CELL BIOLOGY

Investigating heterogeneity in HeLa cells

Multi-omic study uncovers biological variation across 14 HeLa cell samples, which might help to explain the growing concerns about reproducibility issues in cell culture.

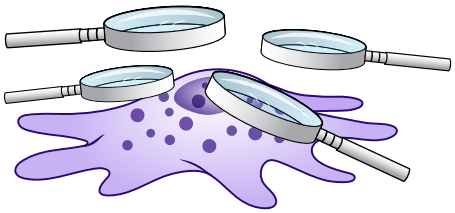
Human cancer cell lines have been widely used as in vitro models for cancer biology and drug discoveries. Ideally, these cells should be genetically stable and maintain concordant proteomic and cellular profiles, so that research outcomes can be readily reproduced between laboratories. However, in practice, results involving cancer cell lines are poorly reproducible owing to technical and biological variations that often have complex and unknown effects.

Genetic changes during culturing and passaging can affect gene expression and eventually cellular functions. In 2018, Ben-David et al. investigated 27 strains of the breast cancer cell line MCF7, and reported genetic heterogeneity and widely varied drug responses to 321 anti-cancer compounds (Ben-David et al., 2018).

Concerns about reproducibility have also been raised for HeLa cells, another popular cell line that has been referenced in over 100,000 publications, because of genome instability. Yansheng Liu, now an assistant professor at Yale University; his postdoc advisor Ruedi Aebersold from the Institute of Molecular Systems Biology, ETH Zurich; and their collaborators conducted a systematic investigation of the biological variations across HeLa cells from 14 stock samples used in 13 international laboratories, as well as their consequences for cellular phenotypes (Liu et al., 2019).

"We are proteomic scientists and therefore have a long-term research interest in reproducibility and variability analysis," says Liu. In 2014, they had the idea of using mass spectrometry to profile the proteomic heterogeneity in cell lines with the "same name." To this end, they used 'sequential-window acquisition of all theoretical fragments' mass spectrometry (SWATH-MS), which can efficiently and reproducibly detect thousands of proteins in cell samples.

The researchers studied three HeLa variants: HeLa CCL2 (the original variant), HeLa S3 (also called CCL2.2), and HeLa Kyoto. Although the whole genomes of HeLa CCL2 and Kyoto have been sequenced, researchers barely know the protein expression heterogeneity, the genomic and proteomic changes caused by successive passaging, and how such changes influence common cell assays.



Multi-omic study helps to explain the heterogeneity in HeLa cells. Credit: Marina Corral Spence/Springer Nature

To alleviate the technical variations, Liu and his colleagues decided to culture the cells under the same conditions for a few passages, instead of using aliquots directly sent from each lab. Making this decision was "one of the practical challenges" Liu mentions.

To reveal genome alterations, they first used array comparative genomic hybridization to quantify gene copy-number variations (CNVs). Strikingly, they observed substantial ploidy changes between the HeLa CCL2 and Kyoto lines, and even among HeLa variants within the same annotation. When taking the passage (P) number into account, they found that P50 HeLa cells can gain or delete entire chromosome copies, in contrast to P7 HeLa cells.

They then studied how CNVs affect gene expression and protein abundance by using their previously established SWATH-MS method. The multilayered dataset facilitates the correlation between CNV and mRNA or protein levels. At a given locus, the transcriptomic and proteomic differences in HeLa variants largely follow the CNV imbalance. Collectively, the gene expression differences between HeLa CCL2 and Kyoto groups are as distinct as between cell lines originating from different tissues. "I think the systematic difference between HeLa Kyoto and CCL2 was quite striking to me. These two HeLa variants are so different in many molecular layers. And if you look at PubMed, many researchers just simply report that they used HeLa cells," Liu notes.

Therefore, the baselines for researchers are that one should clearly report the identity of the HeLa variant in publications, and one should use early passages of HeLa cell lines, or at least similar passages of cells for an entire study, as altered genomic, transcriptomic, and proteomic profiles are apparent between P7 and P50.

Beyond the changes at the genomic and proteomic levels, this study also confirms a tight link between omics heterogeneities and phenotypic differences. For example, the cell-doubling time for different HeLa strains can vary between 17.5 hours and 32.3 hours under the same culture conditions.

The presented multilayered omics information uncovers the reasons underlying observed phenotypic changes, which might help to explain the reproducibility issues that arise in cell culture experiments.

Liu and Aebersold, together with EMBO, are organizing a workshop on the topic of cancer cell lines and reproducibility in 2019.

Lei Tang

Published online: 28 March 2019
https://doi.org/10.1038/s41592-019-0375-1

Research papers
Ben-David, U. et al. Genetic and transcriptional evolution alters cancer cell line drug response. *Nature* **560**, 325–330 (2018).
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