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Synopsis

BEYOND CANCER GENOMES

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CANCER BEYOND THE GENOME

Nowadays there are very few doubts as to the fundamental role genes play in the onset and spread of cancer. Nevertheless, just reading the letters they are made up of[1] doesn't seem to be enough to fully understand it.

Epigenetics, the study of hereditary changes that are not dependent on the DNA sequence, is shaping up to be key in rounding out the landscape, because it explains how it is regulated and organized. This field has allowed scientists to observe the genome not as a linear book but as a **3-D pop-up**, with regulatory sequences, switches and long-distance relationships. Plus, it has helped explain the phenomena of **cancer stem cells**, which are key cells that seem to start the process and resist chemotherapy. In fact, the latest studies show that many cancerous mutations are found not in genes but in the regions that regulate them, what has been called the **dark genome**. And several promising treatments are being developed based on these discoveries.

A group of top experts from around the world came together on 13 and 14 October 2016 to discuss some of the latest, most important advances in this arena at the debate 'Beyond Cancer Genomes. Barcelona Conference on Epigenetics and Cancer', organized by B·Debate – an initiative of Biocat and the "la Caixa" Foundation to promote scientific debate – with the Institute for Research in Biomedicine (IRB Barcelona), and collaboration from the Molecular Biology Institute of Barcelona (CSIC), Institute of Predictive and Personalized Medicine of Cancer (IMPPC), Cancer Epigenetics and Biology Program (PEBC; IDIBELL) and Center for Genomic Regulation (CRG).

CONCLUSIONS

- Mass cancer sequencing projects are showing that many mutations are found not in the genes but in the regions that regulate them, known as the "**dark genome**".
- Epigenetics regulates DNA activity and part of its 3-D structure. Recent studies show that **the genome is structured into insulated, independent neighborhoods, with up to one million switches**.
- Cancer stem cells seem to be the origin of tumors and play a key role in resistance to treatment. **Similar cells may trigger metastasis and be fat dependent**, a clear link to diet.

[1] Genes are made up of DNA, which contains the genetic instructions used by living beings to develop and function. The main purpose of DNA molecules is to store information long-term. DNA is made up of what is known as nitrogenous bases, which are like the letters in a book and act as a sort of code containing the instructions required to produce other cell compounds, such as proteins.

- **Some of the most promising therapies target "Myc"**, a protein altered in cancer patients that controls up to 15% of the genome and until recently was considered untouchable.

THE GENOME: A 3-D POP-UP

The metaphor that compares DNA to a book implies imagining it is a linear story made up of letters. However, is much more complicated. In reality, it is more like a 3-D pop-up where some regions (pages) can end up interacting with others that seem very far away if we are only looking at the sequence. And in this spatial confirmation, it can be folded more or less compactly, showing or hiding its information (thus allowing or prohibiting synthesis of the proteins it codes).

For Richard Young, professor at the Whitehead Institute and MIT in Cambridge and one of the top experts in studying gene regulation, "**The structure of chromosomes is much more important than we used to think**." One of the things that have been seen is that there are **nearly one million switches**, **located throughout the genome**. These areas act as a key to lock or unlock regions of the DNA and allow or prohibit them from being read. However, "Chromosomes also tend to organize themselves into regions of approximately 1 million pairs of bases –letters– that have a similar structure in all cells." These regions form looping structures, separating themselves from neighboring regions so they can independently control the genes they contain. They are what have been called "insulated neighborhoods".

This is important not only in terms of how a healthy cell works, but also in tumor cells. In fact, as Young quoted, "Oncogenes[2] – genes with the potential to cause cancer– are often activated in insulated neighborhoods whose boundaries have been disrupted." Meaning those that have lost their looping structure, their independence.

CANCER STEM CELLS: ORIGINS, RESISTANCE, METASTASIS (AND DIET)

Cancer cells are far from homogenous, both in terms of genetics and epigenetics. **There seems to be a hierarchical structure established inside a tumor, a sort of tree whose roots store what are known as stem cells**. Although it is still somewhat controversial, quite a few studies grant them fundamental properties: based on epigenetic mechanisms, they're the only ones

[2] An oncogene is an abnormal or activated gene that comes from the mutation of an allele in a normal gene. Along with silencing tumor-suppressor genes, oncogenes can be responsible for turning a healthy cell into a malignant one that will develop a specific type of cancer.

able to start a tumor (if other tumor cells are transplanted into mice, the tumor doesn't grow), they divide very slowly and, above all, they're particularly resistant to chemotherapy. This is why cancer can reappear if even one of these cells remains, even though treatment originally seemed to work.

For John Edgar Dick, a professor at the University of Toronto and a pioneer in research in this field, “**If cancer stem cells are so significant, and tumors grow and evolve from them, then their characteristics are the most important**." But studying them is a challenge, as is developing treatments to beat them. "Stem cells have many properties that allow them to escape from therapies," explained Dick. "If they're at the core of tumor evolution, it's difficult to beat Darwin."

METASTASES

In the end, **most cancer deaths are due to metastasis**, the journey cancer cells take through the body. For this to happen, some of them must take on very specific properties: through fundamentally epigenetic mechanisms, they become more "liquid", detaching easily from the tumor mass. But they also have to be able to start a new tumor wherever they nest, so they have to have the characteristics of stem cells.

The group led by Salvador Aznar – ICREA researcher at the Institute for Research in Biomedicine Barcelona (IRB) and scientific leader of this B·Debate along with Eduard Batlle and Raúl Méndez– has tried to identify the cells in a tumor that trigger metastasis, and in the process has come across some remarkable surprises: **the cells responsible "look like adipocytes,"**[3] explained Aznar and, in fact, they have many upregulated genes associated with fat metabolism. Specifically, a receptor called CD36, which imports fatty acids to the cell, is abnormally active in the cells.

The data is both hopeful and alarming: CD36 seems to be necessary to trigger metastasis, and blocking it with antibodies dramatically decreases the process in mice. However, the finding, published in Nature, also leads to questions, like the ones Aznar himself wonders, **"So, is there a link between diet and metastasis? Could that explain the increase in mortality seen recently in some types of cancer?"** That could be the case. "We consume a lot more fatty acids than we think, even in normal, everyday products," said Aznar. It's too soon to extract conclusions but, from the wide range of those possible, one seems particularly worrying: palmitic acid, found in vegetable oils like coconut and palm oil.

Another surprising, cutting-edge study is that led by Eduard Batlle, ICREA researcher and head of the Oncology Program at IRB Barcelona. Metastasis is the most clinically relevant process in colon tumors and his group has observed that "the genes that predict a poor prognosis are expressed not in the tumor but in its surroundings," Batlle explained. It seems that cancer changes its surroundings to make it easier for it to spread and that "gives us a chance to treat it" by focusing on the area around the tumor.

One way could be to attack the TGF beta molecule, which seems to be an important regulator of the process. However, this approach is complex; in the initial stages it may, paradoxically, play a protective role. Batlle's team has attempted to identify which tumors could benefit from this treatment. In the laboratory, the results are promising, and surprisingly better when combined with new forms of immunotherapy.

[3] Adipocytes are the cells that make up adipose or fat tissue.

FROM STEM CELLS TO THERAPY

Although it is still quite controversial, it seems that in many cases tumor stem cells come from the stem cells found in the tissues themselves, those in charge of regeneration. So, explained Andreas Trumpp, professor at the German Cancer Research Center (DKFZ), **these cells "should be protected: as a mutation here becomes amplified a million times**." To do so, a huge proportion of the *most powerful* ones are in a quiescent state, lying dormant (by not dividing, the potential for mutation is minimized). But sometimes (when faced with an infection, blood loss or chemotherapy), they must be activated to replace lost cells. This is when Myc, an essential protein, comes into play.

Myc is what is known as a transcription factor: a protein that bonds with DNA and switches on the expression of one or more genes. In this case, it is so powerful that it modifies activity in up to 15% of our genes. For Trumpp, "**It is the gas pedal of cell activity**," and what stem cells use to come out of their dormant state and regenerate tissue.

And not only stem cells. **The vast majority of tumors show *Myc* activation**. It is a command center; the great target everyone was looking to block.

But that seemed impossible, as explained Laura Soucek, ICREA professor at the Vall d'Hebron Institute of Oncology (VHIO): "When I was a student everyone told me that *Myc* couldn't be treated and **shouldn't even be touched**."

It wasn't treatable because its characteristics and its location inside the cell nucleus made it nearly impossible to reach. And it shouldn't be touched because of its function was so important that the side effects could be devastating. However, Soucek's research uncovered one surprise after another and seems to have taken down all of those hypotheses.

To disprove the first, they used biotechnology. They "designed" transgenic mice adding a gene that synthesized a Myc inhibitor, which could be activated just by adding an antibiotic. In this way, they were able to block its action from the inside. The results? It was surprisingly effective in treating lung tumors and glioblastomas, a particularly aggressive type of brain tumor that currently has no effective therapy. The surprise? Although they still don't know exactly why, **the side effects weren't catastrophic at all**; they were limited and reversible, at least in mice.

The second hypothesis was overturned on its own, as if by a happy coincidence. For a treatment based on Myc inhibition to become a reality, there must be an effective drug, as transgenic patients aren't an option. However, Myc acts in the cell nucleus, which is very hard to reach. The surprise came when the product of the transgene -called Omomyc-, which is large and wasn't even considered a candidate, was shown to have special traits that induce tumor cells to capture and collect it. In fact, studies underway have shown that it can reach the lungs even when administered intranasally. This is what they're working on now, concluded Soucek, **"Going from genetics to pharmacology and making Omomyc a viable therapeutic option to fight cancer**".

FROM SEQUENCING THE WHOLE GENOME TO THE IMPORTANCE OF THE “EPIGENOME”

According to Carlos López Otín, professor at the University of Oviedo and one of the heads of the International Cancer Genome Consortium in Spain, “The possibility of sequencing whole genomes has allowed us to go from hypothesis-based studies to agnostic approaches." His team, which is sequencing the genome of chronic lymphoid leukemia, has made huge steps forward in recent years. They began analyzing the full DNA of 4 patients in 2011 and just four years later, they were able to do so with up to 150 (checking part of their results in nearly 500 more).

Their results have helped identify up to 60 new mutations involved in the development of leukemia, with a curious characteristic: **many of them are found in regions outside of the genes, in the supposedly regulatory areas of the "dark DNA"**. And some of these discoveries are already being used as the basis for experimental treatments. This is why it is so important, says Otín, to sequence full genomes and not just the regions where genes are located.

Not just that. In the transcendental fight against resistance to treatment –"the never-ending story of anti-tumor drugs," says Otín– information on the genome can also be very useful. Cancer's ability to evolve and adapt to therapies could sometimes be predicted from its initial characteristics. "This is another possible use for the cancer sequencing projects: anticipating resistances and mechanisms."

But how can we select and prioritize when it is so complex? Otín calls for three key criteria: **the recurrence of a mutation, how new it is and, surprisingly, "intuition".**