METASTASIS

Methylated clusters

> “CTC clusters from patients and mouse models with breast cancer have a distinct DNA methylation profile”

Circulating tumour cells (CTCs) found in the blood of patients with cancer give rise to metastases. They can exist as single cells or clusters, with the latter associated with higher metastatic potential. Yet, what bestows CTC clusters with this increased capacity to seed metastases is less clear. To this end, Gkountela et al. have identified that CTC clusters from patients and mouse models with breast cancer have a distinct DNA methylation profile from that of single CTCs, which together with the phenotypic difference represent a targetable therapeutic vulnerability.

To explore the biological characteristics of CTC clusters, the authors employed a microfluidic device to isolate both single and clustered CTCs based upon size exclusion from the blood of patients with progressive breast cancer. Positive enrichment for CTCs was confirmed by staining for cell surface protein expression (such as EpCAM). At the same time, spontaneously generated GFP-expressing single CTCs and CTC clusters were captured from three mouse xenograft models orthotopically injected with human breast CTC-derived cell lines or a human metastatic breast cancer cell line. Collectively, this totalled 89 single CTCs and 71 CTC clusters from patients and xenografts for genome-wide single-cell-level DNA methylation analysis.

Across the genome, overall methylation levels were similar between single CTCs and CTC clusters. However, investigation of differentially methylated regions in patient-derived CTCs revealed 1,305 hypomethylated regions in CTC clusters and 2,042 in single CTCs. Of these hypomethylated regions, many were identified as transcription factor binding sites (TFBSs), an observation which could be used to define single CTCs versus CTC clusters from both patients and xenografts. The predominant hypomethylated binding sites in CTC clusters were for stemness-related transcription factors, such as OCT4, NANOG, SOX2 and SIN3A, which regulate proliferation and pluripotency in embryonic stem cells. In contrast, single CTCs were distinguishable by other hypomethylated TFBSs, such as those for MEF2C, JUN, MIXL1 and SHOX2. Furthermore, in a cohort of 789 patients with breast cancer, the CTC cluster-related hypomethylated regions also showed low methylation levels in primary tumours and correlated with poor prognosis.

In line with the DNA methylation findings, single-cell-level RNA sequencing uncovered patient-derived CTC clusters enriched for genes related to cell–cell junctions, proliferation and stemness. Dissimilarly, single CTCs were associated with expression of genes involved in metabolic processes.

With the hypothesis that dissociating clustered CTCs would reverse the epigenetic and transcriptional changes associated with clustering, the authors screened 2,486 US Food and Drug Administration-approved compounds for their ability to reduce the mean cluster size of human breast CTC-derived cells, without compromising cell viability. At the lowest concentrations tested, six compounds were identified as being the most effective at disrupting CTC clusters, specifically the Na^+–K^+ ATPase inhibitors digitoxin and ouabain and the tubulin-binding agents rigosertib, podofilox, colchicine and vincristine sulphate. Notably, treatment with both digitoxin and ouabain resulted in gain of methylation at binding sites for the stemness-related transcription factors and corresponding reductions in the expression of their target genes. Extended analysis suggested these effects were the result of increased intracellular Ca^{2+} and subsequent failure to establish proper cell–cell junctions.

To determine whether the approach of targeting CTC clusters was effective at suppressing metastases formation in vivo, immunocompromised mice were orthotopically injected with a human breast CTC-derived cell line. Primary tumours were allowed to form over a 14-week period before mice were intraperitoneally injected with ouabain daily for 3 weeks. Treatment with ouabain was sufficient to decrease the number of CTC clusters while increasing the number of single CTCs and, importantly, limiting metastatic burden by 80.7-fold.

Overall, this study advocates that CTC cluster formation during metastasis should be prevented; as these cluster-targeting compounds have been used in the treatment of patients with cardiac disorders, they could potentially have immediate clinical utility for patients with breast cancer.

Anna Dart

ORIGINAL ARTICLE Gkountela, S. et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* **176**, 98–112 (2018)