Taking a 3D view of the genome may help treat pediatric brain cancers

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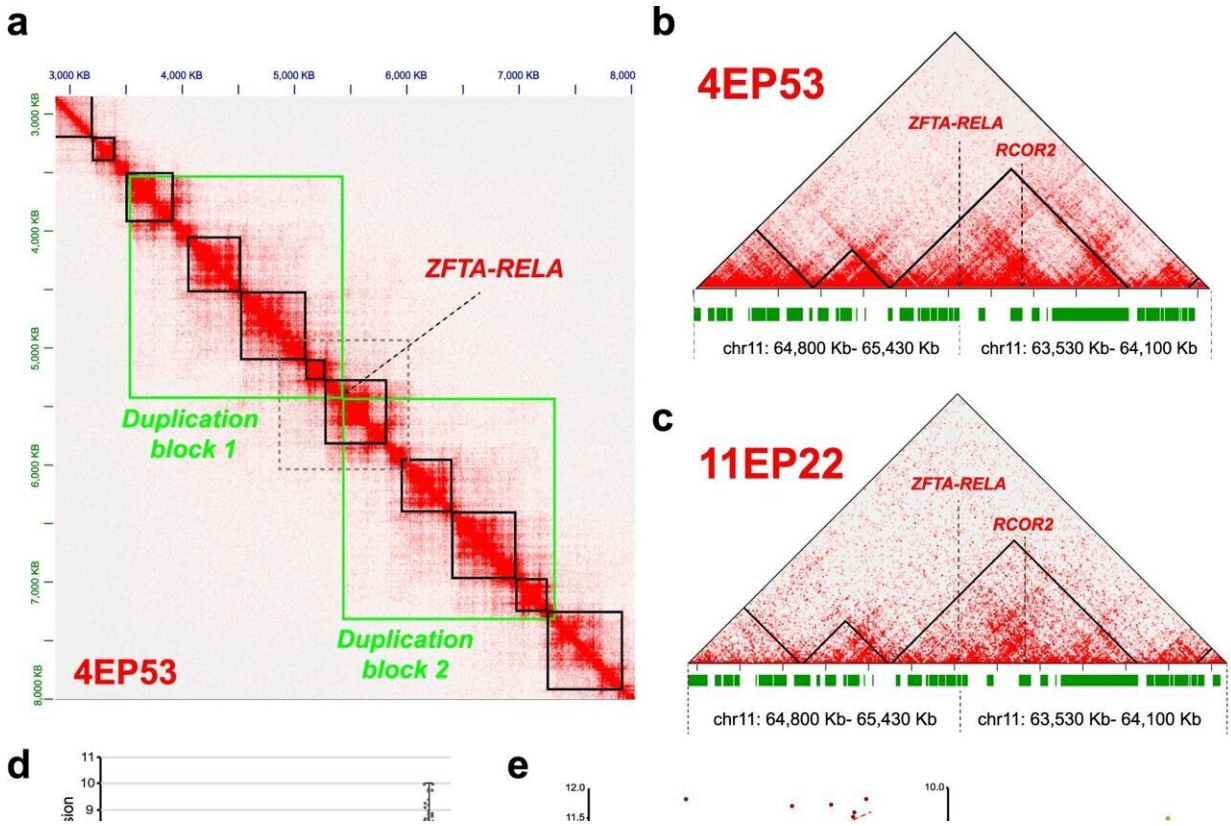

Transcriptional activation of RCOR2 by neo-TADs in RELA ependymoma. a Chromatin contacts in a reconstructed ZFTA tumor genome (sample 4EP53) including the tandem duplication that leads to the ZFTA-RELA fusion (chr1:63532174-65429788, green boxes). Solid black boxes show TADs identified by applying TopDom to the Hi-C data mapped on to the reconstructed tumor genome, including a neo-TAD that spans the DNA breakpoint. b, c Reconstructed genomic locus containing the ZFTA-RELA fusion gene in the ZFTA ependymoma sample 4EP53 (b) and 11EP22 (c). The black boxes/

triangles indicate TADs reported by TopDom when applied to the reconstructed tumor genome. A neo-TAD is identified that spans the DNA breakpoint and places RCOR2 into a new regulatory environment. d Boxplot of RCOR2 gene expression across ependymoma groups using Affymetrix gene expression data (n = 393). RCOR2 is significantly upregulated in ZFTA tumors (ZFTA vs all other tumor classes limma p-val.: 7.62e−27). The center line, box limits, whiskers, and points indicate the median, upper/lower quartiles, 1.5× interquartile range and outliers, respectively. e Correlation between RCOR2 and ZFTA in ZFTA (left side, n = 76, cor = 0.663, p-val = 6.93e−11) and PFA ependymoma samples (right side, n = 200, cor=0.336 p-val = 1.13e−06). f–i shRNA time-course knock-down experiments in ZFTA (EP1NS) and PFA (EPD210FH) ependymoma cell lines using a scrambled control and two shRNA constructs each targeting either RCOR2 in EP1NS (f), RCOR2 in EPD210FH (g), LSD1 in EP1NS (h) and LSD1 in EPD210FH (i). All constructs are GFP tagged and GFP positive cells are sorted by FACS. For panel (f), error bars represent mean ± SD for n = 3 independent experiments (two-tailed paired t test p-val = 0.0018 and 0,0046; shRCOR#1 and shRCOR2#2, respectively). For panels (g–i), normalized data represent mean from n = 2 independent experiments per cell line. j, k Dose–response curves of single-compound treatment with ORY-1001 (j) or Entinostat (k) of ZFTA (EP1NS, BT165 and ST-1) and PFA (EPD210FH, BT214) ependymoma spheroids over a 72-h time-course using Celltiter-Glo cell viability assays. For each sample the results are presented as percentage of the Luminescence signal from control condition (i.e. water for ORY-1001 and DMSO for Entinostat). Error bars represent mean ± SD for n = 3 independent experiments (one-way ANOVA test p-val Nature Communications (2023). DOI: 10.1038/s41467-023-38044-0

Researchers led by Sanford Burnham Prebys assistant professor Lukas Chavez, Ph.D., are leveraging the latest technology to take a never-before-seen look at ependymoma, one of the deadliest pediatric brain tumors. By visualizing how the genome is organized and arranged within tumor cells, they were able to reveal genes in tumors that may be future targets for therapy. The results appear in *Nature Communications*.

"The human genome is made up of many protein-coding genes and an even greater number of noncoding sections, which are all tightly packaged and coiled up to fit inside the tiny nucleus of a cell," says Chavez. "We're using cutting-edge technologies to look at the way that genome is packaged and coiled, which gives us a unique perspective on the mechanisms of gene regulation. This approach helps us understand the link between the shape of the genome and cancer."

Tumors of the brain and spinal cord, including ependymomas, are the most common malignant cancers in children up to age 14 and the leading cause of death by disease during childhood in the United States.

"Ependymomas come in many different genetic and molecular subtypes that can affect how well patients respond to treatment," says Chavez. "The current standard-of-care treatment includes surgery followed by radiation, which bears the risk of long-term, therapy-induced neurological side effects as well as secondary cancers. New, targeted therapeutic options for ependymoma are desperately needed. If successful, our research will lead to new, effective medications to treat these dangerous cancers."

The researchers focused on the most common and aggressive forms of the disease, which occur mainly in young children. They used an emerging technique called 3D genome mapping, which visualizes how genes are organized within the nucleus of cells.

"Science has historically studied the genome in two dimensions, focusing on how genes are arranged in long, linear sequences," says Chavez. "But the genome is a 3D object like anything else, and the way that genes are arranged in space makes a difference in how those genes are expressed in the body."

The researchers studied previously undiscovered loops in the genomes of

ependymoma tumors. These loops change the way that genes are expressed, which in turn produces signals that help tumors grow.

"We've confirmed that configuration of these genes in the loops are essential for ependymoma tumors," says Chavez. "This means that we now have a host of new candidate targets for treatments that we would never have been able to identify without this technology."

The researchers believe that their results will lay the foundation for further studies that will lead to future therapies. The researchers are also planning to look at other pediatric cancers, since many of these diseases have few therapeutic options.

"There are alarming numbers of tumors that we struggle to treat because we simply don't know how they work from a biological standpoint," says Chavez. "This work shows that there's a lot more we still don't know about the genomics of tumors, and unlocking these mysteries may be the key to finally overcoming these aggressive cancers."

More information: Konstantin Okonechnikov et al, 3D genome mapping identifies subgroup-specific chromosome conformations and tumor-dependency genes in ependymoma, *Nature Communications* (2023). DOI: 10.1038/s41467-023-38044-0

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