Study identifies new target for treatment of melanoma

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A Brazilian study shows that inhibition of an RNA named RMEL3, which is encoded by a previously uncharacterized gene (also named RMEL3), can reduce the viability of cultured melanoma cells by up to 95%.

Although RMEL3 is a non-coding RNA and hence does not contain information for protein synthesis, it appears to modulate the main signaling pathways related to cell proliferation and survival. How it does so is not fully understood.

"Our research suggests RMEL3 is expressed in most cases of melanoma. On the other hand, this RNA is rarely found in other kinds of tumor or even in healthy cells, so it's a highly specific therapeutic and diagnostic target of considerable promise for development," said Enilza Espreafico, a professor at the University of São Paulo's Ribeirão Preto Medical School (FMRP-USP) and principal investigator for the FAPESP-funded study.

These findings are derived from PhD research by two recipients of scholarships from FAPESP, Lucas Goedert and Cristiano Gonçalves Pereira, in collaboration with Cibele Cardoso and other researchers in Brazil and abroad. The existence of RMEL3 and its association with melanoma, however, had already been identified in previous research by Espreafico's group.

"At that time, our aim was to identify genes expressed only in cases of melanoma. We used bioinformatics tools to explore databases created from tumor sequencing projects," Espreafico said.

The first analyses revealed the existence of 29 RNA sequences transcribed only in melanoma cells. Three of these appeared most significant to the Ribeirão Preto group: RMEL1, RMEL2, and RMEL3.

The study showed that these three non-protein-coding RNAs are present both in melanoma cell lines and in tumor samples from patients. RMEL3 even appears in skin lesions considered premalignant.

Next, the researchers conducted in vitro experiments to investigate how the presence or absence of these three RNAs changed the cellular phenotype. The first findings showed that inhibition of RMEL3 provided the most intense reduction in the viability of cultured melanoma cells, and this RNA therefore became their preferred target.

To silence RMEL3 in cultured cells, the group deployed RNA interference, a technique that consists of using small molecules of non-coding RNA capable of binding to RNA transcribed from the target gene (in this case, RMEL3) and inducing its degradation.The effects of this procedure were compared in five different cell lines. The first three were melanoma cell lines with a mutation known to be associated with cancer in a gene called BRAF. The fourth line, also a melanoma cell line, lacked the BRAF mutation. The fifth, considered a kind of control group, was an ovarian cancer line that did not express RMEL3 and that also lacked the BRAF mutation.

"BRAF is the main proto-oncogene associated with the development of melanoma. Roughly 60% of cases of this type of cancer involve a mutation of BRAF, which codes for a kinase protein that initiates the MAPK signaling pathway, an important trigger of cell proliferation," Espreafico said.

"The mutation changes a single letter of the genetic code in BRAF, and this results in the substitution of an amino acid in the polypeptide chain, which is sufficient to create the proto-oncogene protein BRAF V600E. In its mutant form, this enzyme is intrinsically active, so the cell enters the replication cycle even without receiving any external signal for proliferation."It was in cultured melanoma cells with the BRAF V600E mutation that inhibition of RMEL3 had the most dramatic effect, reducing cell survival

and proliferation by up to 95%. In the melanoma cell line that lacked the mutation, cell viability decreased by about 40%. In the control cell line, RNA interference had no effect, and the cells continued proliferating normally.

The researchers still cannot say exactly what role this RNA plays in the cell or why it is frequently present in melanoma cells. However, they have found evidence of what happens in the cell when the expression of this RNA is interrupted.

"When we silence RMEL3, there is a decrease in levels of the BRAF oncogene protein and the kinase Akt (pAkt), a key protein in the PI3K cell survival signaling pathway. The opposite effect is observed for the protein PTEN, the main inhibitor of that pathway," Espreafico said.

They have also observed an increase in levels of ACC-pS79, a substrate of the enzyme AMPK, which is a nutrient deprivation sensor.

"This suggests the absence of RMEL3 induces a state that mimics nutrient deprivation, similar to that reported for pharmacological inhibition of BRAF V600E. Consistent with such changes, cell-cycle effectors are altered. There's a decrease in levels of the protein cyclin B1, important in activating cell mitosis. On the other hand, there's an increase in levels of proteins that inhibit the cell cycle, such as p27 and p21. These and other alterations are consistent with the effect we've observed in cells: an increase in cell death and interruption of the cell cycle," Espreafico said.

To understand more about the role of RMEL3 in cells, the researchers are currently performing new in vitro experiments in which its expression is artificially induced in both melanoma cells and healthy cells, where it is not normally expressed. The results are due for publication soon.

The group also set out to find out how frequently RMEL3 is expressed in melanoma cases by analyzing almost 500 samples from patients with this type of cancer from The Cancer Genome Atlas (TCGA), a consortium linked to the US National Cancer Institute that collects genomic, epigenomic and clinical data from patients in several countries.

"We observed that RMEL3 was expressed to a greater or lesser extent in over 90% of the melanoma samples available. Together with the fact that it isn't present in healthy tissue in the rest of the organism, this makes it a very interesting therapeutic target," Espreafico said.

RNA interference itself could have therapeutic uses, she added, noting that several technical barriers would have to be surmounted first.

More information: Lucas Goedert et al. RMEL3, a novel BRAF^{V600E}-associated long noncoding RNA, is required for MAPK and PI3K signaling in melanoma, *Oncotarget* (2016). DOI: 10.18632/oncotarget.9164

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