https://doi.org/10.1038/s41592-023-02016-5

Setting standards for stem cells

New traceability and reporting standards aim to improve transparency in stem cell research.

Pluripotent stem (PS) cells undergo self-renewal and can be differentiated into the three germ layers, thus forming the building blocks of a living system. As such, stem cell research can advance our fundamental understanding of biology and human development. By harnessing the underlying mechanisms that control differentiation, researchers can explore how cells differentiate, self-renew and interact within complex systems.

However, complete understanding of the biology that governs PS cell behavior is lacking. One of the challenges is variability in experimental conditions and methods, which can affect the reproducibility of results. Volpato et al.[1] independently differentiated two induced PS (iPS) cell lines across five laboratories using an established method and reported poor cross-site reproducibility: only 15 differentially expressed genes were found in common between all the laboratories.

Therefore, for scientific findings to be accurate, robust and reproducible, there needs to be a set of guidelines to facilitate the meaningful interpretation of data. This year, the International Society for Stem Cell Research (ISSCR) released recommendations as a two-pronged approach to tackle this issue. The ISSCR is a global, non-profit organization dedicated to promoting rigor and reproducibility in stem cell research.

These recommendations, published online in June 2023, aim to (1) set standards for the characterization of cell lines and (2) improve transparency in reporting human stem cells. Our colleagues at *Nature Cell Biology* have published a Q&A[2] with co-chairs of the steering committee to discuss the importance of these guidelines, which cover a wide range of topics. For example, the ISSCR recommends that all stem cell lines should be subject to detailed characterization at acquisition and a master cell bank with unique cell identifiers must be established before experimental use. This will ensure that the source cell line is stocked, documented and traceable in case of any contamination and handling errors. Cell lines should be authenticated by short tandem repeat analysis, followed by analyses to identify potential transgene expression or mycoplasma infection. This is important as adventitious agents such as transgenes or microbial contaminations can substantially affect cell proliferation and function.

Similarly, cells should be tested periodically during culture and following any manipulations for acquired genetic changes, as stem cells routinely acquire mutations that lead to abnormal karyotypes. In fact, Popp et al.[3] analyzed 72 iPS cell lines and found a high variability in somatic variant load. The study therefore recommended that only 63.9% of these lines be distributed for further research. Although these mutations may be difficult to avoid, we think that transparent reporting will be key to ensuring proper interpretability of the results.

Inherent to iPS cells is their potential to differentiate into functional cell types; variability in this property calls into question the utility of the stem cell line. To avoid misinterpretation of data, the ISSCR suggests that pluripotency in cell lines be quantitatively demonstrated by methods such as flow cytometry and quantitative imaging. When working with cell lines, additional analyses to confirm differentiation into the three germ layers and one or more tissue types must be carried out.

One of the most intriguing applications of stem cells has been in the development of models such as embryoids and organoids, which enable a 3D view into complex molecular mechanisms in vitro. It is important to acknowledge that these systems do not fully recapitulate in vivo identity[4]. Therefore, maintaining transparent standards is especially important for obtaining reliable results in model systems.

The ISSCR recommends that for stem cell models, the original cell line or tissue must be characterized and reported in detail. Researchers must report as much donor metadata as possible while maintaining privacy. In addition, researchers should consider diversity[5] while selecting cell lines for model development, as sex, age, and ethnic, genetic and lifestyle factors may affect the generalizability of results and provide an incomplete picture of the underlying biology.

Models must be further validated by the demonstration of cell functionality and phenotype that recapitulates native tissue. These criteria will be familiar to our authors at *Nature Methods* as we already expect a demonstration of functionality and at least transcriptomic comparison with native tissues or peer-reviewed cell atlas data (for example, as in ref. 6). In fact, to fairly represent the role of iPS cell variability, we typically require that researchers show the reproducibility of their methods across three or more iPS cell lines.

In studies with engineered devices such as microfluidic culture systems, the ISSCR encourages detailed reporting of all fabrication steps and troubleshooting advice. This, too, is already a criterion for publication at *Nature Methods* (for example, as in ref. 7) and we make no exceptions for methods submitted by laboratories from for-profit companies[8]. We also strongly recommend adding a stepwise protocol to supplementary data to ensure that your method or device can be easily reproduced.

So how will stem cell reporting standards apply at *Nature Methods*? The ISSCR has provided a handy checklist for researchers and editors to assess the details of a study. We strongly encourage our authors to use this while conducting their research and preparing their manuscripts. It is ultimately up to the researchers to ensure that they conduct reproducible and transparent research that is in line with regulations from local and national jurisdictions and funding agencies.

We will be watching closely to ensure that papers at *Nature Methods* uphold the highest standards for reproducible and transparent science. We support the recommendations proposed by the ISSCR and we hope that these guidelines become living documents that continue to be updated as new methods and discoveries improve our understanding of stem cell biology.

Published online: 7 September 2023

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