TCABSE-J Application Reports
ISSN: 2583- 2557, *Volume 1, Issue 4, pp 41-44. 2022.*

TCABSE-J
Interdisciplinary Sciences Journal

Design of a minimally invasive stem cell therapy by targeted sonic hedgehog protein engineering for intervertebral disc damage repair.

[1]Devi Lakshmi P. Korangi and [1, 2]Ravikiran S. Yedidi*

[1]The Center for Advanced-Applied Biological Sciences & Entrepreneurship (TCABS-E), Rajahmundry, AP, India; [2]Department of Zoology, Andhra University, Visakhapatnam, AP, India.
(*Correspondence to RSY: tcabse.india@gmail.com).

Spinal cord injury leading to the intervertebral disc [IVD] damage leads to chronic pain and requires surgery with long recovery times. At present, pain management and post surgical physiotherapy are available as options. The long process of healing severely affects the patient's life in different ways. In order to cut down the healing time with the same results we hypothesized that one can genetically program stem cells into IVD producing progenitor cells and deliver them precisely to the site of injury to naturally recover from the damaged IVDs. This method is minimally invasive compared to complex surgery. In this study, the sonic hedgehog [SHH] protein was chosen as the target for genetic engineering to differentiate the stem cells *in vitro* before using them for IVD damage repair. SHH plays a critical role in cell differentiation. We performed a detailed structural analysis of SHH bound to the patched receptor for protein engineering and chemical biology approaches.

Keywords: Stem cells, spinal disc damage, genetic engineering, sonic hedgehog gene, therapy.

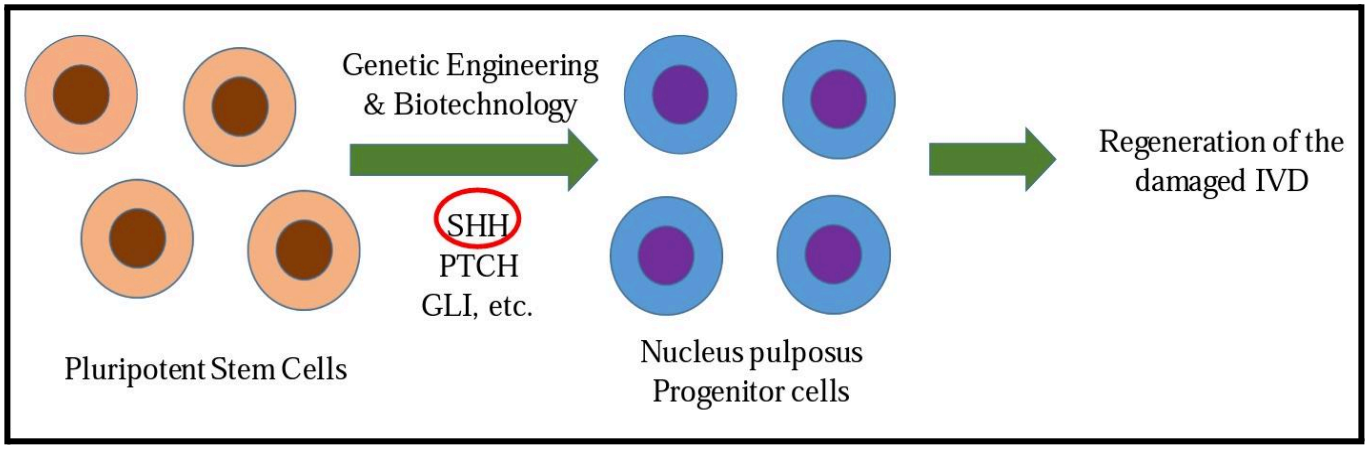

Figure 1. The overall proposed workflow for stem cell therapy treatment to repair the damaged IVDs using genetic engineering and protein engineering protocols.

Citation: Korangi, D. P. and Yedidi, R.S. (2022). Design of a minimally invasive stem cell therapy by targeted sonic hedgehog protein engineering for intervertebral disc damage repair. *TCABSE-J*, Vol. 1, Issue 4: 41-44. Epub: Oct5th, 2022.

ISSN: 2583- 2557, *Volume 1, Issue 4, pp 41-44. 2022.*

Among the major disorders, intervertebral disc (IVD) damage has been a menace throughout the world. Majority of the adults aged 30-50 are affected by IVD damage. IVD is mostly caused due to heavy weight lifting and working continuously for long hours. Human spine is made up of 33 bones called vertebrae. IVD is the disc that is present in between the two vertebrae of the spinal cord except for the first cervical segment. There are 23 discs in the human spine (6-cervical region, 12-thoracic region, 5-lumbar region). Each disc contains a nucleus that acts as a shock absorber, absorbing the impact of the body's activity and keeps the two vertebrates separately acting as strong elastic pivot for each joint segment of the spine. Disc has a tough exterior with a soft, gel-like interior creating cushions between the bones. Each Disc is covered on the two sides by the subchondral plate and cartilaginous endplate. The nucleus consists of nucleus pulposus and the outer region comprises of annulus fibrosus. Nearly 60-80% of the disc consists of water and the remaining primarily consists of type - 2 collagen and proteoglycans.

The common type of disc problem includes, (i) Herniated disc or slipped disc, (ii) Pinched nerve, (iii) Degenerative disc disease, (iv) Spinal stenosis, (v) Sciatica and (vi) Bulging disc. Common symptoms of the IVD damage problem include back pain, arm pain, neck pain, leg pain, muscle weakness, numbness, burning difficulty in walking and sleeping. The available treatment options for these problems are hot cold therapy, physical therapy, steroid injections, yoga, acupuncture, chiropractic manipulations and massage therapy. In severe cases surgery is performed. But these treatment options don't ensure complete cure.

In this study, we hypothesized that one can genetically program pluripotent stem cells into Nucleus pulposus progenitor cells and deliver them precisely to the site of injury to naturally recover from the damaged IVDs (Figure 1). We propose to use the pluripotent stem cells from bone marrow or umbilical cord as a starting point and genetically engineer them into nucleus pulposus progenitor cells that can naturally regenerate the damaged IVD. As a part of the proposed genetic engineering protocol, we are focussing on the sonic hedgehog (SHH) pathway first which interacts with cell receptor proteins such as patched [PTCH] and transcription factors such as gli. SHH is a growth factor protein responsible for cell differentiation during embryonic development. SHH interacts with patched (PTCH) and smoothened (SMO) receptors and triggers the downstream signals. Gli is a transcription factor that relocates into the nucleus and helps the expression of SHH target genes which further help in cellular differentiation. The activation of SHH signaling requires binding of SHH to the PTCH mediated smoothened (SMO) (PTCH-SMO) receptor complex and induction of downstream signaling cascade. This is a heterodimeric receptor complex consisting of two transmembrane subunits, namely PTCH and SMO. They have seven alpha helices that play a major role in the downstream SHH signaling. The PTCH suppressor is normally a molecular transporter. PTCH indirectly inhibits the SMO activity as a response to the binding of SHH with PTCH. SMO is activated and stabilized. The activated SMO initiates the SHH downstream signaling cascade. It generates intracellular signals that regulate several protein kinases, which activates transcription factors such as GLI.

In order to understand the overall organization of the SHH analysis of its core domain structure was performed using computational biology tools. Structure of SHH was downloaded from the protein data bank (PDB ID: 3HO5). The analysis includes evaluation of structure (α helices and β strands) using PyMOL molecular graphics software. Hydrogen bond analysis was performed using PyMOL. All hydrogen bonds with bond lengths less than 3 Å were considered as strong hydrogen bonds (Table 1). Evidently, only 4 out of 13 hydrogen bonds were found to be weaker.

TCABSE-J Application Reports
ISSN: 2583- 2557, *Volume 1, Issue 4, pp 41-44. 2022.*

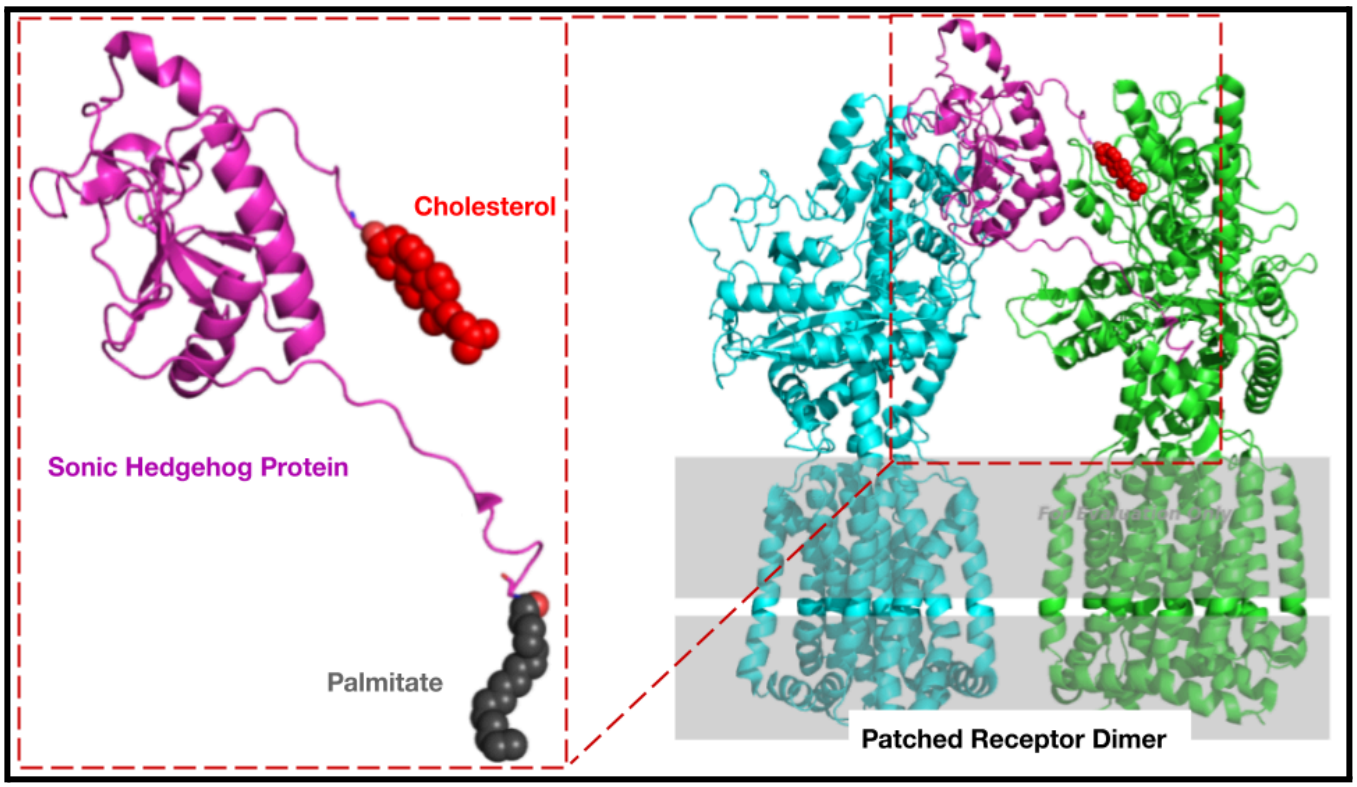

Figure 2. Structure of SHH and PTCH dimer.

SHH is a 45 kilo Dalton protein containing 462 amino acids. SHH consists of an N-terminal and a C-terminal domain with a protease cleavage site in between. After cleavage, the N-terminal domain binds palmitate and cholesterol to become functionally active. Next, we analyzed the binding interactions of SHH with PTCH (PDB ID: 6RVD). The right panel of Figure 2 shows the active complex in which the SHH is bound to PTCH such that the smoothened (SMO) allows the transcription of target genes through GLI1 but when the hedgehog interacting protein (HHIP) binds to SHH then SMO is inhibited so that the downstream transcription of the target genes is also inhibited. The current study revealed the interactions between SHH and HHIP (PDB ID: 3HO5) shedding light on how SHH is regulated by the HHIP from binding PTCH. This mechanism of SHH targeted gene regulation can be achieved by tweaking the interactions between SHH and HHIP to enhance their mutual binding affinities.

SHH	Bond length	HHIP
Tyr44	2.7A	Thr418
Tyr44	2.6A	Asp387
Lys178	3.4A	Asp387
Glu176	2.6A	Gly384
His180	2.9A	Asp383
Glu136	3.0A	Ala311
His133	3.4A	Asp383
Arg153	2.9A	Glu380
Arg153	3.0A	Glu380
Arg123	3.1A	Glu380
Lys87	2.8A	Glu380
Lys87	2.8A	Glu381
Thr125	3.1A	Glu380

Table 1. List of hydrogen bonds between SHH and HHIP.

This strategy will be used as a part of our protein engineering protocol that will be used in future to differentiate stem cells into nucleus pulposus progenitor cells. These differentiated cells will then be tested in animals for their performance in the IVD damage repair. All further research on this project will be published in the future issues of TCABSE-J.

References

1. Pattappa, G., Li, Z., Peroglio, M., Wismer, N., Alini, M., & Grad, S. (2012). Diversity of intervertebral disc cells: phenotype and function. *Journal of anatomy*, *221*(6), 480–496.
2. Sakurai, H., Sakaguchi, Y., Shoji, E., Nishino, T., Maki, I., Sakai, H., Hanaoka, K., Kakizuka, A., & Sehara-Fujisawa, A. (2012). In vitro modeling of paraxial mesodermal progenitors derived from induced pluripotent stem cells. *PloS one*, *7*(10), e47078.
3. Borycki, A. G., Mendham, L., & Emerson, C. P., Jr (1998). Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development (Cambridge, England)*, *125*(4), 777–790.
4. Tendulkar, G., Chen, T., Ehnert, S., Kaps, H. P., & Nüssler, A. K. (2019). Intervertebral Disc Nucleus Repair: Hype or Hope?. *International journal of molecular sciences*, *20*(15), 3622.
5. Fontes, A., Macarthur, C. C., Lieu, P. T., & Vemuri, M. C. (2013). Generation of human-induced pluripotent stem cells (hiPSCs) using episomal vectors on defined Essential 8™ Medium conditions. *Methods in molecular biology (Clifton, N.J.)*, *997*, 57–72.
6. Tang, R., Jing, L., Willard, V. P., Wu, C. L., Guilak, F., Chen, J., & Setton, L. A. (2018). Differentiation of human induced pluripotent stem cells into nucleus pulposus-like cells. *Stem cell research & therapy*, *9*(1), 61.
7. Oh, J., Lee, K. I., Kim, H. T., You, Y., Yoon, D. H., Song, K. Y., Cheong, E., Ha, Y., & Hwang, D. Y. (2015). Human-induced pluripotent stem cells generated from intervertebral disc cells improve neurologic functions in spinal cord injury. *Stem cell research & therapy*, *6*(1), 125.
8. Choi, H., Johnson, Z. I., & Risbud, M. V. (2015). Understanding nucleus pulposus cell phenotype: a prerequisite for stem cell based therapies to treat intervertebral disc degeneration. *Current stem cell research & therapy*, *10*(4), 307–316.
9. Bosanac, I., Maun, H., Scales, S. *et al.* The structure of SHH in complex with HHIP reveals a recognition role for the Shh pseudo active site in signaling. *Nat Struct Mol Biol* 16, 691–697 (2009).

Acknowledgements: The authors thank T*yi*DE-Toronto, Canada for helping to write this manuscript.

Funding: The authors thank TCABS-E, Rajahmundry, India and TyiDE-Toronto, Canada for financial support.

Conflict of interest: The applications report presented here is a currently ongoing project at TCABS-E, Rajahmundry, India. The authors invite collaborations without any conflict of interest.