Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

The role of recombinant basic fibroblast growth factor in de-differentiation of chondrocyte to mesenchymal stem cell-specific immunophenotype

Kondrositlerin mezenkimal kök hücreye spesifik immünofenotipe geri farklılaşmasında rekombinant bazik fibroblast büyüme faktörünün rolü

Hasan SALKIN[1] (ID)

ABSTRACT

Objective: Some changes in genes, protein, and function in chondrocytes can cause them to develop into a fibroblastic morphology. Our study aims to transform chondrocytes in monolayer cultures into a mesenchymal stem cell-like immunophenotype by inducing chondrocyte de-differentiation with recombinant basic-fibroblast growth factor (FGF-b) supplementation.

Methods: In our study, chondrocytes were obtained from Erciyes University Genome and Stem Cell Center as ready-made cell lines. Chondrocytes grown on a standard medium were used as the control group. An experimental group was formed by adding recombinant basic FGF at a concentration of 5 ng/ml to the normal growth medium. MSC-specific surface markers expressions such as CD271, CD166, CD29, CD44, CD73, CD90, and CD105 were compared between groups by flow cytometry. "Muse Annexin-V assay kit" and "Muse Count and viability kit" were used according to the production procedure to compare apoptosis and viability data between groups. Colony-forming potentials in chondrocytes were determined by Colony-forming units-fibroblast assay. Statistical analyzes were performed using the statistics software GraphPad Prism (version

ÖZET

Amaç: Kondrositlerdeki gen, protein ve fonksiyondaki bazı değişiklikler, bunların fibroblastik bir morfolojiye dönüşmesine neden olabilir. Çalışmamız, rekombinant bazik fibroblast büyüme faktörü (FGF-b) takviyesi ile kondrosit de-diferansiyasyonunu indükleyerek tek tabakalı kültürlerdeki kondrositleri mezenkimal kök hücre benzeri bir immünofenotipe dönüştürmeyi amaçlamaktadır.

Yöntem: Çalışmamızda kondrositler Erciyes Üniversitesi Genom ve Kök Hücre Merkezi'nden hazır hücre hattı olarak temin edildi. Kontrol grubu olarak standart bir besiyerinde büyütülen kondrositler kullanıldı. Standart büyüme ortamına 5 ng/ml konsantrasyonda rekombinant bazik FGF ilave edilerek deney grubu oluşturuldu. CD271, CD166, CD29, CD44, CD73, CD90 ve CD105 gibi MKH'ye özgü yüzey belirteç ifadeleri, akış sitometrisi ile gruplar arasında karşılaştırıldı. Gruplar arasında apoptoz ve canlılık verilerinin karşılaştırılması için üretim prosedürüne göre "Muse Annexin-V assay kit" ve "Muse Count and viability kit" kullanıldı. Kondrositlerdeki koloni oluşturma potansiyelleri, Koloni oluşturan birimler-fibroblast tahlili ile belirlendi. İstatistiksel analizler, GraphPad Prism (sürüm 8.02)

[1]Department of Histology and Embryology, Faculty of Medicine, Istanbul Beykent University, Istanbul, Türkiye

İletişim / Corresponding Author : Hasan SALKIN
İstanbul Beykent Üniversitesi, Tıp Fakültesi, Histoloji ve Embriyoloji AD., İstanbul - Türkiye
E-posta / E-mail : hasansalkin@gmail.com

Geliş Tarihi / Received : 08.03.2023
Kabul Tarihi / Accepted : 29.12.2023

DOI ID : 10.5505/TurkHijyen.2024.08624

Salkın H. The role of recombinant basic fibroblast growth factor in de-differentiation of chondrocyte to mesenchymal stem cell-specific immunophenotype. Turk Hij Den Biyol Derg, 2024; 81(4): 399 - 408

8.02). Data with P<0.05 were considered statistically significant.

Results: The addition of recombinant basic-FGF to chondrocyte cultures reduced the percentage of total apoptosis in chondrocytes and increased viability. Our flow cytometry findings showed a significant increase in the expression of MSC-specific surface markers CD105, CD90, CD166, and CD271 in recombinant basic-FGF-treated chondrocytes. On the other hand, a decrease was observed in CD29 and CD73 expressions. There was no statistically significant difference among the groups in terms of CD44 expression.

Conclusion: In conclusion, the addition of recombinant basic-FGF to chondrocyte cultures decreased the percentage of total apoptosis in chondrocytes and increased viability. Our flow cytometry findings showed a significant increase in the expression of MSC-specific surface markers CD105, CD90, CD166, and CD271 in recombinant basic-FGF-treated chondrocytes. The increase in these MSC key markers suggests that recombinant basic FGF may induce chondrocyte differentiation at the level of MSC phenotype. In this study, we hypothesized that recombinant basic FGF might be an effective growth factor in the differentiation of chondrocytes into MSC immunophenotype. De-differentiation of chondrocytes to the MSC immunophenotype by recombinant basic-FGF may render these cells more effective and potential for chondrocyte implantation for regenerative medicine in cartilage damage such as osteoarthritis (OA) or other joint disorders.

Key Words: Chondrocyte, de-differentiation, fibroblast growth factor, apoptosis, mesenchymal stem cells

istatistik yazılımı kullanılarak yapıldı. P<0.05 olan veriler istatistiksel olarak anlamlı kabul edildi.

Bulgular: Kondrosit kültürlerine rekombinant bazik FGF ilavesi, kondrositlerdeki toplam apoptoz yüzdesini azalttı ve canlılığı arttırdı. Akış sitometri bulgularımız, rekombinant bazik FGF ile muamele edilmiş kondrositlerde MKH'ye özgü yüzey belirteçlerinden CD105, CD90, CD166 ve CD271'in ifadesinde önemli bir artış olduğunu gösterdi. Buna karşın CD29 ve CD73 ifadelerinde ise düşüş gözlendi. CD44 ifadesi bakımından gruplara arasında istatistikel olarak anlamlı bir fark bulunmadı.

Sonuç: Sonuç olarak, kondrosit kültürlerine rekombinant bazik FGF ilavesi, kondrositlerdeki toplam apoptoz yüzdesini azaltmış ve canlılığı arttırmıştır. Akış sitometri bulgularımız, rekombinant bazik-FGF ile işlenmiş kondrositlerde MKH'ye özgü yüzey belirteçlerinden CD105, CD90, CD166 ve CD271'in ifadesinde önemli bir artış olduğunu gösterdi. Bu MKH temel belirteçleri üzerindeki artış, rekombinant bazik-FGF'nin MKH fenotipi seviyesinde kondrosit farklılaşmasını indükleyebileceğini düşündürür. Bu çalışmada, rekombinank bazik FGF'nin kondrositlerin MKH immünofenotipine farklılaşmasında etkili bir büyüme faktörü olabileceğini hipotez ettik. Kondrositlerin rekombinant bazik FGF tarafından MKH immünofenotipine geri farklılaşması, bu hücreleri osteoartrit (OA) veya diğer eklem bozuklukları gibi kıkırdak hasarında rejeneratif tıp amacıyla kondrosit implantasyonu için daha etkili ve potansiyel hale getirebilir.

Anahtar Kelimeler: Kondrosit, de-diferansiyasyon, fibroblast büyüme faktörü, apoptoz, mezenkimal kök hücreler

INTRODUCTION

Light microscopy observations of chondrocytes in monolayer culture in the sixties described serious morphological changes to us. Investigations revealed that chondrocytes shifted towards a fibroblast-like morphology in monolayer culture. This phenomenon brought a new perspective to chondrocyte biology,

and this phenomenon is now called de-differentiation (1). De-differentiation is the process by which a cell in a differentiated state is transformed into a less differentiated phenotype in terms of gene, protein, function, and morphology. It has been reported that de-differentiated chondrocytes acquire multipotent character just like mesenchymal stem cells (MSCs), and specific surface markers exhibit similar expressions. Adipogenic, osteogenic, and chondrogenic differentiation abilities, a characteristic unique to MSCs, were observed in de-differentiated chondrocytes similarly to MSCs (1-3). Researchers examined the changes in stem cell-specific surface marker expression in de-differentiated chondrocytes by RT-PCR and flow cytometry. They observed an increase in the expression of MSC-specific markers such as CD105, CD166, and CD90 in chondrocytes remaining in monolayer culture for two weeks. They found a remarkable increase in CD105 gene and protein expression, especially in late-passage chondrocytes (4-6). Microarray analyses performed to date have identified different growth factors released during de-differentiation. In vitro, sub-cultures showed that insulin growth factor1 (IGF-1), IGF-1R, bone morphogenic protein2 (BMP-2), fibroblast growth factor receptor3 (FGFR-3), transforming growth factor beta2 (TGFb2) and TGFB3 expressions were decreased in chondrocytes. On the contrary, it was revealed that there was an increase in the expression of FGF-18, BMP-5, and 8 (7-11). The FGF family contains at least 23 molecules that regulate the differentiation and proliferation of chondrocytes. Among those identified so far, FGF 1, 2, and 18 have been found to play important roles in cartilage regeneration and chondrocyte differentiation (12). Studies show that FGF-2 (basic) stimulates pro-chondrogenesis in chondrocytes and MSCs via SOX9 (13-15). The addition of FGF-18 to articular chondrocytes in culture showed that these chondrocytes increased matrix production and proliferation (16, 17). Given these considerations, it is highly likely that the FGF family may be involved in de-differentiation from the chondrocyte phenotype to the MSC-specific immunophenotype.

Therefore, in this study, we hypothesized that recombinant basic FGF might be effective in de-differentiation from chondrocytes to MSC-specific immunophenotype and increase MSC-specific surface marker expressions. FGF-basic is one of 23 known members of the FGF family. Proteins of this family play a central role during prenatal development, postnatal growth, and regeneration of a variety of tissues, by promoting cellular proliferation and differentiation. Recombinant Human FGF-basic is a 17.2 kDa protein consisting of 154 amino acid residues (13, 14). The aim of our study is to convert by recombinant basic FGF supplementation to chondrocytes in monolayer cultures into an MSC-like immunophenotype by inducing chondrocyte de-differentiation. Thus, it is aimed to produce de-differentiated chondrocytes, which can be applied in degenerative joint disorders such as osteoarthritis and whose in vitro regeneration potential is further increased.

MATERIAL and METHOD

Culture of human chondrocytes

Chondrocytes were obtained from Erciyes University Genome and Stem Cell Center (GENKOK) as a ready-made cell line. The human chondrocyte cell line used was purchased from Merck Millipore (Germany) with catalog number SCC042. Since in vitro experiments were performed on ready-made cell lines, no ethics committee document was needed. The supplied cells were thawed in a water bath at 37˚C and taken into the culture medium. Cells were cultured at 37˚C in DMEM High Glucose (Biological Industries, Israel) containing 10% FBS (Fetal Bovine Serum) (Biological Industries, Israel), 1% penicillin-streptomycin (Lonza, USA) and 1% L-glutamine (Gibco, USA). Chondrocytes were seeded at 8000 cells/cm^2 and cultured in a humid atmosphere in a 5% CO_2 incubator. Chondrocytes were amplified by passage by trypsinization until they reached the required number for the experimental sets.

Application of recombinant basic FGF to chondrocyte cultures

When the cells reached the required numbers, three repetitive sets of experiments were created for flow cytometry, Colony-forming Units-Fibroblast assay, apoptosis, and viability analyses. In the experimental sets, a standard growth medium was used for the control group. As the experimental group, recombinant basic FGF (ThermoFisher, USA, # 13256-029) was added to the growth medium at a final volume of 5 ng/ml. Thus, the control group chondrocytes were cultured with a growth medium and the experimental group chondrocytes were cultured with FGF supplemented growth medium.

Flow Cytometry

Chondrocytes in the third passage were collected and suspended in their culture medium at a density of $1x10^6$ cells/ml. Then, chondrocytes were stained with positive MSC markers such as CD90-FITC, CD44-PE, CD105 PerCP5.5, CD73-APC, CD271-PE, CD166-PerCP5.5 and CD29-APC mouse-anti-human fluorescent antibodies (BD Bioscience, Heidelberg, Germany). After the cells were centrifuged and reconstituted with a washing solution, they were analyzed using a Navios (Beckman Coulter, USA) flow cytometer device. Data were analyzed with KALUZA (Beckman Coulter, USA) software.

Colony Forming Units-Fibroblast (CFU-F) Assay

CFU-F assay was performed to evaluate the clonogenic potential and colony formation potential in culture in the control group and FGF-treated chondrocytes. For this, cells were inoculated into 6 well plates at 100 cells/cm^2 in control and experimental groups. Thus, the cells were allowed to fall into the culture dishes one by one and away from each other. The cells were cultured in their culture medium (growth medium and growth medium + FGF) for two weeks. Colonies formed at the end of the second week were stained with crystal violet (Sigma, USA). Colored colonies were counted and compared between groups.

Annexin-V Assay

Early, late, and total apoptosis percentages in the control group and experimental group chondrocytes were measured at 24, 72, and 120 hours using the Muse Cell Analyzer (Millipore, Germany) device and Annexin V and Dead cell kit (Muse, Millipore, Germany) according to the production procedure. Experiments were run in three repetitions. The percentages of early, late, and total apoptosis were compared between groups.

Viability Assay

Viability percentages and cell numbers of cells in chondrocytes in the control and experimental groups were measured at 24, 72, and 120 hours using the Muse Cell Analyzer (Merck Millipore, Germany) device and cell counting and viability kit (Muse Count and Viability-Merk Millipore, Germany) according to the production procedure. Experiments were run in three repetitions. Viability percentages were compared between groups.

Statistical Analysis

Viability, apoptosis, CFU-F, and flow cytometry findings were presented as mean ± standard deviation in the groups. For comparison of viability and apoptosis parameters, distribution and homogeneity of data between groups were determined by two-way ANOVA. All experiments were performed in triplicate and multiple comparisons between groups were analyzed by Sidak's test. Flow cytometry and CFU-F findings were compared between groups by unpaired t-test. Statistical analyzes were performed using GraphPad Prism (version 8.02) statistical software. Data with $P<0.05$ were considered statistically significant.

RESULTS

Immunophenotypic Analysis

Recombinant basic FGF administration to chondrocyte cultures increased the expression of MSC-specific positive markers such as CD271,

CD166, CD90, and CD105 (P<0.001). There was no statistically significant difference between the groups regarding CD44 expression. CD29 and CD73 expression decreased in chondrocytes with recombinant basic FGF administration (Figure 1). The findings obtained between the groups are presented in Table 1 as mean ± SD, and data with a p-value less than 0.05 were considered statistically significant.

Table 1. Mean ± SD and p values of immunophenotypic markers whose expressions were compared between groups

Antibodies	Groups	Mean	Std. Deviation	P-value
CD29	Growth medium	99.0	0.3	<0.001
	Growth medium + FGF	92.1	1.0	
CD166	Growth medium	88.2	0.2	<0.001
	Growth medium + FGF	99.0	0.2	
CD271	Growth medium	97.0	0.4	=0.001
	Growth medium + FGF	99.2	0.3	
CD90	Growth medium	88.8	0.5	=0.02
	Growth medium + FGF	90.9	0.9	
CD44	Growth medium	94.5	0.5	=0.55
	Growth medium + FGF	94.9	0.9	
CD105	Growth medium	58.0	1.0	<0.001
	Growth medium + FGF	99.2	0.5	
CD73	Growth medium	98.1	0.3	=0.003
	Growth medium + FGF	94.9	0.8	

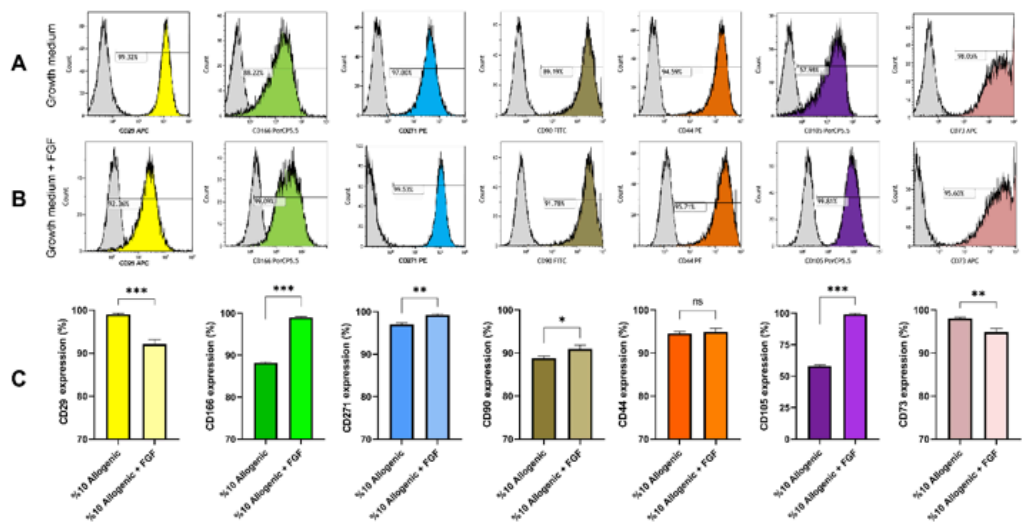

Figure 1. Flow cytometric analyzes of CD29, CD166, CD271, CD90, CD44, CD105, and CD73 surface marker expressions between groups (A-B) after FGF administration in chondrocyte cultures in the 3rd passage. Statistical comparison of surface marker expressions between groups after repeated experiments (C). It was determined that FGF administration induced chondrocyte-MSC immunophenotypic de-differentiation. Histograms in gray represent negative control isotypes

Colony Forming Units-Fibroblast (CFU-F) Assay

Colony formation potential in FGF-treated chondrocyte cultures was significantly reduced compared to the control group (Figure 2). FGF-treated chondrocytes were found to display a more monolayer appearance in culture, while a clustered morphology was observed in control cells. While the number of colonies formed in the control group was 29 ± 2, the number of colonies formed by chondrocytes treated with FGF was determined as 21.7 ± 4.04 (P=0.05).

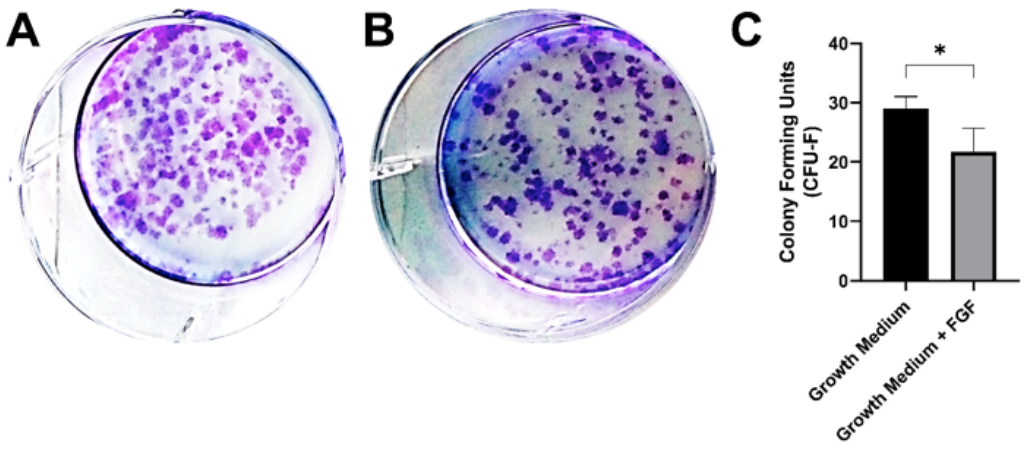

Figure 2. Crystal violet-stained chondrocyte colonies in the control group (A) and FGF-treated chondrocytes (B). Statistical comparison of the numbers of properly morphed and purified colonies between groups obtained as a result of repeated experiments (C).

Annexin-V Apoptosis Assay

Although early apoptosis was found to be significantly increased (P=0.03) in FGF-treated chondrocytes at 24 hours compared to the control group, late apoptosis was found to be significantly reduced (P<0.001) (Figure 3 A and B). Therefore, there was no statistically significant difference between the groups regarding total apoptosis percentages at 24 hours (Figure 3 C). There was no significant difference between the groups regarding early, late and total apoptosis at 72 hours (Figure 3A-C). Remarkable results in terms of apoptosis were seen at the 120th hour. At 120 hours, total apoptosis was found to be dramatically decreased in FGF-treated chondrocytes compared to the control group (P<0.001). These findings showed us that FGF administration has a suppressive effect on apoptosis, especially in long-term chondrocyte cultures (Figure 3 C).

Viability Analysis

The percentage of cellular viability in FGF-treated chondrocytes compared to the control group was statistically significantly increased at 24 and 120 hours, respectively (P=0.003 and P<0.001). Especially at the points where apoptosis decreased in chondrocytes with FGF application, it was determined that cellular viability increased significantly (P<0.001). Our viability findings supported our apoptosis findings. Viability increased where apoptosis decreased (Figure 3D).

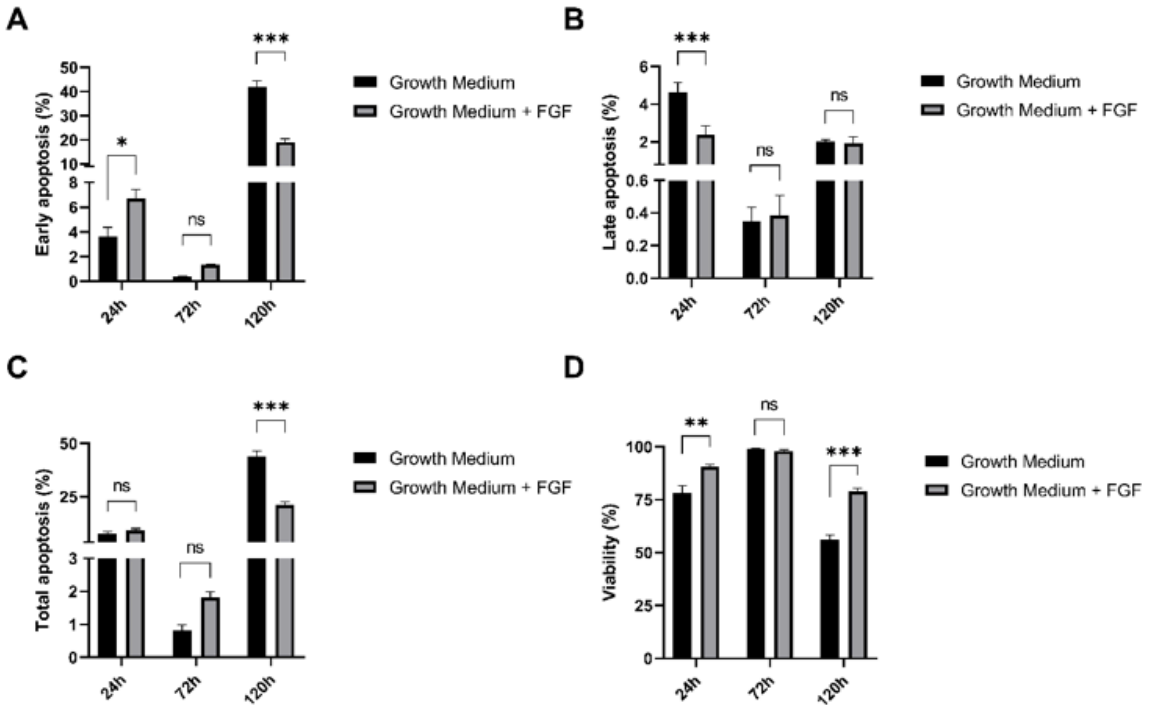

Figure 3. Statistical comparison of the percentages of early apoptosis, late apoptosis, total apoptosis, and viability in the control group and FGF-treated chondrocytes. While FGF administration increased early apoptosis in chondrocytes at 24 hours, it decreased early apoptosis at 120 hours (A). The percentage of late apoptosis was decreased in FGF-treated chondrocytes compared to the control group (B). Total apoptosis in FGF-treated chondrocytes appears to be markedly reduced at the 120th hour (C). Cell viability increases significantly at points where apoptosis is reduced by FGF administration (D).

DISCUSSION

Although studies over the last two decades have evaluated the changes in gene expression patterns on differentiated chondrocytes and de-differentiated chondrocytes in chondrocytes de-differentiation and preservation of chondrocyte properties, the exact mechanism of chondrocyte de-differentiation has still not been elucidated to date (7, 10, 18-19).

The role of growth factors in stimulating chondrocyte proliferation in culture is well known. From the past to the present, many studies have reported that the addition of factors such as transforming growth factor beta1 (TGF-B1), FGF, and insulin-like growth factor1 (IGF-1) to the culture medium increases the proliferation, differentiation, and extracellular matrix formation of chondrocytes (20-24). In our study, unlike the information reported so far, the role of recombinant basic FGF in inducing de-differentiation to MSC-specific immunophenotype surface markers in cultured chondrocytes was investigated. Yamaoka et al. (2010) emphasized the importance of FGF-18 in the de-differentiation of human chondrocytes in culture and reported that FGF-18 is highly expressed in undifferentiated chondrocytes and that FGF-18 up-regulation may be a marker of differentiation. It has also been reported that it may be an important marker in the preservation of chondrocyte properties. Members of the FGF family, FGF-1,5,10,13 and 18, were found to be expressed higher in the later passages compared to the early passages of chondrocyte cultures (25). In our study,

it was determined that chondrocytes treated with recombinant basic FGF induced chondrocyte-MSC immunophenotypic de-differentiation. In addition, our findings show that recombinant basic FGF plays an important role in increasing chondrocyte viability and reducing apoptosis in long-term culture. Cucchiarini et al. (2009) reported that overexpression of FGF-2 in chondrocyte spheroids increased viability and cell division. While the viability of control spheres was 35%, the viability of chondrocyte spheres induced by FGF-2 overexpression viral vectors increased to 92% (26). Results supporting this were also observed in chondrocytes after recombinant basic FGF administration in our study. While the viability was 56% in the control group chondrocytes of our study at the 120th hour, the viability was 80% in the chondrocytes treated with recombinant basic FGF. The increase in viability at the 120th hour in recombinant basic FGF-applied chondrocytes significantly suppressed total apoptosis in the same time period. Our findings show that recombinant basic FGF administration increases viability and reduces apoptosis in chondrocytes.

Many studies have shown that chondrocytes adopt an immunophenotype similar to MSC during de-differentiation. It has been reported that de-differentiated chondrocytes increase the expression of MSC-specific surface markers CD105, CD10, CD166, and CD90 in monolayer culture. Studies show that CD105 expression is more expressed in later passages compared to early passages, especially in chondrocytes (4-6). In addition, Arufe et al. (2009) showed that CD105+ MSC subsets have the potential for chondrogenic differentiation (27). In our study, we found that CD105 expression increased drastically from 58% to 99.2% with FGF application to chondrocytes. Considering that CD105 expression is highly expressed in de-differentiated chondrocytes according to our current literature knowledge, this study shows that recombinant basic FGF application may be very important in chondrocyte-MSC immunophenotypic de-differentiation. Our flow cytometry findings showed a significant increase in the expression of MSC-specific surface markers CD105, CD90, CD166, and CD271 in recombinant basic-FGF-treated chondrocytes. On the other hand, a decrease was observed in CD29 and CD73 expressions. There was no statistically significant difference between the groups in terms of CD44 expression. In a previous study, we showed that TGF-B1 increases CD44 expression in fibroblasts (28). This situation may be one of the possible reasons why CD44 expression did not change in chondrocytes with recombinant basic FGF administration (29). Tanaka et al. (2007) have reported that integrin alpha5beta1 induces hypertrophic differentiation in chondrocytes. Integrin alpha5beta1 is composed of α5 (ITGA5/CD49e) and ß1 (ITGB1/CD29) subunits (29). This actually explains why CD29 expression was decreased in recombinant FGF-treated chondrocytes in our study. In our study, we aimed to de-differentiate chondrocytes and convert them to an MSC-like immunophenotypic profile. Hypertrophy may negatively affect de-differentiation, and therefore, the decrease in CD29 expression with recombinant basic FGF seems to support our hypothesis in our study. Another study reported that CD73 expression was down-regulated, inversely to CD90 expression, in adipose-derived stem cell spheroids induced for chondrocyte differentiation (30). Considering the importance of de-differentiation for a stable cartilage tissue without hypertrophy, the fact that CD73 expression was downregulated by recombinant basic FGF in our study and that it did this in an opposite way to CD90 expression supports the literature. Accordingly, the increased expression of not only CD105 but also MSC-specific surface markers such as CD166, CD271, and CD90 in chondrocytes with recombinant basic FGF application revealed that recombinant basic FGF plays a very active and key factor in chondrocyte de-differentiation. Recombinant basic FGF-treated chondrocytes were found to display a more monolayer appearance in culture, while a clustered morphology was observed in control cells. This suggests that recombinant basic FGF can induce chondrocyte

de-differentiation at the level of MSC phenotype.

In conclusion; in this study, we hypothesized that recombinant basic FGF might be an effective growth factor in the de-differentiation of chondrocytes to the MSC immunophenotype. Our findings supported this hypothesis. De-differentiation of chondrocytes to the MSC immunophenotype by recombinant basic FGF may render these cells more effective and potential for chondrocyte implantation for regenerative medicine in cartilage damage such as osteoarthritis (OA) or other joint disorders. Therefore, there is a need for new studies to be performed in vivo to support these in vitro results. Our results may lead to in vivo studies modeling cartilage damage such as OA.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Cai S, Fu X, Sheng Z. Dedifferentiation: a new approach in stem cell research. Bioscience, 2007; 57: 655-62.

2. Barbero A, Ploegert S, Heberer M, Martin I. Plasticity of clonal populations of dedifferentiated adult human articular chondrocytes. Arthritis Rheum, 2003; 48 (5): 1315-25.

3. Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, et al. Chondrocyte dedifferentiation and osteoarthritis (OA). Biochem Pharmacol, 2019; 165: 49-65.

4. Diaz-Romero J, Gaillard JP, Grogan SP, Nesic D, Trub T, Mainil-Varlet P. Immunophenotypic analysis of human articular chondrocytes: changes in surface markers associated with cell expansion in monolayer culture. J Cell Physiol, 2005; 202 (3): 731-42.

5. Jones E, Schäfer R. Biological differences between native and cultured mesenchymal stem cells: implications for therapies. Methods Mol Biol, 2015; 1235: 105-20.

6. Finnson KW, Parker WL, Chi Y, Hoemann CD, Goldring MB, Antoniou J, et al. Endoglin differentially regulates TGF-ß-induced Smad2/3 and Smad1/5 signalling and its expression correlates with extracellular matrix production and cellular differentiation state in human chondrocytes. Osteoarthritis Cartilage, 2010; 18 (11): 1518-27.

7. Lin Z, Fitzgerald JB, Xu J, Willers C, Wood D, Grodzinsky AJ, et al. Gene expression profiles of human chondrocytes during passaged monolayer cultivation. J Orthop Res, 2008; 26 (9): 1230-7.

8. Goessler UR, Bugert P, Bieback K, Sadick H, Verse T, Baisch A, et al. In vitro analysis of matrix proteins and growth factors in dedifferentiating human chondrocytes for tissue-engineered cartilage. Acta Otolaryngol, 2005; 125 (6): 647-53.

9. Ma B, Leijten JC, Wu L, Kip M, van Blitterswijk CA, Post JN, et al. Gene expression profiling of dedifferentiated human articular chondrocytes in monolayer culture. Osteoarthritis Cartilage, 2013; 21 (4): 599-603.

10. Schnabel M, Marlovits S, Eckhoff G, Fichtel I, Gotzen L, Vécsei V, et al. Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. Osteoarthritis Cartilage, 2002; 10 (1): 62-70.

11. Goessler UR, Bugert P, Bieback K, Sadick H, Baisch A, Hormann K, et al. In vitro analysis of differential expression of collagens, integrins, and growth factors in cultured human chondrocytes. Otolaryngol Head Neck Surg, 2006; 134 (3): 510-5.

12. Ornitz DM. FGF signaling in the developing endochondral skeleton. Cytokine Growth Factor Rev, 2005; 16 (2): 205-13.

13. Lee J, Lee JY, Chae BC, Jang J, Lee E, Son Y. Fully Dedifferentiated Chondrocytes Expanded in Specific Mesenchymal Stem Cell Growth Medium with FGF2 Obtains Mesenchymal Stem Cell Phenotype In Vitro but Retains Chondrocyte Phenotype In Vivo. Cell Transplant, 2017; 26 (10): 1673-87.

14. Handorf AM, Li WJ. Fibroblast growth factor-2 primes human mesenchymal stem cells for enhanced chondrogenesis. PLoS One, 2011; 6 (7): e22887.

15. Dailey L, Ambrosetti D, Mansukhani A, Basilico C. Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev, 2005; 16 (2): 233-47.

16. Davidson D, Blanc A, Filion D, Wang H, Plut P, Pfeffer G, et al. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. J Biol Chem, 2005; 280 (21): 20509-15.

17. Ohbayashi N, Shibayama M, Kurotaki Y, Imanishi M, Fujimori T, Itoh N, et al. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. Genes Dev, 2002; 16 (7): 870-9.

18. Stokes DG, Liu G, Dharmavaram R, Hawkins D, Piera-Velazquez S, Jimenez SA. Regulation of type-II collagen gene expression during human chondrocyte de-differentiation and recovery of chondrocyte-specific phenotype in culture involves Sry-type high-mobility-group box (SOX) transcription factors. Biochem J, 2001;360(Pt 2):461-70.

19. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res, 2005; 23: 425-32.

20. Somoza RA, Welter JF, Correa D, Caplan AI. Chondrogenic differentiation of mesenchymal stem cells: challenges and unfulfilled expectations. Tissue Eng Part B Rev, 2014; 20 (6): 596-08.

21. Hu X, Zhang W, Li X. Strategies to Modulate the Redifferentiation of Chondrocytes. Front Bioeng Biotechnol, 2021; 9: 764193.

22. Nasrabadi D, Rezaeiani S, Eslaminejad MB, Shabani A. Improved Protocol for Chondrogenic Differentiation of Bone Marrow Derived Mesenchymal Stem Cells -Effect of PTHrP and FGF-2 on TGFß1/BMP2-Induced Chondrocytes Hypertrophy. Stem Cell Rev Rep, 2018; 14 (5): 755-66.

23. Huang L, Yi L, Zhang C. Synergistic Effects of FGF-18 and TGF-ß3 on the Chondrogenesis of Human Adipose-Derived Mesenchymal Stem Cells in the Pellet Culture. Stem Cells Int, 2018; 2018: 7139485.

24. Jakob M, Démarteau O, Schäfer D, Hintermann B, Dick W, Heberer M, et al. Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation in vitro. J Cell Biochem, 2001; 81 (2): 368-77.

25. Yamaoka H, Nishizawa S, Asawa Y, Fujihara Y, Ogasawara T, Yamaoka K, et al. Involvement of fibroblast growth factor 18 in dedifferentiation of cultured human chondrocytes. Cell Prolif, 2010; 43 (1): 67-76.

26. Cucchiarini M, Terwilliger EF, Kohn D, Madry H. Remodelling of human osteoarthritic cartilage by FGF-2, alone or combined with Sox9 via rAAV gene transfer. J Cell Mol Med, 2009; 13 (8B): 2476-88.

27. Arufe MC, De la Fuente A, Fuentes-Boquete I, De Toro FJ, Blanco FJ. Differentiation of synovial CD-105(+) human mesenchymal stem cells into chondrocyte-like cells through spheroid formation. J Cell Biochem, 2009; 108 (1): 145-55.

28. Salkin H, Acar MB, Gonen ZB, Basaran KE, Ozcan S. Comparative proteomics analysis of transforming growth factor-beta1-overexpressed human dental pulp stem cell-derived secretome on CD44-mediated fibroblast activation via canonical smad signal pathway. Connect Tissue Res, 2023; 64 (2): 205-18.

29. Tanaka K, Yokosaki Y, Higashikawa F, Saito Y, Eboshida A, Ochi M. The integrin alpha5beta1 regulates chondrocyte hypertrophic differentiation induced by GTP-bound transglutaminase 2. Matrix Biol, 2007; 26 (6): 409-18.

30. Côrtes I, Matsui RAM, Azevedo MS. A Scaffold- and Serum-Free Method to Mimic Human Stable Cartilage Validated by Secretome. Tissue Eng Part A, 2021; 27 (5-6): 311-27.