPRII immunoreactivity was associated with many muscle fibers (Fig. 1A, D and F), and overlapped with the rhodamine-α-bungarotoxin labelled NMJs (Fig. 1E and F). BMPRII immunoreactivity was not detected in nerves (data not shown).

BMP4 mediates motor neuron and muscle interactions

The association of BMPRII with the NMJ indicated that motor neurons may receive a muscle-derived BMP. Among several BMP ligands we examined previously, BMP4 mRNA was highly expressed in differentiated C2C12 muscle cells (Fig. 2B and data not shown). BMP4 mRNA, however, was barely detectable in NG108-15 neuron cells (Fig. 2B) and motor neurons that isolated by laser capture microdissection (Fig. 2A). C2C12 muscle cells and NG108-15 cholinergic neurons were often used as an *in vitro* model for studying motor neuron-muscle interactions (Ling et al., 2005).

If BMP4 is a muscle-derived factor for motor neurons, its proteins are likely to be associated with the NMJ. The location of the BMP4 protein in the soleus muscle was therefore examined using immunohistochemistry. Consistent with this, BMP4-immunoreactivity was associated with muscle fibers, and concentrated beneath the rhodamine-α-bungarotoxin labeled NMJs (Fig. 2C-E). This observation was absence in the denervated soleus muscle (Fig. 2F-H).

The disappearance of BMP4 immunoreactivity after denervation indicated that the expression of BMP4 in muscle cells may be regulated by a motor neuron-derived factor. Agrin is a well known clustering agent for acetylcholine receptor (AchR) that synthesized by motor neurons, anterogradely transported, and released at the nerve terminals (Reist et al., 1992, Gautam et al., 1996). We examined whether agrin can also affect BMP4 expression or localization at the NMJ. Addition of agrin to the medium of differentiated C2C12 muscle cells caused AChR aggregating on the surface of the myotube (Fig. 3D and E). Agrin caused a dose-dependent increase in BMP4 mRNA (Fig. 3A) and protein (Fig. 3B, C and F) expressions. These observations were disappear when agrin was absent from the culture medium (Fig. 3F-H). However, the localization of BMP4 proteins was not affected by agrin, suggesting the targeting of BMP4 to the NMJ may be regulated by other factors that require further investigation (Fig. 3C-H).

BMP4 is produced by Schwann cells and transported in the motor neurons

The locations of BMP4 proteins in sciatic and hypoglossal nerves were examined using immunohistochemistry. Intense BMP4-immunoreactivity was detected along the longitudinal sections of the sciatic nerve (Fig. 4A) and the hypoglossal nerve (not illustrated). Cross sections of the nerves further confirmed that BMP4 immunoreactivity was associated with semiconcentric-like structure of the Schwann cells (Fig. 4B). If muscle fibers and Schwann cells use BMP4 to regulate motor neurons, BMP4 should be axonally transported, like other neurotrophic factors (Jiang et al., 2000, Russell et al., 2000, Wang et al., 2007). The nerves

were therefore double ligated to determine whether BMP4 was transported in the axon. BMP4-immunoreactivity was associated with the proximal and distal portion ligation sites in nerves (Fig. 4E and F), indicating that BMP4 was being anterogradely and retrogradely transported. The immunoreactivity at both ligation sites appeared to be in axons. This was further verified by cutting transverse sections of the nerves, which allows for clear delineation between axonal and Schwann cell proteins (Jiang et al., 2000, Russell et al., 2000, Wang et al., 2007). In the transverse sections, the motor axons can be unambiguously identified (Fig. 4H). The motor axons contained intense BMP4-immunoreactivity (Fig. 4G), with lower levels of immunoreactivity in the Schwann cells that surround them.

Axon damage affects peripheral BMP4 expression

Many peripheral-derived factors are known to change their expression pattern after nerve injury (Ishii, 1989, Friedman et al., 1992, Sendtner et al., 1992, Jiang et al., 2000). These responses are thought to be involved in the regeneration process of nerves. After 18 to 20 hours of ligation in the sciatic nerves, BMP4 mRNA was significantly up-regulated in the soleus muscle (Fig. 5). BMP4 mRNA was down-regulated in ligated sciatic nerves and no difference was observed in the lumbar region of the spinal cord (Fig. 5).

BMP4 may act as a survival factor for motor neurons

BMP4 was expressed by peripheral cells and axonal transported by motor neurons. These properties suggested that BMP4 may act like a classic peripheral-derived survival factor for motor neurons. We therefore examined the survival effect of BMP4 on glutamate-induce excitotoxicity *in vitro*. Differentiated NG108-15 neurons were expose to glutamate (50 μM) for 12 hours, followed by a 8-hour recovery period (conditioned medium) and the effects of BMP4 were examined by counting the number of surviving neurons (Fig. 6A). Addition of BMP4 to the conditioned medium during the recovery period significantly protected NG108-15 neuronal cells from glutamate-induce cell death (Fig. 6C). A greater protection could be achieved by addition of BMP4 in the cultures during both glutamate exposure and recovery periods (Fig. 6C). The presence of only BMP4 in the cultures did not affect neuron survival (Fig. 6B).

DISSCUSSION

BMP4 as a physiological regulator for motor neurons

This study demonstrates BMP family as important regulators for motor neurons. The identification of BMPRII and BMP4 at the neuromuscular system suggests that BMP4 may mediate motor neuron-peripheral interactions. This is in agreement with previous studies using fruit and mice as models. Strong connections among BMP signaling, synaptic growth

and synaptic stabilization at *Drosophila* NMJ have already been established (Marques et al., 2002, McCabe et al., 2003, Eaton and Davis, 2005).

Our data suggested BMP4 as a peripheral-derived factor for motor neurons. Its mRNA was present in muscles and nerves (Fig. 2 and Fig. 5). BMP4 proteins were also detected at the NMJ and Schwann cells (Fig. 2 and Fig. 4). Most importantly, ligation of sciatic and hypoglossal nerves led to the accumulation of BMP4 proteins at both proximal and distal tie (Fig 4). This implies that there is a continuous flow of BMP4 moving up and down the motor axons. The characteristics of axonal transportation is shared by BMP4 and other peripheral-derived neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) (Russell et al., 2000), TGF-β2 (Jiang et al., 2000) and BMP6 (Wang et al., 2007). Unlike BMP4 and TGF-β2 being anterogradely and retrogradely transported by motor neurons, GDNF is mainly transported towards the nerve terminal while BMP6 is largely transported towards the cell bodies of motor neurons. It is not clear why so many peripheral-derived factors are used to communicate with motor neurons. One reasonable explanation is that the peripheral cells may use different factors in different context to regulate different aspects of motor neuron function.

Regulation of muscle-derived BMP4

Muscle-derived signals are essential for both developing and mature motor neurons. Although the target dependence only exists until early postnatal stage (P7 to P10) for the survival of developing motor neurons (Lowrie and Vrbova, 1992), mature motor neurons continue to receive some neurotrophic signals from adult muscle fibers. For instance, adult motor neurons receive muscle derived neurotrophin-4 (NT-4) for nerve growth and remodeling although the expression of NT-4 in muscle fibers requires signals from motor neurons (Funakoshi et al., 1995). Equally, the disappearance of BMP4 immunoreactivity at the NMJ following nerve transaction may suggest that BMP4 expression was regulated by motor neurons in a fashion similar to that of NT-4 (Fig. 2). In addition to agrin, we speculate that other unknown factors may involve in this process. The neuronal regulation of BMP4 may reflect its function in the regulation of neuron survival, axon growth and maintenance of synaptic connections as reported for NT-4 previously (Caroni et al., 1994, Funakoshi et al., 1995).

The up-regulation of BMP4 in skeletal muscles after nerve ligation could also represent a similar physiological function for BMP4 discussed above. Indeed, it has been shown that enhanced BMP4 signaling at dorsal root ganglion is able to induce axon regeneration in an animal model of spinal injury (Parikh et al., 2011). The actions of BMP4 are, nevertheless, context-dependent as manipulation of BMP pathway locally at the injury sites affects astrogliosis (Sahni et al., 2010) and may inhibit axon regeneration (Matsuura et al., 2008).

Regulation of Schwann cell-derived BMP4

Extensive cell death occurs in mature motor neurons only when they are deprived of both muscles and Schwann cells by ventral root avulsion (Koliatsos et al., 1994). This indicates that Schwann cells are at least as important as the target muscles for motor neuron survival. Our studies implicate BMP4 as a Schwann cell-derived factor for motor neurons. In comparison to injured nerves, relatively higher levels of BMP4 mRNA and protein are present in healthy nerves (Fig. 4 and Fig. 5), suggesting Schwann may use BMP4 to communicate with motor neuron in a day-to-day basis. Muscle-derived BMP4, in contrast, is up-regulated after nerve damage, pointing to it as a specialized injury factor that may responsible for the axonal regeneration (discussed above).

The potential of Schwann cell-derived BMP4 being a day-to-day regulator is similar to that observed in ciliary neurotrophic factor (CNTF). In adult rats, CNTF is normally expressed in high quantities in Schwann cells but it reduces dramatically after nerve damage (Sendtner et al., 1992). Mice and humans with mutations of CNTF display a progressive loss of motor neurons and may have a higher risk of developing more severe motor neuron disease (Masu et al., 1993, Giess et al., 2002). These studies together may encourage the idea of considering CNTF and BMP4 as critical modifiers for motor neuron diseases. In fact, recent studies have been supportive to our view as the dysregulation of BMP signaling could be one of the causes of axonopathy in human hereditary spastic paraplegias (Tsang et al., 2009).

Is BMP4 a motor neuron survival factor?

Classical survival factors for motor neurons are derived from their peripherals. The characteristics of BMP4 fit most criteria of being a physiological survival factor for motor neurons although our *in vitro* experiments can only partial answer this question (Fig. 6). Motor neurons are particularly vulnerable to glutamate-induced cell death and inhibition of glutamate release with riluzole prolongs the survival of patients with motor neuron diseases (Lacomblez et al., 1996). Evidence for excitotoxicity in motor neuron diseases also includes elevated glutamate levels in plasma and cerebrospinal fluid and diminished tissue glutamate levels (Shaw et al., 1995). It is known that glutamate excitotoxicity induces an acute morphological change and followed by a massive cell death at the later stage (Choi, 1987). The protective effects of BMP4 during both glutamate exposure and recovery periods in this study may provide a greater implication for treating motor neuron and other neurodegenerative diseases in the future.

In summary, our data support a model that peripheral cells may use BMP4 to communicate with motor neurons. The extent to which peripheral-derived BMP4 affects motor neurons during normal and pathological physiology is not fully understood. Here, we provide some evidence showing that BMP4 may involve in the survival regulation of motor neurons. Future

studies may desire animal models with BMP4 and BMPRII being specific deleted in each cell type. This will help to delineate the actions BMP4 in the neuromuscular system.

FIGURE LEGENDS

Figure 1 BMPRII proteins are detected at NMJs. Cross sections of EDL muscle were stained with an anti-BMPRII antibody (A, C-F) and control IgG (B) antibody. The sections were visualized using a color reaction product (AEC) (A and B) or a green-fluorescence-linked secondary antibody (C-F). Panel A and B were adjacent sections. Panels C to F were the same section, which show the images in phase contrast (C), green channel (D) and red channel (E). The images of green and red channels were merged using Adobe Photoshop softwear (F). "m" indicates muscle fibers. The arrows identify BMPRII immunoreactivity. The arrow heads point to the NMJs labeled by rhodamine-α-bungarotoxin. The scale bars = 100 μm (A, B) and 50 μm (C-F).

Figure 2 BMP4 is expressed by muscles. (A, B) BMP4 mRNA was measured in laser capture isolated spinal motor neurons (A), differentiated NG108-15 neurons and C2C12 muscle cells (B) using real-time PCR. BMP4 mRNA was highly expressed in differentiated C2C12 muscle cells but barely detectable in both neuronal samples. BMPRII mRNA measurements in the laser capture isolated spinal motor neurons was shown as a positive control. The values are the mean ± SEM (n=11 for A and n=8 for B). (C-H) The localizations of BMP4 proteins (green) in normal (C-E) and dernatived (F-H) soleus muscles were examined using immunohistochemistry. The NMJ was labeled by rhodamine-α-bungarotoxin (red). The scale bars = 15 μm.

Figure 3 BMP4 mRNA and protein are up-regulated by agrin. Differeniated C2C12 muscle cells were treated with agrin (A-E) or without agrin (F-H) for 16 hours. The BMP4 mRNA expression level and protein localization was examined using real-time PCR (A) and immunocytochemistry (C-H). The fluorescence intensity of the BMP4 immunoreactivity (green) was quantified using Image J software (B). The values are the mean ± SEM (n=6 for A and n=70 for B; *****, $P < 0.05$ compared to controls using Student's *t* test). The AchR clusters were labeled by rhodamine-α-bungarotoxin (red, arrows). The scale bars = 15 μm.

Figure 4 BMP4 is produced by Schwann cells and transported in the motor neurons. Longitudinal sections (A, E, F) and cross-sections (B-D, G, H) of normal (A-D, H) and ligated (E-G) sciatic nerves were stained with an anti-BMP4 (A, B, E-G), anti-neurofilament to mark axons (H), anti-S100 beta to label myelin sheaths of Schwann cells (C) or non-immune IgG (D). The proximal and distal ligations are marked in (E) by a "p" and a "d", respectively.

(F) is a higher magnification of the proximal region illustrated in (E). The arrows and arrow heads point to Schwann cells and axons respectively. The magnifications of A and E; B-D, G and H are the same. The scale bars = 200 μm (A,E) and 50 μm (B-D, F-H).

Figure 5 Expression of BMP4 mRNA in the neuromuscular system after nerve ligations. Total RNA was isolated from sciatic nerve (n=4), soleus muscles (n=6) and lumbar spinal cords (n=6) of normal and denervated mice. The expression levels of BMP4 mRNA were measured using real-time PCR. The values are the mean ± SEM (*****, $P < 0.05$ compared to controls using Student's *t* test).

Figure 6 BMP4 protects NG108-15 neurons against glutamate-induced excitotoxicity. Differentiated NG108-15 cell was treated with glutamate for 16 hours and followed by an 8-hour recovery period (A). The surviving neurons were counted under a microscope using trypan blue exclusion assay. Cell numbers from at least 60 fields for each group were counted. BMP4 only did not affect neuron survival (B), and showed protective effects during both glutamate treatment and recovery period (C). The values are the mean ± SEM (*****, $P < 0.05$ compared to control groups; $^{\#}$, $P < 0.05$ compared to glutamate treated groups using Student's *t* test).

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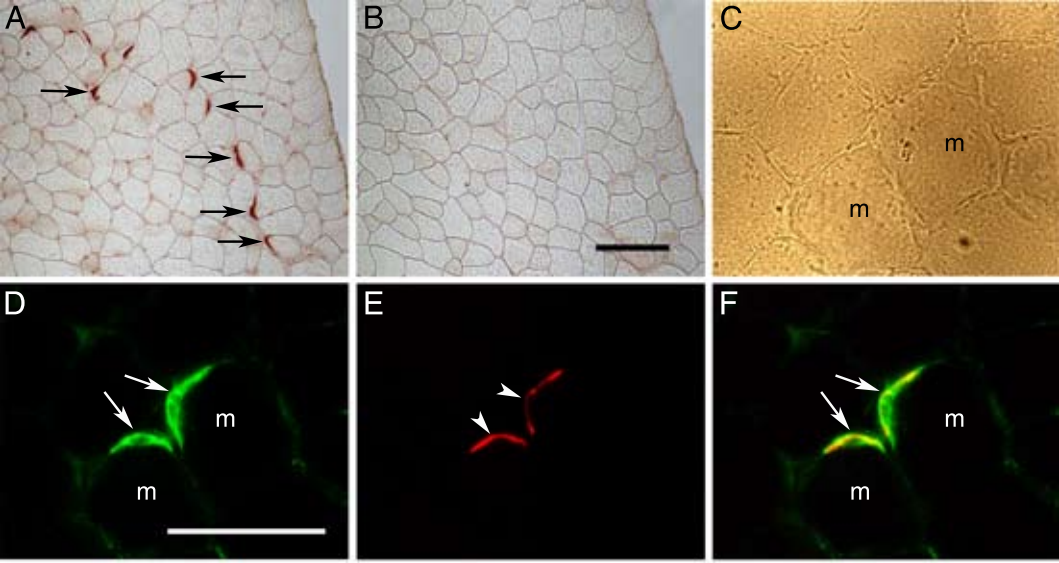
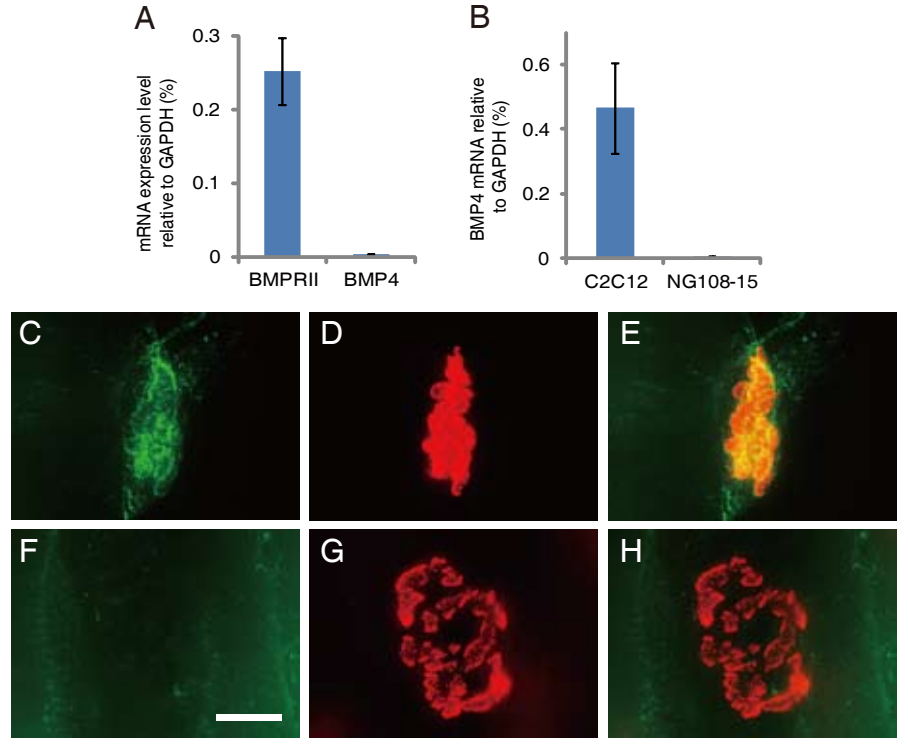
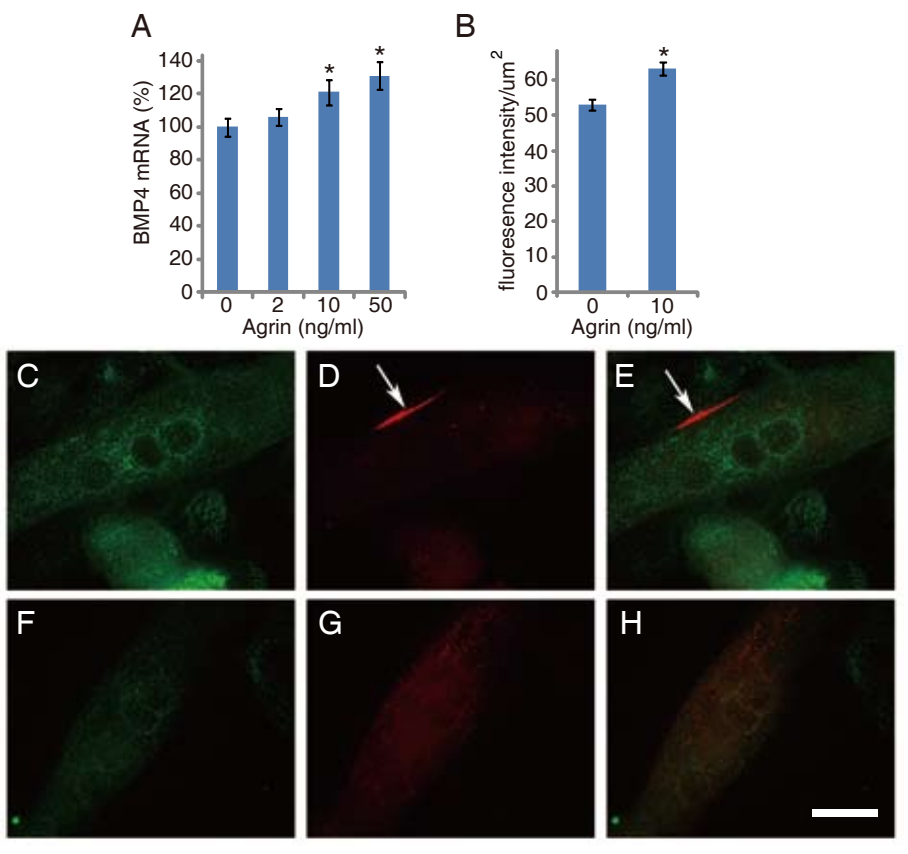
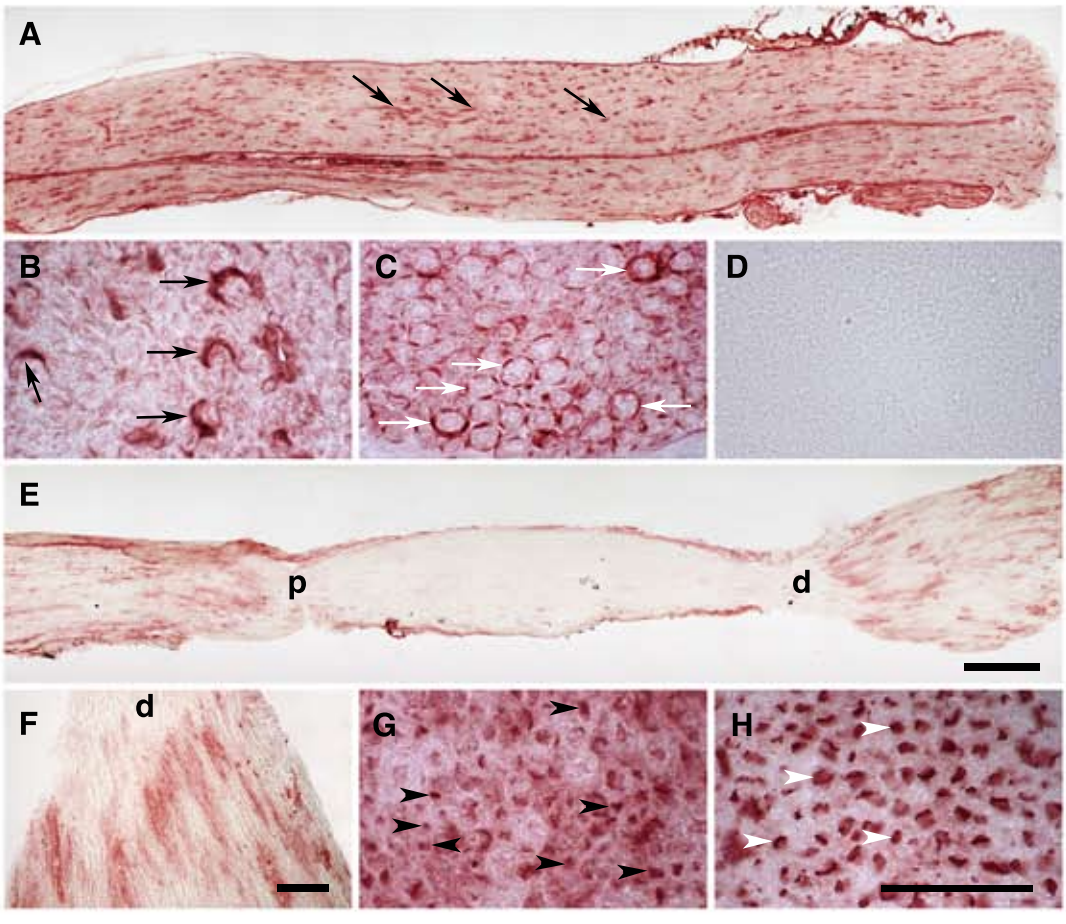
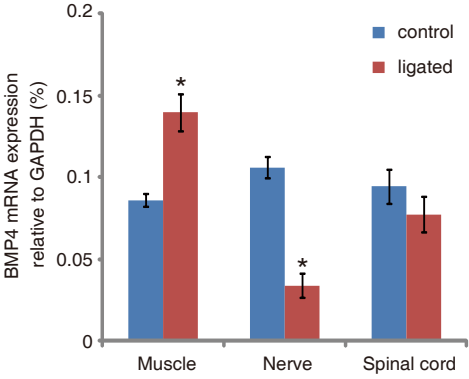

A
B
C
m
m
D
m
m
E
F
m
m

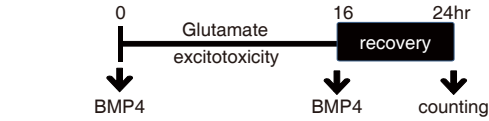

A
mRNA expression level relative to GAPDH (%)
0.3
0.2
0.1
0
BMPRII
BMP4
B
BMP4 mRNA relative to GAPDH (%)
0.6
0.4
0.2
0
C2C12
NG108-15
C
D
E
F
G
H


A
BMP4 mRNA (%)
140
120
100
80
60
40
20
0
0
2
10
50
Agrin (ng/ml)
*
*
B
fluoresence intensity/um2
60
50
40
30
20
10
0
0
10
Agrin (ng/ml)
*
C
D
E
F
G
H

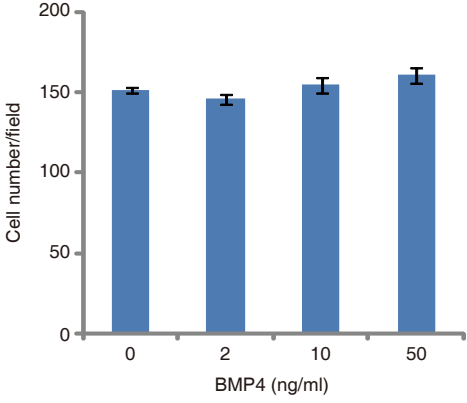

A
B
C
D
E
p
d
F
d
G
H


0.2
0.15
0.1
0.05
0
BMP4 mRNA expression relative to GAPDH (%)
control
ligated
*
*
Muscle
Nerve
Spinal cord

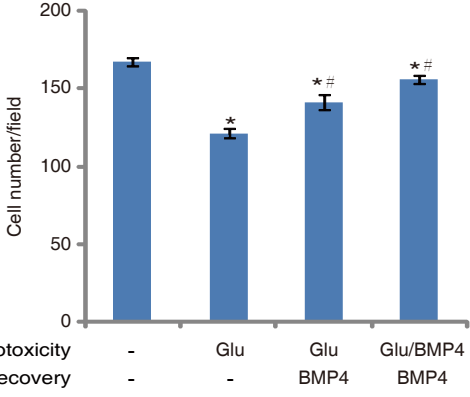
A



B



C



excitotoxicity	-	Glu	Glu	Glu/BMP4
recovery	-	-	BMP4	BMP4

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/30

國科會補助計畫	計畫名稱: 骨形成蛋白於神經肌肉系統功能之研究
	計畫主持人: 王培育
	計畫編號: 98-2320-B-004-003-MY2　　學門領域: 生理

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：王培育	計畫編號：98-2320-B-004-003-MY2
計畫名稱：骨形成蛋白於神經肌肉系統功能之研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	0%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	0	0	0%		
		專書	0	0	0%		
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（本國籍）	碩士生	3	3	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	0%		
		專任助理	0	0	0%		
國外	論文著作	期刊論文	0	0	0%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	1	1	100%		
		專書	0	0	0%	章/本	
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	0%	人次	
		博士生	0	0	0%		
		博士後研究員	0	0	0%		
		專任助理	0	0	0%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	準備發表之論文:BMP4 is a peripheral-derived factor for motor neurons and attenuates glutamate-induced exictotoxicity in vitro

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本計畫為我個人回到國內服務後所執行的第一個國科會計畫，而在這過程中的意義不只是學術上的進展，更重要的是將在國外所學的知識及技術帶回台灣並發揚光大，也期望為台灣培養出有潛力的科技人材，而幾位參與本計畫的學生也即將畢業，希望他們將來對國家有所貢獻。

本研究計畫中以許多不同的技術嘗試了解骨形成蛋白於神經肌肉系統中的功用，除了生理功能上的探討，我們亦發現 BMP4 可以有效的減緩麩胺酸所引發的神經細胞死亡，因此，BMP4 可作為將來開發治療神經退化性疾病的重要標的。而未來的研究可著重於:(1)BMP4 及 BMP 下游訊號路徑的調控, (2)疾病動物模式的測試, (3)其他 BMP 家族成員的作用。希望藉此更了解運動神經元疾病的致病機轉，並且找到適當的治療方式。