EPIGENETICS AND STEM CELLS: A NEW PATH FOR UNDERSTANDING PLASTICITY, DIFFERENTIATION AND IMPRINTING MECHANISMS

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ABSTRACT

The characteristic of plasticity, especially of embryonic stem cells (ESC), confers them differentiation capacity in any cellular type. Differentiation processes occur through loss of this plasticity, specializing cells by blocking the expression of non-related genes. The epigenetics suggests explanations for this capacity, by regulatory processes that are not contained in genome. The elucidation of the processes involved in the cellular differentiation phenomena is fundamental towards the development of cellular therapy techniques and can be extrapolated to studies about other phenomena, like the cellular malignant transformation. This review summarizes and discusses key-points of the recent topic of the stem-cells epigenetics.

KEYWORDS: plasticity, differentiation, imprinting, epigenetics.

RESUMO

Epigenética e células-tronco: um novo caminho para a compreensão dos mecanismos de plasticidade, diferenciação e *imprinting*

A característica de plasticidade, especialmente das células-tronco embrionárias (ESC: *Embryonic Stem Cells*), confere a elas capacidade de diferenciação em qualquer tipo celular. Processos de diferenciação ocorrem através da perda desta plasticidade, especializando células pelo bloqueio da expressão de genes não relacionados. A epigenética sugere explicações para esta capacidade, através de processos regulatórios que não estão contidos no genoma. A elucidação dos processos envolvidos nos fenômenos de diferenciação celular é fundamental em relação ao desenvolvimento de técnicas de terapia celular e pode ser extrapolada para estudos sobre outros fenômenos, como transformação celular maligna. Esta revisão sumariza e discute pontos-chave do recente tópico da epigenética das células-tronco.

PALAVRAS-CHAVE: plasticidade, diferenciação, *imprinting*, epigenética.

RESUMEN

Epigenética y células madre: un nuevo camino para comprender los mecanismos de plasticidad, diferenciación y *imprinting*

La característica de la plasticidad, sobretodo de las células madre embrionarias (ESC: *Embryonic Stem Cells*), les confiere la capacidad de diferenciarse en cualquier tipo celular. Los procesos de diferenciación se producen por la pérdida de la plasticidad, especializando células mediante el

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bloqueo de la expresión de genes no relacionados. La epigenética sugiere explicaciones para esta capacidad, por parte de los procesos regulatorios que no están contenidos en el genoma. La elucidación de los procesos involucrados en los fenómenos de diferenciación celular es fundamental para el desarrollo de técnicas en terapia celular y puede ser extrapolada a estudios sobre otros fenómenos, como la transformación de células malignas. Esta revisión resume y analiza los puntos clave sobre el tema reciente de las células madre en epigenética.

PALABRAS CLAVE: plasticidad, diferenciación, *imprinting*, epigenética.

INTRODUCTION

The phenotypic plasticity is defined as the group of undergone changes suffered by an organism when environmental alterations are imposed on it. These changes can influence behavior, format, activities of the organism, among other characteristics. However, these changes will not always be beneficial, as they can be adaptive or non-adaptive[1,2]. The fact that internal changes occur in response to external ones is described as a way to maintain homeostasis.

These changes can be either reversible or not[1,2]. In the former, a phenomenon called polyphenism is characterized. It allows organisms to change according to environmental factors. One example is the phenotypic alterations in some animals when the year seasons change[3]. When the phenotypic alterations are not reversible, they are maintained over generations as part of the evolutionary process.

The phenotypic plasticity can be noticed both in an organism (individually) and in a population (collectively). In the first case, it is possible to describe the organism adaptation capacity through polyphenism[4]. In the second, the phenotypic plasticity is associated with genetic mutations occurred over generations in the whole population[5].

The role of plasticity can be related to various subjects of biological interest. Evolution process, for example, is connected with the plasticity from life history; in other words, survival strategies of organisms lead to a phenotypic plasticity through a modification on the genome. It implies in the hereditary passage of genetic information, characterizing the evolution. In humans, the plasticity is most commonly noticed with a behavioral change, which includes rapid modifications on hormone levels. This is due to the existence of synaptic connections in which this phenomenon occurs, opening a huge path for studying environmental effects on the nervous system genes, especially those related to the transmission of synaptic signals[1,2].

CELL PLASTICITY AND EPIGENETICS

Cell plasticity cannot be explained only by the information contained in genome. “Extra-genomic” mechanisms regulate the gene expression of cells that, although show the same genetic information, have differential behavior when exposed to distinct environmental conditions. These mechanisms include DNA methylation (that occurs at cytokine-related genes), chromatin remodeling and histones post-translation changes. All these mechanisms, among others that are not well elucidated or not discovered, are called as Epigenetics[6].

Nowadays, researches aimed at the explanation of this plasticity at molecular levels are much appreciated.

The actual epigenetics field is trying to appoint factors involved with DNA modification that controls gene expression. From studying it, differences between actives and inactive genes from organism to organism can be explained, having direct relation with life history and, consequently, with evolution and phenotypic plasticity[1,2].

The epigenetics, although not completely elucidated, suggests explanations for phenomena like normal genotype cells showing malignant phenotypes, phenotypic differences between monozygotic twins ("natural clones"), processes of stem cells differentiation and ageing. These processes are due to plasticity characteristic of cells, especially of embryonic stem cells (ESC) – which show the highest adaptation capacity among the different eukaryotic cellular types[2,7,8].

EPIGENETICS DYNAMICS OF NON-DIFFERENTIATED CELLS

Recent researches are trying to define factors that cause stem cells, both embryonic and adult, to have their differentiation processes stimulated. Due to the necessity of a well-coordinated control between cell plasticity and phenotypic stability, the study of these factors become essential for understanding the cellular and molecular biology of stem cells[9]. Phenotypic stability, essential in processes of tissue engineering, is explained by epigenetics as a consequence of differentiation processes, through methylation of gene promoters, among other epigenetic changes[10].

One of the most important points of understanding cellular plasticity is studying processes involved in cloning. Cloning represents the cell nucleus plasticity, since it is transplanted to a different cellular environment (a oocyte, for example), being able to generate a complete organism[11]. Of course, as seen earlier, this plasticity at cellular level has an epigenetic explanation.

Because ESC have the biggest part of their genes able to be expressed, they show a high capacity of plasticity[12,13]. As the cells suffer differentiation, genes that are not related to the function that the cellular type will play are gradually being silenced through epigenetic mechanisms, as promoter methylation by DNA methyltransferases (DNmt) enzymes. As methylation is passed cell-to-cell during mitosis, the modification is maintained in a way that prevents an aberrant reactivation of pluripotency in physiological conditions[12,14,15].

The detailed knowledge about epigenetics of ESC plasticity and differentiation allow the improvement of biotechnological applications involving stem cells, as cellular therapy – besides cloning, previously mentioned. Cellular therapy using ESC would be based on production of induced pluripotent stem (iPS) cells from already differentiated somatic cells[16]. Thereby, it would be possible to perform the therapy with the own cells of the patient, thus eliminating risk of rejection and the ethical barrier imposed on researches with human stem cells extracted from embryos.

Another option to obtain pluripotent stem cells is the epigenetic reprogramming by the somatic cell nuclear transfer (SCNT) technique. In this method, oocytes receive the entire nucleus of an adult somatic cell, corresponding to an epigenetic modification. Thereby, oocytes give rise to stem cells equivalent to embryonic, having capacity to originate almost any tissue, both *in vitro* and *in vivo*[16]. Epigenetic mechanisms that promote cell division to result in stem cells are not totally elucidated yet. So, it is still not

possible to find plausible explanations to several anomalies in the development of cloned organisms by this technique.

The differentiation capacity of stem cells obtained by SCNT using primate fibroblasts and oocytes was analyzed both *in vitro* and *in vivo*. Cultured blastomeres originated cardiac muscle cells and functional neurons in a relatively easy way. When injected in mouse, non-differentiated cells developed a teratoma – a malignant differentiation that results in one aggregated of distinct kinds of tissues. Although the conclusions showed some negative points, the method represents similar efficiency to the acquisition of ESC by *in vitro* fertilization (IVF), but has the advantage of not involving the ethical barrier on manipulating fertilized embryos[16]. Researches like this one open a path to studies related to regulation of gene expression during ESC differentiation, especially by the epigenetic alterations in mammals.

Although ESC have a greater plasticity, differentiated cells still show a reduced adaptation capacity. The explanation to this fact is found in differences between regulations of two types of vertebrates gene promoters: CG-rich sequences and CG-poor sequences. The former ones are permanently inhibited by methylation; nevertheless just a small portion of these promoters are methylated during cellular differentiation. This last fact allows the replication machinery to act on these regions during any development stage[12,13].

ASPECTS OF EPIGENETICS IN GENOMIC IMPRINTING

Another important natural process related with cellular differentiation is the Genomic Imprinting. Imprinting is the preferential expression of the maternal or the paternal alleles, not both. It is regulated, mainly, by DNA methylation. Genes are determined to be silenced according to sexual chromosomes[17]. It means that certain genes are expressed with presence of the Y chromosome, in animal species. Such genes are responsible for the specialization of germ cells, meaning that an organism responds to phenotypic plasticity according to sex determined by its genome.

There are genes in which the imprinting pattern is conserved among different species. It has been shown, for example, that *H19* exons 1 and 5 are only expressed by the maternal allele in several tissues of many mammalian species – including humans[18]. The expression pattern conservation of this gene in studied species suggests that such gene has an important function in the organism.

Beyond epigenetic mechanisms, the imprinting can occur due to DNA mutations, especially single nucleotide polymorphisms (SNPs). These variations may result in expression of a truncated, non-functional or still in an oncogenic protein. Relating with imprinting, SNPs that result in loss of certain gene expression may interfere in analysis of inhibition by methylation, for example[19,20]. Thus, the occurrence of SNPs is a factor that must be considered in studies about genomic imprinting.

After the fertilization of gametes, it is known that the developed embryo lose its “epigenetic memory”. It means that methylated promoters suffer demethylation in order to promote totipotency to cells. However, certain regions are resistant to this demethylation, which determine the genomic imprinting by epigenetics[18]. The imprinting pattern of *IGF2* (Insulin-like Growth Factor 2) on the exons 2 and 9 were also analyzed, and exon 2 showed bi-allelic expression, while exon 9 showed mono-allelic expression. Based

in previous knowledge that *IGF2* is regulated by multiple promoters, a quantification of transcripts containing exon 2 and exon 9 were proceed, relating with regulatory action of four promoters (P1, P2, P3 and P4). The conclusion was that promoters P2 and P4 can transcribe just exon 9, pointing that these promoters direct *IGF2* imprinted expression pattern.

This is a good example of epigenetic alteration that reduces the viability of cloned organisms. Cloned animal which does not express their imprinted genes correctly may develop tumors and have other kinds of diseases[21,22]. Furthermore, stem cells with this kind of problem are unviable to be used in therapies. So it is necessary to elucidate the integrity of stem cells genomic imprinting before thinking about using them in clinical cases.

FINAL CONSIDERATIONS AND CONCLUSIONS

Conclusions obtained by studying epigenetic regulation of different genes during replication process can be extrapolated to other researches. An example would be cancer studying, in which the prime malignant characteristic is cell immortalization. About 85 to 90% of cancer types show high-level expression of an enzyme called telomerase, which promotes telomere maintenance, thus avoiding senescence and cell death[23,24]. Although immortality is an undesirable characteristic to adult cells, stem cells need to maintain their telomeres in order to origin cells that have a normal life time (about 60 generations). Thereby, there should be a mechanism that allows the expression of this enzyme in ESC and block it in differentiated ones, like the epigenetic silencing mechanisms (probably by methylation) during differentiation processes. This is just one example of several phenomena that may find explanation in epigenetics.

Nowadays, genes involved with maintenance of ESC characteristics have been studied in order to understand the molecular processes involved with stem-cell differentiation (especially embryonic) and to develop some molecular markers that may be used for analysis of cellular differentiation *in vitro*. The expression of *hTERT* (human Telomerase Reverse Transcriptase) is related with capacity of pluripotency, ESC characteristic morphology and raise of proliferative rate. Moreover, inhibition of *hTERT* expression is related with cellular differentiation and loss of pluripotency[25]. Thereby, the block of *hTERT* expression seems to be a phenomenon that occurs during ESC differentiation to all cellular kinds. Lots of genes are already known as participants of plasticity and differentiation processes, and many others are being discovered by new researches.

Two recent researches in humans found a relation between stress states and telomerase expression, which includes *hTERT* subunit. As cited earlier, transmission of synaptic signals can be altered in way to modify gene expression and promote the phenotypic plasticity. Based on it, one of the experiments submitted elderly women to positive-stress conditions and analyzed the variation of telomerase expression of peripheral blood mononuclear cells. Most of the women had altered levels of telomerase expression, characterizing a phenotypic plasticity that can conduce to fast ageing and malignant transformation of the cells[26]. Moreover, the second article shows that meditation have strict relation with telomeres length, and thus with telomerase. This research involved Buddhists, in which the high mindfulness and concentration during a determined

meditation program were capable to decrease the stress levels and then stabilize the gene expression[27]. These conclusions have important implications for the understanding of phenotypic plasticity and epigenetics at molecular level in future studies.

Although phenotypic and molecular plasticity are strictly related, it is possible that this connection is unconsidered in some cases. For example, several variations on the environmental wave vibration do not prevent molecules, such as proteins, from adopting their conformation defined as normal. This is explained by mechanisms developed previously by the organisms that annul such factors, characterizing a plasticity that maintains homeostasis.

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