Regulation of gene expression in tissue engineering, differentiation and bone regeneration of ossifying stem cells

Amirhosein Nezakat Yazdi1, Nastaran Sahraei1, Mehdi Ahmadifar1,2*

1. Department of biology, college of science, University of science and culture, ACECR, Tehran branch, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

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ABSTRACT

Cells that make up the body's tissues are usually three-dimensional architecture, the three-dimensional culture system enables cells to create natural and in vivo interactions which is an ideal environment for 3D (Three-dimensional) cell growth and issues such as exchange of similar food exchanges inside Capillary in living tissue. In tissue engineering discussion, cell scaffolding is highly important and is used for tissue implantation, which will eventually dissolve or demolish after applying to the body. Bone formation can occur via two separate pathways, within the cartilaginous and within the membrane. The vascular endothelial growth factor is a key regulator of angiogenesis. The combination of osteoactivity is a major parameter in the engineering structures and the function of the bones and osteoblasts is very important in maintaining and repairing bone in the laboratory conditions. Also, in this study, we investigated transcription factors, epigenetic reforms, miRNAs, and Sox family in tissue engineering. Between transcription factors, TLX's core receiver is essential for maintaining neural stem cells and NCSs reviver. Epigenetic reforms maybe a major factor that links genetic and environmental factors to the risk of osteoporosis. Micro-RNAs regulate bone and chondrogenic differentiation by targeting important transcription factors and relative pathways during skeleton maturation. The members of the Sox family interact in a wide range of cellular tissues and thus create a variety of effects on cellular metabolism.

Introduction

Tissue engineering is used to create bio-alternatives that can restore, preserve, and improve the function of damaged tissues. The main components in scaffold tissue engineering are cells and growth factors (1). The scaffold is a three-dimensional structure that is used as a framework to guide cells and is an alternative to the extracellular matrix. The cells penetrate the scaffold and begin to grow, differentiate, proliferate and migrate according to the physical and chemical signals around them, and if the environmental conditions are favorable, they secrete an extracellular matrix and create the new tissue (2) (Fig. 1). Nanotechnology is the engineering of functional systems that covers a wide range of topics at the molecular level and focuses on controlling and exploiting the structure of matter at a scale below 100 nanometers.
In recent years, the use of many nanomaterials, including nanocapsules, nanoparticles, and nanofibers with a size of less than 100 nm has attracted the attention of researchers in the diagnosis and treatment of diseases (3).

**Bone tissue engineering**

Biomaterials used in the manufacture of porous scaffolds include natural and synthetic compounds. Biomaterials are obtained from natural sources (plant or animal); collagen, chitosan, agarose, alginate, and fibrins are natural materials discovered for RPT. Chitosans can be the best choice when scaffolds are exposed to a transparent environment because they have antibacterial activity against the risk of bacterial contamination.

**Classification of cell scaffolds:**

1) 3D cell culture scaffold, which is mainly used in renewable medicine or cell behavior studies.

2) 3D tissue engineering scaffolding which is used for tissue growth and reproduction (9, 8, 7).

The main difference is based on whether the scaffolding dissolves after use or not. 3D cell scaffolding is used in the laboratory for cells culture, study, or analysis and is not dissolved or destroyed after usage (8, 7, 6). Tissue engineering scaffolding is used for tissue implantation and tends to dissolve or destroy after application to the body. The porosity of the scaffold varies from nano to micro-level, the number of pores per square centimeter is significantly different and is generally from approximately 50 to 600 (12,11,10). Two types of isotropic and orthotropic architectures are mainly used on three-dimensional scaffolds. Both offers specific stimuli on cells as well as different mechanisms in scaffold structures (14, 13, 8). Scaffolding mechanics are important because they help tissues and cells maintain their integrity and proper function (13, 8, 2). The mechanical properties of scaffolding can be mainly affected by the size, dimensions, and materials used in construction. Studies show that physiological processes can affect the mechanical properties of cell environments. These mechanical properties can cause vulnerabilities and cellular behaviors to differentiate and increase growth and etc. The mechanical properties of 3D scaffolding are generally dependent on the texture and its hardness is from 0.3-1 kPa to an average hardness of 8-17 kPa and above. Therefore, adjusting the mechanical properties for 3D scaffolding design helps to ensure physiological rigidity and mechanical supports; This can help in better cell uptake as usual as well as the remaining intermittent mechanism in dynamic cells vibration (16,15,2).

Figure 1. Growth factors and nanoparticles can repair bone in two ways.

1) They differentiate stem cells from osteoblasts.

2) Both directly cause cells to grow and differentiate.

**Cell scaffolding and stimuli for in vitro modeling**

Experiments performed on 2D cell culture systems have resulted in extensive knowledge in the early life sciences (1); However, the morphology of cells growing in 2D media is significantly different from those of cells in real tissues, because 2D media is usually flat and cells grow only in x and y directions. It is endangered as a result of the interaction of one cell with another and also, this harms gene expression, protein synthesis, and other cellular functions (3,2).

The cells that make up the body's tissues usually have a three-dimensional architecture, and for this reason, three-dimensional cell culture and related tools have been developed in recent decades. The range of the vertical axis of the third dimension is between a few micrometers to centimeters and supports cells for complex three-dimensional interactions with adjacent cells and produce more layers (4,5). Also, a three-dimensional culture system; empowers cells to create intercellularly and in vivo interactions, providing an ideal environment for the growth of 3D cells, and issues such as nutrient exchange similar to capillary exchange in living tissues (1,3,6).

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1 Periodontal Regeneration Technique
2 Two-dimensional
3 Three-dimensional
toxic to cells, easily removed from the body, and have a low immune response due to its presence. If a scaffold is not destroyed quickly enough, the inflammatory response can lead to external scaffold material and impair tissue regeneration (17).

Macroscopically, bones are made up of interconnected trabecular tissues, each with very different mechanical strength and stiffness (18). At the molecular level, the ECM is composed mainly of fibrous macromolecules, glycosaminoglycans, and glycoproteins (19). The bone is first supplied with blood by an arterial network. Inside the brain cavity; The bones and canals of the Haversian carry blood vessels to thin-walled capillaries whose main role is to exchange nutrients and signals between blood cells (20).

Bone formation can occur through two distinct pathways, intra-cartilage and intra-membrane. In each case, the first step is to compress the mesenchymal cells to create a pattern for subsequent bone formation (21). Intracartilage ossification: The direct differentiation of mesenchymal progenitor cells into osteoblasts and intramembrane ossification: Differentiation of mesenchymal progenitor cells into chondrocytes and subsequent hypertrophy is matrix mineralization and replacement with bone tissue. Both pathways of bone formation occur near arteries (22). During intramembranous bone formation, mesenchymal tissue is affected, while in intracartilaginous bone formation, overactive cartilage cells cause blood vessels to absorb and penetrate (23). Primary arteries are associated with invasion of osteoclasts and osteoblasts and, coordination, with overactive cartilage, ECM mineralization, and bone formation; Reabsorbed. VEGF is a key regulator of angiogenesis and consequent bone development; VEGF-A levels also depend on hypoxia-induced reversal (24), resulting in bone mass modulation (24). Creating a functional vessel within the structure can be necessary to create a broad and accurate model that can mimic its tissue. Current vascular strategies include: The use of angiogenic factors is combined with 3D scaffolding and the use of culture systems.

The first angiogenesis-based approach by endothelial precursors using angiogenic agents (angiogenic factors) such as VEGF. Studies show that the 'electrospun poly (caprolactone)' scaffold with the VEGF layer causes the penetration and proliferation of EPCs in the 3D matrix. This process is regulated to endothelial cells and in the field of tissue engineering, special interactions with scaffolding and other cells types are required to optimize vascular formation in vitro (25) (Fig. 2). Osteocytes are distinctly stable osteoblasts located in cavities within the bones matrix. They travel through cells processes to the mineral matrix inside small canals called “canaliculi” that are filled with canicular fluid (26). Although osteocytes play a secondary role in bone formation, they play an important role in homeostasis, mechanization, and transmission mechanics (28, 27). One of the parameters that must be considered when creating vascular bone in vitro is the combination of osteoclast activity within the engineered structures. Osteoclasts for bone regeneration by remodifying the bone matrix and creating physical space for osteoblasts and endothelial cells, new bone tissue with sufficient arteries can be formed. The dissolution of the mineral phase of the bone matrix is mediated by the secretion of hydrochloric acid and the absorption of the organic matrix by secreted enzymes such as cathepsin K and metalloproteinase9 (29).

The ideal in vitro model of bone should consider the possibility of studying its interaction with other tissues, thus creating considerable challenges in replacing different specific tissue environments. Stem cells are an exceptional tool for achieving this complex integration between bones and other tissues due to their multiple capabilities.

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4 extracellular matrix
5 Vascular endothelial growth factor
6 HIF-1
7 Endothelial progenitor cells

Figure 2. 1. Mesenchymal stem cells are extracted from bone marrow, umbilical cord blood, fat cells, or embryonic tissue and transferred to scaffolds along with growth factors. 2. In miRNA-29a cells, by targeting the foxo3a gene, it provides differentiation and formation of cartilage, which is due to the activity of cartilage due to the influence of angiogenic factors such as VEGF. Angiogenesis and repair of damaged tissue occur.
Table 1. List of in vivo studies in which the potential HDPSCs for bone tissue engineering were examined and the experimental model, type of scaffold, bone defect were created and the time and method of identification of each of them were used.

| Author             | Experimental model | Scaffold type          | Defect                      | Evaluation method                                         | Evaluation time |
|--------------------|--------------------|------------------------|-----------------------------|-----------------------------------------------------------|-----------------|
| Miura et al. 17    | Mice Immunological | HA/TCP ceramic         | Skull defect scaffolding    | Histology Immunohistochemistry, in local hybridization and RT-PCR | 8 weeks         |
| LAINO et al. 18     | Mice Immunological | Bone subcutaneous tissue is made by HDPSCs | Implant | Histology | 4 weeks |
| PAPACCIO et al. 19  | Mice Immunological | Bone subcutaneous tissue is made by HDPSCs | Implant | Histology and immunofluorescence | 4 weeks         |
| et al. 20 LAINO     | Mice Immunological | Bone subcutaneous tissue is made by HDPSCs | Implant | Histology | 4 weeks |
| GRAZIANO et al. 21  | Mice Immunological | PLGA membrane           | Subcutaneous transplantation | Histology, immunohistochemistry and immunofluorescence | 30,40,60 days   |
| D'AQUINO et al. 22  | Mice Immunological | Bones woven by HDPSCs are obtained subcutaneously in vitro | PGA-TMC implants and scaffolding | Histology and immunohistochemistry | 4,6,8 weeks     |
| OTAKI et al. 23     | Mice Immunological | HA / TCP powder         | Subcutaneous transplantation | Histology | 7,15 weeks |
| GRAZIANO et al. 24  | Mice Immunological | PLGA membrane           | Subcutaneous transplantation | Immunohistochemical histology, and X-Ray diffraction | 4,8 weeks |
| DE MENDONSA COSTA et al. 25 | Rat | Collagen membrane | Skull defects | Histology | 1,3,4,8 weeks |
| ABE et al. 26       | Mice Immunological | HA scaffolding          | Subcutaneous transplantation | Histology and immunofluorescence | 12 weeks         |
| ZHANG et al. 27     | Mice Immunological | HA / TCP ceramic scaffolding | Subcutaneous transplantation | Histology and real-time PCR | 5,10 weeks |
| MORITO et al. 28    | Mice Immunological | CAP / PLGA scaffolding | Subcutaneous transplantation | Histology | 5,10 weeks |
| D'AQUINO et al. 29  | Human Collagen sponge | Third mill tooth extraction defect | Histology, X-ray, immunofluorescence, and Clinical evaluation | 1,2,3 months |
| KRAFT et al. 30     | Immunological mice | HA / TCP granules       | Subcutaneous transplantation | Histology Immunohistochemistry and histomorphometry | 8 weeks         |
| FEITOSA et al. 31   | Sheep             | Bone necrosis from histology of the femoral head | – | – | 4 weeks         |
| Reference        | Species/Model Type | Procedure/Condition | Control Material | Subsequent Treatment | Tissue Engineering | Follow-up | Notes |
|------------------|--------------------|---------------------|------------------|----------------------|-------------------|-----------|-------|
| CHAN et al.32    | Immunological mice | HA nanofibers       | Subcutaneous     | Histology and X-ray  | 4 weeks           |           |       |
| LI et al.34      | Immunological mice | 3D gelatin          | Subcutaneous     | Histology and X-ray  | 4 weeks           |           |       |
| Pisciotta et al.35| Rat                | Collagen            | Skull defects    | Histology            | 6 weeks           |           |       |
| ABE et al.36     | Immunological mice | HA scaffolding      | Subcutaneous     | Histology            | 12 weeks          |           |       |
| Chen et al.37    | Immunological mice | HA / TCP            | Subcutaneous     | Histology            | 12 weeks          |           |       |
| Kawanaabe et al.38| Immunological mice | B-TCP scaffolding   | Subcutaneous     | Histology            | 8 weeks           |           |       |
| Wang et al.39    | Immunological mice | 3D gelatin          | Subcutaneous     | Histology            | 8 weeks           |           |       |
| Bressan et al.40 | Immunological mice | HA scaffolding      | Eye defects      | Histology            | 3 weeks           |           |       |
| Riccio et al.41  | Rat                | Fibrin             | Skull defects    | Histology            | 4 weeks           |           |       |
| Annibali et al.42| Immunological mice | β-TCP, GDPBP and    | Subcutaneous     | Histology            | 1,2,4,8 weeks     |           |       |
| El-Gendy et al.43| Immunological mice | BIOGLASS (R)        | Intravenous      | Histology            | 8 weeks           |           |       |
| Maraldi et al.44 | Immunological mice | Collagen            | Skull defects    | Histology            | 4,8 weeks         |           |       |
| Alkaisi et al.45 | Rat                | –                  | Mandibular bone  | Histological         | 2,4,6 weeks       |           |       |
| Giuliani et al.46| Human              | Collagen sponge     | Third mill tooth extraction defect | Histology | 6 months/1.3 years |
| Niou et al.47    | Immunological mice | NSC and ISCS        | Subcutaneous     | Histology            | 8 weeks           |           |       |
| Acasigua et al.48| Rat                | PLGA                | Eye defects      | Histology            | 60 days           |           |       |
| Annibali et al.49| Immunological mice | Scaffolding GDPBP and β-TCP | Eye defects | Microsystem tomography 2, 4, 8 and And positron emission | 12 weeks |
| Kim et al.50     | Immunological mice | MBCP                | Subcutaneous     | Histology            | 8 weeks           |           |       |
| Asutay et al.51  | Rat                | Paste HA / TCP      | Eye defects      | Histology            | 8 weeks           |           |       |
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| Authors          | Species     | Material                                | Bone tissue                          | Assay                                      | Duration |
|------------------|-------------|-----------------------------------------|--------------------------------------|--------------------------------------------|----------|
| CAO et al.52     | Small pigs | HA / TCP scaffolding                    | Periodontal bone tissue defects      | HISTOMORPHOMETRY                           |          |
| KUO et al.53     | Small pigs | Scaffolding CSD, α-CSH / ACP and β-TCP  | Mandibular bone defects and defects  | Radiological and clinical                 | 8 weeks  |
| QIAN et al.54    | Immunological mice | HA / TCP scaffolding | Subcutaneous histomorphometry | Histology and implantation                | 2.3 months |
| PETRIDIS et al.55| Rat         | HA-based hydrogel scaffolding           | Eye defects                          | Histology and histomorphometry            | 8 weeks  |
| KWON et al.56    | Rat         | Scaffolding made by computer            | Skull defects                        | Histology and micromechanical tomography  | 4,8,12 weeks |
| JANG et al.57    | Rat         | Hydrogel formation in vivo              | Subcutaneous transplantation         | Histology, reverse transcription PCR and   | 2,4,6 weeks |
|                   |             |                                        |                                      | microscopic computed tomography           |          |
| JAHANBIN et al.58| Rat         | Collagen matrix                         | Jaw bone defect                      | Histology and histomorphometry            | 1,2 months |
| YASUI et al.59   | Immunological mice | Matrix scaffolding MATRIGEL       | Eye defects                          | Immunohistochemistry and microscopic      | 4 weeks  |
|                   |             |                                        |                                      | computed tomography                       |          |
| MONTI et al.60   | Human       | Collagen sponge                         | Third molar tooth extraction defect  | Histology and radiology                   | 60-day evaluation |
| WONGSUPA et al.61| Rabbit      | Scaffolding PCL-BCP                    | Eye defects                          | Histology, histomorphometry, microsomal   | 2,4,8 weeks |
|                   |             |                                        |                                      | tomography and clinical evaluation        |          |
| PAINO et al.62   | Immunological mice | Bone subcutaneous tissue is made by HDPSCs | Mandibular bone defects | Histology Immunofluorescence, X-ray       | 30,40 days |
|                   |             |                                        |                                      | microtomography, Synchrotron enterographic factor, and holotomography |          |
| MA et al.63      | Immunological mice | Scaffolding HA / TCP                | CALVARIAL defect, subcutaneous      | Histology, ELISA and immunofluorescence   | 4,8 weeks |
|                   |             |                                        | implantation and intravenous injection |                                      |          |
| LIU et al.64     | Immunological mice | –                                  | Intravenous injection               | Histology, ELISA histomorphometry and     | 8 weeks  |
|                   |             |                                        |                                      | microscopic computed tomography           |          |
| BEHNAIA et al.65 | Dogs        | Collagen scaffold                      | Mandibular bone defects              | Histology, clinical evaluation and image  | 12 weeks |
|                   |             |                                        |                                      | segmentation                              |          |
| JEON et al.66    | Immunological mice | MBCP scaffolding                     | Subcutaneous transplantation         | Histology Immunohistochemistry, QPCR, and  | 9 weeks  |
|                   |             |                                        |                                      | quantitative testing of alkaline           |          |
|                   |             |                                        |                                      | phosphatase levels                        |          |
| MA et al.67      | Mouse       | –                                    | Intravenous injection               | Histology, ELISA, real-time RT-PCR and    | 4 weeks  |
|                   |             |                                        |                                      | electron microscopy                       |          |
Transcriptional regulators

Transcription factors are protein complexes that participate in regulating gene position and controlling gene expression in NSCs. The diversity in these complexes regulates the final cell phenotype of NSCs. Among the transcription factors, the nuclear Tlx receptor is essential for the maintenance of neuronal stem cells and the self-regeneration of NSCs. The Tlx gene is expressed in sensory neurons as well as postsynaptic neurons. In addition, Tlx3 expression along with two other transcription factors, Phox2b and DRG11, leads to the distinction between two somatic sense circuits (Tlx3 +, DRG1+) from visceral sense circuits (Tlx3 +, Phox2b+). Tlx shows strong but the scattered expression in the subgranular zone, Gadolinium, and the Subventricular Zone, and in the formation of superficial membrane layers in the embryonic brain Nerve (neurogenesis) is effective. The main function of TLX in the adult brain is to prevent premature differentiation of NSCs by controlling the expression of a wide array of genes. In this method, TLX; It keeps NSCs undifferentiated and self-renewable, thereby modulating P53 pathway signaling.

Epigenetic control

The skeletal microstructure of extracellular matrix units of bone includes; Osteocytes, Osteoblasts, Osteoclasts, and liner cells are formed. Osteoclasts and Osteoblasts is very important in maintaining and repairing bones. Bone regeneration is a lifelong process in which new bone tissue is formed and mature bone tissue is reabsorbed. Imbalances in bone formation and bone resorption can lead to bone metabolic diseases. Osteoporosis can occur if the process of bone resorption is faster than the formation of new bone. Osteoporosis is a multifactorial disease that can be regulated by both genetic and environmental factors. In addition to genetic factors, behaviors such as low levels of physical activity, smoking, and caffeine consumption along with nutrients (including dietary calcium intake and vitamin D deficiency) are important determinants of osteoporosis and bone fractures. Evidence suggests that epigenetic modifications can be considered as key mechanisms for linking genetic and environmental factors to the risk of osteoporosis.

Epigenetic modifications can control the transcription process, resulting in self-regeneration and differentiation of stem cells. Epigenetic spatial and temporal methods are effective during the development and differentiation of neuronal stem cells into adult neurons. One of these methods is histone deacetylases, which are involved in regulating differentiation by inhibiting stem cells auto-regeneration transcription. REST increases the expression of specific neuronal genes by regulating transcription. In non-neural cells, it also uses HDAC complexes with its cofactors such as Co-REST, N-CoR.

| Study          | Species | Treatment                          | Bone defects | Histology, microstructural evaluation | Time (weeks) |
|----------------|---------|------------------------------------|--------------|---------------------------------------|--------------|
| FENG et al.68  | Rabbit  | –                                  | Bone defects | Histology, microstructural evaluation  | 8 weeks      |
| L1 et al.69    | Human   | B-TCP scaffold                     | Periodontal bone defects | Clinical and radiological evaluation  | 1,3,9 months |
| HILKENS et al.70 | Immunological mice | HA scaffolding printed with 3D | Subcutaneous transplantation | Histology and scanning electron microscopy | 12 weeks    |
| KANG et al.71  | Immunological mice | Granules HA / TCP and DDM | Subcutaneous transplantation | Histology | Immunohistochemistry, QRT-PCR and microscopic computed tomography | 1.8 weeks |
| SEO et al.72   | Immunological mice | HA / TCP scaffolding | Eye defects | Histology, 6 and 8 weeks or immunohistochemistry, 6-month in situ hybridization and RT-PCR | - |

8 SGZ
9 GD
10 SVZ
11 transcription factor RE1
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and mSin3A to inhibit the expression of neural genes through epigenetic regulation (39).

Methylation is an epigenetic marker and is dependent on transcriptional activation and inhibition (40). At the epigenetic DNA level, it is regulated by methylation, and its most prominent form is the symmetric methylation of cytosine in the 5’ region of CpG dinucleotides. It has a definite role in regulating the transcription of the DNA methylation gene and its chromatin-related rearrangement (31,60).

miRNA regulators

Stem cells are undifferentiated cells and have the potential for multilinear differentiation. Stem cells are generally referred to as adult stem cells, embryonic stem cells (ESCs), and pluripotent stem cells (iPSCs). Adult stem cells are named according to the tissue in which they are formed, such as mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), cardiac stem cells, nerve stem cells (NSCs), cells Endothelial stem cells, and etc. Embryonic stem cells (ESCs) are pluripotent and are derived from the inner cells mass of the blastocyst or Morula precursor embryos (41), whereas iPSCs are produced directly by somatic cells programming. Stem cells have high potential due to their potency and ability to regenerate in clinical treatments (42).

MicroRNAs (miRNAs) are small, non-coding single-stranded RNAs about 21-22 nucleotides long in length that suppress gene expression by inhibiting translation or by promoting mRNA degradation. In the nucleus, primary miRNAs are transcribed to form precursor miRNAs, derived from the DGCR8 RNA binding protein and the enzyme RNase III Drosha. miRNAs enter the cytoplasm and are eventually processed by Dicer to produce adult miRNAs (43). Studies have shown that miRNAs play an important role in the physiological processes and pathogenesis of many diseases. miRNAs are important regulators of stem cells. Studies show that miRNAs regulate stem cells status by directly targeting 3’ UTR. miR-145 suppresses the potency of human ESCs, by suppressing the expression of Oct4, Sox2, and Klf4 (46). However, miR-134, miR-296, and miR-470 target the Sox2, Nanog, and Oct4 coding sequences to power ESCs (47). Studies have shown that the miR-290 family affects the strength and differentiation of ESCs through epigenetic regulation of DNA de novo methylation (48). Another study showed that the transcription factors Sox2, nanog, and transcription factor 3 (Tcf3), with binding sites in the promoter region of most miRNAs, were expressed exclusively in ESCs. These transcription factors also, regulate the expression of miRNAs (49).

miRNAs modulate bone marrow stem cell differentiation

Significant advances have been made in the production of osteogenic cells from adult stem cells. miRNAs regulate bone and chondrogenic differentiation by targeting important transcription factors and relative pathways during skeletal maturation. The ERK-dependent pathway plays an important role in bone differentiation. It can activate second-order transcription factor phosphorylation (RUNX2), enhance ostein expression, and improve alkaline phosphatase activity. miR-138 suppresses the differentiation of human mesenchymal stem cells into osteoblasts by directly targeting FAK (50). miR-23b induces differentiation of human mesenchymal stem cells by suppressing signaling protein kinase A PKA (51).

miRNAs and stem cell potency

miRNAs have the ability to modulate stem cells and generally regulate potency enhancers by directly targeting UTR-3’. miR-145 suppresses the potency of

12 PR-miRNAs
13 EEmiRCs
SOX family

The sox factor family belongs to the SRY\textsuperscript{14} gene family and has a high mobility group (HMG) region that binds to DNA, which is very similar to the sry-box of the HMG-Box of the sox protein family. The DNA-binding region consists of three alpha HMG-Box helixes that occurs specifically in the small DNA groove of this binding and provide a strong curve that changes the DNA architecture. These changes allow them to interact with other transcription factors. Members of the sox family interact in a wide range of cellular tissues, resulting in a variety of effects on cellular metabolism. In invertebrates, soxb1 (sox1, sox2, sox3) factors are widely expressed in proliferating stem cells / proliferating neurons during development and maturation (57).

Conclusion

According to the results, it can be concluded that many genes are involved in different stages of expression of genes involved in the formation of bone tissue, each of which can play many roles in the formation, repairment, or termination of the agent. Negatively involved in the execution of a particular gene.

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\textsuperscript{14} sex-determining region Y

**Table 2.**

| miRNA         | function                          | purpose                  | Reference |
|---------------|-----------------------------------|--------------------------|-----------|
| miRNA-29a     | regulates cartilage differentiation and cartilage formation | FOXO3A                   | 52        |
| microRNA-145  | Power suppression in human ESCs    |                          |           |
| miR-23b       | Makes cartilage differentiation from HMSC |                          | 50        |
| miR-138       | Osteoblast differentiation suppresses human mesenchymal stem cells | FAK targeting            | 51        |
| MiR-290 family or MiR-302 family | increases efficiency | Oct4, Sox2, Klf4          | 53        |
| Human miR-372 | Improves planning efficiency      |                          | 58        |
| miR-17-92 cluster, miR-106b-25 cluster, miR-106a-363 cluster | improves scheduling efficiency | TGF-βR2, p21 | 59        |
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