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Title: Rheumatoid Arthritis and Stem Cell Therapies Perspectives for It

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by systemic inflammation, primarily affecting synovial joints, leading to joint destruction and systemic complications. The pathogenesis involves immune system dysregulation, autoantibody production, and enzymatic imbalances. Enzymes, including matrix metalloproteinases (MMPs), serine proteases, and kinases, play crucial roles in these processes. Their dysregulation contributes significantly to disease progression through the imbalance of pro-inflammatory cytokines, oxidative stress, and extracellular matrix (ECM) degradation. MMPs, such as collagenases (MMP- 1, MMP-8, MMP-13) and gelatinases (MMP-2, MMP-9), are overexpressed in RA, causing cartilage degradation and synovial inflammation. Serine proteases, including neutrophil elastase and cathepsins, exacerbate inflammatory responses and ECM breakdown. Kinases like Janus kinase (JAK), spleen tyrosine kinase (SYK), and mitogen-activated protein kinases (MAPKs) mediate intracellular signaling pathways that promote cytokine production and synovial hyperplasia. Oxidative stress, a hallmark of RA, is intensified by the deficiency of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx). Addressing enzyme dysregulation represents a promising therapeutic avenue. Enzyme engineering, nanotechnology- based enzyme delivery systems, enzyme inhibitors, and recombinant enzyme therapies are being explored to optimize treatment outcomes. Despite challenges like enzyme stability and potential immune responses, advancements in protein engineering, computational modelling, and gene therapy hold promise for personalized medicine approaches in RA, offering more precise and effective treatments by restoring enzymatic balance and preventing joint destruction.

KEYWORDS - Rheumatoid arthritis, MSC, Stem cells, arthritis, MMP-2, MMP-9.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by systemic inflammation, primarily affecting synovial joints. The underlying pathogenesis involves immune system dysregulation, autoantibody production, and enzymatic imbalances leading to joint destruction and systemic complications [1]. Enzymes play a pivotal role in various biochemical pathways, and their dysregulation can contribute to RA progression. In this context, enzyme engineering a field that modifies enzyme activity, stability, and specificity has emerged as a promising therapeutic strategy to counteract disease mechanisms and improve treatment efficacy.

Enzyme engineering involves the rational design or directed evolution of enzymes to enhance their biological functions [2]. In RA, this approach can be applied to modify enzymes involved in inflammatory pathways, oxidative stress, and tissue degradation. For example, matrix metalloproteinases (MMPs) and serine proteases, which are implicated in cartilage degradation, could be engineered to reduce their pathological activity while preserving their physiological roles [3]. Similarly, antioxidant enzymes like superoxide dismutase (SOD) can be modified to enhance their stability and therapeutic potential in mitigating oxidative damage associated with RA [4].

The therapeutic potential of enzyme engineering extends beyond direct enzymatic modification. Nanotechnology-based enzyme delivery systems, enzyme inhibitors, and recombinant enzyme therapies are being explored to optimize treatment outcomes. For instance, engineered chondroitinase ABC, a bacterial enzyme, has shown promise in promoting cartilage repair by breaking down inhibitory molecules in the extracellular matrix [5]. Additionally, enzyme-based drug delivery systems can improve the bioavailability and targeted action of anti- inflammatory drugs, reducing systemic side effects.

Despite its potential, enzyme engineering in RA faces challenges such as enzyme stability in vivo, immune responses against engineered enzymes, and the need for targeted delivery. However, advancements in protein engineering, computational modelling, and gene therapy hold promise for overcoming these hurdles [6]. As research progresses, enzyme engineering could become a key component in personalized medicine approaches for RA, offering more precise and effective treatments.

Pathogenesis Of Rheumatoid Arthritis (Ra)

Its pathogenesis involves three stages: an initial nonspecific inflammatory stage amplified by T- cell activation, a chronic inflammatory stage driven by cytokines such as IL-1, IL-6, and TNF-α, and a tissue damage stage mediated by these cytokines. Fibroblast-like synoviocytes (FLS) play a pivotal role in RA progression, transforming from normal mesenchymal cells into aggressive tumor-like cells that produce matrix metalloproteinases (MMPs), cytokines, and chemokines,contributing to cartilage destruction and joint injury. Autoantibodies such as anti-citrullinated protein antibodies (ACPAs) are central to RA's autoimmune response, targeting citrullinated proteins generated through the enzymatic activity of peptidyl arginine deiminases (PADs). ACPAs form immune complexes with rheumatoid factors (RF), triggering complement activation and amplifying inflammation. Genetic factors like PADI4 and environmental triggers further exacerbate the autoimmune response. In addition to ACPAs, other autoantibodies targeting post-translationally modified proteins, such as carbamylated proteins (CarP), have been identified in RA patients (Figure 1).

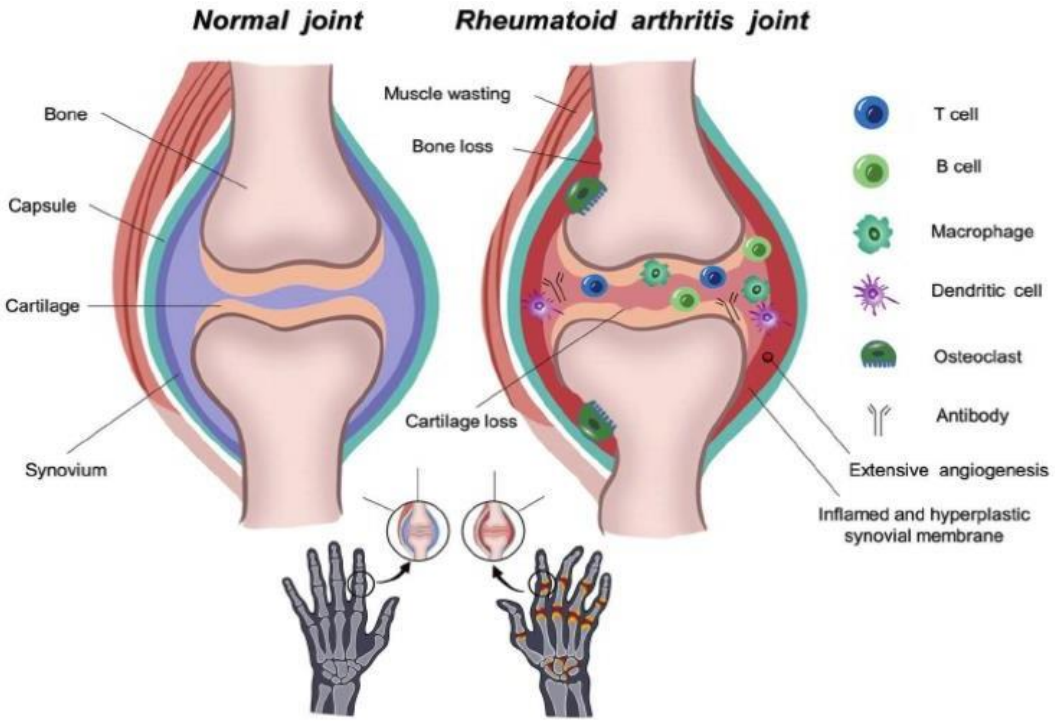

Fig-1: Normal & RA joints, showing joint swelling in RA reflects synovial inflammation due to immune activation. Synovitis is characterized by accumulation of innate & adaptive immune cells (T-cells, Dendritic cells, B-cells, Macrophages & osteoclasts). Pro-inflammatory and bone- destructive factors of immune response led to cartilage and bone loss with synovial thickening, angiogenesis, & muscle wastage

The inflammatory environment in RA is shaped by innate and adaptive immune cells, including dendritic cells, macrophages, T-helper cells (Th1/Th17), B cells, and plasma cells. These cells secrete pro-inflammatory cytokines like IL-1β, IL-6, TNF-α, and MMPs, which contribute to tissue remodelling and bone erosion. Angiogenesis is essential for nutrient supply to hypertrophic joints during inflammation. FLS acquire invasive phenotypes that exacerbate extracellular matrix invasion and promote immune cell recruitment via cytokine secretion. Cytokines such as granulocyte-monocyte colony-stimulating factor (GM-CSF), IL-6, and TNF-α activate endothelial cells and immune pathways .Osteoclast generation is induced by RANKL signalling from monocytes and macrophages, leading to bone erosion at cartilage-bone junctions. The integration of innate immunity with adaptive responses drives chronic inflammation in RA while systemic complications affect multiple organs beyond joints.

Overview of Enzyme Dysregulation in Rheumatoid Arthritis (RA)

The pathogenesis of RA is driven by immune system dysregulation, leading to an imbalance of pro-inflammatory cytokines, oxidative stress, and extracellular matrix (ECM) degradation. Enzymes play a crucial role in these processes, and their dysregulation significantly contributes to disease progression [1].

Several classes of enzymes, including matrix metalloproteinases (MMPs), serine proteases, kinases, and oxidative stress-related enzymes, exhibit altered activity in RA. Their overexpression or deficiency leads to chronic inflammation, cartilage degradation, and bone erosion. Understanding the role of enzymatic dysregulation in RA can help develop targeted therapies aimed at restoring enzymatic balance and preventing joint destruction.

Key Enzymes Implicated in RA Pathogenesis

Matrix Metalloproteinases (MMPs) and Cartilage Degradation, MMPs are a family of zinc-dependent proteolytic enzymes responsible for ECM remodeling and tissue homeostasis. However, in RA, excessive MMP activity leads to cartilage degradation and synovial inflammation [6]. The MMP-1 and MMP-13 collagenases degrade type II collagen, the main structural protein of cartilage. Their overexpression in RA synovium contributes to joint destruction. MMP-3 (stromelysin-1) activates other MMPs and degrades proteoglycans, further weakening the

cartilage matrix. MMP-9 and MMP-14 These enzymes are involved in synovial inflammation and angiogenesis, promoting pannus formation and disease progression (Monaco et al., 2021) [7]. Inhibiting MMP activity has been explored as a therapeutic strategy, but systemic MMP inhibitors often cause side effects due to their broad-spectrum activity. Therefore, selective MMP inhibitors are being developed to target specific enzymes involved in RA pathology [6,7].

Research and Future Directions

Current research is aimed at improving known biomarkers and identifying new biomarkers through the use of high-throughput technologies such as next-generation sequencing. This new method will help to identify more non-coding RNAs and metabolic markers that could greatly improve diagnostic accuracy and prognostic value in early rheumatoid arthritis (RA) [8].

Stem Cell Therapies for Cartilage Regeneration in RA: Mechanisms of stem cell differentiation into chondrocytes

The cartilage tissues and the injuries are considered to be a threat owing to the regeneration ability of the tissues. Stem cells are used to treat those effects the stem cell can perform unique potential functions. There are certain regulators to which stem cells respond to and has multiple applications for it. Stem cell is differentiated by origin, rate of proliferation and tissue type. There are two types of stem cells that are majorly used that is embryonic stem cells and mature stem cells. Embryonic stem cells are pluripotent they can give rise to a whole organism in the body. These cells are valuable as they provide us with the medium to study a disease model and identify the disease.

Mature stem cells are very specific in nature. They can differentiate into other cell types. The bone marrow MSC is the source of MSC. The MSC can include umbilical cord, synovial membrane etc. the bone marrow contains two types that is hematopoietic MSC and MSC. The MSC is highly multipotent, they can differentiate into a chondrocyte cell line. The cell lines can be of endothelial cells or even adipose cells. They can help in DNA damage repair and cell apoptosis and proliferation.

Tissue cartilage is very tight, flexible, oily and it has non- self-healing ability. Cartilage is the specialized tissue of the body as it doesn't have any nerves and veins. The cells that are used for cartilage differentiation include MSCs that are derived from the bone marrow itself (Figure 2). There are some conventional methods that treat the chondrocyte repairs. However, much effort has been made to develop articular cartilage naturally in vitro environments through 3D culture, bioreactors, and mechanical, biochemical stimulation [9].

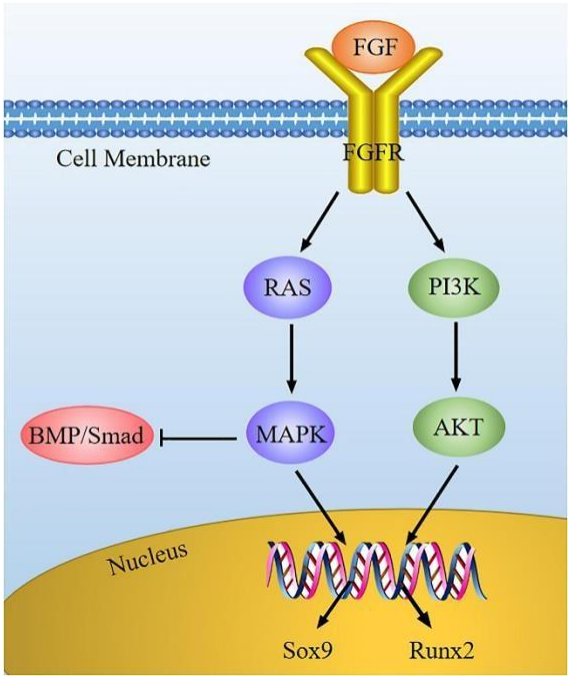

Fig-2: Stimulation of RAS factor from FGFR growth factor [10].

To decrease the disadvantages several other methods have been used, and the period of proliferation has been kept in mind. This is the case of lesions and self-healing ability. For the differentiation of MSC, two sections are important. The stem cells are mature and grow in G1 phase of cell cycle. The cells divide into non-symmetric cornea. And the daughter cells which arise they arise from the parent cells. The daughter cells and the parent cells look similar to each other. The precursor cell now becomes more restricted to differentiation. The cells differentiate asymmetrically and form divalent and trivalent cells. These cells are attached to the stem cell compartment. The cells form a stem cell line, and the mesenchymal cells attach to the bone marrow.
The committed cell lines now convert to single cell lines. The cartilage has the ability to differentiate to other cells in the bone marrow. Scaffolds are present with different chemical composition and structure. The presence of biomolecules is there. Chondrocyte is serum free; it does not have serum. Gene regulation and Sox 9 type collagen 2 are sites for differentiation. The stem cells are extracted from the adipose tissue and incorporated to the cartilage and bone marrow. They attach to the cell receptors attack the target site. TGF-beta is a transforming growth factor that helps in differentiation, and it takes help of the protein that is BMP [bone morphogenic protein] to differentiate. The thin membrane of joints has non adrenal surface called synovial membrane. The MSC basically preserves the membrane. This is the source of articular cartilage. Sox9 is a biochemical marker which reacts with the chondrogenic oligomeric matrix protein, and they are 3 percent in the adult cartilage (Figure 3) [11].

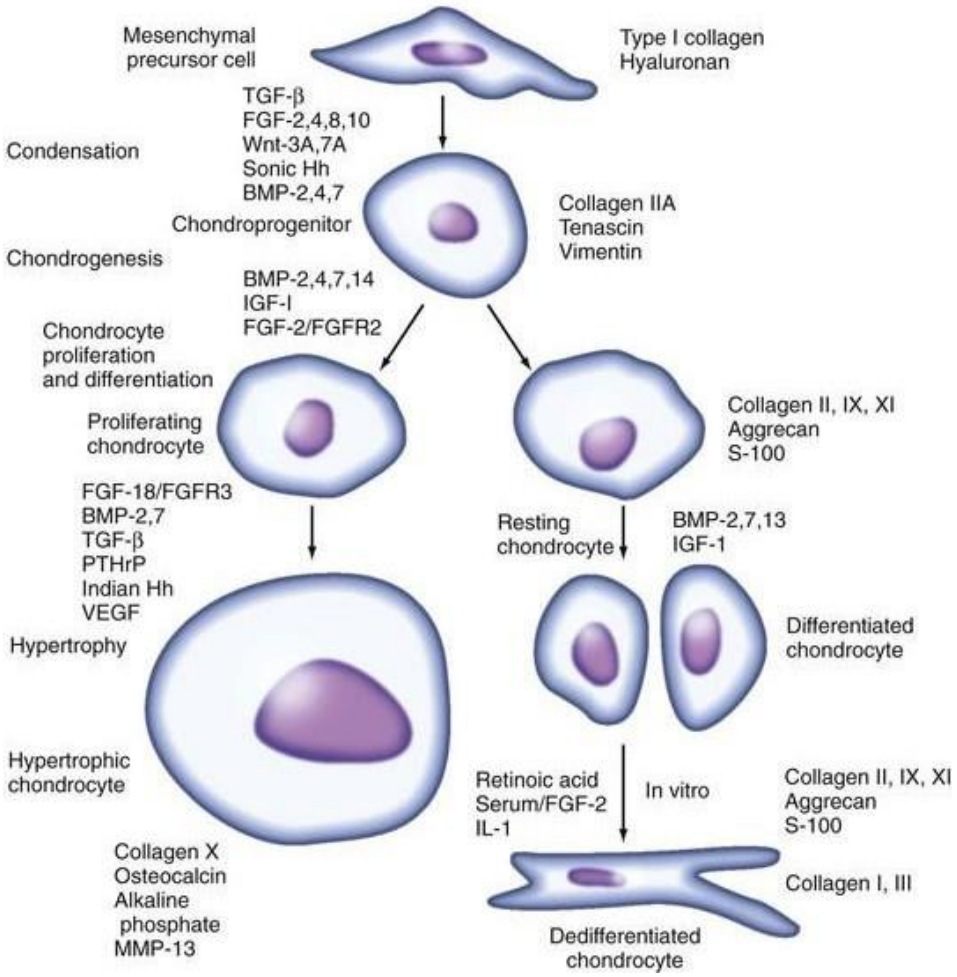

Fig-3: Illustrates differentiation pathway of mesenchymal precursor cells into chondrocytes, highlights key signaling molecules (e.g., TGF-β, BMPs, FGF) and markers associated with various stages, including proliferation, hypertrophy, and dedifferentiation [12].

There are growth factors like collagen 2,9,10,11 which attach to cartilage binding proteins and function as markers of chondrocytes. Matrix cell binding is an important regulator that is involved in cell differentiation and self-renewal. Biomaterials like scaffolds are used in this 3d culture method. The size of scaffold is a adipose derived material. There is RNA silencing process as well as the post-transcriptional regulation part of gene expression. Mi-RNA plays a great role in differentiation of cells of the chondrocytes. Overexpression of mi RNA genes and target genes are smad3 and HDAC. It affects the downregulation of Sox9 genes. It has been studied that Sox9 and mi RNA 496 inhibit over expressions of transcription process. COL2A1 and COL9A2 are the chondrogenic markers that are involved in over expression, and they get involved in bone marrow derived MSC for chondrogenic differentiation.

Role of mesenchymal stem cells (MSCs) in tissue repair

The MSC respond to injuries very fast, and they help in repair. When MSC are supplied in maximum array and applied exogenously to the wounded site. There is the sensing requirement of mediators, cell differentiation and immune cell activation. MSC activates the bactericidal activity and remodulation of the cells in the tissue.
They respond to the post transcriptional modification of the injury and also the efficacy of them. They respond to the microenvironments of the cells and tissues. The functionality of the MSC depends on the expansion and isolation of its environments. The release of protein mediators and transforming growth factors are a function of MSC.

There is an activity of MSC in inflammation and regeneration. They function as anti-inflammatory, auto immune and repairing agents. The efficacy of the MSC gets regenerated by the auto immunity and responsiveness of the environments. The cell-to-cell intact results in potency and efficacy (Figure 4).

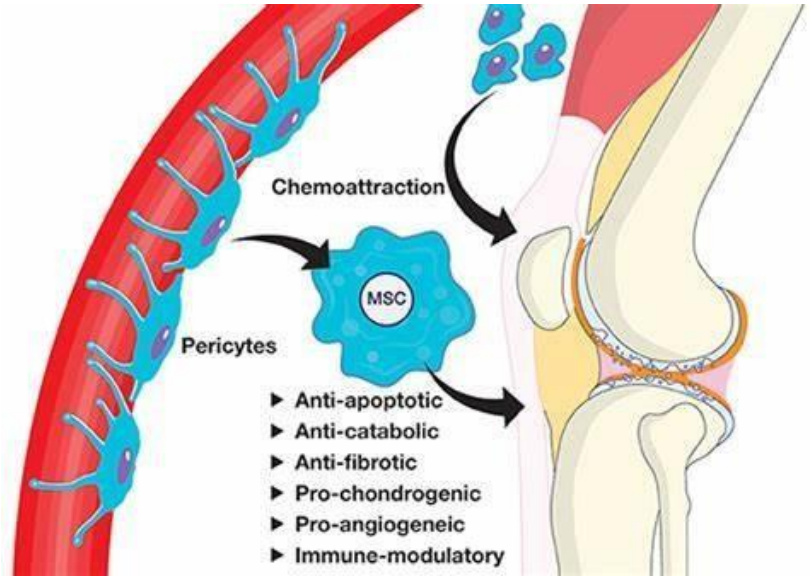

Fig- 4: Mesenchymal Stem Cells (MSCs) being recruited to a joint via chemoattraction. Once there, the MSCs exert anti-apoptotic, anti-catabolic, anti-fibrotic, pro-chondrogenic, pro- angiogenic, and immune-modulatory effects [13].

In the asthma studies MSC bring out an activity as the anti-Inflammatory agents and they supress the cellular events in the lungs. They have many such markers that help in the immunosuppression of the cellular events. The diseased allergen is recruited to the pulmonary insults. The assays of bone regeneration are assayed in the cellular recruitment and bone efficacy. There are many functional assays used to measure the regeneration. The immunosuppressive effect of the T cells is suppressed using MSC.

Proliferative phase is the second phase. It starts on the fourth day after the inflammation. The fibroblast migrates to the other tissues of macrophages. TNF alpha attaches to the chemokines and cytokine barrier breaks due to which the TGF beta has now attacked the receptor. New matrix is produced by the fibroblasts, and it creates a new matrix. The collagen matures and produces fluid in the matrix that strengthens the weakened tissue.
Remodelling is the third phase in wound healing. The MSC are a part of collagen proteins it takes help of certain growth factors that help in transformation of the beta cells. The density is accompanied by macrophages and fibroblasts in the proliferative phases of the cells. The matrix becomes stiff and becomes exposed to the membranes. The metalloproteinase enzymes increase the modifications of the cell matrix. The matrix becomes oriented, and collagen gets bundled and disorganized. The connective tissues are shrinking, and the matrix is full of collagen sheath that helps MSC accumulate in the diseased part. Angiogenesis is stimulated by the vascular endothelial growth factor and new blood vessels are formed. Epithelial cells are formulated by the growth factors. Local factors like the pH, temperature is ensured. As they play an important role in the process.

Chronic injuries are difficult to heal, and they undergo certain variations this can include diabetes and pressure. It follows certain stages of healing. Chronic injuries take a long time to heal as they stay for more than one week. They are affected by remodelling, cell proliferation and certain factorsFuture Directions and Challenges in Enzyme-Based Therapies for RA Enzyme therapies have been a hopeful approach for the treatment of rheumatoid arthritis (RA), an autoimmune disease involving progressive joint damage and chronic inflammation. Recent developments in enzyme engineering are markedly reinforcing the therapeutic potential of enzyme-based treatments. Enzyme engineering is a

pursuit to maximize the specificity and efficacy of therapeutic enzymes, especially those responsible for modulating the pathogenic processes of RA. One of the major targets is matrix metalloproteinases (MMPs), enzymes of crucial importance to cartilage destruction and tissue repair. Protein engineering methods, e.g., directed evolution and computation design, facilitate the creation of more selective and potent MMP inhibitors [14].
The future for enzyme therapies for rheumatoid arthritis (RA) is bright but contains a few problems that must be overcome to enable these therapies to live up to their potential. One of the major paths is the design of more targeted and effective enzymes which can directly target the molecules that cause joint destruction, including matrix metalloproteinases (MMPs). Progress in genetic engineering and protein modification is rendering these therapies more efficient and specific, enabling fewer side effects and improved therapeutic benefits. Furthermore, the integration of enzyme therapies with regenerative strategies, such as stem cell therapy, may provide an even more holistic approach by not only inhibiting further tissue injury but also facilitating repair and immune system regulation. Nonetheless, the major challenges ahead include immunogenicity, by which the body can develop an immune response against the therapeutic enzymes, and bioavailability, through which the enzymes can reach and utilize the inflamed joints in a meaningful way. It will be critical to overcome these challenges through advancements such as PEGylation, nanocarriers, and targeted delivery system to enhance the long-term efficacy of enzyme-based RA therapies. These developments, together with ongoing research, can transform the treatment of RA in the future. These designer enzymes can help target the MMPs that cause joint destruction, minimizing off- target effects and enhancing the overall therapeutic index of the therapy. Enzymes can also be engineered for improved stability and resistance to degradation within the body, overcoming some of the general shortcomings that have been observed in previous enzyme-based therapies. Enzyme engineering is also a potential solution to the hurdles of sustained efficacy since scientists strive to create enzymes that work for longer durations with better tissue penetration [15].

CONCLUSION:

Rheumatoid arthritis (RA) is a complex autoimmune disease driven by a multifaceted interplay of immune dysregulation, inflammation, and enzymatic imbalances, leading to progressive joint destruction and systemic complications. Understanding the intricate roles of enzymes such as matrix metalloproteinases (MMPs), serine proteases, kinases, and antioxidant enzymes is crucial for developing targeted therapeutic strategies. The dysregulation of these enzymes contributes significantly to the pathogenesis of RA, with MMPs degrading the cartilage matrix, serine proteases exacerbating inflammation, kinases amplifying pro-inflammatory signaling, and deficiencies in antioxidant enzymes intensifying oxidative stress. The identification of autoantibodies like anti-citrullinated protein antibodies (ACPAs) further underscores the autoimmune component of RA and its enzymatic underpinnings through peptidyl arginine deiminases (PADs).
The insights gained from studying enzyme dysregulation in RA have paved the way for innovative therapeutic approaches. Enzyme engineering, nanotechnology-based delivery systems, and selective enzyme inhibitors hold promise for restoring enzymatic balance and mitigating joint damage. For instance, targeting specific MMPs while preserving their physiological functions can minimize cartilage degradation without causing systemic side effects. Similarly, enhancing antioxidant enzyme activity through gene therapy or recombinant enzyme therapy may alleviate oxidative stress and reduce inflammation in the synovium. Kinase inhibitors, such as JAK inhibitors, have already revolutionized RA treatment by selectively blocking inflammatory signaling pathways, demonstrating the potential of enzyme-targeted therapies.
However, several challenges remain in translating these advances into clinical practice. Enzyme stability in vivo, potential immune responses against engineered enzymes, and the need for targeted delivery necessitate further research and optimization. Advances in protein engineering, computational modeling, and gene therapy offer opportunities to overcome these hurdles and develop personalized medicine approaches for RA. Future research should focus on identifying novel enzymatic targets, developing more selective and potent enzyme inhibitors, and improving the delivery of therapeutic enzymes to the affected joints. By addressing these challenges, enzyme- targeted therapies hold the potential to transform the treatment landscape of RA, offering more precise, effective, and personalized interventions that can prevent joint destruction, alleviate symptoms, and improve the quality of life for patients with this debilitating disease.

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REFERENCES:

1. Wang X, Zhang Y, Li J. Immune system dysregulation and enzymatic imbalances in rheumatoid arthritis: Pathogenesis and therapeutic implications. J Autoimmune Disord. 2022;45(3):123-35.

2. Bornscheuer UT, et al. Engineering enzymes for improved stability and specificity: Applications in medicine. Trends Biotechnol. 2019;37(4):355-67.

3. Zhang W, et al. Modifying matrix metalloproteinases for therapeutic applications in rheumatoid arthritis. Biochem J. 2020;477(5):1123-35.

4. Chen L, et al. Enhancing antioxidant enzyme stability for oxidative stress mitigation in autoimmune diseases. Antioxid Redox Signal. 2021;34(2):89-103.

5. Kumar S, et al. Chondroitinase ABC and cartilage repair mechanisms in inflammatory joint diseases. Arthritis Res Ther. 2022;24(1):45.

6. Chung C, et al. Advances in protein engineering for autoimmune disease therapy: Challenges and opportunities. Curr Opin Biotechnol. 2021;68:45-55.

7. Monaco C, et al. Role of MMPs in synovial inflammation and angiogenesis during rheumatoid arthritis progression. Rheumatol Int. 2021;41(8):1359-70.

8. Ding Q, et al. Immune cell dynamics and synovial inflammation in rheumatoid arthritis: A mechanistic overview. Nat Rev Rheumatol. 2023;19(1):12-25.

9. Cox J, et al. Development of selective MMP inhibitors for rheumatoid arthritis treatment: Challenges and solutions. Expert Opin Ther Targets. 2020;24(6):555-67.

10. Shi Y, et al. Granzyme B-mediated synovial cell apoptosis and inflammation regulation in rheumatoid arthritis models. Cell Death Dis. 2021;12(4):356.

11. O'Shea J, et al. Janus kinase inhibitors: A breakthrough therapy for autoimmune diseases including rheumatoid arthritis. Nat Rev Drug Discov. 2020;19(11):843-62.

12. Smith R, et al. Antioxidant enzyme deficiencies exacerbate oxidative stress-mediated joint damage in rheumatoid arthritis patients: A clinical correlation study. Radic Biol Med. 2021;172:345-56.

13. Laronha H, Caldeira J. Structure and Function of Human Matrix Metalloproteinases. Cells. 2020;9(5):1076. https://doi.org/10.3390/cells9051076

14. Grillet B, Pereira RVS, Van Damme J, et al. Matrix metalloproteinases in arthritis: towards precision medicine. Nat Rev Rheumatol. 2023;19:363–77. https://doi.org/10.1038/s41584-023-00966-w

15. Khan A, et al. Neutrophil elastase and cathepsins: Their role in inflammatory responses and joint erosion in rheumatoid arthritis patients. Front Immunol. 2022;13:897654.