Drug Repurposing: Verifying Cartilage-Regeneration Efficacy of Aripiprazole using Public Data

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INTRODUCTION: Due to the limited capacity for articular cartilage regeneration, damaged cartilage does not naturally heal over time. Consequently, cartilage damage resulting from various factors like metabolism, genetics, mechanics, and inflammation undergoes gradual deterioration, leading to pain, stiffness, and reduced mobility, ultimately culminating in osteoarthritis. To address this, numerous scientific endeavors have been undertaken, including cell-based therapies, tissue engineering, gene therapy, and mechanical stimulation. In recent years, the concept of drug repositioning, also known as drug repurposing, has emerged as a promising strategy for developing novel treatments, including those for osteoarthritis. This strategy involves the identification of existing drugs or even abandoned ones with therapeutic potential for new medical uses. In contrast to the traditional development of entirely new drugs, this approach offers advantages including a quicker time to market, reduced development costs, and a lower risk of side effects by capitalizing on existing data. Our research aimed to pinpoint drugs that can enhance cartilage regeneration using microarray data obtained from the Gene Expression Omnibus (GEO) public database. We successfully identified several promising drugs for cartilage regeneration among those already commercially available for different purposes. Finally, we conducted experiments using cellular and animal models to validate the potential of Aripiprazole, one of the identified drugs, for promoting cartilage regeneration.

METHODS: To identify drugs that promote cartilage regeneration, cartilage regeneration microarray studies were collected from the Gene Expression Omnibus (GEO) public database. Using GSE69110, GSE107649, GSE11822, and GSE116173 gene sets, a gene expression analysis pipeline was employed in five stages: (1) data preparation (with quality control [QC] included), (2) read alignment, (3) expression quantification, (4) differential expression identification, and (5) biological function profiling. Among the selected Differentially expressed gene (DEGs), only the top 100 genes with the highest magnitudes of fold changes in expression were depicted in a heatmap for each group or cell line. DEGs were identified using StringTie, and the data of drugs were extracted from the Drug-Gene-Interaction (DGI) database. The DGI database was then used to identify a list of candidate drugs applicable to these identified genes. Furthermore, to select a significant drug candidate, commonly tracked drugs among GSE studies were identified and organized. To assess the effectiveness of aripiprazole in cartilage regeneration, we conducted in vitro experiments. Aripiprazole-treated AD-MSCs (Adipose-Derived Mesenchymal Stem Cells) and chondrocytes were subjected to qRT-PCR (quantitative Reverse Transcription Polymerase Chain Reaction) and 3D pellet culture to evaluate cartilage regeneration. Furthermore, bulk-mRNA sequencing was carried out to predict potential pathways involved in this process. To confirm the cartilage regeneration potential in vivo, we mixed aripiprazole with a collagen sponge scaffold and injected it into artificially damaged cartilage in Sprague-Dawley rats.

RESULTS SECTION: To find drugs that promote chondrogenic differentiation of mesenchymal stem cells and aid in cartilage regeneration. A pool of 34 commercially available drugs was tested, and seven drugs were found to significantly increase the mRNA expression of cartilage-promoting genes, SOX9 and COL2, in the first screening. Among these drugs, aripiprazole and irinotecan were chosen for further investigation. In the second screening, aripiprazole and irinotecan were found to increase the expression of cartilage-related genes, including COL2, SOX9, and aggrecan, in hypertrophic chondrocyte media. To assess cartilage formation in a 3D pellet culture environment, the cells were cultured with aripiprazole. Results showed that aripiprazole effectively maintained pellet size, increased cell division (H&E staining), and raised proteoglycan levels (Alcian blue staining), indicating enhanced chondrogenic differentiation. To evaluate cartilage-regeneration in vivo, a cartilage defect model of Sprague Dawley rats was established, and aripiprazole was applied to the defect site. After four weeks, gross appearance scoring and histological analysis revealed effective cartilage regeneration in the aripiprazole treatment group. To investigate the biological mechanism of aripiprazole's cartilage-regeneration effect, mRNA sequencing was performed on mesenchymal stem cells treated with aripiprazole for 48 hours. The expression of 24599 genes related to cartilage development was analyzed. The top five differentially expressed genes (DEGs) were CHIR11, CTSK, NOV, IFT80, and STC1, which were further validated through qRT-PCR. These results suggested that aripiprazole significantly increased the expression of cartilage differentiation-related genes and promoted cartilage regeneration. Overall, the study highlights aripiprazole's potential as a novel drug that can enhance chondrogenic differentiation and promote cartilage regeneration, making it a promising candidate for further research in cartilage-related therapies.

DISCUSSION: Our research underscores the potential of drug repositioning as a promising strategy for the development of novel osteoarthritis treatments. We utilized the publicly accessible GEO database to pinpoint candidate genes associated with cartilage regeneration. Employing DGI, we identified potential drugs that facilitate chondrogenic differentiation. This method led us to validate the efficacy of Aripiprazole, in promoting cartilage regeneration using cell and animal models. Aripiprazole belongs to the class of medications known as third-generation antipsychotics and is currently employed in the treatment of various mental health disorders such as schizophrenia, and bipolar disorder, and administered orally, and intramuscularly. Recent research has also revealed Aripiprazole's anticancer properties and its ability to sensitize radiotherapy in various cancers, including head and neck cancer. We have substantiated Aripiprazole's chondrogenic effects, but further investigation is needed to determine the optimal administration method and dosage. Future studies will be essential to ascertain whether Aripiprazole proves effective in human clinical trials.

SIGNIFICANCE/CLINICAL RELEVANCE: Our discoveries indicate that aripiprazole may possess the capability to induce chondrogenic differentiation in cartilage regeneration. The potential for aripiprazole to recover cartilage defects, a function beyond its initial medical purpose, hints at its potential as a practical and time-efficient option for chemotherapeutic development.

IMAGES AND TABLES:

Figure 1. Differences in the expression of genes involved in chondrogenesis. Hierarchical clustering analysis of differentially expressed genes from GSE107649(A), GSE16173(B), GSE41106(C), and GSE116173(D).

Figure 2. Functional screening of drugs that promote chondrogenic differentiation.

Figure 3. Representative effect of aripiprazole in a rat model of articular cartilage defect. (A) After establishing a cartilage defect model, the knee cartilage was photographed and scored at 6 weeks. (B) Histological analysis using hematoxylin and eosin (H&E), Alcian blue, and safranin-O staining in a euthanized rat model of cartilage defect.