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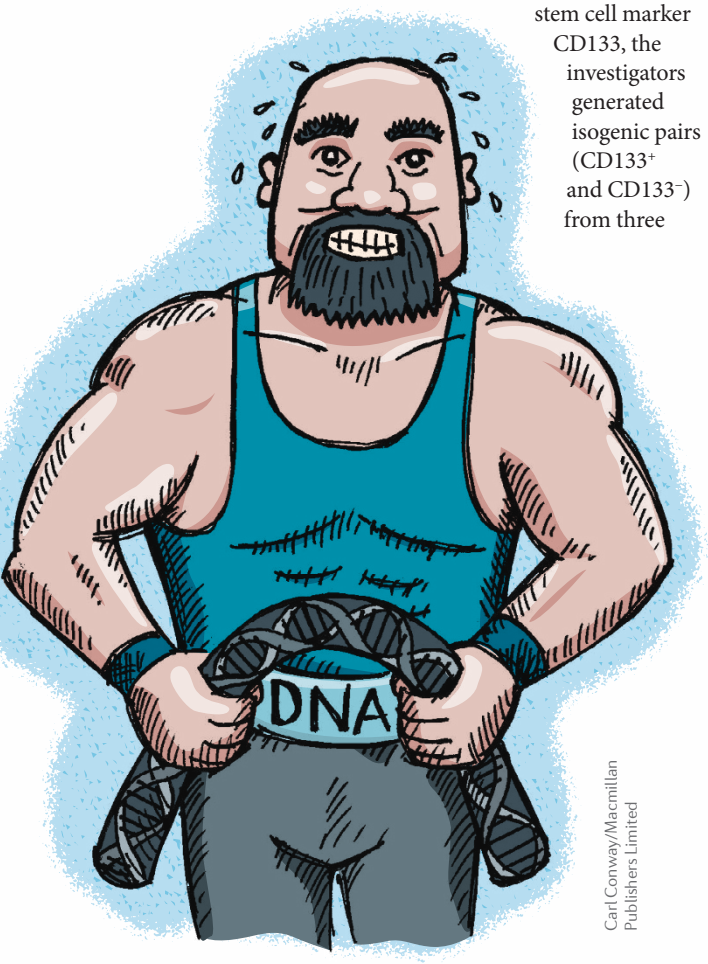
NEUROBLASTOMA

Tumours get super-enhanced

> most neuroblastomas comprise two genetically identical cell types

Super-enhancer-associated networks of transcription factors (TFs) are thought to determine lineage identity; however, their role in intratumoural heterogeneity is unclear. Now, van Groningen *et al.* demonstrate that most neuroblastomas comprise two genetically identical cell types — lineage-committed adrenergic (ADRN) and undifferentiated mesenchymal (MES) cells — with highly divergent phenotypes and gene expression profiles that are defined by unique super-enhancer transcriptional networks.

Using fluorescence-activated cell sorting (FACS) for stem cell marker CD133, the investigators generated isogenic pairs ($CD133^+$ and $CD133^-$) from three patient-derived neuroblastoma cell lines. DNA sequencing confirmed that these isogenic cell types were genetically identical, but gene expression and protein blot analysis revealed highly divergent mRNA and protein expression patterns. $CD133^-$ cells expressed markers of classic ADRN lineage differentiation. By contrast, $CD133^+$ cells expressed high levels of MES markers. Using the expression profiles of isogenic cells to determine MES and ADRN mRNA signatures, unsupervised clustering analysis of 33 neuroblastoma cell lines identified 8 as MES-type and 25 as ADRN-type. In addition, a series of experiments demonstrated that $CD133^+$ and $CD133^-$ cell populations could give rise to heterogeneous populations *in vitro* and *in vivo*, illustrating that ADRN and MES cells can transdifferentiate.



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Next, super-enhancer structures were analysed using chromatin immunoprecipitation–sequencing (ChIP–seq) and bioinformatic analyses. Five ADRN and four MES cell lines clustered into two distinct groups with similar super-enhancer patterns; 276 and 286 super-enhancers were significantly enriched in ADRN and MES cells, respectively, and the super-enhancer patterns were significantly associated with their respective mRNA signatures. By linking differential gene expression and super-enhancer enrichment data, super-enhancer-linked TFs were identified in ADRN and MES cells; this identified 18 MES and 20 ADRN super-enhancer-associated TF-encoding genes that were thought to determine the lineage identity of each cell type. Inducible expression of paired mesoderm homeobox protein 1 (PRRX1) — a MES super-enhancer-associated TF — reprogrammed three ADRN cell lines to a MES state with respect to gene expression and super-enhancer patterns *in vitro*, demonstrating that such TFs might impose lineage identity.

As most patients with high-risk neuroblastoma receive chemotherapy following resection, the effect of treatment on lineage was assessed. MES cells were more resistant to cisplatin, doxorubicin and etoposide than ADRN cells *in vitro*. Importantly, immunohistochemical analysis of tumour biopsies revealed an increased proportion of $PRRX1^+$ cells following chemotherapy in two patients, and in tumours from two patients post-relapse compared with the matched primary tumours, suggesting that MES cells are enriched during chemotherapy and following relapse.

Overall, the results suggest that intratumoural heterogeneity in neuroblastoma is not merely a result of mutagenesis and random drift, but consists of two cell types with consistent epigenetic characteristics and gene expression programmes. "Identification of the core set of TFs that control the identity of these two cell types will allow for further dissection of the regulatory principles of neuroblastoma", explains lead investigator Rogier Versteeg. Moreover, this work might have implications for targeted therapy. "As ADRN-type cells are sensitive to chemotherapy, combined treatment of neuroblastoma patients with chemotherapy and MES-specific drugs might abate lethal relapse", concludes co-lead investigator Johan van Nes.

Conor A. Bradley

ORIGINAL ARTICLE Van Groningen, T. *et al.* Neuroblastoma is composed of two super-enhancer-associated differentiation states. *Nat. Genet.* http://dx.doi.org/10.1038/ng.3899 (2017)