Interactions between genes and the environment. Epigenetics in allergy[1]

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ABSTRACT

Epigenetics is defined as those inheritable changes occurring in gene expression, without actual modification in the genic DNA sequence. Epigenetic factors are chemically stable, potentially reversible, and can be modulated or induced by environmental factors. In the case of allergic disease, epigenetics could explain not only the discordances observed between monozygous twins but also phenomena such as incomplete penetrance, variable expression, gender and progenitor effects, and sporadic cases. In this sense, the hypothesis of hygiene is of great relevance in that it integrates genetic and epidemiological data in the context of environmental exposures.

Among the different epigenetic factors, mention must be made of DNA methylation, covalent histone modifications, and other mechanisms that include different protein complexes and RNA-mediated modifications. The regulatory effect of these phenomena upon immune response has important implications for allergic diseases. At present, different lines of pharmacological research are being conducted, based on the modulation of epigenetic factors, modifying expression of the genes that encode for proteins implicated in allergic processes. Among such modulators, mention can be made of antisense oligonucleotides, ribozymes and interference RNA. The applications of epigenetics to the diagnosis and treatment of allergic disorders offer a very promising future of this specialty.

Key words: Allergy. Epigenetics. Methylation. Silencing. Treatment.

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INTRODUCTION

Monozygous twins have the same genetic composition, with the exception of minor replication errors that may occur during embryonic development[1]. In contrast, dizygous twins have only 50 % of their genetic composition in common. However, phenotypic differences have been described in genetically identical individuals, and historically have been attributed to environmental factors. Given the difficulty of controlling environmental factors in humans, and the bias seen in epidemiological studies, experimental models have been developed in animals, involving controlled environments. These studies have shown that monozygous mice developing in one same controlled environment occasionally also pre-

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sent phenotypic differences. In fact, it is believed that only 30 % of the observed variability can be explained by environmental factors, and that the remaining 70 % must be due to a third factor[2] currently attributed to epigenetics. In this context, epigenetics is defined as those inheritable changes occurring in gene expression, without actual modification in the genic DNA sequence.

There are few transcription factors capable of explaining all cell characteristics. In fact, epigenetics could account for incomplete penetrance and variable expression, as well as those limitations that cannot be explained by the DNA sequence alone. Epigenetics could help identify the molecular effects of environmental factors. In the case of allergic disease, epigenetics could explain not only the discordances observed between monozygous twins – and which in the case of asthma reach 25 % – but also phenomena such as incomplete penetrance, variable expression, gender and progenitor effects, and sporadic cases[3].

In this sense, different interpretations compare epigenetics with a system for reproducing the information "stored on tape" (DNA in this case), or with computer software used to access information stored on a hard disc (represented in this case by DNA). In sum, the difference between genetics and epigenetics could be compared with the difference between writing and reading a book. The text (genetic information) is the same in all copies of the book, though its interpretation will depend on the way and on the emotions with which the reader actually reads to book. In this way, the genotype would not determine a single development but rather a reaction pattern responding differently according to the environmental conditions[3].

In this dialogue between genetic and environmental factors, the hypothesis of hygiene is of great relevance in that it integrates genetic and epidemiological data in the context of environmental exposures. In this way, it has been postulated that both family position and size, and the so-called western life style condition allergic response. The increase in risk of allergic disease associated with the absence of infections, the change in intestinal flora, and increased pollution, or the reduction in risk associated with the absence of vaccines or with certain dietary aspects, contribute to reinforce these interactions. On the other hand, the rapid increase over the last 30-40 years[4] in the incidence of allergic diseases cannot be explained by an increase in number of diagnoses made, and the time elapsed in turn is not considered sufficient to allow for changes in the corresponding genetic markers. An explanation is needed that can integrate the genetic effects and the environment in a time frame consistent with the epidemiological data[3].

Genetic information is encoded for by DNA, which is compacted within the cell nucleus, associated to proteins, forming chromatin. From the cellular perspective, heterochromatin is dense, inaccessible to enzyme action and presents few active genes, while in contrast euchromatin is an open structure, accessible to enzymes and with transcriptionally active genes. A number of epigenetic mechanisms control and regulate modifications in chromatin, making it more or less accessible to transcription actors, and thus contributing to determine the level of expression of different genes.

A very important phenomenon in epigenetics is selective DNA inactivation – a process known as genomic imprinting. Such imprinting can be interpreted as the functional difference observed in certain genes depending on their maternal or paternal origin, after undergoing different "labeling" during gamete genesis. This phenomenon could help explain progenitor influence upon the expression of certain allergic disorders.

The epigenetic factors that intervene in these processes are chemically stable, yet potentially reversible, and modify the phenotype without affecting the genotype. They can be inherited, but at the same time can be modulated or induced by environmental factors. Among the different epigenetic factors, mention must be made of DNA methylation, covalent histone modifications, and other mechanisms that include different protein complexes and RNA-mediated modifications.

DNA METHYLATION

DNA methylation is an epigenetic mechanism allowing the regulation of transcription via the addition of methyl groups to carbon 5 of the nucleotide cytosine. It is the most frequent covalent modification of DNA, and has been referred to as the *prima donna* of epigenetics[5]. This methylation process is carried out via the enzyme DNA (cytosine 5) methyl-transferase EC 2.1.1.37 (DNMT).

DNA methylation takes place in CpG regions known as islands. These are regions with at least 500 base pairs that contain over 55 % of C and G nucleotides[6]. Such regions are basically located at the 5′-terminal.

The genotype of practically all cells in the body is the same, with the exception of the gametes and some immune cells. During cell differentiation, inheritable epigenetic factors control the genomic functions. During subsequent mitotic divisions, other modifications controlled by stochastic epigenetic factors can take place.

A methylation pattern has been described that varies during cell maturation – the pattern becoming consolidated as the gametes mature. After fertilization, DNA methylation takes place. This DNA remains hypomethylated during the first weeks of embryonic development until *de novo* methylation occurs.

The methylation processes prevent transcription factor binding and at the same time favor the binding of transcription inhibiting proteins. The degree of activation of a given gene is generally dependent upon its degree of methylation[7].

COVALENT HISTONE MODIFICATIONS

Chromatin is structured within the cell nucleus in units called nucleosomes, composed of histone octameres around which the DNA is coiled. The histones conforming these octameres are H2A, H2B, H3 and H4. From the structural perspective, histones have different characteristics in terms of their number of amino acids and the concentration of the latter (particularly lysine and arginine), and which condition the different post-translational modifications.

These modifications are found in the lateral chains and fundamentally comprise acetylation, phosphorylation and methylation – though other reactions have recently also been reported, such as ubiquitination and sumoylation. The different modifications in turn have different effects upon transcription factor access to the DNA. As an example, histone acetylation would tend to increase such accessibility[8], while deacetylation (produced by histone deacetylases, HDAc) would close the structure[9]. A hypothesis has been proposed, postulating the existence of a histone code. The combination of histone modifications conforms the so-called epigenome.

OTHER EPIGENETIC MECHANISMS

Among the different epigenetic mechanisms, mention must be made of those mediated by protein complexes. Studies in lower organisms have described different protein complexes with epigenetic effects, such as the Polycomb/Trithorax complex[10], which act through DNA recognition sequences called PREs (Polycomb Response Elements). Other protein complexes such as SWI/SNF[11] act through ATP hydrolysis, altering chromatin structure and giving rise to rotation phenomena secondary to partial nucleosome disruption.

Another important mechanism comprises RNA-mediated epigenetic modifications. Among the multiple types of RNA, mention should be made of siRNA[12], or small interfering RNA molecules. These are double strand molecules that silence recognized sequences via excision, among other mechanisms.

All these described processes do not act isolatedly but in an inter-related manner. Thus, methylated DNA would recruit HDAc through proteins with domains for binding to methylated CpG, in a complex integration system that has not yet been fully elucidated.

TH1/TH2 REGULATION

A clear example of how epigenetic factors modulate cell expression can be found in the immune system. CD4+ cell activation to form Th1 or Th2 cells after antigen exposure is characterized by the secretion of INFγ or IL-4, respectively[13]. The Th1 cells produce INFγ to facilitate late immune responses[14], to promote isotype change of the B cells towards the production of complement-fixing IgG2 antibodies, and to provide protective immunity against intracellular pathogens[15]. On the other hand, Th2 cells produce IL-4, facilitating an isotype change towards the production of IgG4 neutralizing antibodies and IgE associated to allergic processes. In turn[16], these cells regulate Th1 response and afford protection against extracellular pathogens[15].

Apart from other factors, differentiation towards Th1 or Th2 is fundamentally dependent upon the cytokines of the cellular microenvironment in which the naïve CD4+ cells are activated. In this context, Th1 cells require the mediation of IL-12, while the Th2 cells require IL-4[17]. Th2 response is regulated by transcription factor GATA3, which is activated by STAT6. The latter in turn is activated by stimulation of the IL4R receptor in the naïve CD4+ cells[18]. It has been suggested that epigenetic changes of the genes that encode for cytokines may facilitate selective accessibility[19].

In Th2 response, the increase in GATA3 expression is implicated in the modifications experienced by the gene encoding for IL-4, such as DNA methylation and histone acetylation[20]. These phenomena would increase IL-4 transcription factor accessibility. In addition, IFNγ silencing would result from DNA methylation and histone deacetylation[21].

Together with the genes that intervene in the Th1/Th2 response mechanisms, it is important to consider the mechanisms that regulate the genes that encode for proteins related to respiratory pathway remodeling, such as ESE-3, ADAMS33 and SPINK5.

ENVIRONMENTAL FACTORS

As has been commented above, environmental factors are able to regulate these epigenetic changes. These factors include diet. In this sense, it has been reported that folic acid and vitamin B12 influence the availability of methyl groups and co-factors for the formation of S-adenosylmethionine, which is essential for *de novo* DNA methylation. It also has been shown that fish oil-rich diets could induce epigenetic effects. Other important factors are the characteristics of the living environment[22], contact with animals, the administration of certain drugs, or aging[23]. The information on the epigenetic mechanism possibly underlying the relationship between such factors and allergic diseases is still limited, however.

Different techniques are used to study epigenetic factors, including bisulfite pretreatment for differentiation of the methylated sequences, or proteomic methods such as bidimensional electrophoresis – which allows the detection of differences in the protein expression pattern of different cells. The new technologies such as microarrays in turn allow simultaneous analysis of a great number of sequences – thus facilitating the characterization of methylation patterns.

EPIGENETICS AND TREATMENT

At present, one of the most important applications of epigenetics is found in the therapeutic setting. Modifications in dietary habits have been postulated as mechanisms of epigenetic modulation. In addition, inhibitors of both DNMT and of HDAc have been described as possible therapeutic agents with applications in oncology, aging and transplantation. In allergic disease, a number of therapeutic approaches have been described[12]. On one hand, treatments have been designed to modify the expression of genes that intervene in allergic processes, while on the other hand genetic modifications of allergen expression have been described – specifically in the context of food allergies. These modifications are designed to reduce the allergenic character of such allergens.

Current treatments such as beta-adrenergic receptor agonists, corticoids, or antileukotrienes are used to reduce the manifestations of allergic disorders without eliminating the underlying cause. However, modulation of the epigenetic factors would focus on expression of the genes directly implicated in the physiopathology of such diseases. In this context, one of the main mechanisms applied to the treatment of allergic disorders is based on the use of so-called antisense oligonucleotides (AS-ONs)[12]. These are composed of 15-25 nucleotides conforming a single strand that can activate RNases or block ribosomes – thus avoiding the expression of concrete genes. At present, different molecular modifications are used to improve their effectiveness.

The problem facing the use of such molecules in therapeutics is the absence of a specific experimental model of allergy – a situation that complicates their evaluation. In allergic pathology, studies are being made of inhalatory antisense oligonucleotides, which are effective at lower doses, due to the administration route involved. A number of drugs that act against the A1 adenosine receptor are also in the trial phase, while others are targeted to GATA3[24], IL4RA, VLA4 or TNFα.

Other molecules being evaluated for the treatment of allergic disorders are ribozymes and deoxyribozymes, elaborated with genetic engineering techniques, and that splice RNA at specific sites, thereby altering its expression[12]. In this sense, ribozymes against IL5, NFkB and ICAM-1 are being tested.

Lastly, the small interfering RNA molecules (siRNA) commented above are small double-strand RNA molecules that act by incorporating silencing complexes to selectively control the expression of certain genes. Adequate selection of the genes targeted for selective control is one of the keys to the success of treatments of this kind.

To summarize, in the words of Denise Barlow, epigenetics "has always been all what is strange and marvelous, and which cannot be explained by genetics". In effect, epigenetics opens extraordinary perspectives in the diagnosis and treatment of allergic diseases.

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