



Basic Neuroscience

Development of BMP7-producing human cells, using a third generation lentiviral gene delivery system

David W. Chitty^{a,b}, Roger G. Tremblay^a, Maria Ribocco-Lutkiewicz^a, Julie Haukenfrers^a, Bogdan Zurakowski^a, Bernard Massie^c, Marianna Sikorska^a, Mahmud Bani-Yaghoub^{a,b,*}^a Neurogenesis and Brain Repair Group, Neurobiology Program, Institute for Biological Sciences, National Research Council Canada, Ottawa, ON, K1A 0R6, Canada^b Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, K1H 8M5, Canada^c Centre Bioprocédés, Institut de Recherche en Biotechnologie, Conseil National de Recherches du Canada, 6100 Avenue Royalmount, Montréal, Québec, H4P 2R2, Canada

ARTICLE INFO

Article history:

Received 1 December 2010

Received in revised form

13 December 2011

Accepted 14 December 2011

Keywords:

Human amniotic fluid cells

Cerebrospinal fluid

Neuronal differentiation

Signalling pathway

Brain

ABSTRACT

Bone morphogenetic protein 7 (BMP7), a member of the transforming growth factor β (TGF- β) superfamily, plays important roles in the development of various tissues and organs in mouse and human. In particular, BMP7 is critical for the formation of the nervous system and it is considered to have therapeutic potential in brain injury and stroke. One approach to make BMP7 more suitable for therapeutic purposes is the development of efficient vectors that allow the consistent, reliable and cost-effective production of the BMP7 protein.

In this study, we developed an efficient BMP7 delivery system, using a third generation lentiviral vector to produce functional BMP7 protein. The lentiviral transduction of several human cell types, including human embryonic kidney 293 (HEK293) cells, amniotic fluid cells, Ntera2 neurons (NT2-N) and primary neuronal cultures resulted in BMP7 expression. The production of BMP7 protein was achieved for at least 4 weeks post-transduction, as determined by enzyme-linked immunosorbent assay (ELISA). SMAD phosphorylation and neuronal differentiation assays verified the bioactivity and functionality of the lentiviral-based BMP7 protein, respectively. In addition, the intracerebroventricular injection of the lentivirus resulted in exogenous BMP7 expression in both neurons and astrocytes in the mouse brain.

Taken together, this gene delivery system provides a reliable source of functional BMP7 protein for future *in vitro* and *in vivo* studies.

Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.

1. Introduction

Current methods of recombinant protein production utilize bacterial, yeast, and mammalian cell cultures, although, the bacterial and yeast systems are not always regarded as the most suitable for the production of therapeutic factors (Verma et al., 1998; Yin et al., 2007). For example, bacteria are unable to perform post-translation modifications such as glycosylation, phosphorylation, and disulfide-bond formation, which are often necessary to produce biologically active mammalian proteins. Though in yeast systems these events do occur, there are key differences in the processing steps of glycosylation, which may affect proper folding and functionality of expressed mammalian proteins. Therefore, while protein production in bacterial and yeast-based systems can be

quick and relatively inexpensive, mammalian-based systems are the most suitable to ensure proper post-translational processing and secretion of functional mammalian proteins especially those that hold a great therapeutic potential (Yin et al., 2007; Gaillet et al., 2007). Bone morphogenetic proteins (BMPs), which are members of the transforming growth factor β (TGF- β) superfamily belong to this category.

BMPs were originally identified in bone and cartilage (Urist et al., 1968) and later shown to be involved in the development of many organs and have therapeutic effects on many diseases (Miyazono et al., 2010; Zakin and De Robertis, 2010). In particular, BMP7, also known as osteogenic protein-1 (OP-1), plays important roles in the development of bone, kidney and the nervous system in mouse and human. BMP7 functions via a heterotetrameric receptor complex present on the surface of target cells. The BMP receptor complex normally consists of two type I and two type II BMP receptors. Upon binding of extracellular BMP7 to the heterotetrameric receptor complex, phosphorylation of mothers against decapentaplegic homolog (SMAD), leads to its activation and translocation to the nucleus. Once in the nucleus phospho-SMAD 1/5/8 interacts directly with DNA as either a co-activator or repressor of gene

* Corresponding author at: Neurogenesis and Brain Repair Group, Neurobiology Program, Institute for Biological Sciences (IBS), National Research Council (NRC) Canada, 1200 Montreal Rd., Bldg. M-54, Ottawa, ON, K1A 0R6, Canada.
Tel.: +1 613 993 5723.

E-mail address: mahmud.bani@nrc.ca (M. Bani-Yaghoub).

expression (Dudley et al., 1995; Arkell and Beddington, 1997; Gould et al., 2002; Yang et al., 2006).

BMP7 has been extensively studied for its ability to induce bone formation and kidney regeneration and it has been approved by Health Canada and FDA to treat bone fractures, non-unions and spinal fusion (Gould et al., 2002; Chang et al., 2003; Zhu et al., 2004; Gregory et al., 2005; White et al., 2007; Song et al., 2008; Desmyter et al., 2008; Kan and Kessler, 2011). Current data from several laboratories, including ours, suggest that BMP7 also contributes to cell survival and differentiation; hence it may potentially assist in the protection and regeneration of the brain following injury or stroke (Chou et al., 2006; Tsai et al., 2007; Bani-Yaghoob et al., 2008). However, further mechanistic studies, both *in vitro* and *in vivo*, are still needed in support of these functions. Current *in vivo* delivery methods of BMP7 include multiple direct and/or intravenous injections of recombinant human BMP7 (rhBMP7). These delivery methods are often ineffective due to its short half-life and the need for its multiple injections in sensitive tissues (Song et al., 2008). Consequently, safer and more reliable BMP7 delivery methods have yet to be developed for complex tissues such as the brain.

Currently, lentiviral vectors are among the most promising gene delivery tools into mammalian cells. Lentivectors are derived from the human immunodeficiency virus (HIV-1) genome and enable the production of replication-incompetent lentivirus, which can transfer genes up to 8–10 kb in size to both mitotic and post-mitotic cells such as neurons (Broussau et al., 2008; Trono, 2000). In addition, the lentiviral transduction leads to genomic integration of a gene, resulting in stable and long-term gene expression without any selective pressure.

In this study, a full-length cDNA sequence encoding BMP7 was cloned into a third generation lentiviral backbone. Using the BMP7 lentivirus, we generated four different human cell types (HEK293, amniotic fluid cells, NT2 neurons and primary neuronal cultures) that produce functional BMP7. Moreover, BMP7 derived from lentivirally-transduced human cultures (LvBMP7) was shown to be biologically active through BMP signalling, neuronal differentiation assays and *in vivo* studies. The BMP7 expression system developed in this study may serve as a useful tool in the production of biologically active BMP7 for future studies investigating the role of BMP7 in cell fate determination as well as its therapeutic applications.

2. Materials and methods

2.1. Generation of BMP7 lentivector (pLvBMP7-GFP)

A third generation lentiviral transfer vector (pTet07CSII-CMV-GFPq; Broussau et al., 2008) and a commercially-available plasmid containing the full-length cDNA sequence of mouse BMP7 (pCMV-SPORT6-BMP7; OpenBiosystems, Huntsville, AL, USA) were utilized to clone a BMP7 lentivector (Fig. 1). Initially, a 2051 base pair (bp) DNA sequence of mouse BMP7 was cut from pCMV-SPORT6-BMP7 with the restriction endonucleases *AgeI* and *XhoI*. The vector pTet07CSII-CMV-GFP (Blain et al., 2008) was linearized with *AgeI* and *XhoI* to form compatible ends for ligation. The cut BMP7 DNA fragment was ligated into pTet07CSII-CMV-GFPq upstream of an Internal Ribosomal Entry Site (IRES) and the gene encoding for enhanced Green Fluorescent Protein (GFP), using T4 DNA ligase (New England Biolabs, Pickering, ON, Canada). The resulting plasmid, pLvBMP7-GFP, represents a third generation transfer lentivector with the transgenes BMP7 and GFP separated by IRES under the control of a single CMV promoter and flanked only by the HIV-1 Long Terminal Repeats (LTRs). Sequence analysis confirmed that there were no mutations in pLvBMP7-GFP. The

plasmids were prepared from transformed *Escherichia coli* DH5 α chemi-competent cultures, using the Qiagen MiniPrep or MaxiPrep kits, according to the manufacturer's protocols (Qiagen Inc., Mississauga, ON, Canada).

2.2. Transfection and lentiviral production

The human embryonic kidney packaging cell line HEK293SF-PacLV was used to produce the third generation of BMP7 lentivirus and GFP lentivirus. The 293SF-PacLV cell line (Broussau et al., 2008) constitutively expresses the lentiviral proteins *gag/pol* and *rev*, while expressing VSV-G under the control of the transcriptional regulators CymR (cumate-inducible, 50 μ g/mL) and rtTA2s-M2 (doxycycline-inducible, 1 μ g/mL). A total of 1.0×10^7 293SF-PacLV cells were seeded in a 10 cm tissue culture dish (Falcon, VWR, Mississauga, ON), transfected with 30 μ L of Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) and 15 μ g of pLvBMP7-GFP in 6 mL of Freestyle EX media (Invitrogen) and incubated at 37 °C in 5% CO₂. Six hours following transfection, the medium was replaced with 6 mL of fresh Dulbecco's Modified Eagles Medium (DMEM) supplemented with 1 μ g/mL doxycycline and 50 μ g/mL cumate (4-isopropylbenzoic acid) for the induction of VSV-G. The lentivirus-containing medium was harvested (~6 mL/10 cm dish) both 48 and 72 h post-transfection and filtered to remove cell debris with a 10 mL syringe and a 0.45 μ m low protein binding filter (Millipore, Billerica, MA, USA). In parallel experiments, the lentivirus-containing medium was concentrated 30 fold, using sterilized Amicon Ultra-15 centrifugal protein concentrators (Millipore) and 350 fold, using Lenti-X Concentrator (Clontech Laboratories, Mountain View, CA, USA) according to manufacturers' instructions. Both un-concentrated and concentrated viruses were either used immediately for infection or stored at –80 °C in 10% FBS for further use.

2.3. FACS-based titration and lentiviral infection

The human embryonic kidney cell line, HEK293GPG (HEK293) in conjunction with fluorescence-activated cell sorting (FACS) analysis were used to determine the functional viral titer (transduction units (TU)/mL). Briefly, HEK293 cells were seeded in six-well plates at a density of 1.0×10^6 cells/well and incubated at 37 °C in 5% CO₂ for 24 h or until cells were approximately 80–90% confluent (2.0×10^6 cells/well). To remove potential cell debris prior to infection, the medium was replaced with 1.7 mL/well of fresh DMEM with 1% FBS. Serial dilutions were prepared with DMEM in the ratios 1:10 and 1:100 from 30 $\times$ concentrated lentivirus-containing medium and used in parallel with no dilution and control (no infection) samples. Each HEK293-containing well was transduced with 300 μ L of the lentivirus serial dilution. Polybrene was added to a final concentration of 8 μ g/mL to each well and the plates were subsequently incubated at 37 °C in 5% CO₂. Following a 48-h incubation period, the transduction efficiency was evaluated with fluorescence microscopy (via presence of GFP). Infected HEK293 cultures were passed weekly and maintained in HEK293 media (DMEM + 10% FBS + 1 μ g/mL doxycycline).

HEK293 cultures were harvested 48 h post-infection for FACS analysis. Briefly, medium was removed and cells were washed with 1 $\times$ phosphate-buffered saline (PBS). Next, 200 μ L of 0.25% trypsin (Invitrogen) was added to each well and following a short incubation period (1 min) at RT, the cells were resuspended in 1 mL/well of PBS containing 10% FBS, briefly vortexed to dissociate the cells and stored on ice. An aliquot of the sample was counted with a haemocytometer to determine the approximate cell density per well. The samples were immediately analyzed on a MoFlo flow cytometer (DakoCytomation, Copenhagen, Denmark), using Summit software. For each sample at least 40,000 events were collected. The titer of

Download English Version:

<https://daneshyari.com/en/article/6269490>

Download Persian Version:

<https://daneshyari.com/article/6269490>

[Daneshyari.com](https://daneshyari.com)