Researchers decode cancer's genetic control panel one DNA letter at a time

(Vienna, 03.03.2026) – **Scientists in Davide Seruggia's group at St. Anna Children's Cancer Research Institute (St. Anna CCRI), together with collaborators at the Broad Institute of MIT and Harvard, have developed CRISPR-Millipede, a new CRISPR-based method that allows researchers to study regulatory DNA sequences at single-nucleotide resolution. Using this approach, the team uncovered how single mutations in regulatory sequences can help cancer cells evade powerful immune therapies. The study was published in *Nature Communications*.**

Cancer initiation, progression and treatment resistance are driven by genetic mutations. Many cancer-linked mutations affect a gene's coding sequence — the instructions used to build a protein — resulting in proteins with altered shape or function. However, producing a protein requires more than just the coding sequence. Surrounding regulatory DNA regions act as a control panel, providing binding sites for transcription factors that determine when, where and how strongly a gene is expressed.

While the effects of coding mutations can be predicted, understanding how small changes in regulatory sequences influence gene expression has remained difficult. Existing methods can identify short stretches of DNA involved in disease, but lack the resolution to assess the role of individual nucleotides. This has limited our ability to fully understand how mutations in regulatory regions contribute to cancer and therapy resistance.

CRISPR-Millipede: dissecting regulatory regions one nucleotide at a time

To overcome these limitations, the researchers developed CRISPR-Millipede, a new CRISPR-based screening approach that combines dense base editing and flow cytometry with a new computational analysis pipeline. With this approach, scientists now can introduce a dense array of precise changes to individual nucleotides within regulatory regions and test how each mutation affects gene expression at single-nucleotide resolution. The team achieved higher resolution and accuracy than previous similar approaches.

"*Our approach allows us to analyze large cell populations simultaneously and extract meaningful biological insights*," explains Luca Pinello, computational biologist at Harvard and one of the senior authors of this study. "*Importantly, this lets us study regulatory elements at a fraction of the cost of advanced technologies like single-cell RNA sequencing*."

CRISPR-Millipede's high sensitivity also enables scientists to evaluate the relevance of even very rare mutations and discriminate the effects of multiple mutations occurring simultaneously within the same regulatory region.

A potential mechanism for cancer therapy resistance

To demonstrate the power of their method, the researchers focused on a previously unknown regulatory region controlling the expression of CD19, a protein found on the surface of certain blood cells known as B cells. CD19 is a key target of CAR-T cell therapy used to treat B-cell leukemia, where engineered immune cells attack cancer cells carrying this protein.

Using CRISPR-Millipede, the researchers precisely mapped the DNA "landing pads" where transcription factors (proteins that switch genes on and off) attach and regulate CD19 expression. The team found that specific mutations in these regulatory elements can reduce CD19 expression, allowing cancer cells to escape CD19-targeted CAR-T cells.

These findings reveal a previously unrecognized mechanism by which cancer cells could develop resistance to immunotherapy. "*We are already investigating critical regulatory elements controlling other genes involved in immunotherapy, such as PD-L1"* says Sandra Wittibschlager, co-first author of this study.

Expanding the scope of cancer genetics

The results suggest that non-coding, regulatory genomic regions could play a far more active role in cancer biology than previously appreciated. By enabling precise and scalable analysis of these regions, tools like CRISPR-Millipede open new possibilities for understanding how intergenic DNA variants and mutations contribute to disease, potentially informing better advanced therapies.

"*We hope CRISPR-Millepede and the other tools our team develops will be useful to the broader scientific community*," says Seruggia. "*We are only beginning to understand how regulatory DNA shapes disease, and this approach has the potential to reveal the mechanisms behind inherited DNA variants that predispose to childhood leukemia."*

Publication

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biology, cell biology, bioinformatics, and clinical research work together to align the latest scientific and experimental findings with the clinical needs of physicians and sustainably improve the well-being of young patients. www.ccri.at www.kinderkrebsforschung.at

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