SYSTEMIC LUPUS ERYTHEMATOSUS AND EPIGENETICS

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Abstract

Human systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease characterized by autoantibodies to nuclear components with subsequent complex formation and deposition in multiple organs. A combination of genetic and environmental factors is required for disease development[1]. Apoptotic defects and impaired removal of apoptotic cells contribute to an overload of autoantigens that become available to initiate an autoimmune response[2]. Epigenetic factors have significant effects on T-cell functions by modulating its DNA methylation pattern and in patients with active lupus happens gene-specific DNA methylation. Also IL-2 contribute in the pathogenesis by reason of IL-2 regulate the tolerance mechanisms such as the activation induced cell death (AICD) and the induction and maintenance of regulatory T-cells[3]. DNA hypomethylation in CD4+ T-cells causes several gene activations and molecule overexpressions that alters cellular function. Moreover, histone deacetylase inhibitors reverse the skewed expression of multiple genes involved in SLE[2]. 5-azacytidine and other demethylating agents could induce lupus-like autoimmunity in vitro and in vivo. SLE is a predominantly female disease that affects more the female than the male. The etiology of SLE remains incompletely understood, although a number of genetic and environmental factors have been implicated, that may alter epigenetic regulation of gene expression. Epigenetics refers to heritable chromatin-based mechanisms in the regulation of gene expression without changing the DNA sequence. These mechanisms include DNA methylation, histone modification, abnormalities s in ERK pathway signaling, and IL-2 transcriptional irregularity may be the key players through changes on gene expression in the development of this autoimmune disorder.

Key words: SLE,epigenetics,HATS,HDACS

Introduction

One of the mechanism controlling gene expression is suppressing the expression of the gene by methylating the cytokine. It's a rare phenomenon in the lower organisms, but is observed in vertebrates at a percentage of above 10% of the whole number of cytokines. The original methylation is not accidental but occurs in C particular copies, were they have dynucleotides sequence across the DNA length structure

5' ^{m}CpG 3' 3' GpC^{m} 5'

The CpG groups are named also CpG islands.

The active genes are usually non methylated on the cytokines. The methylated situation transferred to the daughter cell with the homocentric way of replicating on only one chain, developing one semimethylated domain[4]. Methylotransferase will add further a methylated group to the newly-synthesized chain[5].
The mechanism of methylating cytokine which modifie the genetic expression, was for a long time on enigma. Today it's known that methylation is not an independent phenomenon of the chromatin with the histones since islands of CpG are prone to the enzyme deacetylase of the histone (HDAC) which develops a complex that modifies the chromatin making it a target, thus, deactivating the surrounding genes[6,7].
The methylation of DNA is noticed to be involved in two other apparitions,that is the gene footprint and the deactivation of the X[7]. The case of gene footprinting is not a common phenomenon and it concerns the silencing of one of the two gene homologue alleles of the chromatin[8,9,10]. It's always the same alllelomorph which is activated by methylation, and is always transferred by one parent[11].
It's known that methylation provokes activation of the gene. Alternatively in hypersensitive DNA polymerase regions where the gene is situated in an epigenetic state[7], and is observed of ten that is a partial demethylation of 5' ^{m}CpG 3' seats within and to the gene[12]. The epigenetic methylation of the cytokine as a mechanism of control of the eukaryotic gene action is a hereditary situation. Thus it can affect the modification against the embryonic development, where the cells follow a certain pathway, developing demorphysised clones. The changes in the prototypes of methylating the DNA during the embryo development have not being fully clarified and, it is hypothesized that the embryonic cells which follow the hereditary procedures , while they develop they have no return back[4,11,12].The expression of a cell is defined by its constituent proteins, which are a result o f specific patterns of gene expressions, and the transcription is suppressed by the recognition of the allele of the basics of the DNA in the promoter region[12]. Activating those factors, provoke a chain of events, which, involve usually changes in the domain of the chromatin, which it then drives the development of an assembly of transcription[14]. Other occurrence which make changes in the expression of the cells, except the genetical mutation processes , one of the epigenetic changes, where it has being found that they play A large interest has being shown on the epigenetic mechanism, in hematology, and oncology, were they are based in epigenetics alleles, however contrary to genetico , are ireversal after pharmaceutical intake. The epigenetic rhythm is determined by the change which provokes the gene expression, and which the realization, with changes, such as modification of histones, changes in methylation and acetylation of the DNA[7,8,12,15,16].

Histone Acetyltransferases-HATS

The acetylation of histones in eykariotic organisms was discovered quite a few years ago, and the identification as well as the characterization of enzymes which create it have revealed their remarkable diversity in different organisms. Histone Acetyltransferases-HATS, is the name for factors which allocate enzyme activation in the transportation of an acetylation team from the acetyl-CoA to the e-amino of the group of amino-acids lysine, that are usually found in the basic region of N-terminal end of histones. The total number of enzymes-HATS are separed in two

categories:type A [8] found in the core and type B found in cytoplasme [19,20]. The HATS of type B are considered to catalyse the acetylation of newly- composed proteins in the cytoplasme , while the HATS of type A are considered that they participate in the nuclear acetylation of the histone related with the transcription, as well.The acetyltransferase of type B, HAT1, was discovered in sacharomyces [21] and it acetylizes the lysin 5 and 12 of histone 4 (H4) in vitro, amino-acids which were known to be found acetylated , in the newly synthesized H4 [22]. The enzyme HAT1 constitutes a part of a multifactor complex whose subunits includes also 14 proteins HAT2 and CAF1 which have been connected with the redevelopment and the aggregation of the components of the chromatin, respectively [23,24]. Despite the particular significance attached to HAT1, the transformation or HAT2 has not presented any problems in the incorporation of H4 to the chromatin [23], which clearly indicates that its action can be replaced by other enzymes when the HAT1 is absent or cannot act. Many of the proteins with HAT activity can acetylized free histones when used in vitro, while others such as the nuclear ones, cannot acetylized their physiologic substrate as they are but only when they are found in the whole complex with other factors, that appear that they are essential for their activity . Another family of acetylates that has been found is the one of MYST, which was named after and shaped by the strong resemblance they bear with the concatenation of proteins MOZ, Ybf/Sas3, Sas2 and Tip60 [25]. The Esa1 of Sacharomyces, the MOF of Drosofilla and the human HBO1 and MORF [26] constitute newer members of family MYST which was discovered later. The strong relationship between the transcriptional activation and acetylation of the histones was clearly indicated when it was discovered that the larger subunit of the complex factors that is connected with TVR (TVR Associated factors-TAFs), Taf1-taf250, allocates the enzyme activity in the acetylation of histones [27]. The complex TFIID can band to the DNA via the TVR factor that recognizes special allles, athough it has been discovered that even TFIID that does not carry TVR it can transcript in vitro .The acetylotransferases are involved in the transcriptional regulation not only via the acetylation of histones but also by transcriptional factors [28].

Histone Deacetylases-HDACS

The acetylation of the histones is a reversal procedure, as the acetyl group can be removed from the action of special enzymes called apocetylase histones- (HDACS), the existence of which was discovered shortly afterwards the presence acetylases [7] The acetylase are categorized in families and the enzymes of human class I, II and III are homologe to the ones of the sacharomyces Rpd3, Hda2 and Sir2, respectively [18]. Acetylase hitones are divided in units in which some of the subunits fuction and regulate rte enzymes. Apocetylase together with the histone acetylases contribute to the acetylation of certain histones as well as the regulation and the differentiation of different megafactors which are responsible for their modification. The interaction between megafactors which advance to specific alleles, has as a result their located action in instigators, which in turn regulate the amount of quantity and the availability of the enzyme [6,29]. The methylation histones is performed by specific enzymes, the methylase histones, HMTs. Recently other enzymes that methylate the histones in specific residues hasve been discovered and it has contributed in the discovery of the transcription mechanism, as when the histones are methylated show significant differences from the methylation known up to the moment as post-translational. The methylation of the histone does not change the total charge of protein and it appears to

be stable modification, concurrently certain enzymes that remove methylgroup have been discovered, but present special action [30,31,32]. The methylation of histones can be classified in two groups, those that methylate the lysine residues[33,34]? Zhang and Reinberg, 2001) and those methylated the arginine residues, such as the family of PRMT. The amino-acid arginin can undergo only - or dj-methylation. The enzymes that end up in the methylation Argjnin residues are separated in two categories: Type I, which leads to a single and asymmetrical di-methylation and Type II, which leads to a single and symmetrtical di-methylation. There five enzymes which involved in the methylation reaction of arginin, and present high degree of maintainance of catalytic region (Zhang and Reinberg, 2001 [33], that are named prmt1-5. The methylation histons is involved in the regulation of transcription mechanisms as much as in the control of suppressive activity on the transcription. The role of methylating histones during transcription is characterized by an additional degree of complexity, the number methylated groups on a residue is related to different attributes. [35]

The Methylation of the aminoacid lysine of histone

The histone lysine that undergo methylation are the Lysine 4,.9,.27 and 36 and 79 on histone the 3 and lysine 20 histone 4. The enzymes that are involved in this the specific transformation bring characteristic region SET, and the characteristic regions that are rich in cytokines that precede, PRESET and follow, Post-set, respectively,to the region where there is enzyme activity. The enzymes that methylated lysine of the histones can be categorized in four large families: the SET1, which includes enzymes which methylate the K4 of I3, the SET2, which includes the enzymes which methylate the K36 of I3, the RIZ and the family SUV39, which is consists of enzymes that can methylate the K9 and K27 of H3 [33] The methylation the K79 of H3 is an exception since the enzyme that is responsible for the modification, DOT1, does not brings the characteristic SET region [35]. The methylation of K79 is related to the activity of the transcription and is considered participate in preventing the spread Heterochromatosis. The first methylated lysine which was discovered in from mammals and brings upon itself an active methylated of K9 of I3 is Suv39h1, counterpart of Su (var) the 3-9 Drosofjla [36] In certain cases where there is a later expressive modification of factors from enzymes which modify the chromatin during methylation of arginin or acetylase have been found and their fuction appears in biological reactions, such as translation [8,37] But for the enzymes from the methylated lysine family with characteristic domain SET, the only known are the histones. Recently other enzymes that methylate the K4 of H3, including the MLL, the ALL, hSET1 and hSMYD3 this [38,39,40,41] have been excluded from humans. These proteins are usually part of mcromolecule structures, which include more the one enzyme activity that modifiy the chromatin. In contrast with heterochromatin situation which was mentioned, the potential role of the modification of the histone in the consseration of the active translational chromatins has not being dified. If the modifications of the histone press for some reaction to the controversial of the translational, the methylation works as aactivation means for the gonads, because contrary to the acetylation, the maethylation of the lysine is stable. In addition, sugnificant levels of methylation of H3-K4 have been observed in the genes of region of b-globulin and in the NF-4 during the cell diffentiation, before the translation, showing that tis specific modification is involved in the resolution and the conservation of one strong active chromatin [41,42] .The cause of myelodysplasiain patients is unknown. Radiation, chemotherapy with agents such as benzide and other

organic compands cause great damage to the majority of patients who have undergone such treatments.

Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease that affects multiple organs, causes mortality and morbidity. In this disease the female to male ratio ranging between 4,3-13,6 to 1 [43]. Male patients with Klinefelter's syndrome (with karyotype 47,XXY) have similar risk to develop the disease compared to females (with 46,XX). This ought to the presence of SLE susceptibility genes on the X chromosome, or the overexpression of an X chromosome gene as a result of loss of random X chromosome inactivation, or both. Strong evidence supports an important role for abnormal T cell DNA methylation in the pathogenesis [44]. The expression of methylation sensitive genes, such as ITGAL (CD11a), TNFSF7 (CD70), PRF1 (perforin) and CD40LG (CD40L), is increased in T cells from SLE patients, similar to normal T cells treated with DNA methylation inhibitors such as 5-azacytidine [45,46,47,48]. The inhibition of DNA methylation with 5-azacytidine caused CD4+ T-cell autoreactivity. The autoreactivity correlated with overexpression of the adhesion molecule LFA-1 (CD11a/CD18) [45] due to demethylation of sequences 5 to the CD11a promoter [49], and overexpressing LFA-1 by transfection caused an identical autoreactivity [50]. Demethylated, autoreactive CD4+ T cells overstimulate antibody production by B cells and kill macrophages [51], relaeasing apoptotic nuclear material that stimulates lupus-like autoantibodies [52]. T-cell hypomethylation correlates with disease activity in SLE, suggesting that DNA hypomethylation may be a key player in the pathogenesis of the disease [53]. Cloned normal T cells treated with the DNA methylation inhibitor 5-azacytidine had increased ITGAL (CD11a) expression. A T cell subset with similar CD11a expression was found in patients with active lupus. This subset contained cells that spontaneously lysed autologous macrophages, similar to 5-azacytidine-treated cells[45]. Subsequent studies revealed that treatment of normal T cells with 5-azaC induced overexpression of several other methylation sensitive genes, such as TNFSF7 (CD70), PRF1 (perforin), and CD40LG (CD40L), all of which are hypomethylated and overexpressed in T cells from lupus patients[54]. Deng et al [56] showed that DNMT1 mRNA expression is reduced in T cells from active lupus patients. This finding suggest that T-cell DNA hypomethylation in lupus might be caused by reduced DNMT1 expression. Luo et al [56] showed that DNMT1 and DNMT3a mRNA levels were significantly lower in CD4+T cells from patients with subacute cutaneous lupus erythematosus (SCLE) than in controls and that DNMT1 expression positively correlated with CD4 T cell DNA methylation in the SCLE patients studied.

Agents or events that modify T cell DNA methylation may induce autoimmunity by causing T cell CD11a overexpression [50,57]. Lu et al [49] demonstrated that specific sequences flanking the CD11a promoter region were hypomethylated in T cells from active lupus patients and normal T cells treated with DNA methylation inhibitors. Patch methylation of these sequences, suppressed the CD11a promoter in reporter constructs, which indicates a functional significance of the methylation changes observed in lupus T cells. Lou et al [56] showed that CD4+ T cell DNA from patients with SCLE was hypomethylated. CD11a mRNA expression was significantly increased in SCLE CD4+ T cells and was negatively correlated with DNA methylation. CD70 is another methylation sensitive gene and a B cell costimulatory molecule overexpressed on CD4 lupus T cells, as well as procainamide and hydralazine treated T cells. It contributes to excessive B-cell stimulation in vtro[46]. Lu et al[58] identified a genetic element that suppresses CD70 expression when methylated. The same genetic element is demethylated in lupus T cells and in T cells treated with

either pprocainamide or hydralazine. This data supports a model in which demethylation of specific genetic elements in T cells, which is caused by decreasing DNMT1 expression or inhibiting its function, contributes to drug-induced and idiopathic lupus through altered gene expression [57]. Oelke et al [46] compared CD70 expression in T cells treated with 2 DNA methylation inhibitors(5-azaC or procainamide) and ERK pathway inhibitors (U0126, PD98059, or hydralazine). On coculture of autologous T and B cells with and without anti-CD70, lupus T cells and T cellstreated with DNA methylation inhibitors and ERK pathway inhibitors overexpressed CD70 and overstimulated B cell immunoglobulin G (IgG) production. IgG synthesis blocked by anti-CD70.

Perforin is a pore-forming cytotoxic molecule that is expressed primarily by NK cells and CD8 T cells. DNA methylation inhibitors increase CD4+ T cell perforin expression. Perforin is overexpressed in CD4+ T cells from patients with active but not inactive lupus [47.] Perforin overexpression in lupus CD4+ T cells is caused by demthylation of the same region in the perforin gene that demethylates in normal CD4+ T cells treated with DNA methylation inhibitors [47], [59]. The perforin inhibitor concanamycin A inhibits the killing of autologous monocytes by lupus CD4+ T cells, which suggests a potential pathogenic role for perforin demethylation and overexpression in lupus CD4+ T cells [47]. Killing of autologous monocytes by a subset of autoreactive CD4+ T cells is one mechanism responsible for accelerated monocyte/macrophage apoptosis observed in lupus patients [60]. Denny et a [52] have demonstrated that induced monocyte/macrophage apoptosis in vivo can exacerbate autoimmunity in a lupus-prone murine model and can induce the production of autoantibodies in non-lupus-prone mice.

Women have 2 X chromosomes; 1 is inactivated by mechanisms that include DNA methylation in order to get the dosage imbalance of X-linked gene with male who has only one X chromosome. Demethylation of sequences on the inactive X chromrosome may cause gene overexpression uniquely in women, which predisposes them to lupus [48].CD40L, which is encoded on the X chromosome, is a methylation sensitive gene. The DNA methylation inhibitor 5-azaC demethylated the CD40L gene and doubled its expression in CD4+ T cells from women but not men, whereas CD70 (an autosomal methylation sensitive gene) is overexpressed equally in men and women. Both copies of the CD40L gene are demethylated in CD4 T cells from women with lupus, and women but not men with lupus overexpress CD40L on CD4+ T cells, whereas both overexpress CD70. This finding suggests that some methylation sensitive genes on the inactive X chromosome demethylate in T cells from women with lupus contributes to CD40L overexpression [48]. This description might help explain the predilection of lupus to affect women and would help explain the gene dose effect described from the X chromosome in lupus patients [61].

Since DNA methylation and histone modifications are mechanistically linked, it is likely that different changes in histone modifications are associated with DNA methylation changes in SLE. Most of the evidence about the role of changes in histone modifications in SLE comes from use of epigenetic drugs. In the case of histone modification , the use of histone deacetylase inhibitors suggests that deacetylation is involved in the skewed expression of certain genes that are associated with the disease. SLE T cells exhibit increased and prolonged expression of cell-surface CD40L, spontaneously overproduce IL-10, but overproduce INF-γ. The deacetylase inhibitor trichostatin A (TSA) significantly reverses this skewed

expression of these genes products [62]. It is likely that this reversion is the result of modification of the histone acetylation status, although the alteration of the acetylation levels of transcription factors cannot be discarded. At any rate, this result not only suggests that histone acetylation might account for this aberrant expression but also that this pharmacological agent may be a candidate for the treatment of this autoimmune disease. Similar results have been obtained with other histone deacetylase inhibitors, such as suberoylanide hydroxamic acids, that are able not only to revert the aberrant expression of certain genes but also to modulate renal disease. [63]. Both H3 and H4 histones are hypomethylated in spleenocytes isolated from MRL/lpr lupus prone mice compared with mice MRL+/+ controls [64]. MRL/lpr mice treated with the histone deacetylase inhibitor TSA or suberoylanilide hydroxamic acid (SAHA) demonstrated improvement in glomerulonephritis and splenomegaly [65,66]. Furthermore, treatment of MRL/lpr splenocytes with histone deacetylase inhibitors in vitro resulted in the reduced expression of several cytokines, which include IL-12, INF-γ, IL-6, and IL-10. These effects on cytokine gene expression were associated with increased histones H3 and H4 acetylation following treatment with histone deacetylase inhibitors [64].

CONCLUDING REMARKS

Both DNA methylation and histone deacetylation are reversible modifications,and inhibitors of each procces exist74.Epigenetic modification ,has led to novel therapeutic approaches in recent years.The area of immunological epigenetics for the better target therapy is open.
Finally improvements in antisense and gene therapy procedures may also allow correction of the molecular defects in autoimmune disorders.

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