

The Mysteries of Metastasis

Researchers investigate the epigenetics behind cancer metastasis using the HumanMethylation450 BeadChip and the HiSeq[®] System.

Introduction

There is a mystery to the process of metastasis, the insidious spread of cancer cells from a primary tumor to other parts of the body. Why do some cancers travel, while others remain contained to the original tumor? Take melanoma for example. Although it is the least common type of skin cancer, it is one of the most feared because of its ability to metastasize quickly and easily throughout the body.

The mystery of metastasis is an important one to solve. Metastatic cancer can change a patient's treatment plan and prognosis radically. Individuals with metastases undergo treatments that are longer, more invasive, and more expensive, and are much less likely to survive their disease.

Manel Esteller, MD, PhD, head of the Grupo de Epigenética del Càncer (Epigenetics of Cancer) at the Bellvitge Biomedical Research Institute, is unravelling the mystery of cancer progression through the study of changes to the epigenomic landscape of tumor cells. His laboratory is devoted to creating epigenomic maps of normal and cancer cells. Their hope is to gain a better understanding of how and why certain tumors spread so efficiently and identify new epigenetic possibilities for cancer therapies.

Dr. Esteller spoke with iCommunity about the importance of the epigenome to our understanding of cancer, how epigenetic information might help stratify patients in clinical trials, and how targeting DNA methylation might help researchers design more personalized treatments in the future.

Q: What sparked your interest in studying cancer from an epigenetic perspective?

Manel Esteller (ME): I am a physician and realized that there were many things that we couldn't achieve in clinical practice to improve cancer patient outcomes. More research was needed, so I went back to graduate school and focused on cancer genetics.

I realized that genetics alone was not enough to explain what was happening in so many cancer patients and became interested in the genomic regulation of cancer cells. I found that although there are many genes involved in tumor suppression, they are often not mutated. That was a big surprise to me. I wondered if there were other mechanisms involved in the activation of tumor suppressor genes, so that they were inactivated, not by mutation, but perhaps by DNA methylation. This type of epigenetic silencing could explain why cells with the same genes can have different phenotypes and different functions.

Q: What types of questions is your lab trying to answer?

ME: The aim of our laboratory is to map the main epigenetic differences between normal cells and disease-associated cells. We focus on cancer, neurogenetic, and cardiovascular disorders. With cancer, we are interested in DNA methylation. We have focused on identifying all the genes that are methylated in cancer cells and have learned that they can behave like tumor suppressors.

Q: What do we know about the role of methylation in cancer development?

ME: It's clear that the genome is important in cancer, however we now realize that there aren't as many cancer mutations as we once thought there would be. In fact, there are many tumors with a very low number of mutational events. Part of the explanation might be that they are undergoing changes in DNA methylation or histone modification.

While we know that DNA is based on 4 crucial elements—adenine (A), cytosine (C), thymine (T), and guanine (G)—that's not exactly true. C can be a plain C, or it can be a methyl-C. The methyl-C adds a methyl group to that element, silencing it, which likely changes the meaning of the sequence it is part of. Usually, a nonmethylated C is associated with active transcription and a methylated C is associated with silencing.

If we look at cancer cells, the methylation patterns look different than the tissue from which the cell was derived. The cell has lost its identity, it has lost its normal DNA mutation profile. Epigenetic alterations might explain the transformation of a normal cell to a cancerous cell. These alterations could be used to predict the behavior of a tumor



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and as potential targets for new drugs to restore a normal or nearnormal epigenome.

Q: What tools have been used to make discoveries concerning the role of the epigenome in cancer progression?

ME: Genetic mutations and epigenetic changes in cancer were discovered around the same time in the 1980s. But genetics had more user-friendly tools. We didn't have the right tools to study epigenetics until many years later. Historically, everything was done by Southern blot using enzymes. In the late 1990s, we started using PCR techniques. That allowed us to distinguish between methylated and nonmethylated sequences. We now use DNA methylation microarrays, such as the Infinium® HumanMethylation450 BeadChip, to study methylation in a similar, comparative way as we study genetic mutations. These arrays have enabled us to make some great advances in studying the epigenetics of cancer.

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Q: Why did you chose melanoma as the focus of your cancer epigenetics research?

ME: We actually study many tumor types. Sometimes the questions we are trying to answer lead us to breast cancer, colon cancer, or glioma. This time it led us to melanoma. Melanoma is a curious tumor because it gives rise to metastasis easily. It has an early dissemination—so it's a good model to use in studying the epigenetic contributions to metastasis.

Q: What epigenetic drug targets have you identified in melanoma that contribute to the cancer cell dissemination process?

ME: Our studies are focused on discovering melanoma markers that we can use to identify subjects with the most prometastatic forms of the disease. In a recent study, we used the HumanMethylation450 BeadChip to obtain the methylation profiles of cell lines derived from the primary tumor and the lymph node metastasis of the same individual.¹ We wanted to find genes that participate directly in metastasis, so we also hybridized additional pairs of primary and metastasic cell lines in colon and breast cancers. We analyzed 482,422 CpGs in the 3 paired cell lines and performed transcriptional analysis on 2 candidate genes. We found different DNA methylation statuses between primary tumors and metastasis in the TBC1D16 gene. It was hypermethylated and downregulated in the paired corresponding metastatic cells. We were able to single that gene out and do a functional in vivo validation of what it was doing. It's an inhibitor of dissemination in melanoma, and likely in breast and colon cancers, too.

Q: What does TBC1D16 control?

ME: *TBC1D16* gene is a GTPase activating protein. It's regulating the activity of oncogenes like KRAS and EGFR downstream. It's able to regulate their activity by trafficking, changing the protein in the membrane across to degradation. Those changes in activity can result in metastasis.

We found that the loss of methylation occurred in the CpGs located around the transcription start site of 2 short isoforms and that patients with hypomethylation of the *TBC1D16-47D* isoform had a poor prognosis. At the same time, their tumors were addicted to BRAF and MEK signaling and could benefit from drugs that target those pathways.

Q: What do you mean when you say that the tumors were "addicted" to BRAF and MEK signaling?

ME: A normal tumor has perhaps 10 pathways that feed that tumor, allowing it to grow and expand. Sometimes there is one pathway, or just a few pathways, that are more important for that tumor's growth. The tumor becomes addicted to those pathways because it needs them to grow and disseminate. If the cells only use a few pathways, you can target those pathways and then the cancer cells cannot escape. It can be a good strategy for treating patients with disseminated disease.

Q: What does the hypomethylation of *TBC1D16-47KD* tell us about a clinical outcome?

ME: *TBC1D16-47KD* is usually silent. When it is hypomethylated, it becomes active and generates metastasis. Identifying the presence of this isoform could be important in a clinical setting. If you look at patients with melanoma, you might not know if the tumor has metastasized. However, if you analyze the primary tumors and find this gene is hypomethylated, there's a good chance that the disease has already disseminated. It could tell a physician that a particular patient needs a more aggressive treatment.

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Q: Is it possible that a similar epigenetic pattern exists in other types of cancer?

ME: It's likely that all tumor types have similar traits and features, and it's possible that *TBC1D16* plays a role in other cancers. However, there's no single molecular event that triggers metastasis. There are likely other epigenetic alterations that induce metastasis.

Q: What could be the value of cancer drugs that target epigenetic methylation sites?

ME: Nearly 90% of patients diagnosed with a primary tumor actually die because of the metastasis. There are some current drugs that have the capacity to change the genome, altering everything slightly. In the process, they help to restore the epigenome to normal. We're interested in a more personalized approach. We're checking the genetic and epigenetic status of patients to see if there is a particular epigenetic drug to which a patient is more sensitive. For example, if a patient has a mutation in histone acetyltransferase, it might be a tumor

that is sensitive to a specific inhibitor. That inhibitor could be the target for a more effective treatment.

I envision a future where epigenetic drugs will be used with classical cancer treatments that target particular genetic mutations that are only present in metastatic cells. Cancer cells are small but they change quickly. They adapt easily to the treatments we throw at them. If we can target a type of cancer specifically, we'll have a better chance of killing the cancer cells and helping those patients.

"We would not have been able to identify the *TBC1D16* gene if the HumanMethylation450 BeadChip didn't also include certain CpG sites, because the *TBC1D16* gene is not a typical main promoter."

Q: Why did you choose the HumanMethylation450 BeadChip for your melanoma study?

ME: The HumanMethylation450 BeadChip is a standard, enabling us to compare the results from my lab with those of another lab. The array is also comprehensive. We can use it to study the minimal regions that regulate the genes, some upstream and downstream regions, and even some noncoding RNAs. We can also cover the main promoters and CpG islands, as well as CpG sites beyond those regions.

We would not have been able to identify the *TBC1D16* gene if the HumanMethylation450 BeadChip didn't also include certain CpG sites, because the *TBC1D16* gene is not a typical main promoter. It's an internal promoter that is usually silenced and only active when cells are ready to metastasize. In fact, the results of this study now lead us to believe that regions beyond the close proximal promotor can also be important in regulation and gene expression.

"Using the HumanMethylation450 BeadChip and NGS has helped us advance our research tremendously."

Q: Could genetic and epigenetic data enable stratification of patients enrolled in melanoma clinical trials?

ME: Patient stratification could be performed easily with simple PCR assays. We could then compare those results to the classical parameters used in melanoma, such as ulceration and the Breslow index. It could give us information, not only about diagnosis, but also about what a patient's response might be to certain drugs.

Q: How have arrays accelerated your research?

ME: Using the HumanMethylation450 BeadChip and NGS has helped us advance our research tremendously. We can perform studies today that were extremely difficult to conduct 5 or 6 years ago. We can now take a global view of what's happening at the level of DNA methylation in a cancer cell. We can compare that with all the data from other receptors to generate profiles that are useful for prognosis and to determine a patient's personal response to different therapies.

With the HumanMethylation450 BeadChip, we can also analyze old formalin-fixed paraffin-embedded (FFPE) material stored at hospitals. The BeadChip is also cost-effective, which is important because performing these kinds of studies is still quite expensive.

Q: How did NGS benefit your studies?

ME: All of our sequencing was performed at a core lab on a HiSeq System. The HiSeq delivered very good sequencing results that are comparable to those I obtain using pyrosequencing or PCR. That gives me the peace of mind that the results I find with the HiSeq System represent what is really happening in the cells.

Using the HiSeq System, we were able to study DNA methylation and easily compare it to copy number variation. I can see if a gene is sufficiently methylated. I can also see if that gene is deleted by just changing the platform in the machine. I can also see 5-hydroxymethylcytosine, a new methylation marker that is likely to be important in the future. It's a comprehensive system.

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Q: What are the next steps in your research?

ME: We want to study regions that are farther away from the gene that still affect gene expression. Sometimes, in three-dimensional regions of DNA, sites that seem far away are, in reality, quite close.

Q: Will you continue to use Illumina systems in the future? ME: We will continue to use the HumanMethylation450K BeadChip and hope to see development of an even bigger array with more CpG sites that we can use to analyze epigenomes cost-effectively. The HumanMethylation450 BeadChip has been an important tool, helping us to understand why cancer cells change, how they disseminate from a primary site, and how they can change to be resistant to a drug. When we better understand all this, we can do more to help cancer patients.

References

 Vizoso M, Ferreira HJ, Lopez-Serra P, et al. Epigenetic activation of a cryptic TBC1D16 transcript enhances melanoma progression by targeting EGFR. Nat Med. 2015: 21(7): 741–750.

Learn more about the Illumina products and systems mentioned in this article:

- Infinium HumanMethylation450 BeadChip, www.illumina.com/ products/methylation_450_beadchip_kits.html
- HiSeq System, www.illumina.com/systems/hiseq_2500_1500.html

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