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REGULATION OF THE CXCL10 EXPRESSION AND INVESTIGATION OF THE RELATIONSHIPS OF THE CXCL10 DNA SEQUENCE VARIATION AND DISEASE

CXCL10 EKSPRESYONUNUN DÜZENLENMESİ VE CXCL10 DNA DİZİ VARYASYONU VE HASTALIK İLİŞKİLERİNİN İNCELENMESİ

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ABSTRACT

Objective: Chemokine proteins are significantly effective in inflammation and immunity. Chemokines are from the family of the chemokine proteins and they organise the leukocyte trafficking through the formation of chemotactic activity in the cells that express the appropriate chemokine receptors. CXCL10 is involved in the CXC chemokine family and is effective in biological events such as chemotaxis, apoptosis, cell growth, and angiostasis through the attachment to the CXCR3 receptor. CXCL10 is pleiotropic due to its effects on different disease groups such as autoimmune disorders, transplantation, infectious diseases, and cancer. The aim of this study was to assess the potential role of CXCL10 on the pathogenesis of various diseases.

Material and methods: The eQTL effects of CXCL10 expression and the regulation of microRNAs (miRNAs) in terms of co-regulated gene clusters were examined. The STRING/GeneMANIA/KEGG PATHWAY/GeneCards was used for the investigation of the gene-protein and pathway interactions; for the detection of miRNA targeting CXCL102, TargetScan/miRDB was used; for the investigation of the association of CXCL10 and miRNA region single nucleotide polymorphisms (SNP) with the diseases, GRASP and GWAS were used; GSEA/MSigDB database was used for gene enrichment analysis

Results: Both the GSEA/MSigDB tool and the gene set enrichment analysis recommended the use of the enriched forms of the genes involved in breast and prostate cancers and in response to inflammation, and to interferon and regulatory T cells (FDR<1E-50). 182 genes (at a 5-fold threshold) that are structurally co-expressed with five additional CXCL genes close to CXCL10 were identified with the use of the CO-Regulation database (CORD. No enrichment was detected for the common targets of

ÖZ

Amaç: Kemokin proteinlerinin, inflamasyon ve bağışıklıkta önemli rolleri vardır. Kemokinler, kemokin proteinleri ailesinin bir üyesi olup uygun kemokin reseptörlerini eksprese eden hücrelerde kemotaktik aktivite üreterek lökosit trafiğinde önemli bir rol oynarlar. CXCL10, CXC kemokin ailesinin bir üyesidir ve CXCR3 reseptörüne bağlanarak kemotaksi, apoptoz, hücre büyümesi, yeni damar oluşumu gibi biyolojik olaylarda etkili olur. CXCL10, otoimmün bozukluklar, transplantasyon, bulaşıcı hastalıklar ve kanser gibi farklı hastalık gruplarında etkileri olması dolayısıyla pleiotropiktir. Çalışmamızda CXCL10'un çeşitli hastalıkların patogenezi üzerindeki potansiyel rolünü değerlendirmek amaçlanmıştır.

Gereç ve Yöntemler: CXCL10 ekspresyonunun eQTL etkileri ve mikroRNA'ların (miRNA'lar); birlikte düzenlenmiş gen kümeleri açısından düzenlenmesi incelendi. Gen-protein ve yolak etkileşimlerinin incelenmesi için STRING/GeneMANIA/KEGG PATHWAY/GeneCards; CXCL102'yi hedefleyen miRNA'ların tespiti için TargetScan/miRDB; CXCL10'u hedeflemek için Blood eQTL Tarayıcı / BIOS / mQTLdb; CXCL10 ve miRNA bölgesi tek nükleotid polimorfizmleri (SNP)'nin hastalıklarla olan ilişkisinin incelenmesi için GRASP ve GWAS, gen zenginleştirme analizi için ise GSEA/MSigDB veri tabanları kullanıldı.

Bulgular: Birlikte eksprese edilen genler için, GSEA/MSigDB veri tabanında yapılan gen seti zenginleştirme analizi sonucunda, immün yanıt ve inflamatuar yanıtta yer alan genler, interferon ve düzenleyici T hücrelerine yanıtta yer alan genler, ve ayrıca meme ve prostat kanserlerinde yer alan genler ile gen setinin genişletilmesi önerildi (FDR<1E-50). CO-Düzenleme Veritabanı (CORD), CXCL10 çevresinde yer alan beş ek CXCL geni de dahil olmak üzere, yapısal olarak birlikte eksprese edilen 182 gen (5 kat eşikte) tanımladı. Birlikte eksprese edilen gen seti, herhangi bir miRNA'nın ortak

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any miRNA in the co-expressed gene sets. The CXCL10 targeting miRNAs were selected, and the TargetScan program was used to identify other target genes in our study. Thus, 80 miRNAs were identified, and the same GSEA analysis was performed for each miRNA target. The association of SNPs with the diseases was investigated for the gene region of each miRNA in the GWAS databases, and an association was detected with the autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, and psoriasis, and with multiple sclerosis, Type 1 diabetes, myasthenia gravis, and allergy/asthma (P<1E-04). For SNPs in CXCL10, no GWAS associations were found; however, SNPs acting as eQTL/meQTL in the blood for CXCL10 had GWAS associations with longevity, aging, inflammatory bowel disease (IBD), and breast cancer (P<1E-04). Although we found no strong evidence for miRNA-mediated CXCL10 expression in our study, strong genetic associations were found associated with inflammatory and immune disorders in the miRNAs neighboring variants.

Conclusion: In conclusion, we suggest that there is a stronger role of CXCL10 in inflammation, autoimmunity, and possibly cancer than its role in transplantation.

Keywords: Genes, disease relationships, autoimmune diseases, bioinformatics, CXCL10

hedefleri için zenginleşmeye sahip değildi. Çalışmamızda CXCL10'u hedefleyen miRNA'lar seçildi ve diğer hedef genleri belirlemek için de TargetScan programı kullanıldı. Böylece 80 miRNA belirlendi ve her bir miRNA'nın hedefleri aynı GSEA analizine tabi tutuldu. GWAS veri tabanlarındaki her bir miRNA gen bölgesi için SNP'lerin hastalıklar ile ilişkileri incelendi ve bu inceleme sonucunda Romatoid artrit, Ankilozan spondilit, Crohn Hastalığı, Psoriasis gibi otoimmün hastalıklar, Multipl Skleroz, Tip 1 diyabet, Miyastenia gravis ve alerji/astım gibi hastalıklar ile ilişki tespit edildi (P<1E-04). CXCL10 içindeki SNP'ler için ise herhangi bir GWAS ilişkisi bulunmadı, ancak CXCL10 için kanda eQTL/meQTL olarak hareket eden SNP'lerin, uzun ömür, yaşlanma, inflamatuar barsak hastalığı (IBD) ve meme kanseri ile GWAS ilişkileri tespit edildi (P<1E-04). Çalışmamızda miRNA aracılı CXCL10 ekspresyonu için güçlü kanıtlar elde edilememiş olsa da, miRNA'ların yakınındaki varyantlar, inflamatuar ve immün bozukluklarla güçlü genetik ilişkiler gösterdi.

Sonuç: CXCL10'un inflamasyon, otoimmünite ve muhtemelen kanserde oynadığı rolün transplantasyondan daha güçlü olduğu sonucuna vardık.

Anahtar Kelimeler: Genler, hastalık ilişkileri, otoimmün hastalıklar, biyoinformatik, CXCL10

INTRODUCTION

Human body has been found to have roughly 50 chemokines (1). Chemokines are the protein molecules with multiple domains, and with the molecular weight of 8-12 kD. The chemokine genes are located at locus 17q11.2-12, while the C-X-C chemokine genes are located at locus 4q13. CXCL10 (C-X-C Motif Chemokine Ligand 10) is a protein-encoding gene, and was first discovered as a chemokine which was induced by interferon (IFN) -γ, and produced by various cell types such as monocytes, neutrophils, endothelial cells, keratinocytes, fibroblasts, and mesenchymal cells (2-8). Various studies in the literature reported that chemokines and particularly CXCL10 are produced by various cell and tissue types, and have pleiotropic effects on various processes including immunity, angiogenesis and organ-specific cancer metastasis (9). The CXCL10 C-X-C-C motif chemokine ligand is a chemokine of the CXC sub-family and is an antimicrobial gene encoding the ligand corresponding to the CXCR3 receptor. Binding of this protein to CXCR3 provides it with pleiotropic effects, such as stimulation of monocytes, induction of migration of natural killer cells and T cells, and modulation of adhesion molecule expression (2). Among the related pathways, there is a signaling with the peptide ligand binding receptors and G protein-linked receptors, and the CXCL10 is effective through this pathway in various human diseases such as chronic inflammation, infectious diseases, immune dysfunction, tumor growth, metastasis, and invasion (10). In addition, CXCL10 has been identified as an important biomarker that predicts disease severity and can be used as a prognostic indicator in multiple diseases. Understanding the role of CXCL10 in the pathogenesis and progression of diseases could underpin its development as a potential biomarker and therapeutic target for related human malignancies (11). The study of the role of CXCL10 in the pathogenesis of infectious diseases has shown its various roles in the pathogenesis and progression of other diseases (12). The use of CXCL10 as a potential therapeutic target in infectious diseases is still a topic under discussion. Specifically, this chemokine controls chemotaxis during the inflammatory response that occurs in allograft rejection after transplantation. Interestingly, there was a strong association between CXCL10 production, immune response, and graft survival after allotransplantation. Increased production of CXCL10 has been observed in patients in various organ transplants. This enhanced production is suggested to be probably due to graft or immune cells and is thought to be associated with an increase in the concentration of circulating CXCL10. The fact that the measurement of the level of CXCL10 in serum and plasma is easy makes it possible to use the detection and quantification of circulating CXCL10 to reveal the immune status of a transplant recipient (13). The production of IFN-gamma and tumor necrosis factor-alpha by Thl lymphocytes located in tissues stimulates the secretion of CXCL10 from various cells and causes the autoimmune process to continue, generating an amplification feedback loop. There is a need for further studies to reveal the contribution of interactions between chemokines and cytokines to the pathogenesis of autoimmune diseases and to evaluate the potential of the utilisation of CXCL10 as a new therapeutic target in various autoimmune diseases (14). We aimed to examine the role of CXCL10 in the pathogenesis and prognosis of diseases using various databases and tools in the present study.

MATERIAL And METHODS

For the investigation of gene-protein and pathway interactions, the STRING (string-db.org)/Gene MANIA (https://genemania.org/)/ KEGG PATHWAY (https://www.genome.jp/kegg/pathway.html) /GeneCards (www.genecards.org) was used; for the detection of miRNAs targeting CXCL102 the TargetScan (www.targetscan.org)/miRDB (www.mirdb.org) was used; Blood eQTL Browser was used for targeting CXCL10 (http://www.genenetwork.nl/bloodeqtlbrowser)/

BIOS (http://genenetwork.nl/biosqtlbrowser) /mQTLdb (http://mqtldb.org); for the investigation of the association of CXCL10 and miRNA region single nucleotide polymorphisms (SNP) with the diseases, GRASP (https://grasp.nhlbi.nih.gov/search.aspx) and GWAS (https://www.ebi.ac.uk/gwas/) were used and GSEA/MSigDB database (https://www.gsea-msigdb.org) was used for gene enrichment analysis.

RESULTS

The GeneMANIA database detected 77.64% of the physical interactions related to CXCL10 (Figure 1). The co-expression interaction with CXCL9 and CXCR3 chemokines was 8.01%. In addition, the pathway interaction of CXCL10 and CXCL9 with the CXCR3 receptor was 1.88%. With GenMANIA, 26 genes that interact with CXCL10 have been identified, including 8 additional CXCL genes around CXCL10. With the use of 5 fold threshold in the common expression analysis on CO-Regulation database or CORD, 182 harmoniously expressed genes were identified including the additional 5 CXCL genes(CXCL2/3/9/9/11/13) found as CXCL10 (CHR4Q21) in the cytogenetic band.

10 MHC region genes including HLA-DRA, DQA1, DPA1, UBD, AIF1, IER3, C2, CFB, TAP2 and HLA-DRB3 were also found to be strongly co-regulated; however, the co-unterparts of beta chain encoding were missing. The most important common expression model was detected in the spleen, as well as in other tissues and cells related to immunity such as neutrophils, thymus and B cells. After selecting the miRNAs targeting CXCL10, 80 miRNAs were identified in the TargetScan program, which is used to identify other target genes, and the same GSEA analysis was performed for the targets of each miRNA. Using the TargetScan (http://www.targetscan.org), miRNAs targeting CXCL10 with a context score lower than -0.30 were selected, and other target genes were identified. In total, 80 miRNAs were determined to target CXCL10. When the gene sets consisting of the targets of these miRNAs were analysed using the same GSEA analysis for each miRNA separately, the results did not reach as much statistical significance as the co-expressed gene set had reached. The Targetscan program that was used to select miRNAs targeting CXCL10 and identify other target genes obtained results close to statistical significance, suggesting that miRNAs play an important role in the regulation of CXCL10 expression (Table 1). The gene set enrichment analysis to detect the set of genes co-expressed in the GSEA/MSigDB tool suggested the enrichment of genes involved in breast and prostate cancers, immune and inflammatory response, and response to interferon and regulatory T cells (FDR<10-50). None of the miRNA targets had enrichment by the co-expressed gene set. We examined the eQTLs/meQTLs for CXCL10 together with the relevant miRNA variants in order to understand the regulation of the CXCL10 (eQTL effects and miRNAs; co-regulated gene sets) expression, the disease associations of CXCL10 sequence variants and their pathophysiological roles (Table 2). As a result of the investigation of the disease associations of SNPs from each miRNA gene regions in GWAS databases, the P was found as < 10-4 for the autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, celiac disease, and psoriasis, and for multiple sclerosis, Type 1 diabetes, myasthenia gravis, Gravis disease, and allergies/asthma. The SNPs within CXCL10 showed no GWAS associations; however, SNPs acting as eQTL/meQTL in the blood for CXCL10 showed GWAS associations with longevity, aging, IBD, and breast cancer (P<10-4) (Table 3).

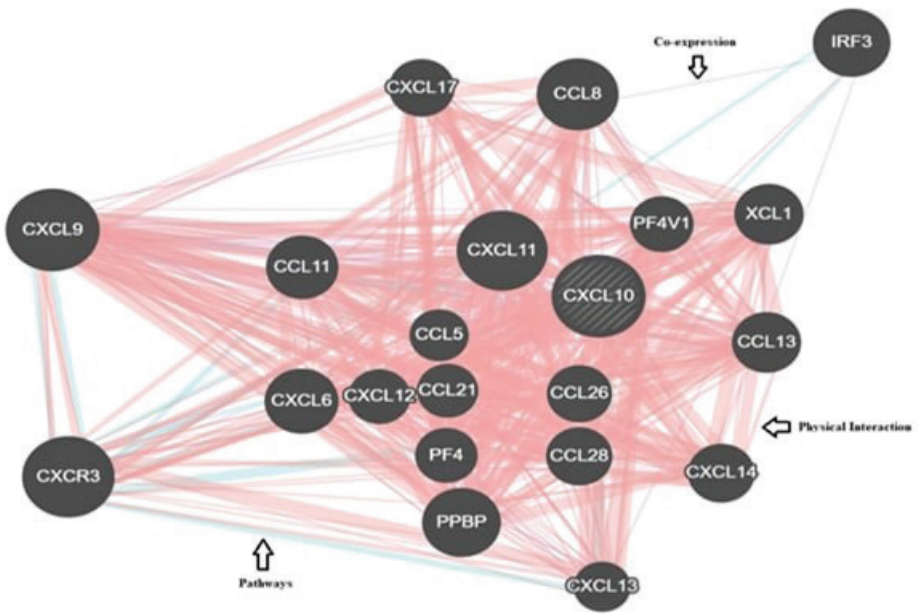

Figure 1: CXCL 10-related physical interactions

Table 1: miRNAs targeting CXCL10

miRNA	Gene name (Gene ID)	Chromosome and coordinates	Conserved binding site for CXCL10	Context ++ score for CXCL10
hsa-miR-503-5p	MIR503 (574506)	chrX: 134546328..134546398	Yes	-0.33
hsa-miR-497-5p	MIR497 (574456)	Chromosome 17, NC_000017.11 (7017911..7018022	Yes	-0.36
hsa-miR-15a-5p	MIR15A (406948)	Chromosome 13, NC_000013.11 (50049119..50049201	Yes	-0.34
hsa-miR-15b-5p	MIR15B (406949)	Chromosome 3 NC_000003.12 (160404588..160404685)	Yes	-0.34
hsa-miR-6838-5p	MIR6838 (102465504)	Chromosome 7 NC_000007.14 (44073378..44073433	Yes	-0.33
hsa-miR-424-5p	MIR424 (494336)	chrX: NC_000023.11 (134546614..134546711	Yes	-0.33
hsa-miR-6828-5p	MIR6828 (102465497)	Chromosome 3 NC_000003.12 (170423103..170423162)	No	-0.47
hsa-miR-6079	MIR6079 (102464830)	Chromosome 1 NC_000001.11 (43838622..43838683)	No	-0.49
hsa-miR-8085	MIR8085 (102465879)	Chromosome 19 NC_000019.10 (44758657..44758721)	No	-0.48
hsa-miR-6731-5p	MIR6731 (102465437)	Chromosome 1 NC_000001.11 (24919345..24919416	No	-0.45
hsa-miR-9500	MIR9500 (103504730)	Chromosome 2 NC_000002.12 (218823090..218823154)	No	-0.46
hsa-miR-135b-3p	MIR135B (442891)	Chromosome 1, NC_000001.11 (205448302..205448398,	No	-0.37
hsa-miR-135b-5p	MIR135B (442891)	Chromosome 1, NC_000001.11 (205448302..205448398,	No	-0.52
hsa-miR-135a-5p	MIR135A1 (406925)	Chromosome 3, NC_000003.12 (52294219..52294308,	No	-0.52
hsa-miR-3591-5p	MIR3591 (100616357)	Chromosome 18, NC_000018.10 (58451080..58451152	No	-0.49
hsa-miR-5587-5p	MIR5587 (100847028)	Chromosome 16, NC_000016.10 (535316..535368)	No	-0.55
hsa-miR-646	MIR646 (693231)	Chromosome 20, NC_000020.11 (60308474..60308567)	No	-0.54
hsa-miR-4524b-5p	MIR4524B 100847008)	Chromosome 17, NC_000017.11 (69099542..69099656)	No	-0.35
hsa-miR-4524a-5p	MIR4524A(100616316)	Chromosome 17, NC_000017.11 (69099564..69099632	No	-0.35
hsa-miR-155-3p	MIR155 (406947)	Chromosome 21, NC_000021.9 (25573980..25574044)	No	-0.41
hsa-miR-4451	MIR4451 (100616349)	Chromosome 4, NC_000004.12 (85722468..85722533)	No	-0.37
hsa-miR-3132	MIR3132(100423039)	Chromosome 2, NC_000002.12 (219549073..219549147	No	-0.35
hsa-miR-197-5p	MIR197 (406974)	Chromosome 1, NC_000001.11 (109598893..109598967)	No	-0.32
hsa-miR-6755-5p	MIR6755 (102465452)	Chromosome 11, NC_000011.10 (86278333..86278398)	No	-0.37
hsa-miR-5006-5p	MIR5006 (100847026)	Chromosome 13, NC_000013.11 (41568286..41568395	No	-0.36

hsa-miR-371a-3p	MIR371A (442916)	Chromosome 19, NC_000019.10 (53787675..53787741)	No	-0.35
hsa-miR-519e-3p	MIR519E (574463)	Chromosome 19, NC_000019.10 (53679940..53680023)	No	-0.34
hsa-miR-33b-3p	MIR33B (693120)	Chromosome 17, NC_000017.11 (17813836..17813931	No	-0.3
hsa-miR-371b-3p	MIR371B (100616185)	Chromosome 19, NC_000019.10 (53787677..53787742	No	-0.3
hsa-miR-4732-5p	MIR4732 (100616385)	Chromosome 17, NC_000017.11 (28861655..28861730	No	-0.38
hsa-miR-936	MIR936 (100126326)	Chromosome 10, NC_000010.11 (104048089..104048186	No	-0.4
hsa-miR-589-3p	MIR589 (693174)	Chromosome 7, NC_000007.14 (5495819..5495917	No	-0.32
hsa-miR-6510-5p	MIR6510 (102466658)	Chromosome 17,NC_000017.11 (41517164..41517217	No	-0.37
hsa-miR-6834-5p	MIR6834 (102465501)	Chromosome 6,NC_000006.12 (33290245..33290325)	No	-0.32
hsa-miR-4710	MIR4710 (100616300)	Chromosome 14,NC_000014.9 (104677694..104677749	No	-0.3
hsa-miR-6739-5p	MIR6739 (102466724)	Chromosome 1,NC_000001.11 (201863373..201863447)	No	-0.4
hsa-miR-6733-5p	MIR6733 (102465439)	Chromosome 1,NC_000001.11 (43171652..43171712	No	-0.37
hsa-miR-3153	MIR3153 (100422936)	Chromosome 9,NC_000009.12 (89312225..89312306)	No	-0.36
hsa-miR-6771-3p	MIR6771 (102465462)	Chromosome 16,NC_000016.10 (50292616..50292675)	No	-0.5
hsa-miR-4251	MIR4251 (100422968)	Chromosome 1,NC_000001.11 (3127975..3128035)	No	-0.44
hsa-miR-5689	MIR5689 (100846998)	Chromosome 6,NC_000006.12 (10439717..10439794)	No	-0.35
hsa-miR-1276	MIR1276 (100302121)	Chromosome 15,NC_000015.10 (85770496..85770578)	No	-0.43
hsa-miR-548ax	MIR548AX (100847063)	Chromosome X,NC_000023.11 (11318614..11318686)	No	-0.37
hsa-miR-548ao-5p	MIR548AO (100847068)	Chromosome 8,NC_000008.11 (41271048..41271143)	No	-0.37
hsa-miR-449a	MIR449A (554213)	Chromosome 5,NC_000005.10 (55170532..55170622)	No	-0.46
hsa-miR-449b-5p	MIR449B (693123)	Chromosome 5,NC_000005.10 (55170646..55170742)	No	-0.48
hsa-miR-34a-5p	MIR34A (407040)	Chromosome 1, NC_000001.11 (9151668..9151777,)	No	-0.46
hsa-miR-34c-5p	MIR34C (407042)	Chromosome 11, NC_000011.10 (111513439..111513515)	No	-0.47
hsa-miR-7106-5p	MIR7106 (102466222)	Chromosome 12,NC_000012.12 (113159113..113159177)	No	-0.4
hsa-miR-3689b-3p	MIR3689B (100500906)	Chromosome 9,NC_000009.12 (134850125..134850272)	No	-0.42
hsa-miR-3689c	MIR3689C (100616333)	Chromosome 9,NC_000009.12 (134849298..134849369)	No	-0.42

hsa-miR-6779-5p	MIR6779 (102465467)	Chromosome 17,NC_000017.11 (38914979..38915042)	No	-0.43
hsa-miR-6780a-5p	MIR6780A (102466195)	Chromosome 17,NC_000017.11 (42708084..42708151	No	-0.42
hsa-miR-30b-3p	MIR30B (407030)	Chromosome 8, NC_000008.11 (134800520..134800607)	No	-0.43
hsa-miR-3689a-3p	MIR3689A(100500846)	Chromosome 9,NC_000009.12 (134849487..134849564)	No	-0.42
hsa-miR-1273h-5p	MIR1273H(102466247)	Chromosome 16,NC_000016.10 (24203116..24203231)	No	-0.44
hsa-miR-6773-5p	MIR6773(102466194)	Chromosome 16,NC_000016.10 (68233426..68233499,)	No	-0.6
hsa-miR-6724-5p	MIR6724(102465433)	Chromosome 21,NC_000021.9 (8205315..8205406)	No	-0.64
hsa-miR-297	MIR297(100126354)	Chromosome 4,NC_000004.12 (110860582..110860647)	No	-0.44
hsa-miR-3942-3p	MIR3942(100500904)	Chromosome 15,NC_000015.10 (35372256..35372364)	No	-0.41
hsa-miR-4789-3p	MIR4789(100616395)	Chromosome 3,NC_000003.12 (175369540..175369621)	No	-0.37
hsa-miR-466	MIR466(100423038)	Chromosome 3, NC_000003.12 (31161704..31161787)	No	-0.42
hsa-let-7g-3p	MIRLET7G(406780)	Chromosome 3, NC_000003.12 (52268278..52268361)	No	-0.49
hsa-let-7a-2-3p	MIRLET7A1(406881)	Chromosome 9, NC_000009.12 (94175957..94176036)	No	-0.49
hsa-miR-6505-3p	MIR6505(102466657)	Chromosome 12, NC_000012.12 (48132797..48132867)	No	-0.31
hsa-miR-8087	MIR8087102465881)	Chromosome 11,NC_000011.10 (27514970..27515047,)	No	-0.35
hsa-miR-570-3p	MIR570(693155)	Chromosome 3, NC_000003.12 (195699401..195699497)	No	-0.33
hsa-miR-4666a-5p	MIR4666A100616308)	Chromosome 1,NC_000001.11 (228462074..228462152)	No	-0.51
hsa-miR-7152-5p	MIR7152102465689)	Chromosome 10,NC_000010.11 (71790747..71790800)	No	-0.58
hsa-miR-4742-3p	MIR4742(100616468)	Chromosome 1,NC_000001.11 (224398227..224398311)	No	-0.54
hsa-miR-411-3p	MIR411(639121)	Chromosome 14, NC_000014.9 (101023325..101023420)	No	-0.42
hsa-miR-379-3p	MIR379(494328)	Chromosome 14, NC_000014.9 (101022066..101022132)	No	-0.42
hsa-miR-1911-5p	MIR1911(100302222)	Chromosome X,NC_000023.11 (114763184..114763263)	No	-0.46
hsa-miR-5192	MIR5192100847087)	Chromosome 2, NC_000002.12 (62205826..62205917)	No	-0.3
hsa-miR-665	MIR665(100126315)	Chromosome 14, NC_000014.9 (100875033..100875104)	No	-0.61
hsa-miR-6830-3p	MIR6830(102465498)	Chromosome 5, NC_000005.10 (132217849..132217918,)	No	-0.38

CXCL10: C-X-C motif chemokine ligand 10

Table 2: CXCL10 eQTLs exon and meQTLs

p value	SNP	SNP Chromosome	SNP Chromosome Position	Exon Chromosome	SNP Allele	Assesed Allele	FDR
5,60E	rs78347618	4	chr_4_76942273_76943101	4	76942687 G/A	A	0.00
1,76E	rs6532172	4	chr_4_76943844_76943970	4	76943907 G/A	A	0.00
6,14	rs71629031	4	chr_4_76943519_76943608	4	76943563 C/T	T	0.00
7,21	Rs6532172	4	chr_4_76944524_76944650	4	76944587 G/A	A	0.00

Perform a quick search for mQTL's across ARIES

Timepoint	SNP	SNP Chr	SNP Pos	A1	A2	CpG	CpG Chr	CpG Pos	beta	Effect Size	p-value	Trans
Adolescence	rs78347618	4	76949076	G	A	cg00809888	4	76862425	-0.40453	0.00335	7.33e-19	N
Birth	rs78347618	4	76949076	G	A	cg04038163	4	76925155	-0.32746	0.02455	7.31e-15	N
Birth	rs78347618	4	76949076	G	A	cg00809888	4	76862425	-0.36992	0.00314	2.37e-16	N
Childhood	rs78347618	4	76949076	G	A	cg00809888	4	76862425	-0.40100	0.00291	8.56e-21	N
Childhood	rs78347618	4	76949076	G	A	cg22252999	4	76996414	-0.23837	0.03478	3.98e-10	N
Childhood	rs78347618	4	76949076	G	A	cg14724265	4	76823661	0.20684	0.01968	2.75e-08	N
Childhood	rs78347618	4	76949076	G	A	cg04038163	4	76925155	-0.36875	0.03244	1.09e-20	N
Middle Age	rs78347618	4	76949076	G	A	cg00809888	4	76862425	-0.42298	0.00384	3.59e-22	N
Middle Age	rs78347618	4	76949076	G	A	cg26767154	4	77069770	-0.21420	0.00210	8.01e-10	N
Middle Age	rs78347618	4	76949076	G	A	cg22252999	4	76996414	-0.21198	0.03325	8.88e-10	N
Pregnancy	rs78347618	4	76949076	G	A	cg22252999	4	76996414	-0.29214	0.02448	1.48e-11	N
Pregnancy	rs78347618	4	76949076	G	A	cg00809888	4	76862425	-0.32682	0.00212	2.14e-13	N

CXCL10: C-X-C motif chemokine ligand 10, eQTLs: Expression quantitative trait loci, meQTLs: Methylation quantitative trait loci, SNP: Single nucleotide polymorphism

Table 3: GWAS associations of eQTLs and meQTLs for CXCL10

eQTL	Associated trait	*P* value	Effect size	PMID	Source
***SDAD1* rs2273**	**Longevity**	**1E-06**	**NA**	**20834067**	**PheGenI**
***ART3* rs12504339**	**Serum albumin levels**	**1.3E-05**	**NA**	**dbGaP**	**PheGenI**
***CXCL9* rs2276886**	**Heart rate**	**1.5E-05**	**NA**	**17903306**	**PheGenI**
***CXCL9* rs2276886**	**Inflammatory bowel disease**	**9.2E-05**	**beta= -0.0766**	**26192919**	**PhenoScanner**
***ART3* rs13139234**	**Serum creatinine level**	**1.1E-05**	**NA**	**20383146**	**GRASP**
***ART3* rs13111494**	**Aging**	**1.3E-05**	**NA**	**22445811**	**GRASP**
***ART3* rs13111494**	**Autism**	**5E-05**	**NA**	**20663923**	**GRASP**
***SCARB2* rs6532244**	**Breast cancer**	**2.1E-04**	**NA**	**17529973**	**PhenoScanner**
***SCARB2* rs894251**	**Body mass index (BMI)**	**1.7E-04**	**NA**	**21935397**	**GRASP**

GWAS: Genome-wide association studies, eQTLs: Expression quantitative trait loci, meQTLs: Methylation quantitative trait loci, CXCL10: C-X-C motif chemokine ligand 10, SDAD1: SDA1 Domain Containing 1, ART3: ADP-Ribosyltransferase 3, CXCL9: C-X-C Motif Chemokine Ligand 9, SCARB2: Scavenger Receptor Class B Member 2

DISCUSSION

CXC chemokine family also involves the CXCL10, which binds to the CXCR3 receptor, acting in biological events such as chemotaxis, apoptosis, cell growth and angiostasis (15). Proinflammatory cytokines have roles in various different processes, including chemotaxis, differentiation and activation of peripheral immune cells, regulation of cell growth, apoptosis and modulation of angiostatic effects (16). Therefore, they have important roles during viral infections, varying from activating immune cells and stimulating their migration to infected areas. Mechanically, the G protein-mediated signaling is activated with the binding of CXCL10 to the CXCR3 receptor, and the intracellular calcium production and actin reorganization are increased by the downstream activation of the phospholipase C-dependent pathway (17). CXCL10 is associated with the diseases such as periapical

periodontitis and viral encephalitis, and interactions between immune cells and microRNAs in the tumor microenvironment and CCR5 pathway in macrophages. The gene ontology (GO) descriptions associated with this gene involve the signaling receptor binding and chemokine activity. CXCL9 is an important paralog of this gene (2). miRNAs suppress the target genes by binding to the binding sites in their 3'unconverted regions after transcription, and thus they ensure the regulation of gene expression. Co-expression analysis was performed to identify the genes that showed similar expression patterns with CXCL10. This similarity suggests co-regulation, either at the transcriptional level (through the same transcription factors) or at the post-transcriptional level (through the same microRNAs). The determination of the hsa-miR-15a-5p binding site as a protected area with the TargetScan program reflects the strong biological relationship of this area. The increase in miR-15a expression may decrease the CXCL10 expression and thus can suppress the activation of abnormal T cells in the immune response, while decreasing the expression of miR-15a can abnormally activate the immune response (18). Particularly the spleen, and the neutrophils, thymus, and B cells as the other immune-associated tissues and cells were found to have the most highly significant pattern of co-expression. The GSEA/MSigDB tool (//software.broadinstitute.org/gsea was used for the gene set enrichment analysis (19). Statistically significant results (FDR q value < 1E-50) were obtained for breast and prostate cancers, immune and inflammatory response, interferon response and regulatory T cells with this program. In our study, GSEA analysis with 269 genes from co-expression analysis using a 2-fold expression change as a threshold yielded similar results for tumor necrosis factor and inflammatory response, as well as interferon gamma and alpha response. Since the co-expression analysis gives statistically highly significant and biologically reasonable results using the TargetScan program (http://www.targetscan.org) it was examined whether the common expression patterns are caused by microRNA (miRNA) mediated regulation (20). For this analysis, miRNAs targeting CXCL10 with a context score lower than -0.30 were selected, and other target genes were identified. In total, 80 miRNAs were determined to target CXCL10. The results did not reach as much statistical significance as in the co-expressed gene set after the same GSEA analysis was performed for each miRNA separately in the gene sets of the miRNA targets. This observation suggested that miRNA-mediated gene expression regulation does not play an important role in the regulation of CXCL10 expression. The investigation of the disease relationships of SNPs within each miRNA gene in GWAS databases showed no genome-wide relationship on the threshold of statistical significance. It has been established that SNPs acting as expression quantitative trait loci (eQTL) or methylation quantitative trait loci (meQTL) correlated with the expression levels of CXCL10 and could be the markers for disease susceptibility mediated by CXCL10 expression variation. CXCL10 eQTLs were obtained from Blood QTL and MeQTLs, and all QTLs were found to be cis, and trans-eQTL or trans-meQTL were not detected. Most CXCL10 eQTLs were also eQTLs for NAAA (N-acylethanolamine acid amidase) downstream from CXCL10. The eQTLs were obtained from Blood QTL Browser and the MeQTLS were obtained from mQTLdb. All QTLs were cis and there was no trans-eQTL or meQTL. All eQTLs and meQTLs were examined for GWAS associations in GWAS catalogue, dbGAP and GRASP databases. The most statistically significant association was with longevity (rs2273), and another SNP (rs13111494) showed an association with aging in a different study.

CONCLUSION

Although we found no strong evidence for miRNA-mediated CXCL10 expression in our study, variants close to miRNAs showed stronger genetic associations with inflammatory and immune disorders. This finding suggests that CXCL10 plays a stronger role in autoimmunity, inflammation and possibly in cancer than its role in transplantation.

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