Eliminating cancer cell immortality

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One of the secrets behind the success of cancer cells is their ability to proliferate indefinitely by keeping long telomeres. In healthy cells, these sections of the genome located at the end of chromosomes shorten during each cellular division. When these telomeres become too short, the cell stops proliferating and enters into the state of senescence. Researchers at the University of Liège have discovered an enzyme that allows cancer cells to maintain the length of their telomeres. The inhibition of this enzyme affects telomeric structures and makes those cells much more sensitive to chemotherapeutic drugs.

In eukaryotic cell, the DNA is not a 'naked" molecule but it goes through several levels of condensation to form chromatin; the chromosome being the most compact form of DNA. In order to reach this highest level of condensation, the DNA wraps around the histone proteins just like thread around a bobbin. This structure offers the genome a certain amount of stability, particularly during cellular division, but DNA cannot be transcribed in this form. For genomic sequences to be transcribed into RNA and then translated into protein, the DNA must first become more "flexible", less condensed, in order to be read. This is where histone deacetylases (HDAC) come in. "The function of this family of 18 enzymes is to open and close the DNA molecule by modifying the chromatin's structure," explains Denis Mottet, FRS-FNRS Research Associate at the Metastasis Research Laboratory, GIGA-Cancer, ULg. As a cancer specialist, he studies histone deacetylases because they are overrepresented in cancer tissues. "We are trying to understand their potential role in different biological processes related to cancer growth, since these enzymes appear to be promising therapeutic targets."

Telomeres and cellular ageing

Denis Mottet and his colleagues began their research on HDAC by inhibiting their activities using pharmacological molecules that could inhibit all 18 members of the family. "But these enzymes are also present in healthy cells and are necessary for several physiological processes. To avoid creating side-effects and
to target certain HDAC more specifically, we worked to determine which members of this enzyme family played the most important role in cancer cells," the scientist continues. Thus for the past decade or so, Denis Mottet has been selectively inhibiting the expression of each of these HDAC members and observing the consequences of these manipulations on cell cultures. "We noticed that when we selectively inhibited histone deacetylase 5 (HDAC5), there was a reduction in proliferation and the cancer cells died," reveals Denis Mottet. "These results suggest that HDAC5 may be a promising therapeutic target, but we still don't fully understand the underlying mechanisms."

Since this family of enzymes is involved in chromatin folding, the scientists wanted to see if HDAC5 played a role in the structure of telomeres, which are located at the ends of chromosomes. "Telomeres are very important since they protect the chromosome from bad events such as the fusion with another chromosome. They are involved in a number of processes such as cellular ageing," says Denis Mottet. In fact, during each cellular division, telomeres shorten and when they become too short, the cell stops dividing and reaches the state of senescence. Cancer cells, on the other hand, keep telomeres constant, continue to proliferate and do not progress to senescence. "Cancer cells have mechanisms which allow them to maintain the structure and length of their telomeres," explains Denis Mottet. Which is why they thought HDAC5 might be involved in some way...

The first objective of the study was to localize the HDAC5 enzyme in cancer cells. "If it was localised in the telomeres, there was a strong chance that it played a role in the structure of these regions," says Denis...
Mottet. To localise the enzyme, the researchers used different types of cancer cell cultures. "We fixed the cells and injected them with antibodies against HDAC5 that we combined with a fluorescent molecule," explains the researcher. "In parallel, we used a probe that recognizes telomeres, also combined with a fluorescent molecule of a different colour." Thanks to these immunofluorescence and fluorescence in situ hybridisation (FISH) techniques, Denis Mottet and his team were able to observe both fluorescent colours in cancer cells with long telomeres. Which means that in these cells, HDAC5 is indeed localized at telomeres.

"The second part of the research was to inhibit this enzyme using an interference technique which depleted its expression in cancer cells and allowed us to observe the specific consequences on telomeres," continues Denis Mottet. To do so, the scientists used cancer cells with telomeres of different lengths. The result: "The inhibition of HDAC5 induced telomere shortening only in cancer cells which already had long telomeres," explains Denis Mottet. Cells with shorter telomeres do not seem to be affected by the absence of the enzyme.

**Massive death in cancer cells deprived of HDAC5**

"These results show that HDAC5 does indeed play a role in maintaining the structure of telomeres in cancer cells," says the researcher. If inhibiting this enzyme affects the telomeres of cancer cells, an obvious question comes to mind: "Are chemotherapeutic agents, which attack the DNA molecule, more effective on cancer cells with shortened telomeres?"

To test this hypothesis, the scientists used cancer cells with long telomeres that seemed to be resistant to agents used in chemotherapy. "Cancer cells that are deprived of HDAC5 are much more sensitive to chemotherapeutic agents and die much more," indicates Denis Mottet. "These results mean that we can propose promising new combined strategies for the cancer-fighting arsenal." This study was published in The FASEB Journal, an American scientific journal, and was selected by its editorial board as the discovery of the month in September 2013 because of its innovative and promising research.
As Denis Mottet and his colleagues have shown, the control mechanisms of telomeres in cancer cells represent an important new cancer treatment possibility, since reducing the length of telomeres should take eliminate cancer cell immortality. And even beyond applications in cancer research, given that telomeres are directly related to cellular ageing, understanding the relationship between HDAC and telomeres could have an important impact in a number of clinical fields such as age-related illnesses.