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Basic Science

Quiescent pluripotent stem cells reside within murine peripheral nerves that can be stimulated to proliferate by recombinant human bone morphogenic protein 2 or by nerve trauma

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Abstract

BACKGROUND: The clinical use of recombinant human bone morphogenic protein 2 (rhBMP-2, Infuse) has been associated with nerve-related complications including new-onset sciatica, and retrograde ejaculation.

PURPOSE: To better understand the interaction of rhBMP-2 and peripheral nerves with the intent of making procedures safer.

STUDY DESIGN/SETTING: Using a mouse model to examine the direct effect of diluted rhBMP-2 (Infuse) on murine sciatic nerves.

METHODS: Animal studies were approved by the Institutional Animal Care and Use Committee. Balb/c mouse sciatic nerves were surgically exposed and 60 ng (in 10 μL) of rhBMP-2 was applied to the nerve. In separate experiments, the sciatic nerves were subjected to mechanical compression using forceps (and not exposed to rhBMP-2). The third group of mice received direct injection of the same amount of rhBMP-2, or sterile saline as a control, into the hamstring area of the posterior thigh without surgery. Mouse limbs with intact sciatic nerve were collected at 24, 48, or 72 hours after treatment for histology processing. A separate set of identically treated sciatic nerves were retrieved from mice at the same time points and cells were isolated by collagenase and trypsin digestion. The isolated cells were cultured in a stem cell medium containing 20% knockout serum and human leukemia inhibitory factor. Immunohistochemical or immunofluorescent cell stains against KLF4, Sox2, c-Myc, and Oct4 were performed on the mouse tissue sections and cell culture slides. In addition, real-time polymerase chain reaction (PCR) was performed to quantify the mRNA expression profiles of the stem cell marker genes in cultured cells.

RESULTS: Profound morphological changes of the mouse sciatic nerves were noted after exposure to rhBMP-2, with a rapid and robust cell proliferation within the nerves followed by migration of these cells into surrounding tissue. Immunohistochemical stain revealed strong nuclear stains of KLF4, Sox2, Oct4, and c-Myc on the overwhelming majority of these proliferating cells in the nerve. Intramuscular injections of rhBMP-2 or willful physical compression of the nerves showed similar cell proliferation effects as the direct application of Infuse to the sciatic nerve. The cells in stem cell culture medium grew steadily without feeder cells and appeared fairly uniform. They were adherent to substrate and were motile. Double fluorescent staining on the cells indicated colocalization

FDA device/drug status: Not applicable.

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of all pairs of the four stem cell markers in the cell nuclei. Real-time PCR confirmed the strong mRNA expressions of KLF4, Sox2, Oct4, and c-Myc in these isolated cells.
CONCLUSION: Exposure to BMP-2 causes a marked proliferation of previously quiescent cells within peripheral nerves. These cells simultaneously express KLF4, Sox2, Oct4, and c-Myc, the transcription factors that confer embryonic pluripotency. Work described in the companion paper reveals some of the differentiation capacity of the cells and their likely clinical significance. In addition, the effects of direct exposure of nerves to rhBMP-2 as described here should clearly illuminate the mechanism of BMP–2-related nerve complications. We would suggest that the use of this agent in proximity to known neural structures should only be done with extreme caution. Published by Elsevier Inc.

Keywords: Mouse; Nerve injury; Peripheral nerve; Pluripotent stem cells; rhBMP-2; Stem cell markers

Introduction

Bone morphogenetic proteins (BMPs), first discovered by Marshall Urist [1–3], are a cluster of powerful cytokines with an ancient lineage. Recombinant human BMP 2 (rhBMP-2) has been successfully manufactured and marketed (Infuse) for the induction of bone formation. It has been widely and successfully used to promote bone formation in human spine [4–7], and more recently in long bones [8–10]. There is abundant literature on the subject of the molecular mechanism of the bone-forming process, with several published reports from Boden and coworkers [11–13], suggesting that the molecule induces a process of membranous bone formation, which generates bone without creation of cartilage intermediates. However, the details of this bone formation process have remained unclear.

With increased popularity and widespread clinical use of BMP-2 (Infuse), some undescribed complications from the use of Infuse were gradually recognized during clinical practice, including such problems as anterior pharyngeal swelling, new onset sciatica, and retrograde ejaculation [14–17]. Animal studies reported by Dmitriev et al. also suggested that when used for spine fusion, BMP-2 may trigger some morphologic changes within the spinal cord and nerve roots that would negatively impact neurologic recovery [14]. It is generally accepted now that the clinical use of BMP should be done with these complications much in mind. Most surgeons now take great care to avoid direct exposure of dura, nerve roots, or the sympathetic chain to the BMP-2 solution during its surgical use. This report describes a likely mechanism for some of these complications, and further describes the discovery of a new population of previously undescribed stem cells.

To better understand the molecular and cellular mechanisms of the BMP–2-related clinical complications, the initial objective of this study was to reveal the biological changes when a peripheral nerve is directly exposed to purified rhBMP-2. Using the commercially available Infuse product, we exposed murine peripheral nerves to small quantities of dilute rhBMP-2 (Infuse), and observed the effects on the nerves, which included the rapid proliferation of cells within the exposed nerves. Further, the proliferating cells within the nerves after Infuse application were isolated for culture and characterized using immunohistochemical stains and real-time polymerase chain reaction (PCR) techniques.

As we will show, these isolated cells unexpectedly express the embryonic stem cell markers KLF4, Sox2, c-Myc, and Oct4. Based on the Yamanaka Nobel Prize-winning studies [18,19], adult cells induced in culture to express these four genes possess the wide differentiation potential of embryonic stem cells. Such cells are often referred to as induced pluripotent stem cells or iPCs [19] and may be created from adult differentiated cells by using retroviral vectors to induce expression of these four genes. Identifying cells expressing these four genes in the present report is a remarkable finding, as such cells are not thought to exist in adult mammals. The data suggest that this is a previously unknown and undescribed variety of pluripotent stem cells that can be obtained from adult animals without the use of retroviruses or any direct manipulation of genes. This current report characterizes these cells, and suggests that they may have uses in regenerative medicine. We suggest that they be referred to as NErve Derived Adult Pluripotent Stem cells or NEDAPS cells.

Materials and methods

Exposure of murine sciatic nerves to rhBMP

All animal procedures were approved by the Institutional Animal Care and Use Committee. A total of 150 female BALB/c mice at 8 weeks of age were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and acclimated to the facility for at least 1 week before commencement of the study. After being anesthetized with intraperitoneal injection of 90 mg/kg ketamine, 8 mg/kg xylazine, and 1%–2% isoflurane (nose cone), the right leg of each animal was shaved, disinfected with povidone-iodine and ethanol, and the sciatic nerve was surgically exposed. BMP-2 (60 ng) in 10 μL (INFUSE Bone Graft, Medtronic Spinal and Biologics, Memphis, TN, USA) was directly applied onto the exposed nerve. The BMP-2 dosage used in the experiments was determined by the results of preliminary experiments. The incision was sutured closed and the animals survived for 24, 48, or 72 hours until being sacrificed for harvest of the nerve, by re-exposing it and excising it for histologic analysis or cell culture. Control mice underwent sham surgery without BMP-2 exposure or physical sciatic nerve disturbance.

A second group of mice was prepared and anesthetized, and the sciatic nerve exposed as above. Instead of applying