MicroRNAs in autoimmune rheumatic diseases

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SUMMARY

The etiology of autoimmune diseases remains largely unknown. In recent years, besides genetic factors, several studies proposed that the epigenome might hold the key to a better understanding of autoimmunity initiation and perpetuation. More specifically epigenetic regulatory mechanisms comprise DNA methylation, a variety of histone modifications, and microRNA (miRNA) activity, all of which act upon gene and protein expression levels. In particular, it is well known that epigenetic mechanisms are important for controlling the pattern of gene expression during development, the cell cycle, and the response to biological or environmental changes. In the present review a description of the most frequent epigenetic deregulations, in particular the role of miRNAs, in rheumatic autoimmune disorders will be investigated.

Key words: *Autoimmune diseases, epigenetics, microRNAs.*

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INTRODUCTION

The mechanisms causing most autoimmune diseases (AID) are still unknown. Genetic factors are surely involved but, although genes and genetic *loci* causing predisposition to autoimmunity are being identified, there are evidences demonstrating that non-genetic factors, including several epigenetic deregulations, also contribute to autoimmune etiologies. At this regard several reviews, pointing the attention to the epigenetic involvement in the pathogenesis of autoimmune disorders, have recently been published (1-4).

Epigenetics is referred to as stable and heritable changes in gene expression that are not accompanied by alterations in DNA sequence. In fact, eukaryotic gene expression requires the regulated activity of transcription factors, but also transcriptional epigenetic mechanisms. Only by studying epigenetic mechanisms it has been possible to explain, at least in part, how cells with the same DNA can differentiate into different cell types and how a phenotype can be passed from one cell to its daughter cells. It is also well known that epigenetic mechanisms are important for controlling the pattern of gene expression during development, the cell cycle, and the response to biological or environmental changes. It is also essential to note that epigenetic regulation is a reversible phenomenon and that, unlike genetic alterations, which are permanent and affect all cells, epigenetic modifications are cell type specific.

In addition, several recent studies have demonstrated that disrupting the epigenetic regulation of transcription plays crucial roles in the development of autoimmune diseases. In the present review a description of the most frequent epigenetic deregulations found in rheumatic autoimmune disorders will be analyzed.

EPIGENETIC REGULATION

Epigenetic regulatory mechanisms comprise DNA methylation, a variety of histone modifications, and microRNA activity, all of which act upon gene and protein expression levels (1-3).

DNA methylation is defined as the post-synthetic addition of methyl groups to cytosine bases, catalyzed by a DNA methyltransferase (DNMT). Interestingly, DNA

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methylation leads to gene silencing and may also interfere with the binding of some transcription factors (5, 6).

Histone modifications regulate gene expression by changing the degree to which gene *loci* are accessible to transcription machinery. The N-terminal tail of histones protrudes from nucleosomal core particles and is subject to an array of post-translational modifications (7). These modifications include, but are not limited to, acetylation, methylation, phosphorylation, ADP-ribosylation, sumoylation and ubiquitination.

MicroRNAs (miRNAs), the main focus of the present review, are small noncoding RNAs that negatively regulate translation and stability of several hundreds messenger RNAs (mRNAs).

In the last years many efforts have been dedicated to the identification of cellular processes that are epigenetically deregulated, as they could contribute to our knowledge of the diseases.

The current state-of-the-art of epigenetics in AID has revealed different profiles according to the disease (1). In particular, alterations in genomic DNA methylation and histone modifications have been implicated in the pathogenesis of systemic lupus erythematosus (SLE), while it has been reported that histone acetylation pattern is affected in rheumatoid arthritis (RA) and the abnormal expression of retroviral elements, from the epigenetically silenced endogenous retrovirus HERV-W, has been associated with the evolution and prognosis of the disease in multiple sclerosis (MS). Furthermore, considering the cell type specificity of epigenetic modification, it has been clearly demonstrated that in SLE both CD4+ T and B cells show an altered pattern of DNA methylation and histone acetylation (8-10), while lymphocytes and synovial fibroblasts have been found to be hypomethylated in RA (11); on the other hand, DNA was found to be methylated in lymphocytes and skin fibroblasts of patients with systemic sclerosis (SSc). There are also evidences that a defective DNA methylation can be found in brain cells of MS patients (3).

Thus, considering the tight relationship between environmental factors and epigenetic mechanisms, it is not surprising that several reports have linked epigenetic dysregulations with both idiopathic AID and chemical/drug-induced AID. This mechanism is probably involved in drug, induced SLE where demethylating drugs such as 5-azacytidine (5-aza), procainamide and hydralazine, can also induce lupus-like autoimmunity, both *in vitro* and *in vivo* (8, 12). An additional important deregulating epigenetic mechanism, the ageing, can give an attractive explanation for age-related AID. Indeed, there are evidences that monozygotic twins are epigenetically indistinguishable early in life but, with age, exhibit substantial differences especially when they have led different lifestyles and have spent less of their lives together (13).

Table I - Autoantibodies and epigenetic modifications.

Post-translational modifications	Autoantigen	Disease	Reference
Acetylation	H4, H2B	SLE	(16, 17)
Deimination/Citrulinisation	collagen, fibrinogen	RA	(18-20)
Histone phosphorylation	U1-snRNP	SLE	(21)
Sumoylation	Sp100	PBC	(22)
Ubiquitination	H2A, Ro52, UBE4A	SLE, pSS, Crohn's	(23-25)
Phosphorylation	H3K9me3S10ph, La/SSB	SLE, pSS	(26, 27)
Methylation	Sm	SLE	(28)
Lipoylation	PDC-E2	PBC	(29)
DNA modifications	demethylated DNA, Z-DNA	SLE SLE	(30) (31, 32)

Abbreviations: SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; PBC, primary biliary cirrhosis; pSS, primary Sjögren's syndrome. From Brooks, et al. Epigenetics and autoimmunity. J Autoimmun 2010; 34: 207-19.

Moreover, several studies have underlined the topic role of X-chromosome inactivation in predisposing women to autoimmunity and neoplasia (4). All the abovementioned findings have also led to the development of epigenetic therapies that are being evaluated (14, 15). Several Authors have found associations between epigenetic histone modifications and disease progression with autoantibody (Abs) production, suggesting that these Abs may be used as biomarkers. In Table I we report specific epigenetic defects that have been found associated with autoantibody production in several autoimmune disorders.

Involvement of MicroRNAs in autoimmune diseases

MicroRNAs (miRNAs) are small noncoding RNAs, about 22 nucleotides long, found in plants and animals, which have emerged as a major class of regulatory genes during development and cell differentiation (33). Their involvement in autoimmunity has been recently reviewed by Carissimi et al. (34). MiRNAs are produced from a long primary transcript, through a series of endonucleolytic maturation steps. In the canonical pathway they are transcribed by RNA polymerase II as long primary miRNA (pri-miRNA) transcripts, which are cleaved by the nuclear-specific multiprotein complex comprised of the RNase III enzyme Drosha (35, 36) liberating an approximately 60-to-80-nucleotide precursor (pre-miRNA) (37). In the cytoplasm the pre-miRNA is further processed by the RNase III enzyme, Dicer, to a double-stranded miRNA that is ~21 nucleotides long (38). Only one strand of the miRNA duplex is subsequently incorporated into a ribonuclear particle (RNP) complex, the RNA-induced silencing complex (RISC), whose catalytic component is a member of the Argonaute family (Ago1-4) (39). Bioinformatic approaches and molecular cloning have identified hundreds of miRNAs in plants, animals and viruses (40). Target predictions support a view in which each miRNA negatively regulates translation and stability of several hundreds messenger RNAs (mRNAs) (41-43). These regulations are exerted through inhibition of individual key targets, fine-tuning of target function and a coordinated regulation of multiple targets. These discoveries confirm that this class of small non-coding RNA molecules represents a novel level of regulatory control over gene expression programs in several organisms (33).

MiRNAs display distinct temporal and spatial expression patterns and are key players in a wide variety of physiological processes such as haematopoiesis, proliferation, tissue differentiation, cell type identity maintenance, apoptosis, signal transduction and organ development (44). The analysis of miRNAs expression and function during haematopoietic lineages development has shed light on complex regulatory mechanisms where miRNAs fine-tune immune system cell differentiation and function. There is evidence that individual miRNAs play a critical role in B and T cell homeostasis and response (45-47), and that T cell selection in the thymus is controlled by a particular miRNA (48).

Furthermore, the fact that miRNAs are involved in the modulation of T cell selection, T cell receptor sensitivity as well as Treg cell development in normal immune response, as also demonstrated in animal models (49-53), suggests that these molecules may also be involved in the development of inflammatory or autoimmune diseases.

In addition, the pioneer knock out of miR-155 in mice revealed an essential role in the acquired immunity for this miRNA. In fact, despite miR-155 null mice develop normally, immune system analysis revealed that miR-155 depletion leads to pleiotropic defects in the function of B cells, T cells and dendritic cells; in particular, these mice are unable to gain acquired immunity in response to vaccination, demonstrating that miR-155 is indispensable for normal adaptive immune responses (45, 46). Recent studies have also demonstrated that miR-21 activation via TCR stimulation is able to negatively modulate TCR signalling strength (34).

To date, few studies on miRNAs have been conducted in AID patients. Nevertheless, it is becoming increasingly clear, from

cell culture and animal studies, that proper miRNAs regulation is critical for the prevention of autoimmunity and normal immune functions (36).

MicroRNAs in rheumatoid arthritis

RA is a systemic autoimmune disorder characterized by chronic synovitis and progressive joint destruction. A characteristic of RA is the degree of histological heterogeneity, mainly regarding both T and B cell lineages activation in the synovial tissue. The relevance and contribution of the miRNA network in the control of synovial gene expression patterns, histo-pathological variants and clinical-pathological subsets, are at present unclear. Supporting the possible involvement of this molecular system, recent studies have highlighted that different miRNAs, potentially involved in the control of genes related to inflammatory pathways or immunological activation, are strongly up-regulated in peripheral blood mononuclear cells (PBMC) and synovial cells from RA if compared with osteoarthritis (OA) or normal individuals. Stanczyk et al. revealed a prominent up-regulation of miR-155 and -146a in RA synovial fibroblasts (SFs) compared to OA affected patients used as control (54). They also demonstrated that the expression of miR-155 in SFs could be further induced by TNF-alpha, interleukin-1 beta, lipopolysaccarides (LPS), poly (I-C), and bacterial lipoprotein. Nakasa et al. confirmed the increased expression of miR-146 in synovial tissue from patients with RA compared to OA and normal controls also by in situ hybridization and immunohistochemistry of tissue sections (55). Recently, Pauley et al. demonstrated a significant increase of miR-146a, -155, -132 and -16 in PBMC from RA patients compared with healthy and disease control individuals, suggesting that miRNAs can be involved at different levels in the pathogenesis of RA (56). Two known targets of miR-146a, TRAF6 and IRAK-1, were examined and despite increased miR-146a expression in RA patients, there was no significant difference in mRNA or protein levels of TRAF6 or IRAK-1 between RA patients and healthy controls. In vitro studies also revealed that repression of TRAF6 and/or IRAK-1 in human monocytic cell line type I (THP-1) resulted in up to an 86% reduction in TNF-α production. Considering that prolonged TNF-α production is known to play a role in RA pathogenesis, these data suggest a possible mechanism contributing to RA pathogenesis where miR-146a is upregulated, but unable to properly regulate TRAF6/IRAK-1, thus leading to prolonged TNF-α production in RA patients. Concerning the possible role of miR-155 in RA, Worm et al. have recently shown that the exposure of mice cultured macrophages to LPS leads to up-regulation of miR-155 and that the transcription factor c/ebp Beta is a direct target of miR-155 (57)**.** Interestingly, these Authors have also found that the expression profiling of LPS-stimulated macrophages combined with over expression and silencing of miR-155 in murine macrophages and human monocytic cells, uncovered marked changes in the expression of granulocyte colony-stimulating factor (G-CSF), a central regulator of granulopoiesis during inflammatory responses. Consistent with these data, Worm et al. have also shown that silencing of miR-155 in LPS-treated mice by systemically administered LNA (locked nucleic acid)-anti-miRNA, results in derepression of the c/ebp Beta isoforms and down-regulation of G-CSF expression in mouse splenocytes. Finally, they reported on miR-155 silencing in vivo in a mouse inflammation model, which underscores the potential of miR-155 antagonists in the development of novel therapeutics for treatment of chronic inflammatory diseases.

We have recently investigated the role of miRNAs in RA with a novel approach (58). In particular, since T-lymphocytes have been reported to play a crucial role in the pathogenesis of RA, we have focused our attention on the role of miRNAs in this cell lineage. Microarray miRNAs profiling of T cells from RA patients showed a dramatic over expression of miR-223 and a significant downregulation of miR-142, -28 and -30e. This was unexpected, since

miR-223 expression is thought to be confined to the myeloid lineage according to most reports. Nevertheless, the role of this miRNA in lymphocytes has not been further investigated thus far. Our study also showed that miR-223 is preferentially expressed in CD4+ T lymphocytes and this expression is independent of treatment, suggesting that it is a feature associated to the disease rather than a consequence of therapy. We recently confirmed these results in a group of patients with early RA (59). On the basis of these observations, we speculate that this aberrant over-expression of miR-223 in RA T-lymphocytes could contribute to the pathogenesis of the disease. Identification of miR-223 targets in T-lymphocytes could therefore contribute to elucidate some of the molecular mechanisms that lead to RA. In Table II miRNAs found to be associated with RA, as well as their targets and target's function, are reported.

MicroRNAs in systemic lupus erythematosus

SLE is a systemic inflammatory autoimmune disease characterized by the presence of autoantibodies against numerous self-antigens including chromatin, ribonucleoproteins, and phospholipids. In 2007, Dai et al. have used microarray analysis to examine miRNAs expression in PBMCs of 23 SLE patients compared to 10 healthy controls. In these SLE patients, seven miRNAs (-196a, -17-5p, -409-3p, -141, -383, -112, and -184) were found downregulated and nine miRNAs (-189, -61, -78, -21, -142-3p, -342, -299-3p, -198, and -298) were upregulated compared to healthy controls (68). In 2008, Dai et al. also reported the miRNAs profile of kidney biopsies from lupus nephritis patients and healthy controls and found 66 differentially expressed miRNAs (36 upregulated and 30 downregulated) in lupus nephritis (69). Te and coll. investigated, by microarray analysis, miRNA expression in both African American (AA) and European American (EA) derived lupus nephritis samples and revealed 5 miRNAs: hsa-371-5P, hsa-423-5P, hsa-638, hsa-1224-3P and hsa-663 that were differentially expressed in lupus nephritis across different racial groups (70). Based on these findings, the authors postulated that these differentially expressed miRNAs might be potential novel biomarkers for SLE as well as help elucidate pathogenic mechanisms of lupus nephritis. Another recent study showed that miR-146a, a negative regulator of the interferon (IFN) pathway, contributes to the pathogenesis of SLE (71). In fact it was possible to demonstrate that, by targeting the key signaling components, decreased levels of miR-146a lead to increased type I IFN pathway activity

Table II - MicroRNAs and rheumatoid arthritis.

miRNA	Target	Function	Cell type	Reference
miR-15a	Bcl-2	Apoptosis	FLS	(60)
miR-16	Bcl-2	Apoptosis	PBMC	(56)
miR-124a	MCP1, CDK2 S	Synoviocyte proliferation	FLS	(61)
miR-132	SirT1	NFkB pathway	PBMC	(62)
miR-140	ADAMTS5	IL-1 response modulation	chondrocyte	(63)
miR-146a	IRAK1, TRAF6 IRF5, STAT1	IFN and TNF pathways	PBMC	(54, 55, 64)
miR-155	C-Maf, Pu-1, MMP-3	Lymphocyte differentiation (TH1) and cellular matrix	FLS, T lymphocytes	(57)
miR-223	RhoB	T lymphocytes	CD4+ T cells, PBMC	(58, 65)
miR-223	?	?	CD4-45+ T cells	(66)
miR-346	IL18	Inflammatory response modulation	FLS	(67)
miR142, 28, 30e	?	?	CD4-45+T cells	(66)

Abbreviations: PBMC, peripheral blood mononuclear cells; FLS, fibroblast like synoviocytes.

in lupus patients. In addition, according to Vinuesa (72), three miRNAs (-181a, -186 and -590-3p) are sufficient to target half of all lupus genes. In a recent study Zhao et al. demonstrated that miR-125a negatively regulates RANTES expression by targeting KLF13 in activated T cells and that the underexpression of miR-125a contributes to the elevated expression of the inflammatory chemokine RANTES in lupus (73). As the introduction of miR-125a into lupus T cells alleviated the elevated RANTES expression, these findings extend the role of miRNAs in the pathogenesis of lupus but also provide potential strategies for therapeutic intervention. Further studies examining larger patient cohorts and different patient populations are needed to determine if the differential expression of these miRNAs in SLE are reproducible. Dicer can become target of autoimmunity giving rise to various types of immunological disorders including autoimmune ones, often in cooperation with viral infections (74) (Table III).

MicroRNAs in Sjögren syndrome

Very recently, in a pilot study, Alevizos et al. (75) showed that miRNAs expression profiles can separate glands of primary Sjögren syndrome (pSS) patients from controls and can distinguish subsets of pSS patients with low or high-grade inflammation. Interestingly, in half of the patients with a focus score of 12, the miR-17-92 cluster, which has been associated with specific types of lymphocytes and lymphocytic pathologies, was downregulated. Authors have also identified two miRNAs with an opposite relationship to inflammation: one increased and the other decreased between the low and high focus score groups. Michael et al. explored the presence of miRNAs in exosomes isolated from parotid and submandibular saliva showing a striking difference between miRNAs profile in saliva obtained from patients and healthy donors (76).

Lu et al. have recently showed that two miRNAs (-574 and -768-3p) are overexpressed in the salivary glands of pSS patients. They reported that these two suspected epithelial cell miRNAs could be used to predict the evolution of the disease. The same authors reported data obtained from their studies of non-obese diabetic (NOD) mice (B6DC) that develop a disease similar to human pSS. They highlighted two miRNAs, -150 and -146, that are upregulated in target tissues and in PBMCs of B6DC mice compared to control mice. The same authors have also reported that miRNA-146a expression is increased in PBMCs and salivary glands of pSS patients (1) (Table IV).

MicroRNAs in systemic sclerosis (SSc)

SSc is a multi-systemic fibrotic disorder associated with high morbidity and mortality. The role of miRNAs in this disease,

Table III - MicroRNAs and systemyc lupus erythematosus.

miRNA	Target	Function	Reference
miR-146a	IRAK1, TRAF6 IRF5, STAT1	IFN and TNF pathways	(71)
miR-181a	PTPN22, SHP2, DUSP5, DUSP6	T cell activation	(72)
miR-186		?	(72)
miR-590-3p	CXCL13	?	(72)

Table IV - MicroRNAs and primary Sjögren syndrome.

miRNA	Targets	Function	Reference
miR-146a	IRAK1, TRAF6 IRF5, STAT1	IFN and TNF pathways	(1)
miR-155	C-Maf, Pu-1, MMP-3	Lymphocyte differentiation (TH1) and cellular matrix	(1, 75)
Cluster mir 17-92	Bcl2, CXCL12	Apoptosis, chemokine expression, B cells accumulation	(1, 75)
miR-574, -768-3p, -164	?	?	(1, 74)

as post-transcriptional regulators of profibrotic genes, has been recently investigated by Maurer (77). The results of this study suggest the primary role of miR-29 cluster, and in particular of miR-29a, on collagen expression regulation at post-transcriptional level, both *in vitro*, on cultured fibroblasts, and *in vivo*, in a murine model of dermal fibrosis induced by bleomycin.

Interestingly, these Authors also demonstrated an additional role of miR-29a that might influence the production of other profibrotic molecules such as PDGF-B, PDGFRβ, thrombospondin and SPARC, as these molecules are predicted targets of miR-29 by in silico analysis. T cells represent a major component of the infiltrate in the skin of SSc patients in the acute inflammatory stage of the disease. Depending on the type and stage of the disease it has been observed changes in the absolute and relative number of T cells subsets in the peripheral blood of SSc patients (78). Moreover an emerging hypothesis for the pathogenesis of fibrotic disorders implicates an altered balance between T helper type 1 (Th1) and T helper type 2 (Th2) with an important prevalence of Th2 type, which notoriously produces profibrogenic cytokines. These cytokines, in fact, can directly stimulate collagen synthesis and fibroblast activation, and induce TGF-β, a powerful modulator of immuno-regulation and extracellular matrix (ECM) accumulation (78, 79). On the basis of these evidences, in order to define the role of circulating T lymphocytes in the pathogenesis of SSc, in a preliminary study, we have analyzed the miRNAs expression profile of CD4+ T lymphocytes from peripheral blood of patients affected by the limited form of SSc compared to healthy donors.

The dysregulated miRNAs identified were associated with different gene products considered to be deeply involved in the pathogenesis of SSc, and with the activity and progression of the disease (*unpublished data*).

In particular, this predictive analysis showed several dysregulated miRNAs involved in expression regulation of various chemokines and chemokine receptors (CXCL5, CCR6), cytokines (IL1, IL16, IL10, IL34), cytokine receptors (TLR8, IL2RB) cytokine receptor antagonists (IL1RN, IL1RAP, IL8RA, IL1RAPL), and growth factors (TGF, PDGF, FGF, HBEGF, IGF, VEGF). In addition, some identified miRNAs seem to be involved in post-transcriptional regulation of several collagen types, different SMAD family members (down stream mediator of TGF β signalling) and transcriptional factors involved in cell survival and anti-apoptotic mechanisms (80) (Table V).

Table V - MicroRNAs and systemic sclerosis.

miRNA	Targets	Function	Cell type	Reference
miR-29a	Type I collagen PDGF-B PDGFRβ Thrombospondin SPARC	Onset and progression of disease: ECM sinthesys, fibroblast activation.	Fibroblasts	(77)
miR-424, -128, -20a, -374, -10a, -95	SMAD family members (SMAD2 SMAD6, SMAD7, TGF-β1receptor, Transforming growth factor alpha (TGFA)	TGF-β signalling, Fibroblasts chemotaxis and activation (a-SMA positive)	Peripheral blood T lymphocytes	(80)
miR-106a, -424, -31, -505, -128	PDGFRA, VEGFC, FGF5, FGF7, IGF1R	Growth factors expression	Peripheral blood T lymphocytes	(80)
miR-374, -95, -505	COL4A1, COL4A2, COL4A6	ECM production	Peripheral blood T lymphocytes	(80)
miR-650, -374a,b, -95, -10a, -128	IL1RN, IL1RAP, IL1RAPL, IL10, IL8RA, IL6	Interleukin signalling	Peripheral blood T lymphocytes	(80)
miR-374a,b, -505	CXCL5, CCR6	Chemokines expression	Peripheral blood T lymphocytes	(80)

CONCLUSIONS

It is now well established that epigenetic mechanisms are important in the control of the pattern of gene expression during the development, the cell cycle, and in response to biological or environmental changes (81). Epigenetic regulatory mechanisms comprise DNA methylation, histone acetylation, and microRNA activity, and dysregulations of each of them can be involved in autoimmune disorders. Several studies demonstrated that miRNAs expression is altered in systemic autoimmune diseases such as RA (82), SLE, pSS and SSc. Next critical steps are to identify the targets of these miRNAs and to determine the mechanisms by which miRNAs regulation/dysregulation contribute to the pathogenesis of these diseases. Some progress has been made, for example it is known that miR-155 can target MMP-3, which could potentially modulate tissue damage, and miR-146a can target TRAF6/IRAK-1 that should suppress inflammatory cytokine production. The first practical aspect of these discoveries is the suppression of arthritis in an animal model by inhibiting the expression of miRNAs-155. This result open the way for future utilization of miRNAs; in fact, further studies are also needed to elucidate if miRNAs could serve as useful disease markers or therapeutic targets. New information is emerging on new therapeutic opportunities (83, 84). The usage of miRNAs as diagnostic tool will be facilitated by the possibility of detecting these molecules in blood serum or plasma. In fact, it has been already observed that particular patterns of circulating miRNAs can be detected at different expression levels in serum of cancer patients compared to healthy controls (85). These observations prompted the hypothesis that circulating miRNAs could constitute a new class of serological biomarkers for several pathologies, including cancer and autoimmune diseases.

Whilst our current knowledge of epigenetic processes is limited, the epigenomics will undoubtedly serve as a tool for diagnosis, prognosis, and therapy in the future.

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