# Needle in a haystack: genome profiling disseminated tumor cells

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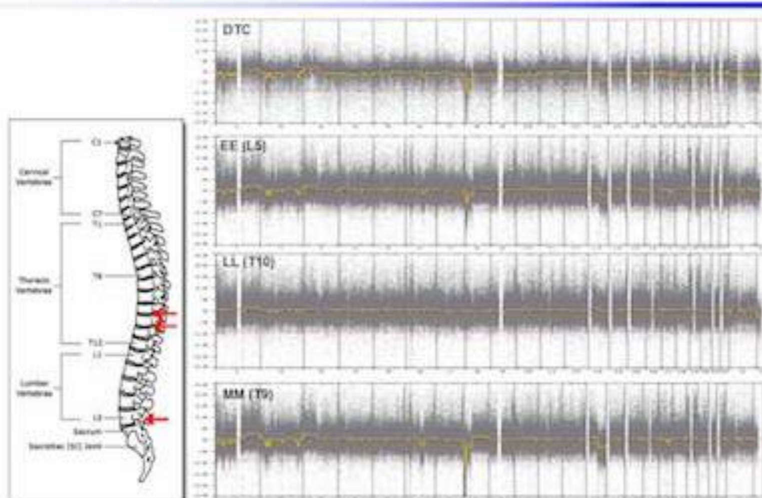


SCNA profile of tumor cells isolated from bone marrow (DTCs), and three metstatic locations along the spine (L5, T10, and T9) in a single patient. The genetic arrays between these samples is very similar.

*Image provided by Dr. Min Fang.*

Successful treatment in nearly every type of cancer is hampered by heterogeneity and evolution within the tumor population. Rare subpopulations are often the cause of cancer recurrence and metastatic disease. In patients with solid tumor types these populations can be isolated from bone marrow and blood, however these bloodborne tumor cells are rare. Cancer cells from a solid tumor circulating in blood have short half-lives of approximately two hours. In some cases these circulating tumor cells, called disseminated tumor cells (DTCs), effectively migrate into bone marrow where they live in a dormant state for years while retaining metastatic potential. These cell populations show promise as a diagnostic tool for treating patients, however their rarity makes consistent genetic analysis challenging. Researchers at Fred Hutch and the SCCA have developed a reliable and consistent method for genomic profiling of these rare populations in samples from prostate cancer patients. These efforts by the Fang and Nelson labs (Clinical Research Division) were recently published in the *Journal of Molecular Diagnostics*.

To work with these limited sample sizes scientists must first amplify the entire genome in a consistent and faithful manner. To demonstrate faithful amplification with limited cell numbers researchers started with a common prostate adenocarcinoma cell line, LNCaP. The entire genome was amplified from 1 or 5 LNCaP cells. The researchers then analyzed genomic alterations in these amplified samples and compared the results to a bulk sample containing roughly two million cells

that had not undergone any PCR amplification. For this comparison both somatic copy number alterations (SCNAs) and single nucleotide polymorphisms (SNPs) were analyzed. SCNAs represent large genetic alterations where at least a single gene or possibly a whole section of a chromosome is deleted or duplicated. SNPs are changes to a single base-pair; usually these changes have no effect but occasionally they alter gene function. In this comparison, SNPs demonstrated a high correlation among all samples. SCNA analysis was also consistent among samples; however, deletions smaller than 1 Mb were difficult to accurately detect in the amplified samples. Interestingly, these same regions were also challenging to identify from samples containing approximately 1000 cells.

Another challenge in detecting rare populations is the sensitivity of an assay in a mixed cell population. To address the limit of detection in this system, cells with a known deletion were mixed at defined ratios with cells containing an intact version of this chromosome. These experiments were performed with two different sets of cells with unique deletions. Using this technique a deletion on a single chromosome was detectible if it was deleted in 40-50% of cells.

Having established this approach in a well characterized prostate cancer cell line, researchers performed this analysis on DTCs isolated from patients with advanced prostate cancer. Many of the SCNAs detected in these samples overlapped between patients and are common to prostate cancer, genes affected included: MYC, BRAC2, RB1, TP53. Importantly, some of the genes affected are not common to prostate cancer, such as: FGFR3, GATA1, and KIF2A. As these samples could not be compared to a bulk' population the results were instead verified using quantitative real-time PCR and the Nanostring nCounter Assay, the latter of which is amplification independent. Excitingly, two of the DTC samples were isolated from patients where metastatic tissue was also available. This allowed researchers to compare the genomic alteration in DTCs to those present in metastatic tumors. This was of particular interest as two models currently exist for the dissemination of prostate tumor cells. One hypothesis is that metastatic tumors arise from cells disseminated late in the growth and progression of the prostate tumor, the assumption underlying this is that tumors cannot initiate in other tissue until accumulating many genetic abnormalities. An alternative hypothesis is that tumor cells disseminate and explant in other tissues at an early disease stage, and progresses independent of the primary tumor. When the SCNA profiles of metastatic tumors and DTCs are compared, their profiles are largely similar, a result that supports early dissemination, whereby the DTCs develop into metastatic disease over time.

This work elucidated a reliable method for faithful amplification of entire genomes from extremely rare cell populations. These studies further identified the mechanism by which metastatic prostate

cancer develops, moreover, this technology is a powerful tool others will use to develop biomarkers that may alter how cancers are diagnosed and treated.

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Wu Y, Schoenborn JR, Morrissey C, Xia J, Larson S, Brown LG, Qu X, Lange PH, Nelson PS, Vessella RL, Fang M. 2016. High-resolution genomic profiling of disseminated tumor cells in prostate cancer. *J. Molec. Diagn.* 18(1): 131-43. doi: 10.1016/j.jmoldx.2015.08.004.