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REGULAR ARTICLE

The progression of cancer and metastasis formation: An epigenetic hypothesis

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Abstract The molecular mechanisms of tumor metastasis remain largely unknown and undefined. A recent model suggests that a minor population of cells (cancer stem cells) is programmed to preferentially metastasize to specific organs based on their gene expression patterns. These cells have the ability to generate tumors after implantation into animal hosts, to self-renew and give rise to non-stem cells. In this paper I hypothesize that epigenetic mechanisms could play an important role in tumor metastasis through the reorganization of the bivalent chromatin marks in cancer stem cells in three phases: 1) the reprogramming of epigenetic marks in differentiation master regulator genes responsible for the differentiation to one particular lineage 2) the resolution of these bivalent chromatin marks forces cells to develop the necessary mechanisms to migrate to a new niche and 3) the epigenetic activation of the tissue-specific genes associated with the specific target organ and, simultaneously, the repression of genes associated with alternative developmental pathways.

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Introduction

Epigenetics is the study of mechanisms that control gene expression in a potentially heritable way [1]. In humans, histone modification represents one prominent form of chromatin remodeling, which results in dynamic and reversible post-translational modifications of the residues at the

N-terminal tails of histones. These modifications are mediated by sets of enzymatic complexes that site-specifically attach or remove chemical groups [2].

The histone modifications described so far include acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation. This epigenetic mechanism has a central role in processes such as DNA repair, DNA

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replication, alternative splicing, and chromosome condensation. In regards to gene regulation, histone modifications are associated with both transcriptional repression and activation. In general, acetylation and deacetylation of the ϵ -amino groups of conserved lysine residues present in histone tails by histone acetylases and deacetylases, respectively, has long been linked to a more relaxed chromatin state, which is conducive to gene expression [3], also playing important roles in the regulation of DNA replication and DNA repair [4]. Methylation by histone methyltransferases correlates with transcriptional activation and repression. Thus, we associate euchromatin with high levels of acetylation and trimethylated H3K4, H3K36, and H3K79, while heterochromatin, on the contrary, is associated with limited acetylation marks and high levels of H3K9, H3K27, and H4K20 methylation.

These mechanisms play a role in the maintenance and differentiation of embryonic stem cells (ES cells) through polycomb groups that establish epigenetic control of important regulator genes that regulate future expression patterns [5]. In these cells, we observed a bivalent chromatin structure in which active and repressive chromatin marks are closely juxtaposed mainly in genes with promoters enriched in CpG islands that correspond with important tissue-specific regulator genes [6]. It is during differentiation that bivalent chromatin profiles are generally resolved, leading to transcriptional activation of tissue-specific genes and the silencing of loci associated with alternative developmental pathways [5]. In summary, ES cells have the potential to differentiate into any cell type in the body, and this potential is related to specific histone modifications [7]. This process has been described in normal ES cells, but the role of the bivalent chromatin structure in the pathophysiology of different diseases, specifically in cancer, has yet to be elucidated.

Tumors contain multiple types of cells, some of which contribute to different processes, such as metastasis. This is a complex, multi-step process that represents the spread of cancer cells from the primary tumor sites to distant organs and tissues. In spite of its clinical importance, the molecular mechanisms driving metastasis remain unknown. However, certain cancers have distinct organ colonization patterns. For example, colon cancer usually metastasizes to the liver, while liver cancer and rectal carcinoma disseminate predominantly to the lungs [8]. This topology of metastasis formation is based, from a genetic perspective, on the existence of metastasis gene signatures expressed by the primary tumors [8,9]. For example, genes such as COX2, ST6GALNAC5, and EGFR ligands mediate breast cancer metastasis to the brain [10], and the MMP1, VCAM1, and GRO1/CXCL1 genes mediate breast cancer metastasis to the lung [9]. These specific processes are carried out by a minor population of cells, cancer stem cells (CSCs), which are programmed to preferentially metastasize to specific organs based on their gene expression patterns [11]. These cells have the ability to generate tumors after implantation into animal hosts, and to self-renew and give rise to non-stem cells [12], showing a dynamic equilibrium between CSCs and non-CSCs that could be shifted in one direction or another by contextual signals within the tumor microenvironment [13]. It is important to highlight that, while CSCs can differentiate into non-CSCs, the reverse process must also be considered [13]. In the last years, a CSC-based model for metastasis has been proposed and suggests that CSCs inherit a unique set of genetic and/or epigenetic changes that

determine the cancer malignancy, metastatic potential, and the tissue tropism that is regulated by cues such as oxygen gradients or other chemo-attractants derived from niche sites [11].

Compelling data indicate that CSCs could have a central role in metastasis, and an improved understanding of this role may lead to novel therapeutic strategies against cancer. Therefore, I hypothesize that the reorganization of the bivalent chromatin marks in a subpopulation of cells within a tumor leads to a differentiation of non-CSCs to CSCs, the latter being responsible for the metastatic process.

The hypothesis

As previously stated, the presence of bivalent chromatin structure at tissue-specific regulator genes plays an important role in the maintenance and differentiation of ES cells. Therefore, the differentiation of ES cells to one particular lineage is an epigenetic phenomenon characterized by the presence of a specific chromatin structure that implies the permanent and irreversible silencing of genes involved in alternative lineages (Fig. 1).

While this epigenetic signature has been described in ES cells, the role of this bivalent chromatin structure in the metastatic process is unknown. As previously mentioned, within a tumor, non-CSCs can be reprogrammed into CSCs given certain contextual signals. I suggest that this transdifferentiation is structured in three phases: First, the transition from non-CSCs to CSCs could be mediated by the reprogramming of epigenetic marks in differentiation master regulator genes responsible for the differentiation to one particular lineage, such that the new CSCs have properties similar to ES cells. Second, this bivalent chromatin signature will be resolved with a pattern of absence of DNA methylation and H3K4me3 in specific cell lineage genes and, simultaneously, a presence of repressive marks (i.e. H3K9me3, H3K27me3, and DNA methylation) in genes involved in the rest of alternative cell lineages; this pattern stimulates differentiation into a specific cell lineage and give a new identity to the CSC. This, in turn, forces cells to develop the necessary mechanisms to migrate to a new niche (i.e. the epithelial–mesenchymal transition or EMT) and, finally, when the CSCs arrive at the new organ, in order to activate the tissue-specific genes associated with this organ, they simultaneously gain H3K4me3 and lose DNA methylation while others, which represent genes associated with alternative developmental pathways, show epigenetic repressive marks, such as H3K9me3, H3K27me3, and DNA methylation.

Evaluation of the hypothesis

I will focus on one type of cancer, for example, colon cancer that, as I have already pointed, usually metastasizes to the liver. Although in vitro cell culture models will be useful in understanding the origins of CSCs (transition from non-CSCs to CSCs) and when and where they arise during cancer initiation and progression, we can use mouse cancer models in order to obtain, first, colon non-CSCs and, second, obtain CSCs after the repeated intraperitoneal administration of the mutagenic agent azoxymethane (AOM) that results in the development of spontaneous tumors [14]. The chromatin analysis could be done through Chromatin immunoprecipitation (ChIP), which is routinely used to examine epigenetic

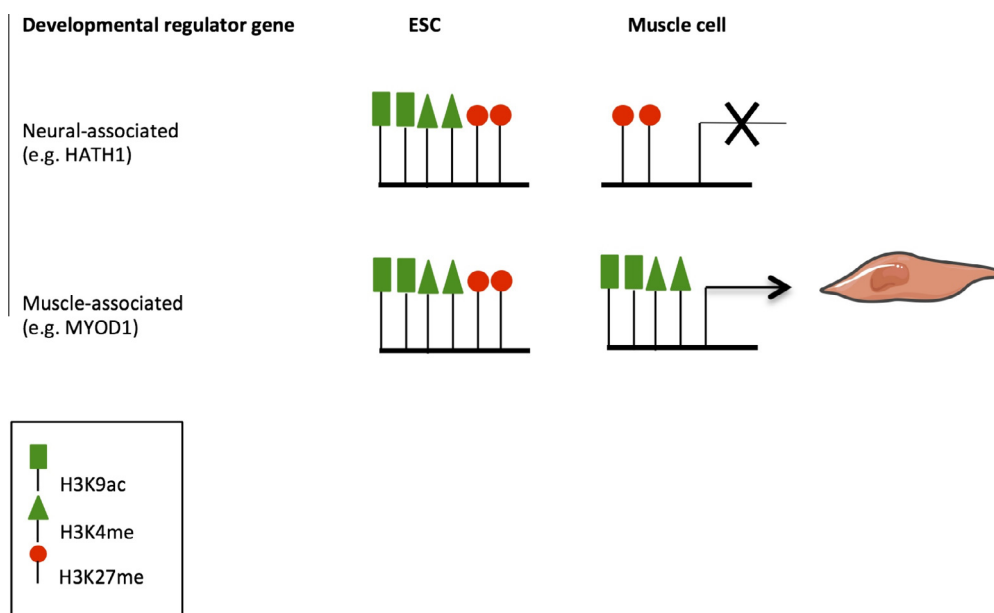


Fig. 1 Bivalent chromatin marks in ES cells and during differentiation to muscle cells.

modification of histones at specific genomic locations. If the hypothesis is right, we will have three different chromatin signatures associated with different cell populations: a temporal bivalent epigenetic one (CSCs), a temporal differentiation signature (new liver-CSCs) and a stable tissue-specific signature (colon non-CSCs). Temporal differentiation signature will be also characterized by the expression of *FOXA3*, *HNF1A*, and *HNF4A*, genes that regulate hepatic genes programs and, therefore, related with functions characteristic of hepatocytes [15]. To analyze the different gene expression signatures associated with each type of cells, we will employ the csSAM, a methodology that enables the analysis of gene expression in complex tissues at a much higher resolution than previously possible [16]. Finally, we have to explore the liver cell population in order to analyze the activation of the tissue-specific genes associated with this organ.

Consequences of the hypothesis and discussion

To date, epigenetic mechanisms have been described that play a key role in different processes, both in healthy and disease states, for example silencing or activating specific genes in cancer or resolving bivalent chromatin profiles in ES cells. But epigenetic mechanisms could provide a link between these two processes, apparently disconnected; that is, what is the role of this bivalent chromatin structure in the metastatic process? We know that CSCs are considered to be the root of cancer and could be responsible for cancer metastasis, but its molecular mechanisms remain largely unknown and undefined. In the present paper I hypothesize that epigenetic mechanisms could play an important role in cancer progression through the reorganization of the bivalent chromatin marks in these CSCs. This process is structured in three hits, in consonance with the well-accepted Knudson hypothesis. The first hit involves the erasing of epigenetic marks in differentiation master regulator genes. This event is following with the second hit: the reorganization of histone marks, that results in a new

temporal cell identity, which forces cells to develop migration mechanisms. Finally, the third hit involves the epigenetic activation of the tissue-specific genes associated with the specific target organ and, simultaneously, the repression of genes associated with alternative developmental pathways. In summary, I think that the identification of the epigenetic reprogramming events in non-CSCs proposed in this paper could have profound implications for cancer therapies targeting CSCs.

Overview box

First Question: What do we already know about the subject?

Cancer represents one of the most lethal pathologies affecting mankind. Although its molecular mechanisms remain largely unknown and undefined, recent studies suggests that CSCs are involved in the metastasis process to specific organs based on their gene expression patterns.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?

Although many studies focus on the genetic signatures that distinguish CSCs from normal tissue stem cells, none has described the epigenetic regulation of this cell population. If the hypothesis is right, we could understand the molecular mechanisms driving metastasis and, therefore, develop new therapeutic strategies based on epigenetic approaches (i.e. by developing new histone deacetylase inhibitors, as EPZ005687, targeting the key enzymes regulating EMT or cancer progression).

Third Question: Among numerous available studies, what special further study is proposed for testing the idea?

In order to evaluate the idea, animal models should be conducted. If we obtain positive results, a detailed study including human cancer samples would be necessary in order to validate or refuse the hypothesis.

Conflict of interest

The author whose name is listed immediately below certify that he has NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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