International Journal of Latest Trends in Engineering and Technology
Vol.(10)Issue(1), pp.105-112
DOI: http://dx.doi.org/10.21172/1.101.17
e-ISSN:2278-621X

STUDY OF CHONDROGENESIS IN MURINE MESENCHYMAL STEM CELLS AS A CELL THERAPY IN CARTILAGE REPAIR BY IN SILICO APPROACH

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Abstract- Mesenchymal stem cells (MSCs) are multipotent cells able to differentiate into several mesenchymal lineages, classically derived from bone marrow. We have focused our attention on the genes responsible for the chondrogenic differentiation of murine MSCs by analysing the interaction of genes and microarray data analysis. For this purpose, softwares like Cytoscape, Polar mapper, Bio interpreter, Gene Spring. This study provides an overall view of the transcriptions factors and their protein interaction involved in the chondrocytic differentiation of MSCs. The proper knowledge about the transcription factors and their mechanism is important for the efficient chondrocytic differentiation and this will be a milestone for the development of cartilage tissue engineering. The identified transcription factors and proteins can be visualised in the invitro conditions and this can increase the efficiency of cartilage formation.
Keywords: Mesenchymal, cytoscape, chondrocytic differentiation,multipotent

1. INTRODUCTION

Bone marrow stromal cells, also known as mesenchymal stem cells or fibroblastic colony-forming units, are multipotent non-hematopoietic stem cells adhering to culture plates. Mesenchymal Stem Cells (MSC) of the bone marrow have the ability to renew and differentiate themselves into multiple lineages of conjunctive tissues, including bone, cartilage, adipose tissue, tendon, muscle, and bone marrow stroma (Renata et al.,2006).In the 1960s and 1970s, Friedstein described the isolation of stromal cells from bone marrow by plastic adherence and the ability to regenerate or support ectopic bone, stroma and hematopoietic tissues (Mathew et al.,2013).MSCs are been derived from bone marrow, adipose tissue, dental pulp ,umbilical cord, blood, placenta, skeletal muscle, tendon and trabecular bone (Danisovic et al.,2012,Guilani et al.,2013). Mesenchymal stem cells (MSCs) have been studied in regenerative medicine because of their unique immunologic characteristics. However, before clinical application in humans, animal models are needed to confirm their safety and efficacy. The mouse is a suitable experimental model system to study the cell biology and biochemical characteristics of MSCs (Sung et al., 2008). The International Society of Cellular Therapy (ISCT) proposed the minimal criteria for defining MSCs as follows: (i) adherence to plastic under standard tissue conditions, (ii) the expression of cell surface markers such as CD73, CD90, and CD105 and the lack of expression of CD34, CD45, CD14 or CD11b, CD79 or CD19, and HLA-DR, and (iii) the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts (Guilani et al., 2013).

Biological processes are often represented in the form of networks such as protein-protein interaction networks and metabolic pathways. The study of biological networks their modelling, analysis, and visualization are important tasks in life science today. An understanding of these networks is essential to make biological sense of much of the complex data that is now being generated. This increasing importance of biological networks is also evidenced by the rapid increase in publications about network-related topics and the growing number of research groups dealing with this area. Most biological networks are still far from being complete and they are usually difficult to interpret due to the complexity of the relationships and the peculiarities of the data. Network visualization is a fundamental method that helps scientists in understanding biological networks and in uncovering important properties of the underlying biochemical processes. The insilico methodology was adopted for integrating biomolecular interaction network of the genes involved in the chondrocytic differentiation. The protein-protein interaction studies were carried out using cytoscape, polar mapper, gene spring, bio interpreter. Several studies of parameters are surveyed, including a search for interaction pathways correlating with gene expression (Renata et al., 2006).

2. MATERIALS AND METHODS

2.1 Tools

- String

STRING is Search Tool for the retrieval of Interacting genes/proteins.

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STRING quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable (Andrea Franceschini et al., 2013). The database currently covers 9'643'763 proteins from 2'031 organisms.

- Cytoscape

Cytoscape is an open source software platform for visualizing complex networks and integrating these with any type of attribute data. Although cytoscape was originally designed for biological research, now it is a general platform for complex network analysis and visualization (James et al., 2006).Cytoscape core distribution provides a basic set of features for data integration, analysis and visualization .

- Polar Mapper

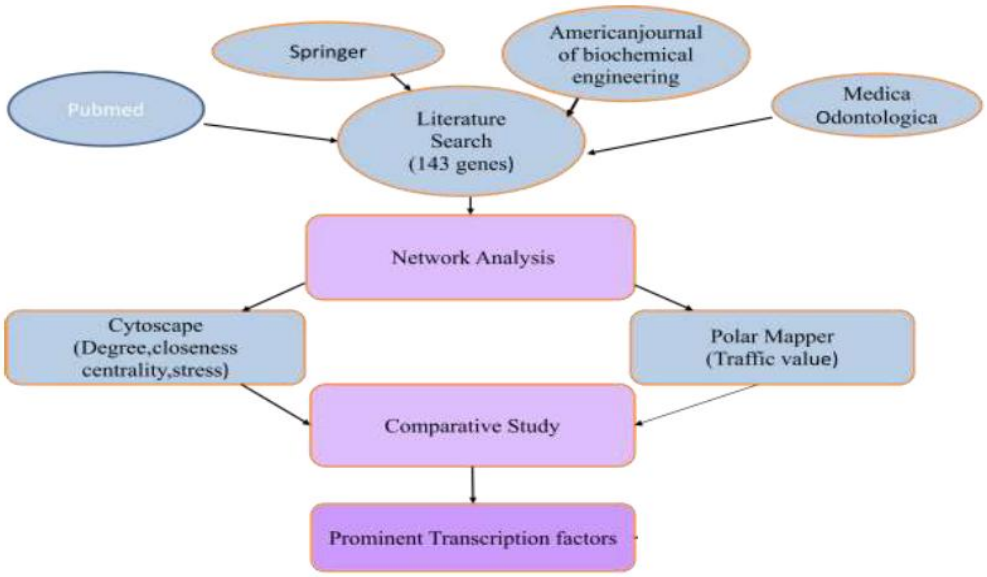
Polar mapper is a computational approach for exposing the architecture of protein interaction networks. It facilitates the system level analysis of mRNA expression data in the context of the underlying protein interaction network.Protein interaction network provides the natural context for interpreting large scale gene expression data. The data is divided into interactome, islands, modules and submodules.

- Bio Interpreter

Bio interpreter is a simple, user-friendly web-based biological interpretation tool for Microarray data analysis. It significantly reduces the biological analysis time from weeks to hours. Simple, quick and effective solution for interpreting Microarray data and to meet the other challenges in biological interpretation like making sense of many gene lists and to get quick updated and comprehensive annotations.

CENTRALITY MEASURES: Degree, closeness centrality, stress, traffic value.

3. FLOW CHART : METHODOLOGY FOR COLLECTION OF GENES



3.1 Collection of transcription factors (TF) relevant to chondrocyte differentiation through literature search

The transcription factors responsible for chondrocyte differentiation through literature search from Springer, PubMed, American Journal of biochemical engineering, Medica Odontologica 1.2**.**The collected transcription factors were visualised using cytoscape and polar mapper to find the genes with maximum expression. The comparative study of these softwares yields prominent genes that play a major role in the mesenchymal stem cell differentiation to chondrocytes.

The genes include various transcription factors and surface markers involved in the chondrocyte differentiation. Various parameters are used for visualisation and analysis of the genes. Cytoscape uses parameters like closeness centrality, degree and stress, whereas polar mapper uses traffic value of islands, modules and submodules.

3.2 Preparation of protein protein interaction (PPI) for TFs using string database

The protein protein interaction of the collected transcription factors was prepared through string database.1069 protein interactions were obtained using the string database. This database predicts functional interactions at an expected level of accuracy of at least 80% for more than half of the genes.

3.3 Analysis of gene network using cytoscape

The protein interactions obtained from string database was used as as input for network analysis for cytoscape. The mimi plug in was used for the network study .The visualization of protein -protein interaction (PPI) network is available at various granularity levels through this software. The parameter used for visualisation of genes are closeness centrality, degree and stress.

3.3.1 Closeness centrality:

$$C_{clo}(v) := \frac{1}{\sum_{w \in V} dist(v, w)}$$

V= Node Value

W= Proximity of the node

The closeness is a centrality property of node. The shortest path length between any node v to the other nodes present in the graph is calculated together with their summation is represented as closeness of any node v. The value obtained by above calculation is then its reciprocal is obtained. High values of closeness show that all other nodes are in proximity to node v. whereas, low values of closeness indicates that all other nodes are distant from node v. When correlated with biological networks, the proteins present in these network will represent their functional importance but, with the possibility to be irrelevant for few other proteins.

3.3.2 Degree:

It represents all the directly connected edges to a single node v, where directly means one to one connection. These direct connections of nodes are also called as first neighbours of node v. Degree distribution A(d) can also be identified by calculating degree, which shows the probability that a selected node has exactly d connections. In biological relevance the degree allows a direct analysis of the regulatory importance of the node. For example, in different types of signalling pathways, proteins with very high degree are interacting with several other signalling proteins, thus suggesting its central regulatory role that is they are likely to be regulatory clusters.

3.3.3 Stress:

It is a parameter for measuring the difficulty with which genes are connected among themselves and with the nearest neighbours. High values of stress indicates that all other nodes are distant from the node c, whereas low values of stress show that all other nodes are in proximity to node v. In various signalling pathways, proteins with less stress value are interacting with several other signalling proteins.

3.3.4 Traffic value

It is an important parameter for finding the protein protein interaction in polar mapper. High traffic value indicates that the protein interaction is good in in the submodules, modules and islands.

3.4 Whole interaction study using Polar mapper

For the whole network analysis, polar mapper was used and as the input the PPI network (String database) was used. The parameter to select the prominent gene was traffic values (TV).The traffic value was identified for the island, modules, and submodules. There were 15 islands, 19 modules and 62 submodules.

Polar mapper is a computational application for exposing the architecture of protein interaction networks. It facilitates the system level analysis of mRNA expression data in the context of the underlying protein interaction network. Protein interaction networks provides the natural context for interpreting large scale gene expression datas.

4. RESULTS

The genes responsible for chondrocyte differentiation was analysed using cytoscape and polar mapper. The culturing of the murine mesenchymal stem cells was carried out through invitro approach. Microarray gene expression studies of the expression difference between osteoarthritic chondrocytes and mesenchymal stem cells during chondrogenic differentiation was analysed using Gene spring and the functions, pathways and diseases associated with the prominently expressed genes was studied by bio interpreter.

5. GENES RESPONSIBLE FOR CHONDROCYTE DIFFERENTIATION.

The genes responsible for chondrocyte differentiation was obtained using the cytoscape and polar mapper. The comparative study of the obtained genes was carried to find out the prominent genes which play a major role in chondrocyte differentiation.

5.1 Collection of transcription factors (TF) relevant to chondrocyte differentiation through literature search.

About 143 genes relevant to chondrocyte differentiation were found through literature search from Springer, PubMed, American Journal of biochemical engineering, Medica Odontologica 1.2.

Table : Collection Of Transcription Factors

SOX5	PFE	FGF-8	IGF1A	WNT3A	Msx2	OP1	CLEC3A
SOX6	IRP	Shh	BMP7	Acan	Fra2	Dlx5	CLECSF1
GDF5	EGF	Wnt14	BMP2	Comp	Mef2c	TCF	STC1
Runx2	ALP	Runx1	BMP4	Dlk1	Alpl	SOST	DSPG3
VEGF	Ihh	Runx3	BGCAN	WNT7A	Prg4	LRP5	SFRP1
TAZ	FGF-18	Msx2	ALK5	Bapx1	Agc1	LRP6	Znf219
Hoxa10	FGF-2	Fra2	BNSP	Col9a1	Ncam1	Dsh	PBX1
c-Myc	Hoxa2	Prg4	FGF10	Col11a1	Tnc	Ctnnbip1	HIF1
NCAM	STAT1	Col2a1	JTK4	CREB	Crtm	HDAC5	HIF1a
SOX9	PDGFB	Col10a1	FLT2	ATF	c-Maf	ADAMTS5	MEF
Spp1	MMP-9	GDF1	ALK2	MAP	ALK1	Sca-1	Ccnd1
Mmp13	MMP-14	ALK4	ALK6	ERK	Smad2	HAPLN1	Tnfrsf19
Pthr1	BMP13	SMAD4	SMAD1	p38	Smad3	CD166	Wnt7b
Nkx3.2	BMP12	SMAD3	SMAD5	LAP	JNK	HOX2	Tcf7
Alpl	GDF11	SMAD2	SMAD8	ALK5	Smad4	SLRR4A	Wnt10a
Acvr2a	Dkk1	FA1	CD90	Klf4	ALK3	WNT11	Fmod
Dusp2	Kremen	Dlk1	Pref-1	Lin-28	CD73	PENK	Postn
Cthrc1	Dcn	Fndc1	Bsp	CD-RAP	PLZF		

5.2 Network Analysis by Cytoscape

The parameters like closeness centrality, degree and stress were considered for the network analysis by cytoscape. The network was built with 688 nodes and 1037 edges.

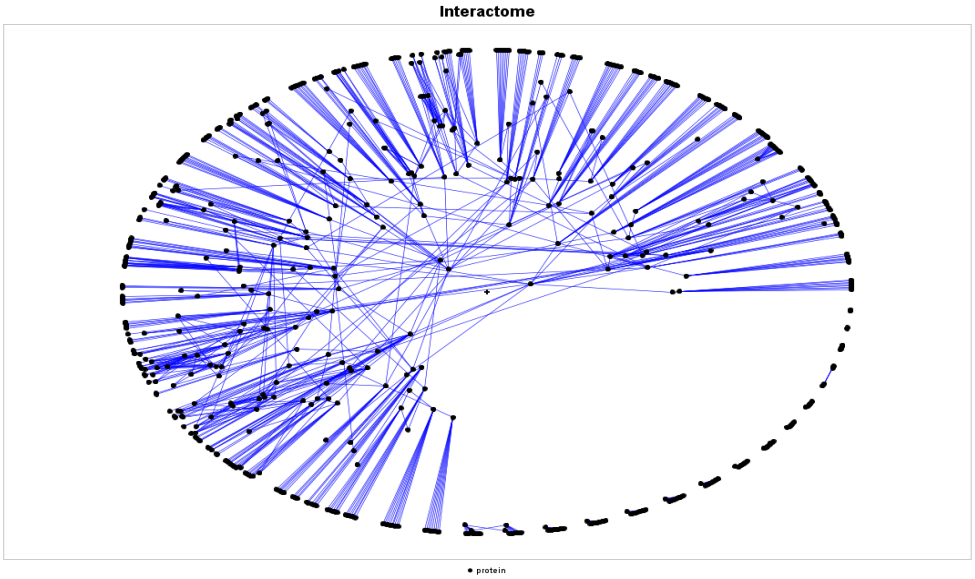
Table: Network Analysis By Cytoscape

GENE NAME	CLOSENESS CENTRALITY	DEGREE	STRESS
Acvr2a	0.214917	16	68616
Smad4	0.239811	16	179314
Runx2	0.241991	16	295456
Col2a1	0.206045	16	138828
Col10a1	0.210784	15	109364
Dkk1	0.210388	14	92408
Col11a1	0.182919	13	32972
Shh	0.204612	13	55458
Ccnd1	0.22495	13	65112

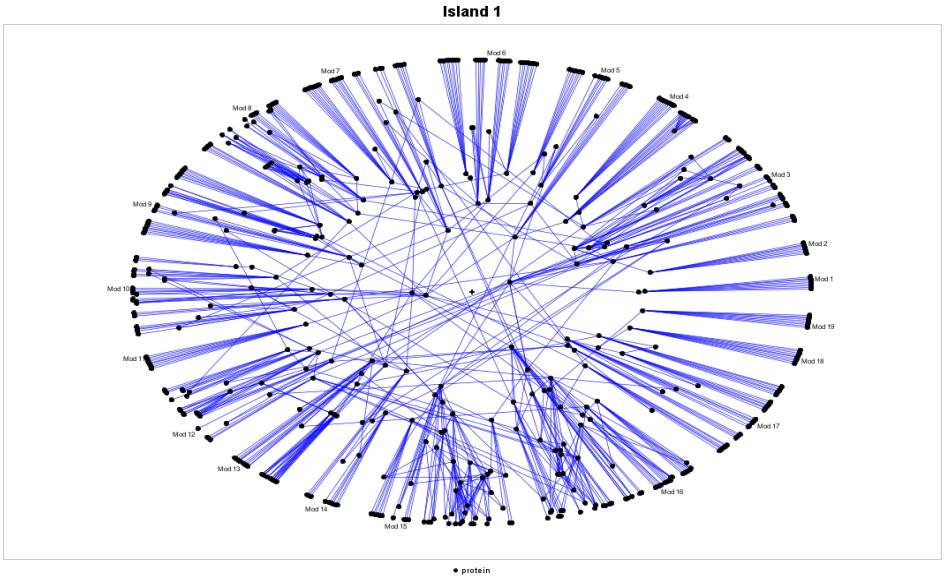
5.3 Network analysis by Polar mapper

The traffic value of the island, module and submodule were obtained and the genes with high traffic values were chosen to find out the genes responsible for chondrocyte differentiation. It was divided into 15 islands,19 modules and 62 submodules.

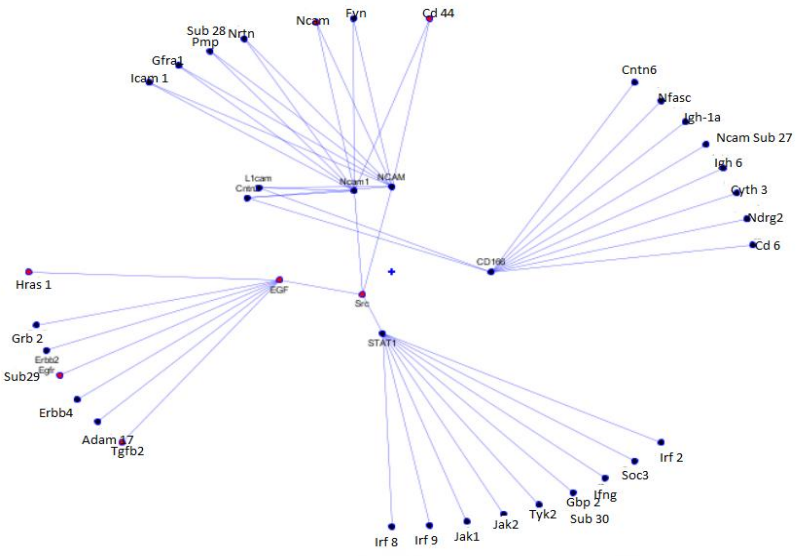
Figure : polar mapper analysis of interactome, island, module and submodule.



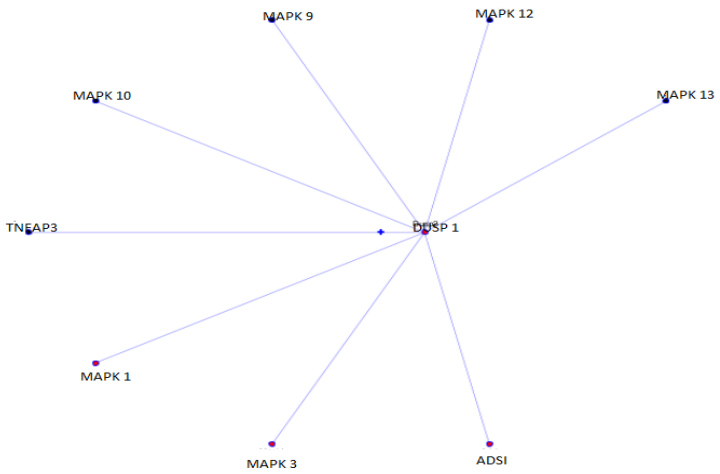
a) Interactome



b) Island 1



c) Module 9



d) Submodule 16

5.2 Comparative Analysis of Cytoscape and Polar mapper.

The genes with high values of closeness centrality, degree and minimum stress in cytoscape was compared with the genes of high traffic values of polar mapper. The comparative analysis resulted in the genes responsible for chondrocyte differentiation.

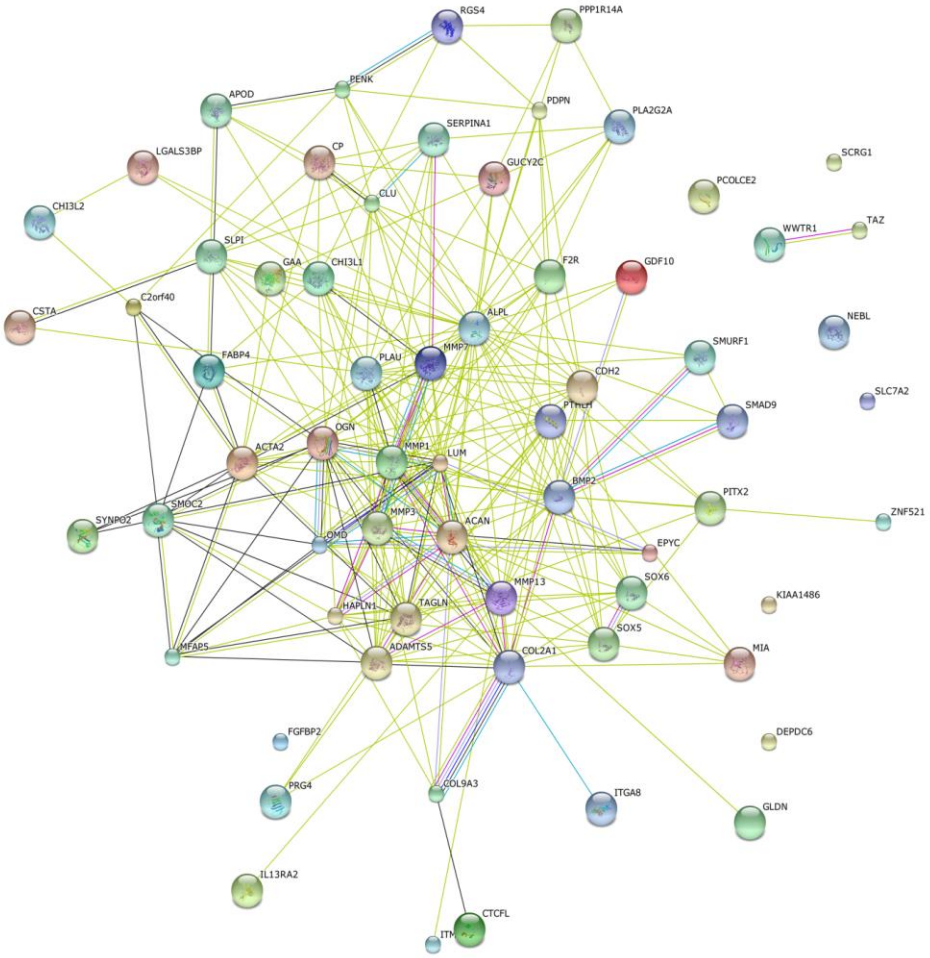
Table : Comparative Analysis Of Cytoscape And Polar Mapper

GENE	**GENE NAME**	**FUNCTION**
Alpl	Alkaline Phosphatase	Dephosphorylation
Acan	Aggrecan	Mediates chondrocyte-chondrocyte and chondrocyte-matrix interaction
TAZ	Tafazzin	Altering cardiolipin
CD166	Activated leukocyte cell adhesion molecule	Implicated in the process of cell adhesion and migration.
SOX6	SRY(Sex determining region y)-box 5	Development of the central nervous system, chondrogenesis and maintenance of cardiac and skeletal muscle cells
SOX5	SRY(Sex determining region y)-box 5	Regulation of embryonic development and in the determination of the cell fate
Lum	Lumican gene	Regulate collagen fibril organization and circumferential growth,corneal transparency, and epithelial cell migration and tissue repair.
Smad9	SMAD Family member 9	Transduces signals from TGF-beta family members.
Pthlh	Parathyroid hormone like peptide gene	Regulates endochondral bone development and epithelial-mesenchymal interactions during the formation of mammary glands and teeth.
Gucy2c	Guanylate cyclase 2c	Receptor for the E.coli heat-stable enterotoxin
Smurf1	SMAD specific E3 ubiquitin protein ligase1	Regulation of cell motility, cell signalling, and cell polarity.
Gaa	Glucosidase alpha acid	Breaks down a complex sugar called glycogen into a simpler sugar called glucose.
Wwtr1	WW domain transcription	Transcriptional coactivator which acts as a downstream regulatory target in the Hippo signalling pathway that plays a pivotal role in organ size control and tumour suppression by

	regulator	restricting proliferation and promoting apoptosis.
ADAMTS5	ADAM Metallopeptidase with thrombospondin type1 motif,5	Functions as aggrecanase to cleave aggrecan, a major proteoglycan of cartilage

5.3 Network analysis

The gene network analysis was done using string database.



5.4 Prominent Transcription Factors In Chondrogenesis Identified Through Network Biology

Table 4.5:Prominent Transcription Factors In Chondrogenesis

GROWTH FACTORS		EFFECT ON CHONDROCYTES/CARTILAGE
TGF-β	Transforming Growth Factor β	Stimulates synthesis of ECM Decreases catabolic activity
BMP-2	Bone Morphogenetic Protein -2	Stimulates synthesis of ECM Increased ECM turnover (increased aggrecan degradation)
BMP-7	Bone Morphogenetic Protein -7	Stimulates ECM synthesis Decrease cartilage degradation
FGF-2	Fibroblast Growth Factors-2	Decreases aggrecanase activity Antagonizes PG synthesis
FGF-18	Fibroblast Growth Factors-18	Increases chondrocyte proliferation and stimulates ECM
ACAN	Aggrecan	Mediates chondrocyte- chondrocyte and chondrocyte-matrix interactions
MMP-7	Matrix metalloproteinase-7	Breakdown of extracellular matrix
COL2A1	Collagen, type II,alpha 1	Adds structure and strength to joints.
MMP-3	Matrix metalloproteinase-3	Involved in wound repair and degrades collagen.
MMP-1	Matrix metalloproteinase-1	Breakdown of extracellular matrix
SERPINA--1	Serpin peptidase inhibitor, clade -A	Dedifferentiation during chondrocyte expansion

MMP-13	Matrix metalloproteinase-13	Breakdown of extracellular matrix
ALPL	Alkaline phosphatase	Proliferation and differentiation

6. CONCLUSION

Chondrogenesis is a multistep process that comprises of several steps: precursor cell condensation, differentiation towards chondrogenic phenotype, secretion of cartilage specific ECM components (collagen type II, aggrecan and others), chondrocytes proliferation in the area of growth plate, further differentiation towards hypertrophy, and replacement of cartilage with the bone tissue. The most important factors currently used in tissue engineering are the members of transforming growth factor β (TGF-β) family, Bone Morphogenetic Protein (BMP), Parathyroid hormone like peptide gene (Pthlh), Fibroblast Growth Factors (FGF), especially FGF-2 and FGF-18, Epidermal Growth Factor (EGF) and Vascular-Endothelial Growth Factors (VEGFs),SERPINA 1,Alkaline phosphatase(ALPL).Sox9 controls the transcription of genes characteristic to the cartilage matrix,such as type II collagen and aggrecan ,and it also suppresses the subsequent formation of hypertrophic chondrocytes.Sox9 induces the expression of coupling factors,L-Sox5 and Sox6,forming a transcription complex, which activates the transcription of cartilage-related genes such as Col2α1.Prechondrocytes differentiate into mature chondrocytes producing abundant aggrecan and collagen fibers(types II,IX and XI).Chondrocytes brgin to express alkaline phosphatase in conjunction with the transcription factors, Indian hedgehog(Ihh) and parathyroid hormone-related receptor(PTHrP-R) and produces type X collagen instead of type II collagen.The summary of the effects of different GFs on chondrocytes/cartilage is presented in the above table

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7. REFERENCE

[1] Adelola O. Oseni, Claire Crowley, Maria Z. Boland, Peter E. Butler and Alexander M. Seifalian:Cartilage tissue engineering: The application of nanomaterials and stem cell technology, Tissue engineering and regenerative medicine,2011.

[2] Andrea Page: Matrix metalloproteinase and the regulation of tissue remodelling, Natural Rev Mol Cell Biol. 2007 Mar; 8(3): 221–233.

[3] Arnold I Caplan: Mesenchymal Stem Cells: Cell-Based Reconstructive Therapy in Orthopedics,Tissue Engineering 2005,Vol 11.

[4] Beatriz Ranera, Ana Rosa Remacha a, Samuel Álvarez-Arguedas a, Tomás Castiella b, Francisco José Vázquez andClementina Rodellar: Expansion under hypoxic conditions enhances the chondrogenic potential of equine bone marrow-derived mesenchymal stem cells, Elseveir 2013, 248-251.

[5] Danisovic, Varga.I, Polak.S: Growth factors and chondrogenic differentiation of mesenchymal stem cells, Elsevier 2012; 69-73.

[6] Giuliani Nicola, Gina Lisignol, Marina Magnani, Costantina Racano,Marina Bolzoni, Angelica Spolzino,Cristina and Franco Aversa: New Insights into Oestrogenic and Chondrogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells and Their Potential Clinical Applications for Bone Regeneration in Pediatric Orthopaedics, Stem cells International 2013,1-12.

[7] Halleux.C, Sottile.V, Gasser J.A and Seuwen : Multi-lineage potential of human mesenchymal stem cells following clonal expansion, Musculoskel Neuron International 2001;71-76.

[8] Hanna Taipaleenmaki et al: Factors Regulating Chondrocytic Differentiation, Medica Odontologica 2010; 17-39.

[9] Heng Zhu and Zi-Kuan Guo : A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone, Nature Protocols 2010, 550 – 560.

[10] Hood, L., and Perlmutter, R.M. The impact of systems approaches on biological problems in drug discovery,Natural Biotechnology 2004 ,22, 1215 – 1217.

[11] Ideker T, Sharan R. Protein networks in disease. Genome Research 2008, 18:644–652.

[12] James Vlasbom et al:GenePro: a cytoscape plug-in for advanced visualization and analysis of interaction networks,Systems biology2006, Vol22,2178-2179.

[13] Mathew and Murphy:Mesenchymal stem cells:environmentally responsive therapeutics for regenerative medicine,Experimental and Molecular Medicine 2013,1-16.

[14] Mehmet and Koyukuk : Algorithmic and analytical methods in network biology, System boilogy medicine 2009

[15] Poetz.O, Schwenk J.M., Kramer. S., Stoll. D., Templin M.F., and Joos:.Protein microarrays: catching the proteome. Mechanical Ageing Development, 2005. 126, 161 – 170.

[16] Renata Aparecida Camargo Bitten court et al: Isolation of bone marrow mesenchymal stem cells, Acta Ortop Bras-2006.

[17] Sung J.H, Yang, Park.B., Choi J.-W, Joh,. Kwon, Chun: Isolation and Characterization of Mouse Mesenchymal Stem Cells, Elseveir 2008; 2649–2654.

[18] Sylwia Bobis, Danuta Jarocha and Marcin Majka et al: Mesenchymal stem cells: characteristics and clinical applications, Folia Histochemica et Cytobiologica 2006,Vol. 44, No. 4, 215-230.