Personalized Medicine JOURNAL

Medical Journal/6 year/No,24/150000Rials/2022 Winter/ ISSN 2717-3860

The Future of Medicine is Personalized

Journal Information

Name	Personalized Medicine Journal
Abbreviated name	PMJ
Date of first issue published	February 2019
Concessionaire	AmitisGen Med TECH Group
Release period	Quarterly

Editorial Board Information

Phone number: 009821-88985293

Address: 3rd Floor, No. 2, Italia Street, Tehran, Iran

Postal Code: 1416673744

E-mail: info@personalizedmedicinejournal.com

Personalized Medicine Journal
Winter 2022,Volume 6, Issue 24
Table of Content

http://pmjournal.ir Review Article

Winter 2022, Volume 7, Issue 24 (1-7)

The Role of DNA Methylation in Development and Progression of Rheumatoid Arthritis

Fawziah Mohammed[1], Seyed Akbar Moosavi[2*]

[1] Medical Laboratory Sciences.Faculty of Allied Health Sciences, Kuwait University. Kuwait.
[2]School of Paramedical Sciences, Iran University of Medical Sciences, Tehran, Iran.

DOI: 10.22034/pmj.2022.252438

**Corresponding author: Seyed Akbar Moosavi, School of Paramedical Sciences, Iran University of Medical Sciences, Tehran, Iran. Email: seyedmoos@gmail.com*

Submitted: 2021-12-10
Accepted: 2022-02-15

Keywords:
Rheumatoid Arthritis
DNA methylation
Epigenetics
Treatment

Abstract:

Rheumatoid arthritis (RA) is a chronic autoimmune disease of unknown etiology that results in progressive joint destruction and ultimately to disability. Currently effective biologic therapies, exist for approximately 40% of patients, but disease activity remains inadequately controlled in others. Therefore, it is crucial to identify specific markers that predict therapeutic response in various patients, prior to the initiation of therapy. DNA methylation , as a epigenetic factor, is increasingly being explored as a potential theranostic biomarker. It has been suggested that DNA methylation might contribute to RA development, nonetheless , with conflicting results. Epigenetic modules have provided a possible interface through which genetic and environmental risk factors contribute to the susceptibility and pathogenesis of RA. Hence, epigenetic regulators may provide promising drug targets to develop novel therapeutic drugs for tailored treatment of RA patients. Here we review the current knowledge regarding the role of DNA methylation in RA and indicate its potential therapeutic implications.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial hyperplasia and joint destruction (1). Its onset is progressive and invasive, which can lead to joint deformity and disability. The disease can cause a wide variety of symptoms, clinical forms and prognoses in patients(2). While Its incidence begins to increase at the age of 25 years; it reaches a plateau at the age of 55. Furthermore, its prevalence is more than six times greater in women with 60 to 64 year-old than those in 18 to 29-year-old (3). In the industrialized world, RA affect more individuals than other countries. One third of people of all ages are also may affected by the disease at some point of their lifetime (4). The production of autoantibodies such as rheumatoid factor, and anticitrullinated protein antibodies (ACPA) are considered the hallmark of the disease that in turn supports an autoimmune etiology whereby an immune response is directed against an, as yet, unknown autoantigen. Although autoantibodies are an important characteristic of RA autoimmunity, some affected individuals may lack these autoantibodies in their blood (5, 6). The disease is complex and involves many environmental factors that trigger disease in genetically susceptible individuals (7).

Major advancements in understanding the development of RA originate from studies investigating the expression and regulation of pro-inflammatory cytokines within the affected synovial tissue. Of these pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, is a pivotal factor in the inflammatory cascade which led to its identification as a target for therapeutic intervention (8). TNF increases inflammation and tissue damage mediated by T cells, B cells, fibroblasts and macrophages in affected joints and also has systemic effects that can lead to comorbidities such as cardiovascular disease (9). Other cytokines such as IL-1 and IL-6 have similar roles in promoting the activation of T cells, B cells and osteoclasts while IL-17 promotes the infiltration of T cells and recruitment of monocytes and neutrophils, which also contribute to synovitis (10). B-lymphocytes are involved in the production of autoantibodies such as rheumatoid factor (RF) and antibodies against cyclic citrullinated peptide (anti-CCP). Differences in expression of anti-CCP and RF, rate of disease manifestation and variability of response to therapy cause heterogeneity of RA patients indicating, variation in pathophysiological

mechanisms, implication in the disease development and progression (11, 12).

In recent years, numerous studies have started to focus on the role of epigenetics in RA and investigate its contribution to the heterogeneity of patients (13). DNA methylation and histone modifications, are important epigenetic factors that affect the expression of immune-related genes and inflammation progression have become promising mechanisms to explain the pathogenesis of RA (14). Epigenetic changes in RA have been studied both in mononuclear cells of peripheral blood as well as other type of immune cells such as monocytes, T-cells and B-cells (15). In addition the epigenetic modifications in the RA synovial fibroblasts (RASFs) are of particular interest because of their aggressive phenotype, which remain stable for several passages in cell culture (16). RASFs are clue cells of joint damage and inflammation development due to pro-inflammatory and catabolic molecules synthesis, promoting abnormal proliferation and invasiveness (17). Numerous studies have found that methylation in immune cells may lead to RA progression through coordinated control of immune cell differentiation and function (18). In this review we aim to collate the current knowledge on DNA methylation in autoimmunity with a particular focus on RA, its role in altering gene expression in different cells that contribute to the pathogenesis of RA, and discuss its therapeutic and diagnostic potential.

Molecular Mechanism of RA

RA primarily affects synovial joints, in which the balance between recognition of pathogens and avoidance of self-attack is impaired and the immune system attacks and destroys healthy tissue (19). Additionally, there is increased recruitment and migration of immune cells from the bloodstream into the target tissue, including synovial membrane or synovial fluid (3, 20). Consequently, such an influx of activated immune cells producing an enhanced level of pro-inflammatory cytokines that leads to the progressive erosion of articular cartilage (21). Leukocytes, including T cells, B cells and phagocytes, are the main types of immune cells in the rheumatoid synovium (22). In fact, macrophages and granulocytes produce chemokines, pro-inflammatory cytokines, and reactive oxygen species, which are associated with classical inflammation. Besides, B lymphocytes play critical roles in the pathogenesis of RA (23). They are the main source of ACPAs and RF which involve in the formation of immune complexes as well as complement activation in the joints (24). B cells associated with the pathogenesis of RA disease not only activated by the presentation of antigens but also play a pivotal role in the development of the disease due to the production of antibodies, anti-self-antibodies and cytokines. In addition to the role of soluble pro inflammatory molecules and activity markers, such as TNF, interleukin (IL)-6 and C-reactive protein, in the pathogenesis of RA, local synovial cellular interactions drive the key processes of long-term cellular proliferation and destruction of the rheumatoid joint (25, 26).

influencing factors in RA

People born with certain genes, called HLA class II genotypes, are more likely to develop RA (27). Although it is undeniable that genetic factors play the major role in the susceptibility of the illness , nonetheless, the low concordance rate (12–20%) observed in monozygotic twins suggests that environmental factors may also play a significant role in the pathogenesis of RA (28). Environmental factors such as smoking and infections may have affect the incidence of the disease as well as the rate of progression and severity of the RA (29). Moreover, other known environmental factors such as latent viral infections, sex hormones and deficiency of vitamin D may influence the disease (30). It is thought that these environmental factors influence epigenetic modifications, which in concert with the individual genetic susceptibility status result in the development of RA symptoms. Genetic heterogeneity however, does not explain all the features of illness (31, 32). Thus, investigation of epigenetic factors and mechanisms associated with the progression of the disease and response to treatment is increasingly important. Nevertheless, Investigating the epigenetic landscape can provide novel therapeutic targets (5, 33).

Treatment for arthritis rheumatoid

The main treatment goals are to control inflammation, ease pain, and reduce disability linked to RA (34). Current treatment guidelines recommend that patients initially treated with a combination of corticosteroids and disease-modifying antirheumatic drugs (DMARDs) to slow down disease progression and reduce synovitis along with disability (35). Though many people with RA need to take more than one drug to combat the disease. This is because drugs work in different ways to reduce the symptoms(36). There are three types of DMARD:

•conventional synthetic DMARDs (sometimes called csDMARDs)

•biological therapies (sometimes called bDMARDs).

•targeted synthetic DMARDs (sometimes called tsDMARDS)

Some DMARDs include hydroxychloroquine (Plaquenil), leflunomide (Arava), sulfasalazine (Azulfidine), or tofacitinib (Xeljanz) (37, 38). Although steroids are sometimes known by their full name: corticosteroids, it helps to reduce the pain, stiffness and inflammation caused by RA (39). The most common anti-inflammatory steroids

include hydrocortisone (Cortef), methylprednisolone (Medrol), and prednisone (Deltasone) (40). Non-steroidal anti-inflammatory drugs (NSAIDs) can be used to help control symptoms of pain, swelling or stiffness. They commonly used in combination with painkillers (41).

Biologic therapies are genetically engineered human proteins that specifically target inflammatory cytokines, such as TNF-α and IL-6 or immune pathways, such as CTLA-4 costimulatory pathways, or B cells (42). Though when bone damage from the arthritis become severe or pain is not controlled with medications, surgery is an option to restore function to a damaged joint (43).

Mechanism of DNA methylation

Epigenetics refers to chemical modifications that influence gene regulation without changing the DNA sequence (44). These alterations include DNA methylation and post-translational modifications of histone proteins(45). This review will focus mainly on studies of DNA methylation as a biomarker of response to treatment in RA. DNA methylation is a heritable epigenetic marker involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (Fig1) (4, 46). However, more than 98% of DNA methylation occurs in a CpG dinucleotide context in somatic cells, while as much as a quarter of all methylation appears in a non-CpG context in embryonic stem cells (ESCs) (47).

DNA methylation is a more stable biomarker than gene expression, and aberrant methylation has been reported in several cancers (48). DNA methylation has also been found to predict response to therapy; for example, an epigenome-wide association study (EWAS) identified methylation signatures as a predictive response to anti-EGFR, a common therapeutic for metastatic colorectal cancers (49, 50).

Impaired DNA methylation in RA

Whole genome analysis of aged individuals has highlighted a number of hypomethylated regions that may contribute to age-related diseases, including some autoimmune diseases such as RA (51). The first evidence to suggest that DNA methylation may play a role in aging and autoimmunity came from studies investigating the effect of the DNA methyltransferase inhibitor 5-azacytidine that can induce symptoms associated with autoimmunity (52). There is emerging evidence of the interrelationship between DNA methylation and inflammation in regulating immune pathways. For example, the cytokine, IL-6, has been reported to increase the expression of DNMT1, levels of which correlate with DNA methylation in T cells (53). Studies have shown that the extent of methylation regulates migration, differentiation, and activation of T-cells. T cell activation leads to demethylation of the interleukin-2 promoter, resulting in interleukin-2 production (54). Such impairment of DNA methylation may play a role in RA pathogenesis. Specifically for RA, differential DNA methylation has been demonstrated in the IL-6 promoter (55). In 2008, Nile and colleagues found that IL-6 promoter methylation reduced transcriptional activity and identified a single CpG within the IL-6 promoter that was key to regulating IL-6 gene expression (53).

Recent studies confirmed a global DNA hypomethylation in T-cells and monocytes of RA patients compared to healthy individuals (56). In CD4+ T cells, 383 hyper- and 785 hypo-methylated genes were identified in RA patients ($p < 3.4 \times 10\text{-}7$), including three regions within HLA that were frequently

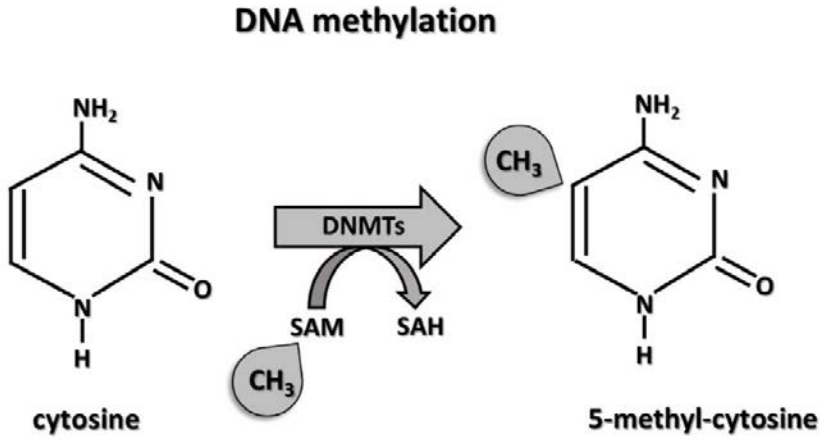

Fig1. DNA methyltransferases (DNMTs), responsible for the transfer of a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA (46).

hypomethylated (57). Genome-wide analysis of DNA methylation by microarrays revealed its impact in B-cells on the early stages of patients who have not yet received treatment compared to healthy donors. Genome-wide methylation change was also found in T and B (58). A study identified 150 and 113 CpG loci with unique methylation characteristics in T and B lymphocytes in patients with ERA.

Rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) are involved in the release of inflammatory mediators and matrix degrading enzymes, which are key effector cells leading to synovial inflammation and destruction of bone and cartilage (59). The changes in DNA methylation in RA-FLS play important roles in the pathogenesis of illness. Hypomethylation in RA-FLS may be caused by the downregulation of DNMT1 and DNMT3A after inflammatory environmental stimulation (60).

DNA methylation as a biomarker in RA

Biologic drug therapies represent a huge advance in the treatment of RA (61). However, effective treatment of disease trol is achieved in only 30% of patients, making identification of biomarkers of response a research priority. Since DNA methylation appears to have a role in RA pathogenesis, it may also be a suitable biomarker of treatment response (62). Just as serologic and genetic studies indicate that there may be more than one sub-type of RA with a wide range of responses to biologic treatment, differences in baseline methylation status may also serve as a marker of varied disease subtypes that might respond better to therapies targeting the particular pathway involved (63). DNA methylation may thus provide a biomarker of subsequent treatment response (64). It is urgent to find novel markers to augment the diagnostic accuracy, prediction of disease onset , and its progression. For example Methylation levels of SHROOM1 in ERA are substantially increased, hence it can be applied as an early diagnostic biomarker (65). Additionally, identification of aberrant DNA methylation may change disease onset which in turn it might lead to a better understanding of the risk factors that contribute to disease development and thus result in the identification of specific biomarkers for disease analysis(66). Recently, reverse transcriptase (RT)-PCR assays have been developed to quantify the number of Foxp3+ cells within RA tissue samples (67).

It should be noted that there may be other appropriate biomarkers of response. Micro-RNAs (miRNA) are small, noncoding RNA structures that act as regulators of gene expression (68). There is increasing evidence that implicates dysregulation of miRNA in blood, T cells, and synovial fibroblasts in inflammation and joint destruction are found in RA patients. There have been a number of successful therapies for patients, however , a proportion of patients fail to respond to conventional therapy (69). Ideally, it would be useful to identify this fraction of nonresponders earlier in the course of the disease, to provide better treatment regimes that are tailored towards the individual patient. It is becoming clear that RA patients display a differentially methylated genome when compared to healthy individuals (70). This raises the possibility that measuring DNA methylation patterns of responders and nonresponders may lead to the use of DNA methylation as a predictive biomarker for treatment response (71).

CONCLUSIONS

In recent decades, many studies have shown that epigenetic mechanisms are involved in the regulation of all biological processes in the body from impregnation to death. In recent years, a major advancement has taken place in understanding the role of DNA methylation in the pathogenesis of RA, hence it can be used as effective biomarker in the disease process (72). It is hoped that the progress made in identifying epigenetic mechanisms occurring in cancer can also be exploited in inflammatory disease for other disease assessment. Unfortunately, studies on the clinical use of epigenetic drugs modulating aberrant DNA methylation patterns in RA are at a very early stage. More research should be conducted on DNA methylation in regard to treatment and diagnosis of cancer and proliferative diseases (73). Moreover, identified differential methylation genes can be applied as useful biomarkers to predict disease progression and severity and also provide potential therapeutic targets for RA. Epigenetic modifications as drug targets could provide a new direction of pharmacological research for the development of novel drugs that alleviate symptoms of high toxicity, low efficiency, and high cost of the current medical care. For example, demethylation of FOXP3 is used as a biomarker to evaluate the therapeutic drug response, which provides a direction for the precision treatment of RA (74). It is crucial to find new DNA methylation biomarkers that can be used in everyday practice to detect early onset of RA before the induction of irreversible joint destruction . These knowledges collectively may not only delay or reduce disease progression but also it decreases the costs of health care (75).

REFERENCES

1.McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. New England Journal of Medicine. 2011 Dec 8;365(23):2205-19.
2.Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. The Lancet. 2017 Jun 10;389(10086):2338-48.
3.Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu. Rev. Immunol. 19, 163–196 (2001).

4.Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. Jama. 2018 Oct 2;320(13):1360-72.
5.McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. The Lancet. 2017 Jun 10;389(10086):2328-37.
6.Okada Y, Eyre S, Suzuki A, Kochi Y, Yamamoto K. Genetics of rheumatoid arthritis: 2018 status. Annals of the rheumatic diseases. 2019 Apr 1;78(4):446-53.
7.Ai R, Laragione T, Hammaker D, Boyle DL, Wildberg A, Maeshima K, Palescandolo E, Krishna V, Pocalyko D, Whitaker JW, Bai Y. Comprehensive epigenetic landscape of rheumatoid arthritis fibroblast-like synoviocytes. Nature communications. 2018 May 15;9(1):1-1.
8.Tseng CC, Lin YZ, Lin CH, Li RN, Tsai WC, Ou TT, Wu CC, Sung WY, Yen JH. Genetic and epigenetic alteration of the programmed cell death 1 in rheumatoid arthritis. European Journal of Clinical Investigation. 2019 Oct;49(10):e13094.
9.Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y, Cao L. DNA methylation, its mediators and genome integrity. International journal of biological sciences. 2015;11(5):604.
10.Mazzone R, Zwergel C, Artico M, Taurone S, Ralli M, Greco A, Mai A. The emerging role of epigenetics in human autoimmune disorders. Clinical epigenetics. 2019 Dec;11(1):1-5.
11.Qiu H, Wu H, Chan V, Lau CS, Lu Q. Transcriptional and epigenetic regulation of follicular T-helper cells and their role in autoimmunity. Autoimmunity. 2017 Feb 17;50(2):71-81.
12.Bullock J, Rizvi SA, Saleh AM, Ahmed SS, Do DP, Ansari RA, Ahmed J. Rheumatoid arthritis: a brief overview of the treatment. Medical Principles and Practice. 2018;27(6):501-7.
13.Tam LS, Gu J, Yu D. Pathogenesis of ankylosing spondylitis. Nature Reviews Rheumatology. 2010 Jul;6(7):399-405.
14.Glant TT, Mikecz K, Rauch TA. Epigenetics in the pathogenesis of rheumatoid arthritis. BMC medicine. 2014 Dec;12(1):1-5.
15.Ospelt C. Epigenetic biomarkers in rheumatology-the future?. Swiss Medical Weekly. 2016;146:w14312.
16.Hardy RS, Hülso C, Liu Y, Gasparini SJ, Fong-Yee C, Tu J, Stoner S, Stewart PM, Raza K, Cooper MS, Seibel MJ. Characterisation of fibroblast-like synoviocytes from a murine model of joint inflammation. Arthritis Research & Therapy. 2013 Feb;15(1):1-5.
17.Lefevre S, Meier FM, Neumann E, Muller-Ladner U. Role of synovial fibroblasts in rheumatoid arthritis. Current pharmaceutical design. 2015 Jan 1;21(2):130-41.
18.Nasonov EL, Lila AM. Rheumatoid arthritis: achievements and unresolved issues. Terapevticheskii arkhiv. 2019 May 15;91(5):4-7.
19.Hitchon CA, El-Gabalawy HS. Suppl 1: the synovium in rheumatoid arthritis. The open rheumatology journal. 2011;5:107.
20.Laria A, Lurati A, Marrazza M, Mazzocchi D, Re KA, Scarpellini M. The macrophages in rheumatic diseases. Journal of inflammation research. 2016;9:1.
21.Ciechomska M, Wilson CL, Floudas A, Hui W, Rowan AD, van Eden W, Robinson JH, Knight AM. Antigen-specific B lymphocytes acquire proteoglycan aggrecan from cartilage extracellular matrix resulting in antigen presentation and CD 4+ T-cell activation. Immunology. 2014 Jan;141(1):70-8.
22.Bogdanos DP, Smyk DS, Rigopoulou EI, Mytilinaiou MG, Heneghan MA, Selmi C, Gershwin ME. Twin studies in autoimmune disease: genetics, gender and environment. Journal of autoimmunity. 2012 May 1;38(2-3):J156-69.
23.Shi J, van Steenbergen HW, van Nies JA, Levarht EN, Huizinga TW, van der Helm-van AH, Toes RE, Trouw LA. The specificity of anti-carbamylated protein antibodies for rheumatoid arthritis in a setting of early arthritis. Arthritis research & therapy. 2015 Dec;17(1):1-6.
24.Ciechomska M, O'Reilly S. Epigenetic modulation as a therapeutic prospect for treatment of autoimmune rheumatic diseases. Mediators of inflammation. 2016 Aug 10;2016.
25.Carmona-Rivera C, Carlucci PM, Moore E, Lingampalli N, Uchtenhagen H, James E, Liu Y, Bicker KL, Wahamaa H, Hoffmann V, Catrina AI. Synovial fibroblast-neutrophil interactions promote pathogenic adaptive immunity in rheumatoid arthritis. Science immunology. 2017 Apr 14;2(10):eaag3358.
26.Klein K, Kabala PA, Grabiec AM, Gay RE, Kolling C, Lin LL, Gay S, Tak PP, Prinjha RK, Ospelt C, Reedquist KA. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. Annals of the rheumatic diseases. 2016 Feb 1;75(2):422-9.
27.Abbasi M, Mousavi MJ, Jamalzehi S, Alimohammadi R, Bezvan MH, Mohammadi H, Aslani S. Strategies toward rheumatoid arthritis therapy; the old and the new. Journal of cellular physiology. 2019 Jul;234(7):10018-31.
28.Frisell T, Saevarsdottir S, Askling J. Family history of rheumatoid arthritis: an old concept with new developments. Nature Reviews Rheumatology. 2016 Jun;12(6):335-43.
29.Conigliaro P, D'Antonio A, Pinto S, Chimenti MS, Triggianese P, Rotondi M, Perricone R. Autoimmune thyroid disorders and rheumatoid arthritis: A bidirectional interplay. Autoimmunity Reviews. 2020 Jun 1;19(6):102529.
30.van der Woude D, van der Helm-van AH. Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Practice & Research Clinical Rheumatology. 2018 Apr 1;32(2):174-87.
31.van der Woude D, van der Helm-van AH. Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Practice & Research Clinical Rheumatology. 2018 Apr 1;32(2):174-87.
32.Suzuki A, Kochi Y, Okada Y, Yamamoto K. Insight from genome-wide association studies in rheumatoid arthritis and multiple sclerosis. FEBS letters. 2011 Dec 1;585(23):3627-32.
33.Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA—the citrullinated enolase connection. Nature Reviews Rheumatology. 2010 Dec;6(12):727-30.
34.Singh JA, Saag KG, Bridges Jr SL, Akl EA, Bannuru RR, Sullivan MC, Vaysbrot E, McNaughton C, Osani M, Shmerling RH, Curtis JR. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis & rheumatology. 2016 Jan;68(1):1-26.
35.Scott DL. Biologics-based therapy for the treatment of rheumatoid arthritis. Clinical Pharmacology & Therapeutics. 2012 Jan;91(1):30-43.
36.Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. The Lancet. 2017 Jun 10;389(10086):2338-48.
37.McInnes IB, Schett G. Pathogenetic insights from the treatment of

rheumatoid arthritis. The Lancet. 2017 Jun 10;389(10086):2328-37.

38.Bullock J, Rizvi SA, Saleh AM, Ahmed SS, Do DP, Ansari RA, Ahmed J. Rheumatoid arthritis: a brief overview of the treatment. Medical Principles and Practice. 2018;27(6):501-7.

39.Oliver J, Plant D, Webster AP, Barton A. Genetic and genomic markers of anti-TNF treatment response in rheumatoid arthritis. Biomarkers in Medicine. 2015 Jun;9(6):499-512.

40.Rein P, Mueller RB. Treatment with biologicals in rheumatoid arthritis: an overview. Rheumatology and therapy. 2017 Dec;4(2):247-61.

41.Zampeli E, Vlachoyiannopoulos PG, Tzioufas AG. Treatment of rheumatoid arthritis: unraveling the conundrum. Journal of autoimmunity. 2015 Dec 1;65:1-8.

42.Taylor PC, Abdul Azeez M, Kiriakidis S. Filgotinib for the treatment of rheumatoid arthritis. Expert Opinion on Investigational Drugs. 2017 Oct 3;26(10):1181-7.

43.Kerschbaumer A, Sepriano A, Smolen JS, van der Heijde D, Dougados M, van Vollenhoven R, McInnes IB, Bijlsma JW, Burmester GR, de Wit M, Falzon L. Efficacy of pharmacological treatment in rheumatoid arthritis: a systematic literature research informing the 2019 update of the EULAR recommendations for management of rheumatoid arthritis. Annals of the rheumatic diseases. 2020 Jun 1;79(6):744-59.

44.Stricker SH, Köferle A, Beck S. From profiles to function in epigenomics. Nature Reviews Genetics. 2017 Jan;18(1):51-66.

45.Marchal C, Miotto B. Emerging concept in DNA methylation: role of transcription factors in shaping DNA methylation patterns. Journal of cellular physiology. 2015 Apr;230(4):743-51.

46.Nair N, Wilson AG, Barton A. DNA methylation as a marker of response in rheumatoid arthritis. Pharmacogenomics. 2017 Sep;18(14):1323-32.

47.Renauer P, Coit P, Jeffries MA, Merrill JT, McCune WJ, Maksimowicz-McKinnon K, Sawalha AH. DNA methylation patterns in naïve CD4+ T cells identify epigenetic susceptibility loci for malar rash and discoid rash in systemic lupus erythematosus. Lupus science & medicine. 2015 Sep 1;2(1):e000101.

48.Blanch M, Mosquera JL, Ansoleaga B, Ferrer I, Barrachina M. Altered mitochondrial DNA methylation pattern in Alzheimer disease–related pathology and in Parkinson disease. The American journal of pathology. 2016 Feb 1;186(2):385-97.

49.Ouchi K, Takahashi S, Yamada Y, Tsuji S, Tatsuno K, Takahashi H, Takahashi N, Takahashi M, Shimodaira H, Aburatani H, Ishioka C. DNA methylation status as a biomarker of anti-epidermal growth factor receptor treatment for metastatic colorectal cancer. Cancer science. 2015 Dec;106(12):1722-9.

50.Nervi C, De Marinis E, Codacci-Pisanelli G. Epigenetic treatment of solid tumours: a review of clinical trials. Clinical epigenetics. 2015 Dec;7(1):1-20.

51.Cribbs A, Feldmann M, Oppermann U. Towards an understanding of the role of DNA methylation in rheumatoid arthritis: therapeutic and diagnostic implications. Therapeutic advances in musculoskeletal disease. 2015 Oct;7(5):206-19.

52.Jin Z, Liu Y. DNA methylation in human diseases. Genes & diseases. 2018 Mar 1;5(1):1-8.

53.Sun B, Hu L, Luo ZY, Chen XP, Zhou HH, Zhang W. DNA methylation perspectives in the pathogenesis of autoimmune diseases. Clinical Immunology. 2016 Mar 1;164:21-7.

54.de Andres MC, Perez-Pampin E, Calaza M, Santaclara FJ, Ortea I, Gomez-Reino JJ, Gonzalez A. Assessment of global DNA methylation in peripheral blood cell subpopulations of early rheumatoid arthritis before and after methotrexate. Arthritis research & therapy. 2015 Dec;17(1):1-9.

55.Ishida K, Kobayashi T, Ito S, Komatsu Y, Yokoyama T, Okada M, Abe A, Murasawa A, Yoshie H. Interleukin-6 gene promoter methylation in rheumatoid arthritis and chronic periodontitis. Journal of periodontology. 2012 Jul;83(7):917-25.

56.Park SH, Kim SK, Choe JY, Moon Y, An S, Park MJ, Kim DS. Hypermethylation of EBF3 and IRX1 genes in synovial fibroblasts of patients with rheumatoid arthritis. Molecules and cells. 2013 Apr;35(4):298-304.

57.Hammaker D, Whitaker JW, Maeshima K, Boyle DL, Ekwall AK, Wang W, Firestein GS. LBH gene transcription regulation by the interplay of an enhancer risk allele and DNA methylation in rheumatoid arthritis. Arthritis & rheumatology. 2016 Nov;68(11):2637-45.

58.Guderud K, Sunde LH, Flåm ST, Mæhlen MT, Mjaavatten MD, Lillegraven S, Aga AB, Evenrød IM, Norli ES, Andreassen BK, Franzenburg S. Rheumatoid Arthritis Patients, Both Newly Diagnosed and Methotrexate Treated, Show More DNA Methylation Differences in CD4+ Memory Than in CD4+ Naïve T Cells. Frontiers in immunology. 2020:194.

59.Guo S, Zhu Q, Jiang T, Wang R, Shen Y, Zhu X, Wang Y, Bai F, Ding Q, Zhou X, Chen G. Genome-wide DNA methylation patterns in CD4+ T cells from Chinese Han patients with rheumatoid arthritis. Modern rheumatology. 2017 May 4;27(3):441-7.

60.Sellars M, Huh JR, Day K, Issuree PD, Galan C, Gobeil S, Absher D, Green MR, Littman DR. Regulation of DNA methylation dictates Cd4 expression during the development of helper and cytotoxic T cell lineages. Nature immunology. 2015 Jul;16(7):746-54.

61.Viatte S, Plant D, Han B, Fu B, Yarwood A, Thomson W, Symmons DP, Worthington J, Young A, Hyrich KL, Morgan AW. Association of HLA-DRB1 haplotypes with rheumatoid arthritis severity, mortality, and treatment response. Jama. 2015 Apr 28;313(16):1645-56.

62.Viatte S, Plant D, Bowes J, Lunt M, Eyre S, Barton A, Worthington J. Genetic markers of rheumatoid arthritis susceptibility in anti-citrullinated peptide antibody negative patients. Annals of the rheumatic diseases. 2012 Dec 1;71(12):1984-90.

63.Viatte S, Massey J, Bowes J, Duffus K, arcOGEN Consortium, Eyre S, Barton A, Worthington J, Loughlin J, Arden N, Birrell F. Replication of associations of genetic loci outside the hla region with susceptibility to anti–cyclic citrullinated peptide–negative rheumatoid arthritis. Arthritis & rheumatology. 2016 Jul;68(7):1603-13.

64.Han GG, Lee JY, Jin GD, Park J, Choi YH, Kang SK, Chae BJ, Kim EB, Choi YJ. Tracing of the fecal microbiota of commercial pigs at five growth stages from birth to shipment. Scientific reports. 2018 Apr 16;8(1):1-9.

65.Lange CP, Campan M, Hinoue T, Schmitz RF, van der Meulen-de Jong AE, Slingerland H, Kok PJ, van Dijk CM, Weisenberger

DJ, Shen H, Tollenaar RA. Genome-scale discovery of DNA-methylation biomarkers for blood-based detection of colorectal cancer. PloS one. 2012 Nov 28;7(11):e50266.

66.Nair N, Wilson AG, Barton A. DNA methylation as a marker of response in rheumatoid arthritis. Pharmacogenomics. 2017 Sep;18(14):1323-32.

67.Plant D, Webster A, Nair N, Oliver J, Smith SL, Eyre S, Hyrich KL, Wilson AG, Morgan AW, Isaacs JD, Worthington J. Differential methylation as a biomarker of response to etanercept in patients with rheumatoid arthritis. Arthritis & Rheumatology. 2016 Jun;68(6):1353-60.

68.Maeshima K, Stanford SM, Hammaker D, Sacchetti C, Zeng LF, Ai R, Zhang V, Boyle DL, Muench GR, Feng GS, Whitaker JW. Abnormal PTPN11 enhancer methylation promotes rheumatoid arthritis fibroblast-like synoviocyte aggressiveness and joint inflammation. JCI insight. 2016 May 19;1(7).

69.Svendsen AJ, Gervin K, Lyle R, Christiansen L, Kyvik K, Junker P, Nielsen C, Houen G, Tan Q. Differentially methylated DNA regions in monozygotic twin pairs discordant for rheumatoid arthritis: an epigenome-wide study. Frontiers in immunology. 2016 Nov 17;7:510.

70.Karouzakis E, Raza K, Kolling C, Buckley CD, Gay S, Filer A, Ospelt C. Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. Scientific reports. 2018 May 9;8(1):1-6.

71.Svendsen AJ, Gervin K, Lyle R, Christiansen L, Kyvik K, Junker P, Nielsen C, Houen G, Tan Q. Differentially methylated DNA regions in monozygotic twin pairs discordant for rheumatoid arthritis: an epigenome-wide study. Frontiers in immunology. 2016 Nov 17;7:510.

72.Tabares P, Berr S, Langenhorst D, Sawitzki B, Ten Berge I, Tony HP, Hünig T. Short-term cytokine stimulation reveals regulatory T cells with down-regulated Foxp3 expression in human peripheral blood. European journal of immunology. 2018 Feb;48(2):366-79.

73.Moosavi A, Ardekani AM. Role of epigenetics in biology and human diseases. Iranian biomedical journal. 2016 Nov;20(5):246.

74.Availableonline:https://www.epigenomics.com/wpcontent/uploads/2016/06/approval_pm_eng.pdf (accessed on 22 August 2016).

75.Nair N, Plant D, Verstappen SM, Isaacs JD, Morgan AW, Hyrich KL, Barton A, Wilson AG, MATURA investigators. Differential DNA methylation correlates with response to methotrexate in rheumatoid arthritis. Rheumatology. 2020 Jun 1;59(6):1364-71.

http://pmjournal.ir Review Article

Winter 2022, Volume 7, Issue 24 (8-13)

Epithelial-mesenchymal transition and its role in breast cancer metastasis

Avan Saeed Mohammed[1], Ghazal Ghajar[2*]

[1] Department of Biology, College of Science, University of Duhok, Duhok,Iraq.
[2]Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.

**Corresponding author: Ghazal Ghajari, Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran. Email: ghajari.ghazal74@ gmail.com*

DOI: 10.22034/pmj.2022.252439

Submitted: 2021-11-28
Accepted: 2022-02-25

Keywords:
Epithelial
Mesenchymal Transition
EMT
Breast Cancer
Signaling Pathway

Abstract:

Breast cancer is the most common cancer in women and distant site metastasis is the main cause of death in breast cancer patients. Epithelial-mesenchymal transition (EMT) is defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. EMT is a vital process for large-scale cell movement during morphogenesis at the time of embryonic development. Tumor cells usurp this developmental program to execute the multi-step process of tumorigenesis and metastasis. Understanding the biological intricacies of the EMT may provide important insights that lead to the development of therapeutic targets in pre-invasive and invasive breast cancer, and could be used as biomarkers for identifying tumor subsets with greater chances of recurrence, metastasis, and therapeutic resistance leading to death. The purpose of this article is to investigate the association between EMT and breast cancer.

INTRODUCTION

Breast cancer is the most common cancer in women and ranks second among cancer deaths in women (1). Developing metastasis is the main cause of death in breast cancer patients (2). The intrinsic classification of Perou and Sorlie1, reported in 2000, distinguished four subtypes of breast cancer: luminal A and luminal B (expressing the estrogen receptor (ER)), basal-like and human epidermal growth factor receptor 2 (HER2)- enriched (without ER expression). Basal-like breast cancer cells are constitutively more invasive. In addition, HER2-enriched tumors are also more likely to develop metastatic disease (3). In 2018, an estimated 2.1 million women were newly diagnosed with breast cancer, approximately one new case diagnosed every 18 seconds; additionally, 626,679 women with breast cancer died (4).

The epithelial to mesenchymal transition (EMT) is a complex program in which epithelial cells acquire a mesenchymal phenotype and motility through a cascade of biological events (5). The EMT process involves the formation of motile cells from epithelial cells that are not themselves motile such as the mesenchymal cells (6). For almost two decades the prospect that recapitulation of the developmental process called epithelial to mesenchymal transition (EMT) may play an important role in carcinoma progression has been vigorously debated. The concept of EMT was developed in the field of embryology but has recently been extended to tumor progression and metastasis (7). There are three types of EMT programs; type 1 relates to embryogenesis, gastrulation, and neural crest formation; type 2 is related to tissue regeneration and wound healing; and type 3 is associated with malignancy, invasion, and metastasis (8). The first EMT event after implantation occurs at gastrulation when the embryonic layers are defined. The EMTs that are associated with implantation, embryo formation, and organ development are organized to generate diverse cell types that share common mesenchymal phenotypes. This class of EMTs neither causes fibrosis nor induces an invasive phenotype resulting in systemic spread via the circulation (8, 9). Type 2 EMT is represented by EMT re-engaged in the context of inflammation. It is associated with wound healing, tissue regeneration, and organ fibrosis. In this process, tissue fibroblasts are generated from epithelial or endothelial cells during injury and chronic inflammation (10). Type 3 EMTs occur in neoplastic cells that have previously undergone genetic and epigenetic changes, specifically in genes that favor clonal outgrowth and

the development of localized tumors. These changes, notably affecting oncogenes and tumor suppressor genes, conspire with the EMT regulatory circuitry to produce outcomes far different from those observed in the other two types of EMT (11). Type 3 EMT plays, in fact, a crucial role in the development process of localized tumors: the cancer cells convert to a mesenchymal phenotype to move to the invasive front of the tumors (12). Although these three types of EMT represent considerably different biological processes, some genetic elements and mechanisms of regulation may be similar and well conserved. Studies suggest that in some situations, following the migration of a cancer cell that has undergone EMT to a distant site, a reverse process of mesenchymal to epithelial transition (MET) occurs. The MET is a state when a mesenchymal tumor cell reverts to the epithelial phenotype, especially in distant metastatic sites (13).

Regulation of epithelial cell plasticity during EMT is increasingly implicated in the progression of carcinoma (14). Epithelial cells that undergo EMT lose their epithelial cell characteristics to acquire a mesenchymal phenotype and become migratory and invasive (15). The existence of EMT changes in clinical breast cancer places EMT at the center of malignancy. Because breast cancer is a heterogeneous disease in terms of tumor histology, clinical presentation, and response to therapy and because breast cancer-related deaths are primarily due to metastatic progression, a deeper understanding of the mechanisms that underlie the EMT program in breast tumors will lead to the development of better therapeutic strategies (16, 17).

Molecular mechanisms of epithelial to mesenchymal transition

The process of EMT requires the coordination of a complex network of extracellular and intracellular signals involving factors for initiation and feedback mechanisms for a continuum of changes that occur within cells during the transition from a less epithelial to a more mesenchymal phenotype (18). EMT can be induced during in vitro cell culture under the influence of extracellular matrix components and growth factors, such as scatter factor/hepatocyte growth factor, transforming growth factor-beta (TGFbeta), epithelial growth factor family members, insulin-like growth factors 1 and 2, and fibroblast growth factors (19). Hypoxia also induces EMT. The EMT is generally induced in epithelial cells by heterotypical signals, specifically those released by the mesenchymal cells that constitute the stroma of normal and neoplastic tissues. Signal transduction pathways such as Hedgehog, Wnt, Notch, and integrin signaling can also coordinate EMT programs. Several transcription factors induce EMT through transcriptional control of E-cadherin, including SNAI1 (zinc finger protein snail 1), SNAI2, ZEB1 (zinc finger E-box-binding homeobox 1), ZEB2, TWIST, FOXC1 (forkhead box protein 1), FOXC2, TCF3 (transcription factor 3 - also known as E47), and GSC (homeobox protein goosecoid) (20, 21).

Wnt pathway

The Wnt pathway plays a critical role in cell proliferation and oncogenesis. Beta-catenin is a downstream signaling molecule that is activated by WNT signaling (22). Beta-catenin has a dual role in EMT:

1.A bridge to enhance cell-cell adhesion when bound to cadherin complexes in adherens junctions

2.A transcription cofactor with DNA-binding proteins of the Tcell factor (TCF)/lymphoid enhancer factor (LEF) family (22, 23).

Therefore, beta-catenin is considered an appropriate and ideal target for studying the molecular basis of EMT and malignant cancer formation (24). The Wnt pathway and loss of E-cadherin from adherens junctions activate b-catenin, which in turn induces several EMT-inducing transcription factors as well, such as Slug, Twist1, and Goosecoid. The Wnt and tyrosine kinase receptor pathways also modulate Snail nuclear transport and degradation through GSK3b (25). Several up-regulated target genes of the Wnt/b-catenin signaling pathway, such as fibronectin4 and matrix metalloproteinase-7 (MMP-7), 5 are correlated with mesenchymal phenotype and invasiveness (26).

Notch signaling pathway

It is believed that the processes that govern the acquisition of EMT are stimulated and regulated by many stimuli, signal transduction pathways, and transcription factors. Recently, the Notch signal pathway has been found to be a key regulator in the induction of EMT (27). The notch signaling path has two important roles: 1. maintaining a balance between cell proliferation, differentiation, and apoptosis 2. Preservation of progenitor cell population and determination of cell fate (28). The Notch pathway is induced by and required for TGF-b-induced EMT and modulates the EMT process by activating the nuclear factor-kB (NF-kB) pathway or by modulating the activity of TGF-b signaling itself. Notch activation in endothelial cells results in phenotypic, morphological, and functional changes consistent with mesenchymal transformation. Notch signaling is initiated when a Notch ligand binds to an adjacent Notch receptor between two neighboring cells. In mammals, the Notch family consists of 4 transmembrane receptors (Notch-1-4) and 5 ligands [Delta-like protein (Delta-like) 1, Delta-like 3, Delta-like 4, protein jagged (Jagged) 1, and Jagged-2] (29, 30).

Hypoxia has received considerable attention as an inducer of tumor metastasis. Notch serves as a critical intermediate in conveying the hypoxic response

into EMT. Recent research shows that hypoxia-induced Jagged 2 promotes breast cancer metastasis and self-renewal of cancer stem-like cells (31).

Hedgehog signaling pathway

Hh signaling controls tissue construction and remodeling by regulating the viability and migratory activity of various types of Hh responsive progenitor cells. The Hh signaling pathway is considered to have a vital role in vertebrate development, the homeostatic process, and tumorigenesis. Recent studies have found that the Hh signaling pathway is abnormally activated in small cell lung, breast, prostate, colorectal and pancreatic cancer. Significantly, the Sonic Hh (Shh) signaling pathway has been shown to contribute to tumor metastasis by inducing EMT in breast cancer (32, 33).

The Hh protein family consists of Hh ligands (Sonic-SHH, Indian IHH, and Desert-DHH) which bind cell surface transmembrane receptor Patched (PTCH) (34). Upon activation, these molecules bind with the transmembrane receptor known as Patched1 (PTCH1). Binding-induced alteration in structural conformation of PTCH1 leads to release of Smoothened (SMO) which mediates downstream activation of GLI family. SHH-mediated activation of GLI1 induces Snail, a major driver of EMT in basal cell carcinoma21. Furthermore, GLI1 stimulates Snail, represses E-cadherin, and enhances nuclear translocation of β-catenin to induce EMT in skin cancers22. SHH-GLI1-Snail axis stimulates EMT in ovarian, pancreatic, and neuroendocrine cancers as well. However, the association of Hedgehog signaling with EMT markers needs further exploration in breast cancer. Hedgehog signaling mediates EMT during embryonic development as well as cancer metastasis. During mammary morphogenesis, the Hedgehog pathway acts as a key regulator in epithelial-mesenchymal interactions and tubule maturation (35, 36).

TGF-β Signaling

The most classical experimental model is the induction of EMT by TGF-β in epithelial cell culture. Upon TGF-β induction, the type II receptor (TGFR2) is activated and phosphorylates the type I receptor (TGFR1), thereby activating the Smad pathway and inducing EMT (37, 38).

EMT and Its Plasticity Features

Breast cancer originates from epithelial tissue, which includes the following features: intact tight and adherent junction and “sheet-like” morphology with apical-basal polarity. Mesenchymal cells are characterized by loosely associated cells and disorganized cellular layers that lack polarity and tight cell-to-cell adhesion proteins (39). The morphology of mesenchymal cells is better adapted to cell migration. EMT is typically characterized by the loss of epithelial cell adhesion protein E-cadherin and cytokeratins and the gain of mesenchymal-associated molecules N-cadherin, Vimentin, and fibronectin. The process is described as “cadherin switching”, i.e., down-regulation of E-cadherin and up-regulation of N-cadherin (40) (Figure 1).

EMT and Breast Cancer

Both classical histological and molecular subtyping of breast cancers have identified the impact of the EMT on breast cancer prognosis (41). Clinical-histologic studies of basal-like breast cancers show that they are among the most aggressive and deadly breast cancer subtypes, displaying a high metastatic ability associated with mesenchymal features (42). The metastatic process includes different steps through which tumor cells have to exit from the primary tumor evading the basement membrane and the surrounding tissue, enter the bloodstream or lymphatics, migrate to a distant site

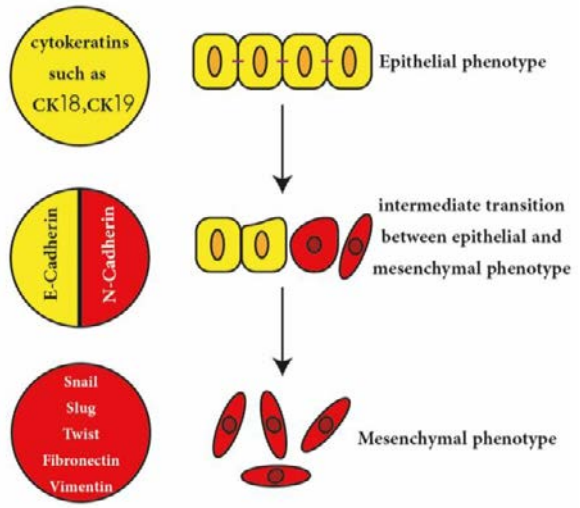

Figure 1. Schematic of the epithelial to mesenchymal transition (EMT).

and colonize to form metastasis. The other crucial step for metastasis is cancer dissemination, which involves circulating tumor cells (CTCs). Even the presence CTCs in the blood of metastatic breast cancer patients has been shown to be an independent predictor of progression-free survival and overall survival, the nature and the biological feature of these cells are still poorly understood (43).

Numerous mediators of EMT have been discovered, including transcription factors, signaling molecules, and microRNAs (miRNAs). A common theme among oncogenic EMT inducers is their crucial role in type I EMT. It has become increasingly evident that improper activation of developmental EMT inducers in adults gives rise to an out-of-context EMT-like program that contributes to the progression of breast cancer, as well as other cancers (44). A few examples of transcription factors and signaling pathways known to play a role in both type I and type III EMT include SIX1, Twist1 (TWIST1), Snail1 (SNAI1), and Ladybird homeobox (LBX1), and the Wnt and transforming growth factor-β (TGF-β) signaling pathways. Much of the evidence for a possible role of EMT in the progression of breast cancer has arisen from studies of in vitro culture of epithelial cell lines. However, EMT has largely been described as a cell culture phenomenon without direct clinical evidence or clear molecular markers in breast carcinoma (45, 46).

studies from Papadaki et al. and Kallergi et al. also detected EMT markers such as pan-cytokeratin, Twist, and Vimentin in CTCs from early and metastatic breast cancer patients. Although specific markers for CTCs still require validation within a larger clinical setting, current evidence supports the hypothesis that EMT is involved in the metastatic process in human breast cancer. However, due to the biological heterogeneity of CTCs, the technical difficulty still remains in the detection and isolation of CTCs (47, 48).

Biomarkers for EMT in breast cancer

A variety of biomarkers have been used to demonstrate EMT in breast cancer such as:

•E-cadherin: A change in the expression of E-cadherin is the typical epithelial cell marker of EMT. Suppression of E-cadherin function or expression leads to mesenchymal morphology and increased cell migration and invasion (49).

•Cluster of differentiation (CD) 44: a cell-surface protein that modulates cellular signaling by forming co-receptor complexes with various receptor tyrosine kinases. It plays an important role in the metastasis of breast cancer (50).

•Discoidin domain receptor 2 (DDR2): an atypical receptor tyrosine kinase. It is the collagen-specific receptor that reflects adaptation to the altered ECM microenvironment associated with the EMT. In breast cancer, DDR2 expression correlates with increased invasiveness, thus demonstrating its utility in identifying EMT (51).

•β-catenin: a cytoplasmic plaque protein that plays an important role in EMT (52).

•Vimentin: an intermediate filament that is used as a marker of mesenchymal cells to distinguish them from epithelial cells (53).

•α-smooth muscle actin (α-SMA): one of the six actin family members. Cells expressing α-SMA contribute to EMT in embryogenesis and to wound healing in normal epithelial cells. In cancer, evidence that the EMT is associated with α-SMA is mostly confined to breast cancer, where α-SMA is largely detected in breast tumors of the ‘basal phenotype’ (54).

MicroRNAs

miRNAs are an evolutionarily conserved class of small non-coding RNAs that control gene expression by targeting mRNAs by binding to the 31-untranslated region (3′UTR), leading to reduced translation of proteins, or degradation of the target mRNAs (55). MicroRNAs (miRNAs) have recently been described as crucial regulators of EMT and metastasis. The most frequently cited EMT-related miRNAs are those belonging to the miR-200 family, which consists of miR-200a/b/c, miR-141, and miR-429 (56). The miR-200 family, which suppresses EMT drivers ZEB1 and ZEB2, is selectively expressed in the sarcomatous component of metaplastic breast cancers. Furthermore, overexpression of miR-29a suppressed the expression of tristetraprolin, a regulator of epithelial polarity and metastasis, and led to EMT and metastasis in cooperation with oncogenic Ras signaling. Another miRNA involved in breast cancer metastasis and invasion in the context of the EMT is miR-10b. miR-10b is associated with mesenchymal features and invasive properties in breast cancer when overexpressed, through translational inhibition of HOXD10 (transcription factor associated with Wilms tumor) and upregulation of RHOC protein levels, enabling matrix extracellular degradation. miR-506, which is a novel miRNA, was found to be significantly related to breast cancer patient survival. It suppressed the expression of mesenchymal markers in the MDA-MB-231 human breast cancer cell line (57, 58).

These powerful programmatic regulators are poised to become important predictive/prognostic markers (59).

CONCLUSION

This review summarizes the evidence for the growing implication of EMT in the progression of breast carcinoma. EMT is a complex, stepwise phenomenon that occurs during embryonic development and tumor progression and involves major reprogramming

of gene expression that leads to alterations in cell fate and behavior. During the EMT, tumor cells acquire invasive traits through overexpression, mutation, or amplification of oncogenes and also repression of tumor suppressors, leading to the aberrant expression of signaling pathways. Validating biomarkers related to EMT in patient models will be highly crucial for identifying patients at risk of developing drug resistance and metastasis. In closing, it is indisputable that studies related to oncogenic EMT have critically contributed to, and will continue to contribute to, our understanding of the most devastating aspect of breast cancer and metastatic dissemination (60).

REFERENCES

1.Waks AG, Winer EP. Breast cancer treatment: a review. Jama. 2019 Jan 22;321(3):288-300.
2.Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer: Targets and Therapy. 2019;11:151.
3.Britt KL, Cuzick J, Phillips KA. Key steps for effective breast cancer prevention. Nature Reviews Cancer. 2020 Aug;20(8):417-36.
4.Creighton CJ, Chang JC, Rosen JM. Epithelial-mesenchymal transition (EMT) in tumor-initiating cells and its clinical implications in breast cancer. Journal of mammary gland biology and neoplasia. 2010 Jun;15(2):253-60.
5.Gyamfi J, Lee YH, Eom M, Choi J. Interleukin-6/STAT3 signalling regulates adipocyte induced epithelial-mesenchymal transition in breast cancer cells. Scientific reports. 2018 Jun 11;8(1):1-3.
6.Fenizia C, Bottino C, Corbetta S, Fittipaldi R, Floris P, Gaudenzi G, Carra S, Cotelli F, Vitale G, Caretti G. SMYD3 promotes the epithelial–mesenchymal transition in breast cancer. Nucleic acids research. 2019 Feb 20;47(3):1278-93.
7.Olea-Flores M, Juárez-Cruz JC, Mendoza-Catalán MA, Padilla-Benavides T, Navarro-Tito N. Signaling pathways induced by leptin during epithelial–mesenchymal transition in breast cancer. International journal of molecular sciences. 2018 Nov;19(11):3493.
8.Scimeca M, Antonacci C, Colombo D, Bonfiglio R, Buonomo OC, Bonanno E. Emerging prognostic markers related to mesenchymal characteristics of poorly differentiated breast cancers. Tumor Biology. 2016 Apr;37(4):5427-35.
9.Yang F, Takagaki Y, Yoshitomi Y, Ikeda T, Li J, Kitada M, Kumagai A, Kawakita E, Shi S, Kanasaki K, Koya D. Inhibition of dipeptidyl peptidase-4 accelerates epithelial–mesenchymal transition and breast cancer metastasis via the CXCL12/CXCR4/mTOR axis. Cancer research. 2019 Feb 15;79(4):735-46.
10.Sethi S, Sarkar FH, Ahmed Q, Bandyopadhyay S, Nahleh ZA, Semaan A, Sakr W, Munkarah A, Ali-Fehmi R. Molecular markers of epithelial-to-mesenchymal transition are associated with tumor aggressiveness in breast carcinoma. Translational Oncology. 2011 Aug 1;4(4):222-6.
11.Zhang N, Zhang H, Liu Y, Su P, Zhang J, Wang X, Sun M, Chen B, Zhao W, Wang L, Wang H. SREBP1, targeted by miR-18a-5p, modulates epithelial-mesenchymal transition in breast cancer via forming a co-repressor complex with Snail and HDAC1/2. Cell Death & Differentiation. 2019 May;26(5):843-59.
12.Thiery JP, Lim CT. Tumor dissemination: an EMT affair. Cancer cell. 2013 Mar 18;23(3):272-3.
13.Li GY, Wang W, Sun JY, Xin B, Zhang X, Wang T, Zhang QF, Yao LB, Han H, Fan DM, Yang AG. Long non-coding RNAs AC026904. 1 and UCA1: a "one-two punch" for TGF-β-induced SNAI2 activation and epithelial-mesenchymal transition in breast cancer. Theranostics. 2018;8(10):2846.
14.Hass R, von der Ohe J, Ungefroren H. Potential role of MSC/cancer cell fusion and EMT for breast cancer stem cell formation. Cancers. 2019 Oct;11(10):1432.
15.Khaled N, Bidet Y. New insights into the implication of epigenetic alterations in the EMT of triple negative breast cancer. Cancers. 2019 Apr;11(4):559.
16.Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. The Journal of clinical investigation. 2009 Jun 1;119(6):1420-8.
17.He X, Xue M, Jiang S, Li W, Yu J, Xiang S. Fucoidan promotes apoptosis and inhibits emt of breast cancer cells. Biological and Pharmaceutical Bulletin. 2019 Mar 1;42(3):442-7.
18.Roussos ET, Keckesova Z, Haley JD, Epstein DM, Weinberg RA, Condeelis JS. AACR special conference on epithelial-mesenchymal transition and cancer progression and treatment.
19.Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, Stanger BZ, Yang J, Weinberg RA. Upholding a role for EMT in breast cancer metastasis. Nature. 2017 Jul;547(7661):E1-3.
20.Pires BR, Mencalha AL, Ferreira GM, de Souza WF, Morgado-Díaz JA, Maia AM, Corrêa S, Abdelhay ES. NF-kappaB is involved in the regulation of EMT genes in breast cancer cells. PloS one. 2017 Jan 20;12(1):e0169622.
21.Neelakantan D, Zhou H, Oliphant MU, Zhang X, Simon LM, Henke DM, Shaw CA, Wu MF, Hilsenbeck SG, White LD, Lewis MT. EMT cells increase breast cancer metastasis via paracrine GLI activation in neighbouring tumour cells. Nature communications. 2017 Jun 12;8(1):1-4.
22.Fuxe J, Vincent T, Garcia de Herreros A. Transcriptional crosstalk between TGFβ and stem cell pathways in tumor cell invasion: role of EMT promoting Smad complexes. Cell cycle. 2010 Jun 15;9(12):2363-74.
23.He X, Xue M, Jiang S, Li W, Yu J, Xiang S. Fucoidan promotes apoptosis and inhibits emt of breast cancer cells. Biological and Pharmaceutical Bulletin. 2019 Mar 1;42(3):442-7.
24.Huang P, Chen A, He W, Li Z, Zhang G, Liu Z, Liu G, Liu X, He S, Xiao G, Huang F. BMP-2 induces EMT and breast cancer stemness through Rb and CD44. Cell death discovery. 2017 Jul 17;3(1):1-2.
25.Hu T, Li C. Convergence between Wnt-β-catenin and EGFR signaling in cancer. Molecular cancer. 2010 Dec;9(1):1-7.
26.Lourenco AR, Ban Y, Crowley MJ, Lee SB, Ramchandani D, Du W, Elemento O, George JT, Jolly MK, Levine H, Sheng J. Differential contributions of pre-and post-EMT tumor cells in breast cancer metastasis. Cancer research. 2020 Jan 15;80(2):163-9.
27.Wang Z, Li Y, Kong D, H Sarkar F. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Current drug targets. 2010 Jun 1;11(6):745-51.
28.Leong KG, Niessen K, Kulic I, Raouf A, Eaves C, Pollet I, Karsan A. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. The Journal of experimental medicine. 2007 Nov 26;204(12):2935-48.
29.Shao S, Zhao X, Zhang X, Luo M, Zuo X, Huang S, Wang Y, Gu S, Zhao X. Notch1 signaling regulates the epithelial–mesenchymal transition and invasion of breast cancer in a Slug-dependent manner. Molecular cancer. 2015 Dec;14(1):1-7.
30.Suman S, Das TP, Damodaran C. Silencing NOTCH signaling causes growth arrest in both breast cancer stem cells and breast cancer cells. British journal of cancer. 2013 Nov;109(10):2587-96.
31.Hui M, Cazet A, Nair R, Watkins DN, O'Toole SA, Swarbrick A. The Hedgehog signalling pathway in breast development, carcinogenesis and cancer therapy. Breast Cancer Research. 2013 Apr;15(2):1-4.
32.Kolliopoulos C, Lin CY, Heldin CH, Moustakas A, Heldin P. Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFβ-induced EMT in breast cancer. Matrix Biology. 2019 Jul 1;80:29-45.
33.Addison JB, Voronkova MA, Fugett JH, Lin CC, Linville NC, Trinh B, Livengood RH, Smolkin MB, Schaller MD, Ruppert JM, Pugacheva EN. Functional hierarchy and cooperation of EMT master transcription factors in breast cancer metastasis. Molecular

Cancer Research. 2021 May 1;19(5):784-98.
34.Maroufi NF, Amiri M, Dizaji BF, Vahedian V, Akbarzadeh M, Roshanravan N, Haiaty S, Nouri M, Rashidi MR. Inhibitory effect of melatonin on hypoxia-induced vasculogenic mimicry via suppressing epithelial-mesenchymal transition (EMT) in breast cancer stem cells. European Journal of Pharmacology. 2020 Aug 15;881:173282.
35.Yin S, Cheryan VT, Xu L, Rishi AK, Reddy KB. Myc mediates cancer stem-like cells and EMT changes in triple negative breast cancers cells. PloS one. 2017 Aug 17;12(8):e0183578.
36.Feng XH, Derynck R. Specificity and versatility in TGF-β signaling through Smads. Annu. Rev. Cell Dev. Biol.. 2005 Nov 10;21:659-93.
37.Massagué J. TGFβ signalling in context. Nature reviews Molecular cell biology. 2012 Oct;13(10):616-30.
38.Drago-García D, Espinal-Enríquez J, Hernández-Lemus E. Network analysis of EMT and MET micro-RNA regulation in breast cancer. Scientific reports. 2017 Oct 19;7(1):1-7.
39.Wu HT, Zhong HT, Li GW, Shen JX, Ye QQ, Zhang ML, Liu J. Oncogenic functions of the EMT-related transcription factor ZEB1 in breast cancer. Journal of translational medicine. 2020 Dec;18(1):1-0.
40.Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Molecular oncology. 2011 Feb 1;5(1):5-23.
41.Micalizzi DS, Ford HL. Epithelial–mesenchymal transition in development and cancer. Future oncology. 2009 Oct;5(8):1129-43.
42.Hass R, von der Ohe J, Ungefroren H. Potential role of MSC/cancer cell fusion and EMT for breast cancer stem cell formation. Cancers. 2019 Oct;11(10):1432.
43.Mego M, Mani SA, Cristofanilli M. Molecular mechanisms of metastasis in breast cancer—clinical applications. Nature reviews Clinical oncology. 2010 Dec;7(12):693-701.
44.Micalizzi DS, Farabaugh SM, Ford HL. Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. Journal of mammary gland biology and neoplasia. 2010 Jun;15(2):117-34.
45.Kar R, Jha NK, Jha SK, Sharma A, Dholpuria S, Asthana N, Chaurasiya K, Singh VK, Burgee S, Nand P. A "NOTCH" deeper into the epithelial-to-mesenchymal transition (EMT) program in breast cancer. Genes. 2019 Dec;10(12):961.
46.Papadaki MA, Kallergi G, Zafeiriou Z, Manouras L, Theodoropoulos PA, Mavroudis D, Georgoulias V, Agelaki S. Co-expression of putative stemness and epithelial-to-mesenchymal transition markers on single circulating tumour cells from patients with early and metastatic breast cancer. BMC cancer. 2014 Dec;14(1):1-0.
47.Kallergi G, Papadaki MA, Politaki E, Mavroudis D, Georgoulias V, Agelaki S. Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. Breast Cancer Research. 2011 Jun;13(3):1-1.
48.Peng Y, Li H, Fu Y, Guo S, Qu C, Zhang Y, Zong B, Liu S. JAM2 predicts a good prognosis and inhibits invasion and migration by suppressing EMT pathway in breast cancer. International immunopharmacology. 2022 Feb 1;103:108430.
49.Brown RL, Reinke LM, Damerow MS, Perez D, Chodosh LA, Yang J, Cheng C. CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. The Journal of clinical investigation. 2011 Mar 1;121(3):1064-74.
50.Bill R, Christofori G. The relevance of EMT in breast cancer metastasis: Correlation or causality?. FEBS letters. 2015 Jun 22;589(14):1577-87.
51.Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. The Journal of clinical investigation. 2009 Jun 1;119(6):1429-37.
52.Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, Stanger BZ, Yang J, Weinberg RA. Upholding a role for EMT in breast cancer metastasis. Nature. 2017 Jul;547(7661):E1-3.
53.Khaled N, Bidet Y. New insights into the implication of epigenetic alterations in the EMT of triple negative breast cancer. Cancers. 2019 Apr;11(4):559.
54.Rhodes LV, Martin EC, Segar HC, Miller DF, Buechlein A, Rusch DB, Nephew KP, Burow ME, Collins-Burow BM. Dual regulation by microRNA-200b-3p and microRNA-200b-5p in the inhibition of epithelial-to-mesenchymal transition in triple-negative breast cancer. Oncotarget. 2015 Jun 30;6(18):16638.
55.Creighton CJ, Gibbons DL, Kurie JM. The role of epithelial–mesenchymal transition programming in invasion and metastasis: a clinical perspective. Cancer management and research. 2013;5:187.
56.Neelakantan D, Zhou H, Oliphant MU, Zhang X, Simon LM, Henke DM, Shaw CA, Wu MF, Hilsenbeck SG, White LD, Lewis MT. EMT cells increase breast cancer metastasis via paracrine GLI activation in neighbouring tumour cells. Nature communications. 2017 Jun 12;8(1):1-4.
57.Yu J, Xie F, Bao X, Chen W, Xu Q. miR-300 inhibits epithelial to mesenchymal transition and metastasis by targeting Twist in human epithelial cancer. Molecular cancer. 2014 Dec;13(1):1-2.
58.Xiang Y, Liao XH, Yu CX, Yao A, Qin H, Li JP, Hu P, Li H, Guo W, Gu CJ, Zhang TC. MiR-93-5p inhibits the EMT of breast cancer cells via targeting MKL-1 and STAT3. Experimental cell research. 2017 Aug 1;357(1):135-44.
59.Yu J, Xie F, Bao X, Chen W, Xu Q. miR-300 inhibits epithelial to mesenchymal transition and metastasis by targeting Twist in human epithelial cancer. Molecular cancer. 2014 Dec;13(1):1-2.
60.Campbell K. Contribution of epithelial-mesenchymal transitions to organogenesis and cancer metastasis. Current opinion in cell biology. 2018 Dec 1;55:30-5.

http://pmjournal.ir Review Article

Winter 2022, Volume 7, Issue 24(14-18)

Androgens in Prostate Cancer: A Review Articles

Nazar Shabila[1], Ghasem Ghorbani Vale Zaghard[2*]

[1]Department of Community Medicine, Hawler Medical University, Erbil, Iraq.

[2]Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran.

**Corresponding author: Ghasem Ghorbani Vale Zaghard, Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran. Email: ghasem71.ghorbani@gmail.com.*

DOI: 10.22034/pmj.2022.252440

Submitted: 2021-11-16
Accepted: 2022-02-07

Keywords:
Androgen
Prostate Cancer
Testosterone
AR
Androgen Deprivation Therapy

Abstract:

Prostate cancer represents a major health problem in men worldwide. Androgens are required for the growth and maintenance of the prostate. The androgen-signaling axis plays a pivotal role in the pathogenesis of prostate cancer. Clinical treatments that target steroidogenesis and the androgen receptor (AR) successfully postpone disease progression. The role of androgens and AR signaling has been well characterized in metastatic prostate cancer, where it has been shown that prostate cancer cells are exquisitely adept at maintaining functional AR signaling to drive cancer growth. This review summarizes the current information regarding the role of androgens in prostate cancer.

INTRODUCTION

Prostate cancer is one of the most common cancers observed in men globally and accounts for 7% of newly diagnosed cancers in men globally (15% in developed regions) (1). In addition, more than 1.2 million new cases are diagnosed and global prostate cancer-related deaths exceed 350,000 annually, making it one of the leading causes of cancer-associated death in men. Prostate cancer can often be cured with definitive local intervention (surgery or radiation), but once cancer metastasizes, it is incurable (2). Our most effective regimens for treating metastatic prostate cancer have arisen from pioneering experiments, in which suppression of testicular testosterone production was shown to cause tumor regression. Prostate cancer risk increases strongly with age and >85% of newly diagnosed individuals are >60 years of age (3).

It is a very well-known fact that prostate cancer and male sex hormones are strongly interrelated. The male sex hormones are collectively known as androgens, a word derived from the Greek Andros, man, and gennan, to produce (4). Since Huggins and Hodges first demonstrated the responsiveness of prostate cancer to androgen deprivation, it has been clear that prostate cancer is dependent on androgen receptor activation (AR) for growth and survival (5). Androgens bind to the androgen receptor (AR) to activate AR signaling and promote the development of prostate cancer. The observations that prostate development depends on androgens and AR signaling and that nearly all prostate cancer cells are critically dependent upon androgens and AR signaling for growth is the basis for the hypothesis that androgens and AR signaling play a causative role in prostate tumorigenesis; however, little is known about this process (6). It is now evident that the majority of prostate cancers express the androgen receptor (AR) throughout the disease, and, in recent years, deeper interrogation into the molecular basis of androgen signaling has offered a better understanding of how AR specifically directs cancer cell behavior (7). The initially androgen-dependent prostate cancer tumor eventually progresses regardless of the patient's hormonal status. Hence, prostate cancer, like most cancers, progresses and recurs after hormone therapy and chemotherapy to a lethally resistant phenotype despite initially encouraging therapeutic responses. However, treatment resistance is inevitable, and prostate cancer can continuously develop even without testosterone from the testes. Thus, castration-resistant prostate cancer (CRPC) was deemed to be hormone-refractory prostate cancer (HRPC) (8). Androgen deprivation therapy (ADT) was first used by Huggins and Hodges to efficiently postpone the development of prostate cancer in clinical settings. Since then, the androgen-AR-signaling axis has moved to the center stage of prostate cancer management (9).

The purpose of this article is to review the mechanisms of androgen action and its relation to prostate cancer.

Mechanism of androgen action

Androgens are responsible for the differentiation and maturation of the male sexual organs as well as the male secondary sexual characteristics (10). The biosynthesis of all steroid hormones begins with 27-carbon cholesterol, which undergoes stepwise modification by a small complement of enzymes first to 21- carbon steroids (progestins) and subsequently to 19-carbon androgens (11). Testosterone is the most important circulating androgen and its production by the testis is regulated by negative feedback regulated by the luteinizing hormone (LH) and the luteinizing hormone-releasing hormone (LHRH) via the gonad-hypothalamus- pituitary axis. Their actions are mediated by the androgen receptor (AR), a ligand-dependent nuclear transcription factor (12). The activity of the AR is controlled at multiple stages due to ligand binding and induced structural changes assisted by the fold some, compartmentalization, recruitment of coregulators, posttranslational modifications, and chromatin remodeling, leading to subsequent transcription of androgen-responsive target genes (13). The androgen receptor (AR) can be weakly stimulated by high concentrations of multiple steroids including weak androgens produced by the adrenal gland such as androst-4-ene-3, 17-dione (androstenedione), and dehydroepiandrosterone. Androgen binding to AR leads to nuclear translocation of AR, and a ligand-bound AR protein forms a complex with transcriptional coregulators to regulate target gene transcription. AR is expressed in various tissues to achieve specific physiological functions. One target of androgens is skeletal muscle, and supraphysiological doses of androgens increase muscle mass and strength. The AR weighs ~ 110 kDa and is found on the X chromosome. The activated steroid-receptor complex binds to specific DNA segments called hormone-responsive elements, which are located in promoters of hormone-regulated genes. The receptor-DNA complex will associate with transcriptional components and co-activators to promote gene transcription (13, 14, and 15).

The Androgen Signaling Axis

Testosterone and DHT mediate their actions by binding to AR, a 110-kDa phosphoprotein and a member of the nuclear receptor transcription factor superfamily (16) (Fig. 1). The gene for AR is located on the X chromosome (q11- 12) and expresses a 110-kDa protein that is 919 amino acids in length, encoded by eight exons. Common in resemblance to other nuclear hormone receptors, the structure of AR is comprised of four separate functionally distinct domains: an amino-terminal domain (NTD),

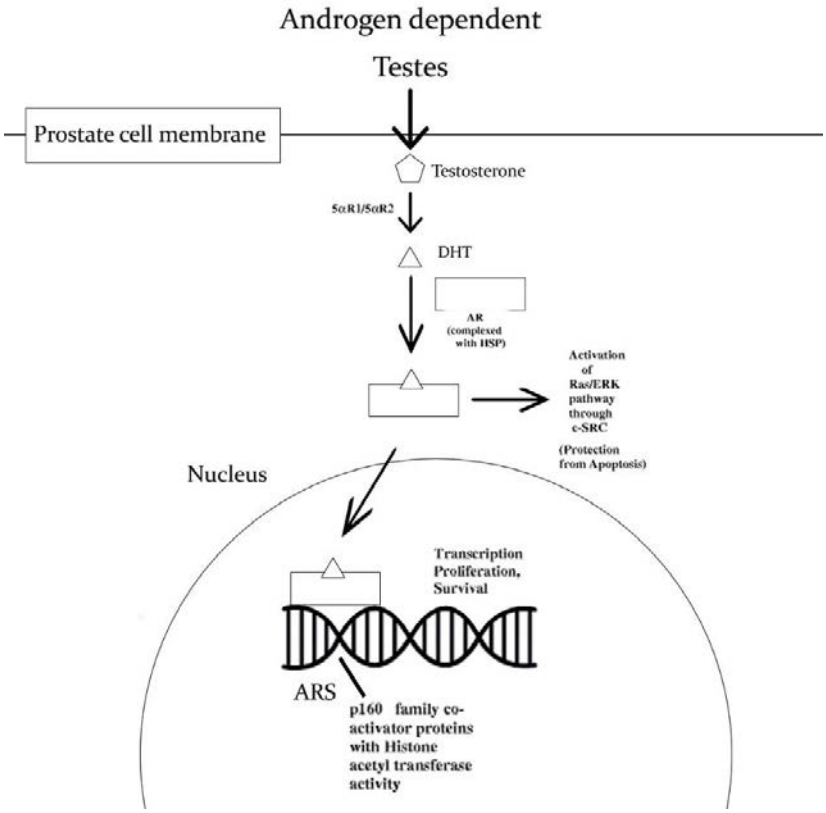

Fig. 1. Schematic representation of androgen action in prostate cancer.

a carboxy-terminal ligand-binding domain (LBD), a DNA-binding domain (DBD), and a flexible hinge region, which joins the LBD and the DBD (17). DHT is stronger than testosterone because it breaks down more slowly than AR and forms a compound in AR that is more resistant to degradation. In the basal, unliganded state, the AR exists in the cytoplasm in a complex with heat shock proteins (Hsp) and immunophilin chaperones such as Hsp90, 70, 56, and 23 (18). This complex is critical for the generation of a high-affinity, ligand-binding conformation of the AR. By binding to androgens, changes in the composition of this complex occur and lead to the transfer of AR to the nucleus. AR is dimerized in the nucleus and binds to androgen response elements (AREs) that are targeted in the promoter and enhancer regions of the gene. In order to activate the transcription of target genes, ARE-bound AR relies on the activity of coactivator proteins, which include the p160 family (SRC-1, GRIP1/ TIF2, RAC3/pCIP/ACTR/AIB1/TRAM1), P/CAF, CBP, Tip60, and p300 (19). These coactivators have the inherent activity of histone acetyltransferase (HAT), through which they can be directed to histone and other proteins. Also, AR can specifically recruit the AR-associated (ARA) coactivators, ARA70, ARA55, and ARA54. These large multi-protein complexes interact with the basal transcriptional machinery to regulate the level of transcription in target genes (20).

A separate ligand-dependent, nongenotropic function of AR also exists. Androgen binding can result in AR mediated activation of the Ras/extracellular signal-related kinase (ERK) pathway through nongenotropic activation of the c-Src nonreceptor tyrosine kinase. In some types of cells, androgen stimulation induces complex formation between AR and c-Src, as well as the estrogen receptor (ER) β-subunit. Importantly, AR-mediated cSrc activation can lead to increased cellular proliferation and protection from apoptosis (21, 22).

Role of androgens in prostate cancer

Because androgens are required for the growth and survival of malignant prostate cells, androgen ablation therapy either in the form of medical or surgical castration is initially effective in inhibiting the growth of these cancer cells in most patients as indicated by reduced expression of its target gene, prostate-specific antigen (PSA), and concomitant tumor regression (23). However, this prostate cancer relapse with a more aggressive and metastatic phenotype that is resistant to hormonal therapy and ultimately causes the death of the patient (24).

The direct correlation between serum androgens, especially testosterone, and the risk of prostate cancer stems from the landmark studies by Huggins and Hodges, who reported regression of metastatic prostate cancer after reduction of serum testosterone levels and progression of metastatic disease and symptoms in a patient who was treated with exogenous testosterone (25). Testosterone from the testes is the major androgen used by prostate cancer cells before ADT and is synthesized in Leydig cells in the testis. There are at least two theories to explain the relationship between prostate cancer and serum testosterone levels: the “suppression theory”, which proposes that prostate cancer cells secrete an androgen inhibitor, and the “saturation theory”, which suggests that serum levels of androgens above a sufficiently low baseline are sufficient to stimulate the growth of prostate cancer. ADT deprives the body of testosterone from the testis and reduces the circulating testosterone to less than 50 ng/dL (~1.7 nM) (25, 26, and 27).

Luminal epithelial AR plays a suppressive role during adult prostate homeostasis, but it plays a very different role in cancerous prostate tissue, which is composed primarily of luminal epithelial cells. AR gene amplification has been observed in approximately 30% of castration-resistant patients with recurrent prostate cancer (28). However, AR gene amplification does not always lead to an increase in AR protein levels. Higher levels of AR protein can result from gene amplification and also from increased transcription rates, or stabilization of the mRNA or protein. Increased AR expression sensitizes prostate cancer cells to low levels of androgen and promotes progression from hormone-dependent to CRPC. AR mutations have been identified in its ligand-binding domain, as well as the amino terminus and DNA-binding domain. These mutations are usually related to AR gain-of-function and are linked to CRPC (29).

Despite the central role of androgens in established prostate cancer, whether androgens are responsible for the initiation of prostate cancer has been more controversial. The fact that aging, one of the strongest risk factors for prostate cancer is associated with a gradual decline in testosterone levels does not preclude a pathogenic role for androgens, given the long preclinical phase of prostate cancer (30, 31).

Androgen deprivation therapy (ADT)

Androgen deprivation therapy (ADT) remains the most effective therapy for metastatic prostate cancer. ADT reduces the levels of androgen hormones, with drugs or surgery, to prevent the prostate cancer cells from growing (32). The pharmaceutical approaches include antiandrogens and chemical castration. However, androgen depletion is usually associated with the recurrence of prostate cancer. The therapeutic efficacy of ADT is due to upregulation in the expression of proapoptotic genes that are normally repressed by androgen receptor activation (33). Medical ADT with long-acting gonadotrophin-releasing hormone (GnRH) agonists is currently the most commonly used

ADT as they are considered to be equally effective in reducing testosterone levels as orchiectomy, with less psychological effect. An interesting feature of CRPC is that, despite low levels of systemic androgen after castration, active AR signaling is maintained in these recurrent prostate cancers. AR amplification/ overexpression has been suggested by many studies as one of the mechanisms leading to ADT failure. Newer androgen-based therapies include abiraterone acetate, an inhibitor of cytochrome P-450 17A1 (CYP17A1), a key enzyme in androgen biosynthesis which is expressed at extra-gonadal sites (34).

ADT is well established to have important clinical benefits, including improvement in survival, when used in the appropriate clinical context. ADT has long been the standard treatment option for metastatic disease and is mandatory in symptomatic patients because it reduces disease-associated morbidity and improves the quality of life, and innovative androgen-based therapy improves survival (35).

CONCLUSION

Prostate cancer afflicts patients mentally and physically, even though it is not lethal in most patients. Numerous studies have defined the importance of the androgen/AR signaling axis in prostate development, homeostasis, and established prostate cancers (36). Many clinical trials and animal studies support the hypothesis that age-related decline in androgen levels is positively associated with the initiation of human prostate cancer; however, few studies have focused on deciphering the mechanism(s) that underlie this association. The hope is that these ongoing efforts will translate into greater precision in AR targeting and novel therapeutic options in the near future for men with prostate cancer (37).

REFERENCES

1.Toivanen R, Shen MM. Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. Development. 2017 Apr 15;144(8):1382-98.McNeal JE. Normal and pathologic anatomy of prostate. Urology. 1981;17:11-6.

2.Pernar CH, Ebot EM, Wilson KM, Mucci LA. The epidemiology of prostate cancer. Cold Spring Harbor perspectives in medicine. 2018 Dec 1;8(12):a030361.

3.Tafuri A, Porcaro AB, Shakir A, Migliorini F, Verratti V, Brunelli M, Cerruto MA, Antonelli A. Serum testosterone and obesity in prostate cancer biology: a call for health promotion in the ageing male. Aging Clinical and Experimental Research. 2021 May;33(5):1399-401.

4.H Lajis N, Abas F, Othman I, Naidu R. Mechanism of anti-cancer activity of curcumin on androgen-dependent and androgen-independent prostate cancer. Nutrients. 2020 Mar;12(3):679.

5.Chatterjee P, Schweizer MT, Lucas JM, Coleman I, Nyquist MD, Frank SB, Tharakan R, Mostaghel E, Luo J, Pritchard CC, Lam HM. Supraphysiological androgens suppress prostate cancer growth through androgen receptor–mediated DNA damage. The Journal of clinical investigation. 2019 Oct 1;129(10):4245-60.

6.Fujita K, Nonomura N. Role of androgen receptor in prostate cancer: a review. The world journal of men's health. 2019 Sep 1;37(3):288-95.

7.Ghashghaei M, Paliouras M, Heravi M, Bekerat H, Trifiro M, Niazi TM, Muanza T. Enhanced radiosensitization of enzalutamide via schedule dependent administration to androgen-sensitive prostate cancer cells. The Prostate. 2018 Jan;78(1):64-75.

8.Pierorazio PM, Ferrucci L, Kettermann A, Longo DL, Metter EJ, Carter HB. Serum testosterone is associated with aggressive prostate cancer in older men: results from the Baltimore Longitudinal Study of Aging. BJU international. 2010 Mar;105(6):824.

9.Kalra R, Bhagyaraj E, Tiwari D, Nanduri R, Chacko AP, Jain M, Mahajan S, Khatri N, Gupta P. AIRE promotes androgen-independent prostate cancer by directly regulating IL-6 and modulating tumor microenvironment. Oncogenesis. 2018 May 25;7(5):1-5.

10.Zhang C, Li P, Wen Y, Feng G, Liu Y, Zhang Y, Xu Y, Zhang Z. The promotion on cell growth of androgen-dependent prostate cancer by antimony via mimicking androgen activity. Toxicology letters. 2018 May 15;288:136-42.

11.Lombardi AP, Vicente CM, Porto CS. Estrogen receptors promote migration, invasion and colony formation of the androgen-independent prostate cancer cells PC-3 through β-catenin pathway. Frontiers in Endocrinology. 2020 Apr 9;11:184.

12.Berchuck JE, Viscuse PV, Beltran H, Aparicio A. Clinical considerations for the management of androgen indifferent prostate cancer. Prostate cancer and prostatic diseases. 2021 Sep;24(3):623-37.

13.Lamb DJ, Weigel NL, Marcell M. Androgen receptors and their biology.

14.Bakouny Z, Yekedüz E, Braun DA, Berchuck JE, Hirsch L, Utkan G, Lee Y, Trinh QD, Choueiri TK, Ürün Y. Neurotoxicities of novel non-steroidal anti-androgens for prostate cancer: A systematic review and meta-analysis. Critical reviews in oncology/hematology. 2021 Oct 1;166:103463.

15.A.H. Davies, H. Beltran, A. Zoubeidi, Cellular plasticity and the neuroendocrine phenotype in prostate cancer, Nat. Rev. Urol. 15 (2018) 271–286.

16.Hou Z, Huang S, Li Z. Androgens in prostate cancer: A tale that never ends. Cancer Letters. 2021 Sep 28;516:1-2.

17.Bakouny Z, Yekedüz E, Braun DA, Berchuck JE, Hirsch L, Utkan G, Lee Y, Trinh QD, Choueiri TK, Ürün Y. Neurotoxicities of novel non-steroidal anti-androgens for prostate cancer: A systematic review and meta-analysis. Critical reviews in oncology/hematology. 2021 Oct 1;166:103463.

18.Hao L, Dong Y, Zhang JJ, He HG, Chen JG, Zhang SQ, Zhang QJ, Wu W, Han CH, Shi ZD. Melatonin decreases androgen-sensitive prostate cancer growth by suppressing SENP1 expression. Translational Andrology and Urology. 2022 Jan;11(1):91.

19.Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domenico M, Bottero D, Vitale F, Migliaccio A, Auricchio F. Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a nontranscriptional receptor action. The Journal of cell biology. 2003 May 12;161(3):547-56.

20.Rao A, Moka N, Hamstra DA, Ryan CJ. Co-Inhibition of Androgen Receptor and PARP as a Novel Treatment Paradigm in Prostate Cancer—Where Are We Now?. Cancers. 2022 Feb 4;14(3):801.

21.Anjaly K, Tiku AB. Caffeic acid phenethyl ester induces radiosensitization via inhibition of DNA damage repair in androgen-independent prostate cancer cells. Environmental Toxicology. 2022 Jan 10.

22.Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, De Wit R, Mulders P, Chi KN, Shore ND, Armstrong AJ. Increased survival with enzalutamide in prostate cancer after chemotherapy. New England Journal of Medicine. 2012 Sep 27;367(13):1187-97.

23.Lu Y, Zhang Z, Yu H, Zheng SL, Isaacs WB, Xu J, Sun J. Functional annotation of risk loci identified through genome-wide association studies for prostate cancer. The Prostate. 2011 Jun 15;71(9):955-63.

24.Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, Wiley KE, Isaacs SD, Johng D, Wang Y, Bizon C.

Germline mutations in HOXB13 and prostate-cancer risk. New England Journal of Medicine. 2012 Jan 12;366(2):141-9.
25. Sharifi N, Auchus RJ. Steroid biosynthesis and prostate cancer. Steroids. 2012 Jun 1;77(7):719-26.
26. Fontana F, Anselmi M, Limonta P. Molecular mechanisms and genetic alterations in prostate cancer: From diagnosis to targeted therapy. Cancer Letters. 2022 Mar 8:215619.
27. Mirzakhani K, Kallenbach J, Rasa SM, Ribaudo F, Ungelenk M, Ehsani M, Gong W, Gassler N, Leeder M, Grimm MO, Neri F. The androgen receptor—lncRNASAT1-AKT-p15 axis mediates androgen-induced cellular senescence in prostate cancer cells. Oncogene. 2022 Feb;41(7):943-59.
28. Suarez-Almazor ME, Pundole X, Cabanillas G, Lei X, Zhao H, Elting LS, Lopez-Olivo MA, Giordano SH. Association of Bone Mineral Density Testing With Risk of Major Osteoporotic Fractures Among Older Men Receiving Androgen Deprivation Therapy to Treat Localized or Regional Prostate Cancer. JAMA network open. 2022 Apr 1;5(4):e225432-.
29. Knudsen KE, Scher HI. Starving the addiction: new opportunities for durable suppression of AR signaling in prostate cancer. Clinical Cancer Research. 2009 Aug 1;15(15):4792-8.
30. Grossmann M, Zajac JD. Management of side effects of androgen deprivation therapy. Endocrinology and Metabolism Clinics. 2011 Sep 1;40(3):655-71.
31. Ryan CJ, Smith MR, De Bono JS, Molina A, Logothetis CJ, De Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, Carles J. Abiraterone in metastatic prostate cancer without previous chemotherapy. New England Journal of Medicine. 2013 Jan 10;368(2):138-48.
32. Westaby D, Maza MD, Paschalis A, Jimenez-Vacas JM, Welti J, de Bono J, Sharp A. A new old target: Androgen receptor signaling and advanced prostate cancer. Annual review of pharmacology and toxicology. 2021 Aug 24;62.
33. Spitzer M, Huang G, Basaria S, Travison TG, Bhasin S. Risks and benefits of testosterone therapy in older men. Nature reviews Endocrinology. 2013 Jul;9(7):414-24.
34. Formaggio N, Rubin MA, Theurillat JP. Loss and revival of androgen receptor signaling in advanced prostate cancer. Oncogene. 2021 Feb;40(7):1205-16.
35. Evans AJ. Treatment effects in prostate cancer. Modern Pathology. 2018 Jan;31(1):110-21.
36. Dai C, Heemers H, Sharifi N. Androgen signaling in prostate cancer. Cold Spring Harbor perspectives in medicine. 2017 Sep 1;7(9):a030452.
37. Crawford ED, Heidenreich A, Lawrentschuk N, Tombal B, Pompeo AC, Mendoza-Valdes A, Miller K, Debruyne FM, Klotz L. Androgen-targeted therapy in men with prostate cancer: evolving practice and future considerations. Prostate cancer and prostatic diseases. 2019 Mar;22(1):24-38.

http://pmjournal.ir Original Article

Winter 2022, Volume 7, Issue 24 (19-22)

miR3-22-p as a Novel Biomarker in Rheumatoid Arthritis

Maryam Hassanpoor[1*], Bahareh Abbasi[2]

[1]Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.
[2]National Institute of Genetic Engineering and Biotechnology.

**Corresponding author: Maryam Hassanpoor, Department of Cell and Molecular Biology, Faculty of BIological Sciences, Kharazmi University,Tehran, Iran. Email: Maryamhassanpoor92@gmail.com*

DOI: 10.22034/pmj.2022.252479

Submitted: 2021-11-14
Accepted: 2022-02-18

Keywords:
Rheumatoid arthritis (RA)
Micro RNA
MiR 22-3p
Gene Expression
Autoimmune Disease

Abstract:

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune. Early diagnosis of RA remains challenging. A significant portion of RA patients also experience unremitting symptoms despite treatment. miRNA are involved in the regulation of autoimmunity- and inflammation-related processes. In this study, we evaluated the expression of miR-22-3p in serum of RA patients as a novel biomarker. Expression level of this gene in the blood serum of 30 people with RA compared with 30 healthy individuals by the qRT-PCR method. Results showed levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control ($p<0.0001$). We suggest that miR 22-3p can be used as a biomarker in early detection and screening.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disorder that manifests as asymmetric polyarthritis of small and large joints that may lead to joint and per articular structural damage and the consequences of systemic inflammation. Recent advances have resulted in better diagnostic criteria, improved serologic testing, novel new drugs, and better guidelines to manage patients with RA (1). The disorder is most typical in women and occurs at any age. It affects about 0.5 ~ 1.0% of the population worldwide (2). The aetiopathogenesis of rheumatoid arthritis is thought to result from a multistep process, where environmental factors induce a pathological activation of the immune system in susceptible individuals (3). MicroRNAs (miRNAs) are small non-coding RNAs that have been implicated as potential biomarkers or therapeutic targets in autoimmune diseases (4). MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in numerous biological processes such as cell differentiation and homeostasis, through the regulation of gene expression (5). Since their discovery, they have been implicated in cancer, viral, neurodegenerative, and autoimmune diseases. Binding to complementary sequences on messenger RNA (mRNA), miRNAs generally function to suppress the translation of target proteins, however, they have also been shown to control the rate of transcription. Furthermore, under certain conditions and in specific cell types, they can, in fact, induce gene expression (6). A number of studies have reported that dysregulated miRNA expression influences immune regulation, enhances pro-inflammatory signaling pathways, and leads to the overproduction of pro-inflammatory cytokines in RA (7-8). Among the miRNAs, miR-22-3p is a 22-nucleotide noncoding RNA that was originally identified as a tumor suppressor in HeLa cells. miR-22-3p is located at a fragile cancer-relevant genomic region in chromosome 17 (17p13.3), close to p53. miR-22-3p might induce complex changes and extensive cooperation with p53 (9). Its expression has since been detected in a variety of tissues, including the liver, breast, lung, skin, and gastric cancer. Several studies have also shown that miR-22-3p is associated with many important biological processes, including neuroprotection, tumorigenesis, and various other tumor progressions (10). However, the roles of miR-22-3p in the progression of various tumors are inconsistent. In some studies, miR-22-3p was reported to act as an oncogene, promoting malignancy in breast cancer, lung cancer, and multiple myeloma (11-12). While several reports have also shown that it may act as a tumor suppressor in gastric cancer and esophageal squamous cell carcinoma (13). Many

miRNAs discovered in several cells, tissues, and body fluids have been confirmed that are involved in the pathogenesis of RA (14). A study demonstrated that miR-22-3p promoted fibroblast-like synoviocyte (FLS) proliferation and interleukin (IL)-6 production by targeting Cyr61 (15).In this study, we evaluated the expression of miR-22-3p in serum of RA patients as a novel biomarker. For this purpose, we evaluated the expression level of this gene in the blood serum of 30 people with RA compared with 30 healthy individuals by the qRT-PCR method.

MATERIALS AND METHODS:

The samples used in this experiment included 30 people with rheumatoid arthritis and 30 healthy people as a control group which was received from Shariati Hospital in Tehran. All RA patients fulfilled the 2010 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) criteria. All subjects gave informed consent and the study protocol was approved by local medical ethics committees. For miRNA extraction, RNA from freshly sera samples was isolated using Plasma/Serum RNA Purification Mini Kit (Norgenbiotek Cat. 55000, Canada) according to the manufacturers. According to the kit protocol, the cDNA was synthesized using BONmiR High Sensitivity MicroRNA 1st Strand cDNA Synthesis kit (STEMCELL Technology, Iran). qPCR reaction was performed using a BON microRNA QPCR Master mix kit (STEMCELL Technology, Iran), a universal reverse primer (CGAGGAAGAAGACGGAAGAAT), and a specific design primer (AAGCTGCCAGTTGAAGAACTGTA). U6 was used as an internal reference, and the relative expression of RNAs was calculated by the $2^{-\Delta\Delta Ct}$ method. All statistical differences analysis and correlation analysis were performed using GraphPad Prism 8 statistical software (GraphPad Software Inc., San Diego, USA). Differences between two groups were utilised by the Mann-Whitney U-test to compare quantitative variables. All tests were two-tailed, and a p-value <0.05 was considered statistically significant.

RESULTS:

We first assessed the expression levels of circulating miR-22-3p in patients with RA and healthy control. As shown in Figure 1, levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control ($p<0.0001$). The other clinical characteristics of all participants are summarized in Table I. Moreover, further analysis demonstrated that the level of circulating miR22-3p was not associated with age or gender. Also, no significant relationship was observed between the expression of this gene and clinical parameters.

DISCUSSION:

In this study, we evaluated the expression of miR22-3p in the blood serum of people with rheumatoid arthritis as a diagnostic biomarker. Results showed levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control

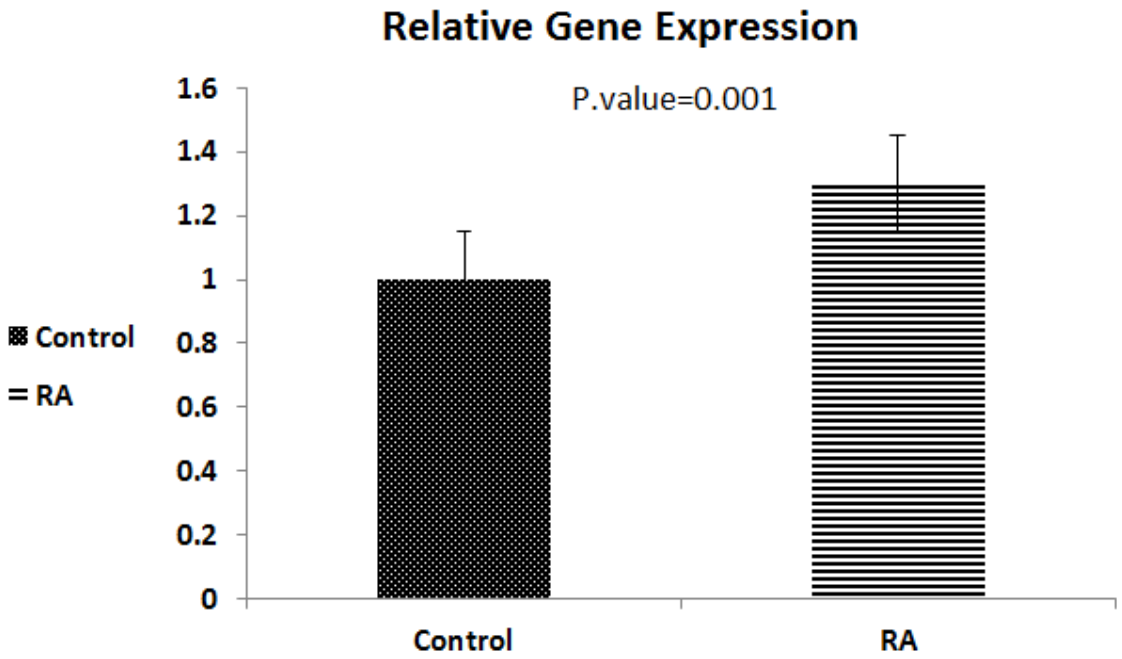

Fig1. Relative miR22-3p expression between RA and healthy control group, circulating miR22-3p in serum RA patient 1.34 fold more than healthy control group.

Table1. Compared and measured clinical parameters.

parameter	RA	Control	sig
Number	30	30	-
age	58±8.6	55±12.7	-
sex (male/female)	11/19	20/10	-
RF(IU/ml)	33±5.3	-	-
anti-CCP (RU/ml)	31±1.1	-	-
Relative expression miR22-3p	1.94	1.1	0.001

($p<0.001$). Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune condition that induces inflammation, stiffness, rigidity, and lack of mobility in the joints and affects the peripheral joint synovial membrane1. It has been characterized by erosive synovitis, penetration of inflammatory cells into the synovium or membrane existing in the synovial joints that line the joint capsules and produce synovial fluid for the joints in the hands and feet is the first structure affected (16). The pathogenesis of RA is complex and involves an intricate interplay between host factors (genetic susceptibilities, aberrant immune response, abnormal metabolic enzymes and sex hormones) and environmental triggers (bacterial or viral infection). Clinically, early diagnosis of RA remains challenging. A significant portion of RA patients also experience unremitting symptoms despite treatment. It is therefore crucial to explore the molecular mechanisms to identify novel diagnostic markers and mechanism-driven therapeutics for RA (17-18). Accumulating studies have shown that miRNA are involved in the regulation of autoimmunity- and inflammation-related processes, including nuclear factor κ-B and Toll-like receptor signaling, cytokine expression, and immune cell proliferation and differentiation (19). several reports have demonstrated that miRNA play an important role in the pathogenesis of a variety of autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, type I diabetes and RA (20-22). miR-22-3p is located at a fragile cancer-relevant genomic region in chromosome 17 (17p13.3), close to p53. miR-22-3p might induce complex changes and extensive cooperation with p53 (9). Its expression has since been detected in a variety of tissues, including the liver, breast, lung, skin, and gastric cancer. In this study, it was shown that miR 22-3p can be used as a diagnostic biomarker, although it is suggested that this study be performed on more samples.

REFERENCE:

1.Cush JJ. Rheumatoid arthritis: early diagnosis and treatment. Medical Clinics. 2021 Mar 1;105(2):355-65.

2.Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmunity reviews. 2005 Mar 1;4(3):130-6.

3.Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, Gabay C, Finckh A, Strowig T. Prevotella copri in individuals at risk for rheumatoid arthritis. Annals of the rheumatic diseases. 2019 May 1;78(5):590-3.

4.Cunningham CC, Wade S, Floudas A, Orr C, McGarry T, Wade S, Cregan S, Fearon U, Veale DJ. Serum miRNA signature in rheumatoid arthritis and “at-risk individuals”. Frontiers in immunology. 2021 Mar 3;12:126.

5.O’Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in endocrinology. 2018 Aug 3;9:402.

6.Vasudevan S. Posttranscriptional upregulation by microRNAs. Wiley Interdisciplinary Reviews: RNA. 2012 May;3(3):311-30.

7.Bogunia-Kubik K, Wysoczańska B, Piątek D, Iwaszko M, Ciechomska M, Świerkot J. Significance of polymorphism and expression of miR-146a and NFkB1 genetic variants in patients with rheumatoid arthritis. Archivum immunologiae et therapiae experimentalis. 2016 Dec;64(1):131-6.

8.Liu F, Liang Y, Zhao Y, Chen L, Wang X, Zhang C. Meta-analysis of association of microRNAs genetic variants with susceptibility to rheumatoid arthritis and systemic lupus erythematosus. Medicine. 2021 Apr 30;100(17).

9.Hussein NA, Kholy ZA, Anwar MM, Ahmad MA, Ahmad SM. Plasma miR-22-3p, miR-642b-3p and miR-885-5p as diagnostic biomarkers for pancreatic cancer. Journal of cancer research and clinical oncology. 2017 Jan;143(1):83-93.

10.Pandey AK, Zhang Y, Zhang S, Li Y, Tucker-Kellogg G, Yang H, Jha S. TIP60-miR-22 axis as a prognostic marker of breast cancer progression. Oncotarget. 2015 Dec 1;6(38):41290.

11.Ahmad HM, Muiwo P, Ramachandran SS, Pandey P, Gupta YK, Kumar L, Kulshreshtha R, Bhattacharya A. miR-22 regulates expression of oncogenic neuro-epithelial transforming gene 1, NET 1. The FEBS Journal. 2014 Sep;281(17):3904-19.

12.Yang C, Ning S, Li Z, Qin X, Xu W. miR-22 is down-regulated in esophageal squamous cell carcinoma and inhibits cell migration and invasion. Cancer cell international. 2014 Dec;14(1):1-6.

13.Wang X, Yu H, Lu X, Zhang P, Wang M, Hu Y. MiR-22 suppresses the proliferation and invasion of gastric cancer cells by inhibiting CD151. Biochemical and biophysical research communications. 2014 Feb 28;445(1):175-9.

14.Evangelatos G, Fragoulis GE, Koulouri V, Lambrou GI. MicroRNAs in rheumatoid arthritis: From pathogenesis to clinical impact. Autoimmunity Reviews. 2019 Nov 1;18(11):102391.

15.Lin J, Huo R, Xiao L, Zhu X, Xie J, Sun S, He Y, Zhang J, Sun Y, Zhou Z, Wu P. A novel p53/microRNA-22/Cyr61 axis in synovial cells regulates inflammation in the rheumatoid arthritis. Arthritis & rheumatology. 2014 Jan;66(1):49-59.

16.Butola LK, Anjanker A, Vagga A, Kaple MN. Endogenous factor and pathophysiology of rheumatoid arthritis: an autoimmune disease from decades. Int J Cur Res Rev. 2020 Nov;12(22):34-40.

17.Wang D, Li Y, Liu Y, Shi G. The role of autoreactive T cell in the pathogenesis of rheumatoid arthritis and implications for T cell targetedvaccine therapy. Minerva Med. 2015;106:157-167.

18.Zhang X, Singla S, Pound J, et al. Identification of follicular helper Tcells as a novel cell population potentially involved in the pathogenesis of Rheumatoid Arthritis. J Immunol. 2015;194:121.

19.Aune TM, Crooke PS, Patrick AE, Tossberg JT, Olsen NJ, Spurlock CF. Expression of long non-coding RNAs in autoimmunity and linkage to enhancer function and autoimmune disease risk genetic variants. J Autoimmun. 2017;81:99-109.
20.Zhang F, Gao C, Ma XF, et al. Expression profile of long noncoding RNAs in peripheral blood mononuclear cells from multiple sclerosis patients. CNS Neurosci Ther. 2016;22:298-305.
21.Wu GC, Pan HF, Leng RX, et al. Emerging role of long noncoding RNAs in autoimmune diseases. Autoimmun Rev. 2015;14:798-805.
22.Kaur S, Mirza AH, Brorsson CA, et al. The genetic and regulatory architecture of ERBB3-type I diabetes susceptibility locus. Mol Cell Endocrinol. 2016;419:83-91.

http://pmjournal.ir Reviwe Article

Winter 2022, Volume 7, Issue 24 (23-27)

Investigating the link between MS and the EBV virus

Bahareh Abbasi[1], Almas Araghi[2*], AmirHossein Akbari Aghababa[3]

[1]National Institute of Genetic Engineering and Biotechnology.
[1]Department of Microbiology, Faculty of Biological Sciences, North Tehran Islamic Azad University, Tehran, Iran.
[2] Department of Microbiology, Faculty of Biological Science, North Branch, Islamic Azad University, Tehran, Iran.

**Corresponding author: Almas Araghi, Department of Microbiology, Faculty of Biological Sciences, North Tehran Islamic Azad University, Tehran, Iran. Email: almasaraghi@yahoo.com.*

DOI: 10.22034/pmj.2022.252441

Submitted: 2022-11-23
Accepted: 2022-02-12

Keywords:
Multiple Sclerosis,
Epstein–Barr virus
MS
B-cells
Infection

Abstract:

Multiple sclerosis (MS) is a disease of the central nervous system characterized by inflammation, demyelination, and neuronal damage. Epstein–Barr virus (EBV) is a human DNA herpes virus infecting more than 90% of the world's population. EBV is the etiological agent of infectious mononucleosis (Pfeiffer's disease). Major predisposing factors for MS are certain tissue types (e.g., HLA DRB1*15:01), vitamin D deficiency, smoking, obesity, and infection with Epstein-Barr virus (EBV). This review summarizes current knowledge on the association between EBV and MS.

INTRODUCTION

Multiple sclerosis (MS) is a disease affecting the central nervous system (CNS), with inflammation and demyelination of nerves, eventually resulting in nerve damage and disabilities (1). Multiple sclerosis (MS) is presently regarded as a disease with multifactorial etiology, comprising genetic as well as environmental influences (2). Already more than a century ago, Pierre Marie did state that "the cause of insular (multiple) sclerosis is intimately connected with infectious diseases" (3). MS can take different courses, most often in the form of relapsing-remitting (RR) cycles of disease activity or more rarely as a primary-progressive (PP) disease. RR MS can progress over many years and may eventually develop into a secondary-progressive (SP) disease (4). Over time, the majority of relapsing-remitting MS (RRMS) patients enter a progressive disease course in which there is a gradual worsening of clinical disability with or without superimposed relapses and eventually become secondary-progressive MS (SPMS) (5). MS often leads to severe disability, although the symptoms and clinical courses are extremely diverse from malignant forms with mortality within a few years to benign forms with few symptoms and very slow progression (6). Although MS risk is associated with environmental, neuroimmune, and genetic factors, the exact causative factor for MS is not known. The environmental risk factors most consistently linked to MS risk are infection with Epstein-Barr virus (EBV), sun exposure/vitamin D deficiency, and smoking (7). Specific environmental exposures are relevant to both triggering MS and modulating disease course. Virus infection is one crucial environmental factor. Of all viruses considered in MS pathogenesis, EBV, a highly B cell-tropic virus, is the best-studied (8).

Evidence supporting the role of Epstein-Barr virus (EBV) infection in multiple sclerosis (MS) comes from ecological studies, observational epidemiological studies, co-occurring pathologies, and experimental laboratory-based research (9). Epstein-Barr virus, the prototype of the gammaherpesviruses, is a linear, double-stranded, 184 kb DNA virus that has a primary tropism for resting B cells (10). Epstein–Barr virus (EBV) infection results in a lifelong persistence of the virus in the host's B-lymphocytes and has been associated with numerous cancers including Burkitt's lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma (11). Early age at primary EBV infection is typically asymptomatic, but primary infection during adolescence or adulthood often manifests as infectious mononucleosis, which has been associated with a two- to threefold increased risk of MS. (12).

This review summarizes current knowledge on the

association of EBV and MS including a discussion of equivocal findings.

Etiology and Epidemiology of MS

No consensus about MS etiology exists at present and theories range from idiopathic loss of self-tolerance, over molecular mimicry to chronic virus infections. Factors involved in pathogenesis broadly group into three categories: Immune factors, Environmental factors, and Genetic associations (13). Genetic factors influencing the development of MS are in particular major histocompatibility class II (MHC II) alleles, of which some increased susceptibility (e.g., human leukocyte antigen (HLA) DRB1*15:01), while others decrease susceptibility (14). Environmental factors, including latitudinal gradients in different countries, have been well-studied phenomena. Vitamin D deficiency has been considered a possible etiology for the noted predisposition of the population in higher latitudes being affected. Different infections, including Epstein Barr virus (EBV), may also play a role. There are likely complex interactions between various environmental factors with patient genetics, and understanding these pathways is an area of ongoing research (15, 16). Smoking increases the risk of MS, but some other uses of tobacco may actually reduce the risk of MS. Other environmental compound exposures have been found to affect MS susceptibility and recently, propionic acid and the composition of the intestinal microbiota have been reported to influence or be influenced by MS (17). Virus infections have for long been suspected to be involved in MS development. Most investigations have focused on EBV, which remains the most likely candidate for a causative virus, but other viruses may also play a role. Characteristic features of MS are inflammatory foci in the CNS and intra thecal synthesis of immunoglobulins (Ig), measured as an IgG index, oligo clonal bands (OCBs), or specific antibody indexes. (18).

Approximately 400,000 individuals in the United States and 2.5 million individuals worldwide have multiple sclerosis. The disease is three-fold more common in females than in males. While the age of onset is usually between 20 to 40 years, the disease can present at any age. Almost 10% of the cases present before the age of 18 (19). The prevalence and incidence of MS in Iran are reported to range from 5.3 to 89/ 100,000and 7 to 148.1/ 100,000, respectively. At the moment, Iran is well known for its high prevalence of MS in the world, whereas 15 years ago, it was assumed based on the MS slope hypothesis that Iran could be a low-risk area for MS with an incidence of less than 5 per 100,000 people (20).

Epstein - Barr virus (EBV)

EBV is a member of the Human Herpes Virus (HHV) family, which also includes Herpes Simplex Virus (HSV) 1 and 2, Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), HHV 6 and 7, and Kaposi Sarcoma Virus (KSV) (21). EBV was discovered in the early 1960s in lymphoma cells cultivated from tumor biopsies obtained by Burkitt in African children with jaw tumors (22). Like other herpesviruses, the EBV has a latency phase following primary infection. It infects epithelial cells, enters the circulating B lymphocyte, and persists for the life in a latent state. According to epidemiological studies, the EBV is estimated to be positive in more than 90% of the world's population (23). Primary infection usually occurs through contact with infected saliva and is asymptomatic in young children, but in up to 40% of adolescents and adults, it results in infectious mononucleosis (IM), an acute and usually self-limited lymphoproliferative disease of a few weeks duration (22, 24).

As a counter-measure to host immune responses, EBV has evolved a multitude of immune evasion mechanisms, counteracting both host cell intracellular anti-viral processes and host extracellular innate and adaptive immune responses. Cellular anti-viral pathways are many and EBV devotes a large part of its genome to the control of cellular anti-viral apoptosis mechanisms and to immune evasion (25, 26). There are two main EBV genotypes, type 1 and type 2, or A and B, respectively, distinguished by the differences in the EBNA-2 gene, since the divergence in EBNA-2 reveals only 54% homology between the two types. EBV types 1 and 2 can further be subdivided into different virus strains (27).

Following primary infection, EBV persists for the life of the host in B-lymphocytes, in which the EBV double-stranded DNA forms an episome, typically present as a single copy at a frequency of 1 to 50 per million B-lymphocytes (28). Decreased capacity for immune control of EBV may, in some cases manifest itself as a tendency to develop EBV-related diseases, including infectious mononucleosis (IM), various cancers, MS, and other relapsing-remitting autoimmune diseases (e.g., systemic autoimmune diseases) (29).

EBV and MS

In MS, much evidence indicates a role for EBV and specifically that EBV-infected B cells have entered the CNS at some point of disease development. As described above, some of the major characteristics of MS are the presence of an elevated IgG index and OCBs in the CNS, representing various B cell clones synthesizing Abs in the CNS (30). EBV appears to be involved across the clinical spectrum of MS, including early pediatric-onset MS, established relapsing-remitting (RRMS), and progressive forms (PMS), as well as in patients with both mild and severe disease courses (31). Many studies have revealed increased

amounts and increased frequencies of EBV Abs in MS, however, such studies are hampered by the nearly ubiquitous presence of EBV in adults. Moreover, the results seem to depend somewhat on the EBV Ags used and the assay methodology. Among healthy individuals infected with EBV, MS risk increases monotonically by several folds with increasing serum titers of anti-EBNA complex and anti-EBNA-1 antibodies. Results of preliminary studies suggest that the presence of EBV in plasma and antibodies to the lytic antigen BZLF1 may also predict an increased MS risk, but these associations are weaker than those observed for antibodies to EBNA-1 (32, 33). In situ hybridization and PCR studies on brain material from MS patients have in some cases indicated the presence of EBV DNA in lesions, but other studies have yielded negative results. Immuno-histochemical studies are few, but one study has demonstrated the presence of EBV Ags in post-mortem brain tissue of MS patients (34). Infectious mononucleosis is the clinical manifestation of acute EBV infection. It is more common in adolescents and adults as compared to younger children, in whom primary EBV infection is more often clinically silent. MS and infectious mononucleosis share a similar prevalence distribution, following a latitude gradient: prevalence generally rises with increasing distance to the equator, in both the southern and the northern hemispheres. Late infection with EBV, evidenced by the occurrence of infectious mononucleosis, is therefore considered a possible risk factor for MS (35).

In a prospective nested study of 62439 women, who were followed for years to determine whether elevation in serum antibodies titers to EBV capsid antigen (VCA), nuclear antigen (EBNA, EBNA-1, and EBVA-2), diffuse and restricted early D Antigen (EA-D) and early R Antigen (EA-R) precede the occurrence of MS and its symptoms. 18 cases of MS with blood collected before disease onset, were compared with their matched controls, these women had higher serum geometric mean titers (GMT) of antibodies to EBV but no cytomegalovirus (CMV) (another member of the herpes family). Elevations were significant for antibodies to EBNA-1, EBNA-2, and EA-D. The strongest association was found for antibodies to EBNA-2; a four-fold difference in titers was associated with a relative risk (RR) of MS of 3.9. Significant but generally weaker elevations in anti-EBV antibodies were also found in an analysis of 126 cases of MS with blood collected after disease onset and their matched control (36).

Genes within the human leukocyte antigen (HLA) complex have long been known to play a crucial part in the development of MS and other autoimmune diseases. Genome-wide association studies identified the HLA allele DRB1*15:01 (HLA-DR15) as the strongest genetic risk factor for MS. Interestingly, symptomatic primary EBV infection, IM, has been found to synergize with this main genetic risk factor HLA-DR15, leading to a 7-fold increase in MS risk. The underlying mechanism of this synergistic effect is, however, largely unknown. Efforts to unravel this interaction have so far been hampered by the lack of an adequate model to study this interaction in vivo (37).

EBV specific T-cells and autoreactive B cells in MS

Aside from B-cell-related pathologies, loss of normal function in the effector T-cell population may also underlie MS disease progression. The frequency of EBNA-1 specific CD4+ memory T cells was strikingly elevated in MS patients compared to healthy EBV carriers. Furthermore, these EBNA-1 specific T cells showed increased proliferative capacity and enhanced interferon-gamma production in healthy individuals, EBV infection is kept under control by CD8+ cytotoxic T-cells, which kill off the EBV-infected lymphoblastoid cell lines (38). Cell-mediated immune mechanisms, involving T and NK cells, are of pivotal importance in controlling the proliferation of EBV-infected B cells. Since specific cytotoxic CD8+ cells are primed to recognize and eliminate infected cells which present latent proteins of EBV, hereafter are referred to as latency-specific T-cells (39). The mechanisms leading to tolerance in the majority of individuals versus the induction of autoimmunity and disease in others are not even rudimentarily understood.

Several studies have used synthetic EBV peptides to investigate T Cell immunity to EBV in MS, with conflicting results. Studies using panels of HLA class I restricted EBV peptides have found an increased frequency of reactive CD8 T-cells in MS patients, in CIS but not established MS, or no increase in either CIS or MS patients. In one study, MS patients had an increased CD4 T cell response to peptides derived from EBNA-1 (40, 41, and 42).

EBV control relies to a large extent on T cells and NK cells. It could therefore be hypothesized that MS patients have a deficiency in the cellular immune control of EBV and possibly also other viruses. CD8 T cell infiltration of MS brain lesions has been demonstrated in several studies but defective T cell control of EBV has also been reported in MS patients. This could indicate an imbalance in the T cell control of EBV in MS patients, and one study has actually found increased programmed death (PD) 1 on CD8 T cells resulting in decreased cytolytic activity against EBV-infected B cells, while PD1 has also been reported to be increased on regulatory T cells (43).

A scenario referred to as Pender's hypothesis is that EBV may infect autoreactive B lymphocytes, which would become latently infected B memory cells that could circulate to the organ in which their antigen is expressed and act as antigen-presenting

cells for autoreactive CD4+ T cells. The thus activated autoreactive CD4+ T cells would then cause the actual organ damage in MS but also other autoimmune diseases associated with EBV (44).

Vaccination

Vaccinating against EBV could be vaccinating against MS. Nevertheless, due to the long incubation period, trials demonstrating that EBV vaccination in early childhood abrogates MS in later life appear challenging. It should also be noted that EBV vaccines that would not prevent EBV infection but rather delay it to an older age might be harmful, given the increased risk of MS associated with EBV infection later in life. Still, though there is currently no approved EBV vaccine available, a prophylactic EBV vaccine could be a means for primary prevention of MS (45).

MS and personalized medicine

The therapeutic approach to multiple sclerosis (MS) requires a personalized medicine frame beyond the precision medicine concept, which is not currently implementable due to the lack of robust biomarkers and a detailed understanding of MS pathogenesis. Personalized medicine demands a patient-focused approach, with disease taxonomy informed by characterization of pathophysiological processes. Important questions concerning MS taxonomy are: when does MS begin? When does the progressive phase begin? Is MS really two or three diseases? Does a therapeutic window truly exist? Newer evidence points to a disease spectrum and a therapeutic lag of several years for benefits to be observed from disease-modifying therapy. For personalized treatment, it is important to ascertain the disease stage and any worsening of focal inflammatory lesions over time (46, 47).

CONCLUSION

MS has traditionally been regarded as an autoimmune disease. However, the occurrence of autoantibodies (AuAbs) in MS (e.g., myelin basic protein (MBP) and major oligodendrocyte glycoprotein (MOG) Abs) is limited to only some patients and the pathogenic role of AuAbs remains debatable, while the search for autoantigens (AuAgs) in MS continues (48). there is convincing epidemiological evidence that EBV infection is a strong risk factor for MS development, although the mechanisms remain elusive. The epidemiological data suggest that MS risk could be markedly reduced by preventing EBV infection, which could only be possible with a hypothetical vaccine that confers permanent sterile immunity against EBV or, less effectively, by causing an iatrogenic EBV infection in early childhood, when the adverse effect of infection on MS risk seems mitigated (49). Assuming EBV really acts as a cofactor in the pathogenesis of MS, there might be an opportunity for preventive strategies such as vaccinations. Hopefully one day, the following statement of Pierre Mariewill become a reality: “I have little doubt, in fact, gentlemen, that in the employment of such a substance as the vaccine of Pasteur or lymph of Koch the evolution of insular (multiple) sclerosis will someday be rendered absolutely impossible” (50).

REFERENCES

1. Lassmann H. Multiple sclerosis pathology. Cold Spring Harbor perspectives in medicine. 2018 Mar 1;8(3):a028936.
2. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. Current opinion in neurology. 2018 Dec 1;31(6):752-9.
3. Dobson R, Giovannoni G. Multiple sclerosis–a review. European journal of neurology. 2019 Jan;26(1):27-40.
4. Langille MM, Rutatangwa A, Francisco C. Pediatric multiple sclerosis: a review. Advances in Pediatrics. 2019 Aug 1;66:209-29.
5. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology. 2018 Feb 1;17(2):162-73.
6. Simonsen CS, Flemmen HØ, Lauritzen T, Berg-Hansen P, Moen SM, Celius EG. The diagnostic value of IgG index versus oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis. Multiple Sclerosis Journal–Experimental, Translational and Clinical. 2020 Jan;6(1):2055217319901291.
7. Bar-Or A, Pender MP, Khanna R, Steinman L, Hartung HP, Maniar T, Croze E, Aftab BT, Giovannoni G, Joshi MA. Epstein–Barr virus in multiple sclerosis: theory and emerging immunotherapies. Trends in molecular medicine. 2020 Mar 1;26(3):296-310.
8. Guan Y, Jakimovski D, Ramanathan M, Weinstock-Guttman B, Zivadinov R. The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to in vivo imaging. Neural regeneration research. 2019 Mar;14(3):373.
9. Fernández-Menéndez S, Fernández-Morán M, Fernández-Vega I, Pérez-Álvarez A, Villafani-Echazú J. Epstein–Barr virus and multiple sclerosis. From evidence to therapeutic strategies. Journal of the neurological sciences. 2016 Feb 15;361:213-9.
10. Hauser SL, Chan JR, Oksenberg JR. Multiple sclerosis: prospects and promise. Annals of neurology. 2013 Sep;74(3):317-27.
11. Tracy SI, Kakalacheva K, Lünemann JD, Luzuriaga K, Middeldorp J, Thorley-Lawson DA. Persistence of Epstein-Barr virus in self-reactive memory B cells. Journal of virology. 2012 Nov 15;86(22):12330-40.
12. Fernández-Menéndez S, Fernández-Morán M, Fernández-Vega I, Pérez-Álvarez A, Villafani-Echazú J. Epstein–Barr virus and multiple sclerosis. From evidence to therapeutic strategies. Journal of the neurological sciences. 2016 Feb 15;361:213-9.
13. Chamberlain SA, Szöcs E. taxize: taxonomic search and retrieval in R. F1000Research. 2013;2.
14. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nature Reviews Neurology. 2017 Jan;13(1):25-36.
15. Tarlinton RE, Martynova E, Rizvanov AA, Khaiboullina S, Verma S. Role of viruses in the pathogenesis of multiple sclerosis. Viruses. 2020 Jun;12(6):643.
16. Duscha A, Gisevius B, Hirschberg S, Yissachar N, Stangl GI, Eilers E, Bader V, Haase S, Kaisler J, David C, Schneider R. Propionic acid shapes the multiple sclerosis disease course by an immunomodulatory mechanism. Cell. 2020 Mar 19;180(6):1067-80.
17. Brown J, Quattrochi B, Everett C, Hong BY, Cervantes J. Gut commensals, dysbiosis, and immune response imbalance in the pathogenesis of multiple sclerosis. Multiple Sclerosis Journal. 2021 May;27(6):807-11.

18.Mirza A, Forbes JD, Zhu F, Bernstein CN, Van Domselaar G, Graham M, Waubant E, Tremlett H. Surveying the gut microbiota in multiple sclerosis: a systematic review (2008-2018). InMULTIPLE SCLEROSIS JOURNAL 2018 Oct 1 (Vol. 24, pp. 345-345). 1 OLIVERS YARD, 55 CITY ROAD, LONDON EC1Y 1SP, ENGLAND: SAGE PUBLICATIONS LTD.
19.Dilokthornsakul P, Valuck RJ, Nair KV, Corboy JR, Allen RR, Campbell JD. Multiple sclerosis prevalence in the United States commercially insured population. Neurology. 2016 Mar 15;86(11):1014-21.
20.Azami M, YektaKooshali MH, Shohani M, Khorshidi A, Mahmudi L. Epidemiology of multiple sclerosis in Iran: A systematic review and meta-analysis. PloS one. 2019 Apr 9;14(4):e0214738.
21.Guan Y, Jakimovski D, Ramanathan M, Weinstock-Guttman B, Zivadinov R. The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to in vivo imaging. Neural regeneration research. 2019 Mar;14(3):373.
22.Jacobs BM, Giovannoni G, Cuzick J, Dobson R. Systematic review and meta-analysis of the association between Epstein–Barr virus, multiple sclerosis and other risk factors. Multiple Sclerosis Journal. 2020 Oct;26(11):1281-97.
23.Tzellos S, Farrell PJ. Epstein-Barr virus sequence variation—biology and disease. Pathogens. 2012 Dec;1(2):156-74.
24.Münz C, editor. Epstein Barr virus volume 2: one herpes virus: many diseases. Springer; 2015 Oct 1.
25.Correale J, Gaitan MI. Multiple sclerosis and environmental factors: the role of vitamin D, parasites, and Epstein–Barr virus infection. Acta Neurologica Scandinavica. 2015 Jul;132:46-55.
26.Jouanguy E, Béziat V, Mogensen TH, Casanova JL, Tangye SG, Zhang SY. Human inborn errors of immunity to herpes viruses. Current opinion in immunology. 2020 Feb 1;62:106-22.
27.Hassani A, Khan G. Epstein-Barr virus and miRNAs: partners in crime in the pathogenesis of multiple sclerosis?. Frontiers in Immunology. 2019 Apr 3;10:695.
28.Laurence M, Benito-León J. Epstein–Barr virus and multiple sclerosis: Updating Pender's hypothesis. Multiple sclerosis and related disorders. 2017 Aug 1;16:8-14.
29.Majerciak V, Yang W, Zheng J, Zhu J, Zheng ZM. A genome-wide Epstein-Barr virus polyadenylation map and its antisense RNA to EBNA. Journal of virology. 2019 Jan 4;93(2):e01593-18.
30.Arrambide G, Tintore M, Espejo C, Auger C, Castillo M, Río J, Castilló J, Vidal-Jordana A, Galán I, Nos C, Mitjana R. The value of oligoclonal bands in the multiple sclerosis diagnostic criteria. Brain. 2018 Apr 1;141(4):1075-84.
31.Makhani N, Lebrun C, Siva A, Narula S, Wassmer E, Brassat D, Brenton JN, Cabre P, Carra Dallière C, De Seze J, Durand Dubief F. Oligoclonal bands increase the specificity of MRI criteria to predict multiple sclerosis in children with radiologically isolated syndrome. Multiple Sclerosis Journal–Experimental, Translational and Clinical. 2019 Mar;5(1):2055217319836664.
32.Veroni C, Serafini B, Rosicarelli B, Fagnani C, Aloisi F. Transcriptional profile and Epstein-Barr virus infection status of laser-cut immune infiltrates from the brain of patients with progressive multiple sclerosis. Journal of neuroinflammation. 2018 Dec;15(1):1-9.
33.Marcucci SB, Obeidat AZ. EBNA1, EBNA2, and EBNA3 link Epstein-Barr virus and hypovitaminosis D in multiple sclerosis pathogenesis. Journal of Neuroimmunology. 2020 Feb 15;339:577116.
34.Ruprecht K, Wildemann B, Jarius S. Low intrathecal antibody production despite high seroprevalence of Epstein–Barr virus in multiple sclerosis: a review of the literature. Journal of neurology. 2018 Feb;265(2):239-52.
35.Marrodan M, Alessandro L, Farez MF, Correale J. The role of infections in multiple sclerosis. Multiple Sclerosis Journal. 2019 Jun;25(7):891-901.
36.Ahmed SI, Aziz K, Gul A, Samar SS, Bareeqa SB. Risk of multiple sclerosis in Epstein–Barr virus infection. Cureus. 2019 Sep 19;11(9).
37.Läderach F, Münz C. Epstein Barr Virus Exploits Genetic Susceptibility to Increase Multiple Sclerosis Risk. Microorganisms. 2021 Nov;9(11):2191.
38.Lünemann JD, Tintoré M, Messmer B, Strowig T, Rovira Á, Perkal H, Caballero E, Münz C, Montalban X, Comabella M. Elevated Epstein–Barr virus-encoded nuclear antigen-1 immune responses predict conversion to multiple sclerosis. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2010 Feb;67(2):159-69.
39.Donati D. Viral infections and multiple sclerosis. Drug Discovery Today: Disease Models. 2020 Sep 1;32:27-33.
40.Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nature Reviews Neurology. 2017 Jan;13(1):25-36.
41.Serafini B, Rosicarelli B, Veroni C, Mazzola GA, Aloisi F. Epstein-Barr virus-specific CD8 T cells selectively infiltrate the brain in multiple sclerosis and interact locally with virus-infected cells: clue for a virus-driven immunopathological mechanism. Journal of virology. 2019 Nov 26;93(24):e00980-19.
42.Rafiee N, Ravanshad M, Asadi B, Kianfar R, Maleki A. Investigation of IL-2 and IFN-γ to EBV Peptides in Stimulated Whole Blood among Multiple Sclerosis Patients and Healthy Individuals. Intervirology. 2021;64(4):203-8.
43.Houen G, Trier NH, Frederiksen JL. Epstein-Barr Virus and multiple sclerosis. Frontiers in immunology. 2020:3315.
44.Ruprecht K. The role of Epstein-Barr virus in the etiology of multiple sclerosis: a current review. Expert Review of Clinical Immunology. 2020 Dec 1;16(12):1143-57.
45.Balfour HH, Schmeling DO, Grimm-Geris JM. The promise of a prophylactic Epstein–Barr virus vaccine. Pediatric research. 2020 Jan;87(2):345-52.
46.Giovannoni G. Personalized medicine in multiple sclerosis. Neurodegenerative disease management. 2017 Nov;7(6s):13-7.
47.Klineova S, Lublin FD. Clinical course of multiple sclerosis. Cold Spring Harbor perspectives in medicine. 2018 Sep 1;8(9):a028928.
48.Hohlfeld R, Dornmair K, Meinl E, Wekerle H. The search for the target antigens of multiple sclerosis, part 1: autoreactive CD4+ T lymphocytes as pathogenic effectors and therapeutic targets. The Lancet Neurology. 2016 Feb 1;15(2):198-209.
49.Fugl A, Andersen CL. Epstein-Barr virus and its association with disease-a review of relevance to general practice. BMC family practice. 2019 Dec;20(1):1-8.
50.Laurence M, Benito-León J. Epstein–Barr virus and multiple sclerosis: Updating Pender's hypothesis. Multiple sclerosis and related disorders. 2017 Aug 1;16:8-14.

http://pmjournal.ir Review Article

Winter 2022, Volume7, Issue 24 (28-32)

A Review of The Role of Exosomes in Prostate Cancer

Rafid A Abdulkareem[1], Seyed Majid Hashemi Fard[2*], Masoomeh Kohandani[2]

[1]Genetic engineering and biotechnology Institute, Baghdad University, Iraq.
[2]Department of Biology, Payame Noor University, Tehran, Iran.
[3] Master of Science in Cellular and Molecular Biology, Department of cell and Molecular Biology, Karazmi University, Tehran, Iran.

Corresponding author: Seyed Majid Hashemi Fard, AmitisGen Lab, Food and Drug Administration of Iran Ministry of Health and Medical Education. Tehran, Iran. Email: majidhf65@gmail.com.

DOI: 10.22034/pmj.2022.252442

Submitted: 2021-11-03
Accepted: 2022-02-15

Keywords:
Exosomes
Prostate Cancer
Extracellular Vesicles
Biomarker
PCa

Abstract:

Prostate cancer (PCa) is the most common solid tumor in men. While patients with local PCa have better prognostic survival, patients with metastatic PCa have relatively high mortality rates. Exosomes (and other extracellular vesicles) are now part of the cancer research landscape, involved both as players in pathophysiological mechanisms, as biomarkers of the cancer process, and as therapeutic tools. Exosomes contain miRNAs, mRNAs, and proteins with the potential to regulate signaling pathways in recipient cells. Accumulating evidence indicates that exosomes play important roles in cell communication and tumor progression and are suitable for monitoring PCa progression and metastasis. we review the role of exosomes and exosomal microRNAs in biological processes of prostate cancer progression for treatment personalization.

INTRODUCTION

Prostate cancer (PCa) is the second most frequent tumor in males. PCa is a high prevalence in developing countries. Most individuals with prostate cancer present with locally advanced or metastatic disease at diagnosis thus limiting the effectiveness of conventional therapies. Prostate-specific antigen (PSA) is a widely utilized biomarker for PCa screening, nonetheless, it does not provide precise and accurate diagnostic and prognostic information (1, 2). Importantly, the tumors of many patients with prostate cancer are refractory to androgen therapy and progress to metastatic castration-resistant disease (3). An effective treatment course for prostate cancer patients requires predictive biomarkers in metastatic castration-resistant prostate cancer that support individual therapy (4). Like all epithelial cancers, prostate cancer (PCa) has not escaped the exosomal fever that has affected both researchers and clinicians for the last twenty years with the development of extracellular vesicles (EVs) individualization and counting techniques, and omics characterization techniques (5).

During cancer development, signal transmission between cells plays a vital role in tumor formation, progression, and metastasis. Exosomes are small extracellular vesicles (EV) ranging from 50 to 150 nm in diameter. Exosomes have a double membrane structure with various cargo contents, such as miRNAs, mRNAs, proteins, lipids, and viral particles (6). Over the last decade, exosome research has rapidly expanded, and the number of coherent publications has gradually increased (7). Exosomes are present in various biological fluids, for instance, blood, urine, milk, semen as well as saliva, and can be purified from the cell growth medium. The biological function of an exosome depends on the contents of the cargo, for instance, miRNAs, viral particles, mRNAs, proteins, or lipids (8). The complex signaling pathway network between exosome-mediated cancer cells and the tumor microenvironment (TME) is considered a key factor in the progression of cancer at all stages (9). It has been shown that urinary markers can aid in the decision-making process regarding whether to carry out a prostate biopsy and in the design of a therapeutic strategy. Urinary exosomes and their cargo, especially miR-21 and miR-375, have become an emerging source of biomarkers in the detection

and prognosis of PCa (10).

The main objective of this review is to describe recent progress in exosome research focusing on the potential role of exosomes as novel biomarkers for PCa.

Exosomes: Structure and Function

Exosomes are small (from 30 to 120 nm in diameter) extracellular vesicles (EVs). Their lipid bilayer membrane, with a width of 5 nm, protects them from the negative action of RNases and proteases. Exosomes comprise a lipid bilayer membrane and encapsulated molecules. Components of the membrane include lipids and proteins (11). Exosomes have longer retention in circulation in comparison to polymersomes or liposomes (12). According to the International Society of Extracellular Vesicles (ISEV), the term "extracellular vesicles" is the appropriate terminology for heterogeneous populations of vesicles isolated from cell culture supernatants or physiological fluids (13). Exosome shedding is a process with a wide range of important regulatory functions. Their discovery in sheep reticulocyte maturation gave rise to the idea that exosomes may function as a trash bin for unnecessary and redundant proteins and therefore could be an alternative pathway for lysosomal degradation (14). Exosomes are released by the exocytosis of multivesicular bodies (MVBs), developed from early and then late endosomes. Those naturally occurring membrane particles mediate intercellular communication by delivering molecular information between cancer and stromal cells, especially cancer-associated fibroblasts (CAFs) (14, 15). Exosomes are present in body fluids, including the plasma, cerebrospinal fluid, and urine. As a material "transport carrier" in the circulated body fluids, exosomes play an important role in a variety of physiological and pathological processes due to their ability to carry a variety of proteins, nucleic acids, and lipids, transporting the contents to surrounding cells for inter-cell communication (16).

In vivo studies in mice have shown that some exosomes can directly deliver mRNA to recipient cells, especially under the stimulation of acute or chronic infections (17). Exosomes also have immunoregulatory activities including antigen presentation and immune tolerance. Exosomes carrying MHC class II complexes that bind to tumor-specific antigens were able to significantly inhibit tumor growth in mice (18). These exosomes may indirectly activate naïve T cells and B cells by interacting with antigen-presenting cells, and may also promote the proliferation of CD4+ T cells (19). Recent studies point out that exosomes released from the tumor microenvironment can regulate (also by tethering TGF) a proliferation, a reduction of apoptosis, promotion of angiogenesis, and, finally, evasion of immune surveillance. Moreover, exosomes can provide candidate biomarkers for prostate cancer, contribute to tumor progression and, after a loss of environment homeostasis, promote tumor metastasis (19, 20).

Role of Exosomes in Cancer

In recent years, research has focused on the usefulness of exosomes in diagnosing cancer patients as well as monitoring their responses to therapy (21). Because of stability, exosomes are easily harvested from a variety of accessible body fluids. This makes them attractive targets for developing new methods for detecting cancer (22). The use of exosomes and exosomal cargo for cancer diagnostics requires the identification of the most commonly deregulated genes for a specific cancer type (23). Among the different types of exosomes, tumor cell-derived exosomes play an essential role in the invasion and metastasis of cancer cells. Tumor cell-derived exosomes can transmit tumor metastasis signals, determine the direction of cancer cell metastasis, and promote epithelial-mesenchymal transformation (EMT) and angiogenesis. In some tumors, cells may release higher quantities of exosomes/microvesicles when compared to normal cells. This increase in exosomes release may be caused by enhanced proliferation rates of cancer cells or cell damage triggered by chemotherapy (24). Moreover, changes in the environmental conditions, like hypoxia, also accelerate this release and can increase invasiveness. Exosomes favor cancer progression by modulating different processes, like the immune response and angiogenesis stimulation, invasion, and resistance (25). Some exosomes also have immunomodulatory functions and cancer treatment potential (22, 26, and 27).

Prostate cells release diverse types of membrane vesicles into extracellular environment. These vesicles released from prostate epithelial cells at times described as 'prostasomes' correspond to bigger sized (30-200nm) vesicles as compared to the exosomes. PCa exosomes are thought to favor the microenvironment for the cellular transformation into tumors, and a large part of such exosomes are also released to prostatic secretions like urine and blood (28). the release of exosomes in biofluids could have major advantages to shed light on complex mechanisms of tumor progression and treatment response. The intercellular exchange of genetic and non-genetic signals via extracellular vesicles (herein, named exosomes) is an emergent tool in personalized cancer medicine (29).

Exosome Functions in Prostate Cancer

Most deaths of advanced prostate cancer patients are due to the metastasis of prostate cancer. Exosomes derived from tumors can be taken by the cells of specific organs and assist in the formation of the pre-metastatic niche. Prostate cancer has metastatic organotropism of

the bone (30). Bone metastasis is the most common type of metastasis from advanced prostate cancer (PCa). Pyruvate kinase M2 (PKM2) is transported through exosomes from PCa cells into BMSCs (bone marrow stromal cells) (31). This feature is a novel mechanism via which primary tumor-originated exosomes enhance premetastatic niche formation (32). PCa-derived exosomes upregulate PKM2 expression, which ultimately upregulates CXCL12 expression (C-X-C motif chemokine ligand-12) in BMSCs thus inducing a pre-metastatic niche. Targeting the exosome-triggered CXCL12 axis abrogates exosome-stimulated bone metastasis indicating the therapeutic potential of targeting exosome-derived PKM2 (33, 34). Exosomes are key biomarkers for the early diagnosis of PCa, personalized treatment, and prognosis of patients (35). Exosomes in the blood and urine of PCa patients were reported to contain unique PCa-specific components, which are the source of biomarkers for PCa metastasis identified 36 exosomal miRNAs and proteins as candidate biomarkers for PCa in clinical studies (36). In prostate cancer, plasma vesicles, isolated using the precipitation-based ExoQuick method identified miR-1290 and miR-375 as potential prognostic biomarkers in castration-resistant prostate cancer (CRPC), since their level correlates with poorer overall survival ($p < 0.004$) (37). Prostate cancer-derived exosomes contained TGF-β which induced the conversion from bone marrow mesenchymal stem cells to fibroblasts. Exosomes can prepare a pre-metastatic niche. For example, exosomal miR- 21, miR-375, and miR-141 help cancer cells overcome the low-androgen conditions in distant metastatic organs (38).

RNA expression analysis of urine-derived and PCa cell line-derived exosomes revealed that the known RNA markers for PCa, such as the TMPRSS2:ERG fusion gene and prostate cancer antigen 3 (PCA3), can be detected in exosomes by reverse transcriptase-polymerase chain reaction (39). The TMPRSS2:ERG fusion transcripts were detected in urinary exosomes from two patients with high Gleason scores but not in those from two patients with low Gleason scores. PCA3 mRNA was detected in exosomes derived from all patients (40). exosomal miR-26a derived from PCa cells significantly changed the expression of epithelial-mesenchymal transition (EMT)- related factors and inhibited the metastasis and tumor growth of PCa. Exosomal integrin avb3 can also increase PCa aggressiveness. These biologically active molecules in exosomes are promising key biomarkers for PCa diagnosis, metastasis detection, individualized treatment, and patient prognosis (41, 42).

Invasion and Metastasis of Prostate Cancer

Tumor metastasis is a complicated process, including vascular leakiness and an alteration of the microenvironment, in which exosomes are also involved (42). Initially, exosomes begin an epithelial-mesenchymal transition (EMT) via miRNAs by losing their junction and adhesion ability. Thus, epithelial tumor cells obtain mesenchymal cell properties and are responsive to malignancy (43). The tumor microenvironment contributes to the regulation of prostate cancer progression through proliferation, angiogenesis, and metastasis, and it also regulates immunity (44). Exosomes released from the TME regulate proliferation, reduce apoptosis, promote angiogenesis, and regulate immune escape, thus promoting the invasion and metastasis of PCa (45).

Exosomes in prostate cancer therapy

EVs can be used as carriers to deliver therapeutic agents to tumor cells, leading to an effective tumor cell killing, while minimizing the side effects of the drugs (46). Exosomes can be used as a delivery vector to target cancer cells and the contents can escape the attack by the immune system (47). Adipose-derived stromal cells (ASCs) derived exosomal miR-145 could reduce the activity of Bcl-xL and promote prostate cancer cell apoptosis via the caspase-3/7 pathway. Therefore, ASCs-derived exosomes can be used in prostate cancer therapy (48). Qi et al. confirmed that drug-loaded exosomes enhanced cancer cell targeting under an external magnetic field and suppressed tumor growth (49). Saari et al. confirmed that cancer cell-derived EVs can be used as effective carriers of Paclitaxel to autologous prostate cancer cells by increasing its cytotoxicity (50).

The simultaneous application of either radiation technology or nuclear medicine with exosomes are promising tools for the realization of the enhancement of targeting strategies using radiation technology (51). Exosomes are also utilized in tumor vaccination. Tumor-derived exosomes often contain tumor-specific antigens to activate dendritic cells which induce the antitumor response of T lymphocytes (52).

In personalized medicine, customized treatment depends on information about the molecular characteristics of the cancer signature, namely personalized diagnostics. Biomarkers in personalized diagnostics can be divided into several subgroups according to their application: screening, early diagnosis, prognosis, prediction, monitoring, and companion diagnostics (53). In contrast to invasive tissue biopsy, exosomes are effective biomarkers in the diversified diagnosis of personalized medicine. Secondly, exosomes are akin to vessels enriched with much information about the parental cells, and the cargoes in exosomes are protected by the phospholipid bilayer from degradation by proteinases and nucleases. Consequently, biomarkers at a relatively low expression are much easier to be detected through

isolating exosomes. For instance, some biomarkers such as PCA3 and TMPRSS2 are mRNAs not easily detected in body fluids but appear in exosomes in prostate cancer (54).

CONCLUSIONS

Exosomes are small vesicles (50–100 nm) secreted by almost all tissues, representing their tissue origin. By isolating these exosomes, several problems of biomarker discovery from complex body fluids can be largely solved. Many biological molecules are encapsulated in the exosomes from prostate cancer such as miRNAs, lncRNAs, and proteins and their expression levels differ from those of normal prostate cells. The unique characteristics of exosomes such as high stability and high biocompatibility imply that they are potential effective drug delivery systems. However, further studies on the translation of EVs into clinical therapies should be conducted to design standards for exosome classification and manipulation. In summary, exosomes are prospective tools for the development of diagnosis, as well as therapy of PCa, however, further studies should explore the clinical application of exosomes (55, 56).

REFERENCES

1. Sasaki T, Sugimura Y. The importance of time to prostate-specific antigen (PSA) nadir after primary androgen deprivation therapy in hormone-naïve prostate cancer patients. Journal of clinical medicine. 2018 Dec;7(12):565.
2. Siegel Rebecca L, Miller Kimberly D. Jemal Ahmedin. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34.
3. Ye Y, Deng M, Zhao D, Jiang L, Chen D, Wu Z, Wang Y, Li Z, Yang Z, Li J, Zhou F. Prostate cryoablation combined with androgen deprivation therapy for newly diagnosed metastatic prostate cancer: a propensity score-based study. Prostate cancer and prostatic diseases. 2021 Sep;24(3):837-44.
4. Ingrosso G, Detti B, Scartoni D, Lancia A, Giacomelli I, Baki M, Carta G, Livi L, Santoni R. Current therapeutic options in metastatic castration-resistant prostate cancer. InSeminars in Oncology 2018 Oct 1 (Vol. 45, No. 5-6, pp. 303-315). WB Saunders.
5. Kretschmer A, Tilki D. Biomarkers in prostate cancer–current clinical utility and future perspectives. Critical reviews in oncology/hematology. 2017 Dec 1;120:180-93.
6. Fujita K, Nonomura N. Urinary biomarkers of prostate cancer. International Journal of Urology. 2018 Sep;25(9):770-9.
7. Foj L, Ferrer F, Serra M, Arévalo A, Gavagnach M, Giménez N, Filella X. Exosomal and non-exosomal urinary miRNAs in prostate cancer detection and prognosis. The Prostate. 2017 May;77(6):573-83.
8. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic acids research. 2012 Jan 1;40(D1):D1241-4.
9. Fujita Y, Yoshioka Y, Ochiya T. Extracellular vesicle transfer of cancer pathogenic components. Cancer science. 2016 Apr;107(4):385-90.
10. Kumar S, Lombard DB. Functions of the sirtuin deacylase SIRT5 in normal physiology and pathobiology. Critical reviews in biochemistry and molecular biology. 2018 May 4;53(3):311-34.
11. Pullan JE, Confeld MI, Osborn JK, Kim J, Sarkar K, Mallik S. Exosomes as drug carriers for cancer therapy. Molecular pharmaceutics. 2019 Apr 5;16(5):1789-98.
12. Di C, Zhang Q, Wang Y, Wang F, Chen Y, Gan L, Zhou R, Sun C, Li H, Zhang X, Yang H. Exosomes as drug carriers for clinical application. Artificial Cells, Nanomedicine, and Biotechnology. 2018 Nov 12;46(sup3):S564-70.
13. Qin J, Xu Q. Functions and application of exosomes. Acta Pol Pharm. 2014 Mar;71(4):537-43.
14. Guo W, Gao Y, Li N, Shao F, Wang C, Wang P, Yang Z, Li R, He J. Exosomes: New players in cancer. Oncology reports. 2017 Aug 1;38(2):665-75.
15. Witwer KW, Buzás EI, Bemis LT, Bora A, Lässer C, Lötvall J, Nolte-'t Hoen EN, Piper MG, Sivaraman S, Skog J, Théry C. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. Journal of extracellular vesicles. 2013 Jan 1;2(1):20360.
16. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nature reviews immunology. 2002 Aug;2(8):569-79.
17. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. The Journal of experimental medicine. 1996 Mar 1;183(3):1161-72.
18. Clayton A, Mason MD. Exosomes in tumour immunity. Current oncology. 2009 May;16(3):46-9.
19. Lynch S, Santos SG, Campbell EC, Nimmo AM, Botting C, Prescott A, Antoniou AN, Powis SJ. Novel MHC class I structures on exosomes. The Journal of Immunology. 2009 Aug 1;183(3):1884-91.
20. Sharrow SO, Mathieson BJ, Singer A. Cell surface appearance of unexpected host MHC determinants on thymocytes from radiation bone marrow chimeras. The Journal of Immunology. 1981 Apr 1;126(4):1327-35.
21. Huang T, Deng CX. Current progresses of exosomes as cancer diagnostic and prognostic biomarkers. International journal of biological sciences. 2019;15(1):1.
22. Soung YH, Ford S, Zhang V, Chung J. Exosomes in cancer diagnostics. Cancers. 2017 Jan;9(1):8.
23. Makler A, Narayanan R. Mining exosomal genes for pancreatic cancer targets. Cancer genomics & proteomics. 2017 May 1;14(3):161-72.
24. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics–Clinical Applications. 2015 Apr;9(3-4):358-67.
25. Falcon-Perez J. Exosome profiling: potential in cancer diagnosis and stratification. InEndocrine Abstracts 2017 May 3 (Vol. 49). Bioscientifica.
26. Zhai LY, Li MX, Pan WL, Chen Y, Li MM, Pang JX, Zheng L, Chen JX, Duan WJ. In situ detection of plasma exosomal microRNA-1246 for breast cancer diagnostics by a Au nanoflare probe. ACS applied materials & interfaces. 2018 Oct 23;10(46):39478-86.
27. Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M. Sorting it out: regulation of exosome loading. InSeminars in cancer biology 2014 Oct 1 (Vol. 28, pp. 3-13). Academic Press.
28. Lázaro-Ibáñez E, Neuvonen M, Takatalo M, Thanigai Arasu U, Capasso C, Cerullo V, Rhim JS, Rilla K, Yliperttula M, Siljander PR. Metastatic state of parent cells influences the uptake and functionality of prostate cancer cell-derived extracellular vesicles. Journal of extracellular vesicles. 2017 Dec 1;6(1):1354645.
29. Kharmate G, Hosseini-Beheshti E, Caradec J, Chin MY, Tomlinson Guns ES. Epidermal growth factor receptor in prostate cancer derived exosomes. PLoS One. 2016 May 6;11(5):e0154967.
30. Vlaeminck-Guillem V. Extracellular vesicles in prostate cancer carcinogenesis, diagnosis, and management. Frontiers in oncology. 2018 Jun 13;8:222.
31. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Liang M, Kohli M. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC genomics. 2013 Dec;14(1):1-4.
32. Wang X, Wang X, Zhu Z, Li W, Yu G, Jia Z, Wang X. Prostate carcinoma cell-derived exosomal MicroRNA-26a modulates the

metastasis and tumor growth of prostate carcinoma. Biomedicine & Pharmacotherapy. 2019 Sep 1;117:109109.
33.Krishn SR, Singh A, Bowler N, Duffy AN, Friedman A, Fedele C, Kurtoglu S, Tripathi SK, Wang K, Hawkins A, Sayeed A. Prostate cancer sheds the αvβ3 integrin in vivo through exosomes. Matrix Biology. 2019 Apr 1;77:41-57.
34.Dai J, Escara-Wilke J, Keller JM, Jung Y, Taichman RS, Pienta KJ, Keller ET. Primary prostate cancer educates bone stroma through exosomal pyruvate kinase M2 to promote bone metastasis. Journal of Experimental Medicine. 2019 Dec 2;216(12):2883-99.
35.Osaki M, Okada F. Exosomes and their role in cancer progression. Yonago acta medica. 2019;62(2):182-90.
36.Liu CM, Hsieh CL, Shen CN, Lin CC, Shigemura K, Sung SY. Exosomes from the tumor microenvironment as reciprocal regulators that enhance prostate cancer progression. International Journal of Urology. 2016 Sep;23(9):734-44.
37.Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Liang M, Kohli M. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC genomics. 2013 Dec;14(1):1-4.
38.Steinbichler TB, Dudás J, Riechelmann H, Skvortsova II. The role of exosomes in cancer metastasis. InSeminars in cancer biology 2017 Jun 1 (Vol. 44, pp. 170-181). Academic Press.
39.Sánchez CA, Andahur EI, Valenzuela R, Castellón EA, Fullá JA, Ramos CG, Triviño JC. Exosomes from bulk and stem cells from human prostate cancer have a differential microRNA content that contributes cooperatively over local and pre-metastatic niche. Oncotarget. 2016 Jan 26;7(4):3993.
40.Lorenc T, Klimczyk K, Michalczewska I, Słomka M, Kubiak-Tomaszewska G, Olejarz W. Exosomes in prostate cancer diagnosis, prognosis and therapy. International Journal of Molecular Sciences. 2020 Jan;21(6):2118.
41.Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. Blood, The Journal of the American Society of Hematology. 2011 Mar 31;117(13):3648-57.
42.Zhang HL, Qin XJ, Cao DL, Zhu Y, Yao XD, Zhang SL, Dai B, Ye DW. An elevated serum miR-141 level in patients with bone-metastatic prostate cancer is correlated with more bone lesions. Asian journal of andrology. 2013 Mar;15(2):231.
43.Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. science. 2011 Mar 25;331(6024):1559-64.
44.Saber SH, Ali HE, Gaballa R, Gaballah M, Ali HI, Zerfaoui M, Abd Elmageed ZY. Exosomes are the driving force in preparing the soil for the metastatic seeds: lessons from the prostate cancer. Cells. 2020 Mar;9(3):564.
45.Bijnsdorp IV, Geldof AA, Lavaei M, Piersma SR, van Moorselaar RJ, Jimenez CR. Exosomal ITGA3 interferes with non-cancerous prostate cell functions and is increased in urine exosomes of metastatic prostate cancer patients. Journal of extracellular vesicles. 2013 Jan 1;2(1):22097.
46.Yim N, Ryu SW, Choi K, Lee KR, Lee S, Choi H, Kim J, Shaker MR, Sun W, Park JH, Kim D. Exosome engineering for efficient intracellular delivery of soluble proteins using optically reversible protein–protein interaction module. Nature Communications. 2016 Jul 22;7(1):1-9.
47.Takahara K, Ii M, Inamoto T, Nakagawa T, Ibuki N, Yoshikawa Y, Tsujino T, Uchimoto T, Saito K, Takai T, Tanda N. microRNA-145 mediates the inhibitory effect of adipose tissue-derived stromal cells on prostate cancer. Stem cells and development. 2016 Sep 1;25(17):1290-8.
48.Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T, Angevin E. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nature medicine. 2001 Mar;7(3):297-303.
49.Qi H, Liu C, Long L, Ren Y, Zhang S, Chang X, Qian X, Jia H, Zhao J, Sun J, Hou X. Blood exosomes endowed with magnetic and targeting properties for cancer therapy. ACS nano. 2016 Mar 22;10(3):3323-33.
50.Saari H, Lázaro-Ibáñez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, Yliperttula M. Microvesicle-and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells. Journal of Controlled Release. 2015 Dec 28;220:727-37.
51.Chaput N, Taïeb J, Schartz N, Flament C, Novault S, André F, Zitvogel L. The potential of exosomes in immunotherapy of cancer. Blood Cells, Molecules, and Diseases. 2005 Sep 1;35(2):111-5.
52.Viaud S, Théry C, Ploix S, Tursz T, Lapierre V, Lantz O, Zitvogel L, Chaput N. Dendritic cell-derived exosomes for cancer immunotherapy: what's next?. Cancer research. 2010 Feb 15;70(4):1281-5.
53.Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366: 883–92.
54.van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev. 2012; 64: 676–705.
55.Pan J, Ding M, Xu K, Yang C, Mao LJ. Exosomes in diagnosis and therapy of prostate cancer. Oncotarget. 2017 Nov 14;8(57):97693.
56.Duijvesz D, Luider T, Bangma CH, Jenster G. Exosomes as biomarker treasure chests for prostate cancer. European urology. 2011 May 1;59(5):823-31.

نشریه پزشکی شخصی

فصلنامه پزشکی/سال ششم/شماره بیست و چهارم/قیمت: ۱۵۰۰۰۰ زمستان۱۴۰۰/ شماره شاپا ۳۸۶۰-۲۷۱۷

آینده علم پزشکی، شخصی محور است