

Differentiation of Mesenchymal Stem Cells Towards a Nucleus Pulposus-like Phenotype In Vitro: Implications for Cell-Based Transplantation Therapy

Risbud, Makarand V. PhD*; Albert, Todd J. MD*; Guttapalli, Asha MS*; Vresilovic, Edward J. MD, PhD†; Hillibrand, Alan S. MD*; Vaccaro, Alexander R. MD*; Shapiro, Irving M. PhD*

Spine: December 1st, 2004 - Volume 29 - Issue 23 - p 2627-2632

doi: 10.1097/01.brs.0000146462.92171.7f

Basic Science

Abstract

In Brief

Author Information

Objective. Because mesenchymal stem cells can differentiate into chondrocyte-like cells, we ask the question, can mesenchymal stem cells commit to the nucleus pulposus phenotype?

Background. Back pain, a significant source of morbidity in our society, is linked to degenerative changes of the intervertebral disc. Absence of suitable graft tissue limits therapeutic approaches for repair of disc tissue. For this reason, there is considerable interest in developing “tissue engineering” strategies for the regeneration of the nucleus pulposus.

Methods. Rat mesenchymal stem cells were immobilized in 3-dimensional alginate hydrogels and cultured in a medium containing transforming growth factor- β 1 under hypoxia (2% O₂) and normoxia (20% O₂). Mesenchymal stem cells were examined by confocal microscopy to evaluate their viability and metabolic status after labeling with Celltracker green, a thiol sensitive dye, and Mitotracker red, a dye sensitive to the mitochondrial membrane potential. Flow cytometry, semiquantitative reverse transcription polymerase chain reaction and Western blot analysis were carried out to evaluate phenotypic and biosynthetic activities and the signaling pathways involved in the differentiation process.

Results. Under hypoxic conditions, mesenchymal stem cells formed large aggregates and exhibited positive Celltracker and Mitotracker signals. Glucose transporter-3, matrix metalloproteinase-2, collagen type II and type XI, and aggrecan mRNA and protein expression was upregulated, whereas there was no change in the levels of decorin, biglycan, fibromodulin, and lumican. Hypoxia maintained the expression of CD44 (hyaluronan receptor), ALCAM (CD166), and endoglin (transforming growth factor- β receptor). Likewise, expression of β 3 and α 2 integrin was upregulated. Transforming growth factor- β treatment increased MAPK activity and Sox-9, aggrecan, and collagen type II gene expression. Basal levels of the phosphorylated MAPK isoform ERK1/2, but not p38, were higher under hypoxic conditions than normoxia, and its activation was further augmented by treatment of cells with transforming growth factor- β . In hypoxia, transforming growth factor- β sustained phosphorylated p38 expression for an extended time period. Pharmacological inhibition of ERK1/2 and p38 enzymatic activity resulted in a decrease in Sox-9, aggrecan, and collagen type II mRNA levels.

Conclusions. Our results indicate that hypoxia and transforming growth factor- β drive mesenchymal stem cell differentiation towards a phenotype consistent with that of the nucleus pulposus. Measurement of selected signaling molecules and response to specific inhibitors suggest involvement of MAPK signaling pathways. It is concluded that mesenchymal stem cells could be used to repopulate the damaged or degenerate intervertebral disc.

When cultured in hypoxia and in the presence of transforming growth factor- β , mesenchymal stem cells commit to a nucleus pulposus-like lineage. During this differentiation process, these environmental factors modulate the MAPK signaling pathways. It is suggested that mesenchymal stem cells could be used to repopulate the damaged or degenerate intervertebral disc.

From the *Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania, and the †Department of Bioengineering, Drexel University, Philadelphia, Pennsylvania.

Supported by Cervical Spine Research Society “Medtronic Sofamor Danek Named” research award A62801.

The manuscript submitted does not contain information about medical device(s)/drug(s).

Professional Organization funds were received to support this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Address correspondence and reprint requests to Makarand V. Risbud, PhD, Department of Orthopaedic Surgery and Graduate Program in Cell and Tissue Engineering, Thomas Jefferson University, 1015 Walnut Street, Suite 501 Curtis, Philadelphia, PA 19107; E-mail: makarand.risbud@jefferson.edu

© 2004 Lippincott Williams & Wilkins, Inc.

Source

Differentiation of Mesenchymal Stem Cells Towards a Nucleus Pulposus-like Phenotype In Vitro: Implications for Cell-Based Transplantation Therapy

Spine29(23):2627-2632, December 1st, 2004.

Full-Size

Email

+ Favorites

Export

View in Gallery

Email to a Colleague

Colleague's E-mail is Invalid

Your Name: (optional)

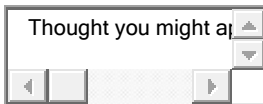
Your Email:

Colleague's Email:

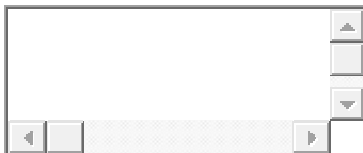
Separate multiple e-mails with a (;).

Message:

Thought you might a



Send a copy to your email



Your message has been successfully sent to your colleague.

Some error has occurred while processing your request. Please try after some time.

Email to a Colleague

Colleague's E-mail is Invalid

Your Name: (optional)

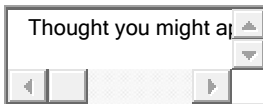
Your Email:

Colleague's Email:

Separate multiple e-mails with a (;).

Message:

Thought you might a



Send a copy to your email



Your message has been successfully sent to your colleague.

Some error has occurred while processing your request. Please try after some time.

Add Item(s) to:

An Existing Folder

A New Folder

Folder Name:

Description:

The item(s) has been successfully added to "".



Article Tools

[View Full Text](#)

[Article as PDF \(790 KB\)](#)

[Article as EPUB](#) [Article as EPUB](#)

[Print this Article](#)

[Email To Colleague](#)

[Add to My Favorites](#)

[Export to Citation Manager](#)

[Alert Me When Cited](#)

[Get Content & Permissions](#)

Share this article on:

Email to a Colleague

Colleague's E-mail is Invalid

Your Name: (optional)

Your Email:

Colleague's Email:

Separate multiple e-mails with a (;).

Message:

Send a copy to your email

Your message has been successfully sent to your colleague.

Some error has occurred while processing your request. Please try after some time.

Export to

- End Note
- Procite
- Reference Manager
- Save my selection

Article Level Metrics

Advertisement

Article Keywords

Keywords

mesenchymal stem cell, nucleus pulposus, differentiation, hypoxia, TGF- β , signaling, MAPK

Search for Similar Articles

You may search for similar articles that contain these same keywords or you may modify the keyword list to augment your search.

Related Links

Articles in PubMed by Makarand V. Risbud, PhD

Articles in Google Scholar by Makarand V. Risbud, PhD

Other articles in this journal by Makarand V. Risbud, PhD

Related Videos

Data is temporarily unavailable. Please try again soon.

Content Links

[Feedback](#)

[Sitemap](#)

[Follow us on Facebook](#)

[Follow us on Twitter](#)

[RSS Feeds](#)

[LWW Journals](#)

[Resources](#)

[Privacy Policy \(Updated October 4, 2017\)](#)

[Terms of Use](#)

[Open Access Policy](#)

[Subscribe to eTOC](#)

[Contact Wolters Kluwer Health, Inc.](#)

Email:

customerservice@lww.com

Phone:

800-638-3030 (within the USA) 301-223-2300 (outside of the USA)

Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Enter your Email address:

Continue

Wolters Kluwer Health may email you for journal alerts and information, but is committed to maintaining your privacy and will not share your personal information without your express consent. For more information, please refer to our Privacy Policy.

Bottom of Form