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A Genetic Analysis of Rheumatoid Arthritis

Introduction to Rheumatoid Arthritis: Classification and Diagnosis

Rheumatoid arthritis is a chronic inflammatory disorder that affects mainly synovial joints. The disease is responsible for the destruction of articular cartilage and ankylosis of the joints. Classified as an autoimmune disorder, rheumatoid arthritis can also cause inflammation in other organs such as the blood vessels, skin and lungs. Symptoms of rheumatoid arthritis include joint pain and stiffness, swollen hands, fatigue, loss of appetite, appearance of rheumatoid nodules (firm lumps located under the skin near joints).

Diagnosis of rheumatoid arthritis is usually performed by imaging. Imaging techniques that are used include X-ray imaging, and magnetic resonance imaging or MRI. Nevertheless, it is difficult to diagnose the disease in its early stages using imaging techniques. Indeed, since there is only very minor changes in the joints in its early stages, rheumatoid arthritis can be misdiagnosed and confused with many other diseases such as gout, osteoarthritis, and Lyme disease. Blood tests are also performed to diagnose rheumatoid arthritis. Immunological studies are performed to test the presence of rheumatoid factor (an autoantibody). This test is however not specific. To begin with, a negative result in this immunological test does not necessarily dismiss the possibility of having rheumatoid arthritis. This is the case of seronegative arthritis, which affects about 15% of patients. Furthermore, this test is more likely to yield accurate results in the later stages of the

illness. The test for the rheumatoid factor is more likely to be negative in the first year of the illness. Finally, the rheumatoid factor can also be identified in other illnesses like Hepatitis C and Sjögren's syndrome. As a result of this low specificity, other blood tests have been developed, such as tests detecting the presence of anti-citrullinated protein antibodies which have a high specificity of 95%.

Rheumatoid arthritis approximately affects up to 1% of the general adult population worldwide. It affects mostly women in between forty and sixty years old. The causes of rheumatoid arthritis are still unknown and no cure has been discovered to treat the disease. However, genetic methods and approaches can be used to identify the genes that are responsible for the disease and to cure the disease. This paper will provide a genetic analysis of rheumatoid arthritis by discussing genome-wide association studies conducted on the disease, and by elaborating on treatments of the disease using genetic approaches such as stem cell therapy and gene therapy.

Genome Wide Association Study of Rheumatoid Arthritis

Rheumatoid arthritis is not a single-gene Mendelian disease: there are multiple genes that are involved in promoting rheumatoid arthritis. Therefore, it is very complicated to figure out what the cause of the disease is. To understand what causes a complex disease such as rheumatoid arthritis, a genome-wide association study must be performed. A GWAS is a study that examines genetic variants in different individuals and attempts to underline an association between a variant

and a trait. A GWAS usually finds a correlation between SNPs (single-nucleotide polymorphisms) and major diseases.

Multiple GWA studies have confirmed that rheumatoid arthritis is strongly associated with the HLA region on chromosome 6p21: the gene HLA-DRB1 has an odds ratio of 2.88 and a $P_{GWAS}<10^{-299}$. The HLA region encompasses many major histocompatibility complexes (MHCs) which are genes that code for proteins that have the immunoregulatory function of allowing the immune system to distinguish between body cells and foreign cells. A mutation in this region therefore could impair the immune system's ability to distinguish between body cells and foreign cells, resulting in an autoimmune disease. Non-HLA genes have also been identified as risk factors for rheumatoid arthritis. The disease is associated with the gene PTPN22 ($P_{GWAS}< 9.1\times10^{-74}$ and Odds Ratio=1.94) which codes for the protein tyrosine phosphatase non-receptor 22 that is known to exhibit regulatory activities for T cells. A mutation in this gene can cause a negative regulation of T-cell activation. Rheumatoid arthritis is also associated with the gene CCR6 ($P_{GWAS}< 3.3 \times 10^{-7}$ and Odds Ratio=1.13) that codes for the Chemokine Receptor 6 protein which regulates the migration of inflammatory and regulatory T cells.

The GWA studies have identified a total number of 31 rheumatoid arthritis risk loci. It is believed that 60% of the disease is due to genetics. However, only 16% of the genetic background of the disease is known from GWA studies. This indicates that environmental factors could also

be associated with the disease. The main environmental factor that induces rheumatoid arthritis is thought to be smoking.

Treating Rheumatoid Arthritis Using Genetics

There is no current cure for rheumatoid arthritis. Surgery can be performed in the early stages of the disease to remove the inflamed synovia and prevent a quick destruction of the tissues and joints. Joint replacement surgery may also be performed if the joints are severely affected. In later phases of the disease, surgery cannot be performed due to its inefficiency, so the disease is treated with painkillers and anti-inflammatory drugs such as steroidal drugs or Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Steroidal drugs suppress swelling, minimize tissue damage, reduce pain and decrease inflammation by decreasing the activity of the immune system. NSAIDs provide analgesic and antipyretic effects and can be prescribed in higher doses to provide anti-inflammatory effects. Although these treatments suppress the symptoms of the disease, they do not stop the progression of joint destruction. Also, due to the suppression of the immune system, these drugs have many side effects such as causing an infection from outside sources. Disease-modifying antirheumatic drugs (DMARDs) have been confirmed to halt the progress of rheumatoid arthritis. If the arthritis is uncontrolled or toxic effects arise from usage of DMARDs, biological agents such Interleukin 1 Receptor Antagonist (IL-1Ra) can be used. However, some patients develop a resistance to the standard DMARD therapy. Also, the use of biological agents

have many significant limitations like systemic side effects, a lack of curative response and the need to be regularly re-administered.

Gene Therapy may be used to enhance the delivery and efficiency of biological agents. Since administering biological agents like IL-1Ra requires to be regularly re-administered, a gene therapy approach could be developed to provide a stable and effective long-term delivery of these biological agents. In order to successfully use gene therapy to treat rheumatoid arthritis vectors and targets must be chosen, a gene delivery method must be analyzed and methods to regulate transgene expression must be investigated. The vectors that are mostly used are viral vectors, which include retroviruses, adenoviruses and adeno-associated viruses. Viral vectors are used instead of non-viral vectors because of their efficiency, even though the use of non-viral vectors decreases host toxicity compared to viral vectors. The main target that has been proposed for gene therapy is the restoration of the balance between immunosuppressant cytokines and proinflammatory cytokines such as interleukin 1 or tumor necrosis factors. Three gene delivery methods have been proposed for treating rheumatoid arthritis. The first is an in vivo gene delivery, which involves injecting a transgene vector into the tissue. The second is an ex vivo gene delivery, which involves harvesting target cells, performing an in vitro transduction and reimplanting them into the host. The third is another ex vivo approach which involves injecting an immune cell carrying the therapeutic gene into the host's inflamed joint. Regulating gene expression is very important to the success of gene therapy. Indeed, the gene therapy techniques rely on the ability to

activate and deactivate the therapeutic genes depending on the state of the disease. Drug-inducible promoters can control gene expression. For example, the antibiotic tetracycline can induce transcription or inhibit transcription.

Gene therapy has, however, many limitations. First of all, the immune system could identify the gene delivery vectors as an antigen and produce antibodies to attack them. Moreover, the genetic causes of rheumatoid arthritis are not completely understood, as only 16% of the genetic background of the disease is known. Furthermore, gene therapy can induce a tumor if the DNA is integrated in the wrong part of the genome. Also, the rapid division of cells does not allow gene therapy from achieving long term results. Finally, gene therapy is a very expensive type of treatment.

Stem cells may be used to treat rheumatoid arthritis patients that are resistant to DMARD therapy. Stem cells would be useful to cure diseases such as rheumatoid arthritis because stem cells can heal damaged tissues and can modify the immune system. Preclinical evidence have shown that stem cell therapy induces great healing in animals expressing rheumatoid arthritis. Indeed, some companies, like Vet-Stem, use stem cells in horses exhibiting arthritis and joint deformities to facilitate their recovery. The stem cells that could be used to treat rheumatoid arthritis are allogeneic mesenchymal stem cells that are harvested from human umbilical cords. There are many reasons why these stem cells are best harvested specifically from human umbilical cords. To begin with, umbilical cord tissue has a large supply of mesenchymal stem cells.

Furthermore, these stem cells are collected without going through any invasive procedure. Finally, allogeneic mesenchymal stem cells can be injected into any patient as they are immune system privileged, meaning that they do not provoke an inflammatory immune response. The mechanism of action of these stem cells is that they are injected into an inflamed tissue and they produce anti-inflammatory agents. Furthermore, these stem cells allow the production of T-cells, which are a type of immune cells that control the activity of the immune system: they protect the organism against immunological self-attack. Mesenchymal stem cells act locally and therefore do not produce anti-inflammatory agents and suppress the immune response of a patient's whole body. Genetic therapy on the stem cells is sometimes required for mesenchymal stem cells. Mesenchymal stem cells are able to prevent disease progression but might not always be able to restore injured cartilage. Therefore, using genetic therapy, mesenchymal stem cells first undergo chondrogenic differentiation and then are introduced to the damaged site. This type of treatment is undergoing phase two of clinical trials. It has already been screened for safety and is now being tested for efficacy.

Rheumatoid arthritis thus appears to be a potential candidate for stem cell therapy. Nevertheless, stem cell therapy has many limitations. Multipotency is usually observed in vivo as adult stem cells are difficult to grow in vitro. Stem cell therapy, just like gene therapy, is a very expensive type of treatment. The immune system might reject the transplant if there is no good match for the stem cells.

Conclusion

The causes and treatments of rheumatoid arthritis seem to lie in the scope of genetics. Since rheumatoid arthritis is believed to be caused by multiple genes, genome wide association studies must be performed to identify the multiple risk loci associated with the disease. GWA studies have identified 31 rheumatoid arthritis risk loci. Many of these risk loci are genes that have already been known to be involved in autoimmune disorders, confirming that rheumatoid arthritis is an autoimmune disease. Since standard treatment for rheumatoid arthritis are not completely efficient, genetic approaches can be developed to cure the disease. Gene therapy is an approach that relies on introducing a therapeutic gene into the host via viral and non-viral vectors. Stem cell therapy is another approach that relies on inserting a multipotent stem cell into the inflamed area of a joint in order to alter the inflammatory immunological response of the body. Nevertheless, these treatments have many limitations and challenges. These include the expensive cost of the treatments, the possibility of an immune reaction rejecting the treatment, and long term side effects of these treatments.

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