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SilenciX cell lines for valuable insights into cellular pathways

SilenciX cell lines offer a fast and economical approach for performing studies of biological pathways as well as drug screening within the cellular environment of a stably silenced target.

The availability of cellular models with silenced genes offers the possibility of hands-on studies based on RNA interference (RNAi). Access to such cell lines helps the researcher focus on experimental questions without the need to first construct a cellular model. tebu-bio offers SilenciX cell lines—human adherent cells that have been modified by nonviral and safe transfection for long-term silencing of genes by RNAi.

Most studies that use RNAi depend on either transient cell transfections of small interfering RNA (siRNA) duplexes or of plasmids encoding them, or on transient infection with viruses expressing short hairpin RNA (shRNA) constructs. In contrast, SilenciX cell lines allow stable and virus-free expression of shRNA for months.

tebu-bio SilenciX technology allows us to create robust and reliable cellular models for drug discovery and screening. More than 20 stably silenced cell lines have been made and are now available from tebu-bio. tebu-bio also provides a service that will create cell lines with any silenced gene of a researcher's interest. The cell lines may be HeLa cells or any other human adherent cell line. The silencing efficacy is validated by relative real-time PCR quantifying expression compared to that in control cell lines expressing an irrelevant shRNA. We also supply control cell lines for comparative studies.

The efficacy of the SilenciX technology has been demonstrated in HeLa cells for proteins involved in DNA damage and repair pathways[1]. Here we illustrate the potential of the SilenciX technology in the expression analysis of human genes.

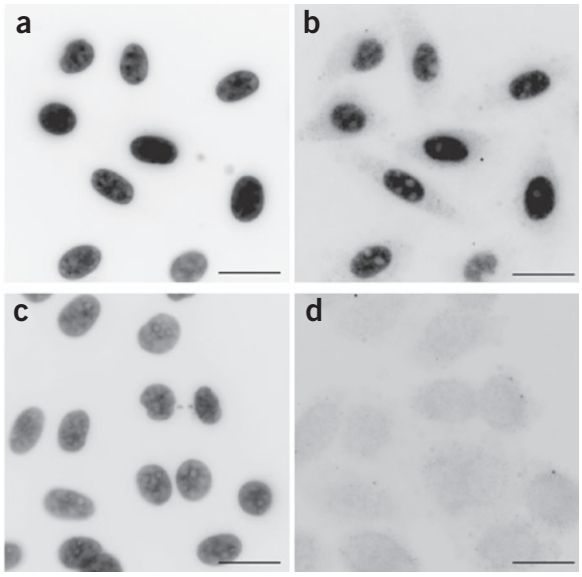

Figure 1 | Immunocytochemical staining of DNA-PKcs HeLa SilenciX cells 163 days after transfection. (**a–d**) Control HeLa SilenciX cells (**a,b**) and DNA-PKcs HeLa SilenciX cells (**c,d**) DAPI-stained (**a,c**) or stained for DNA-PKcs (**b,d**). Scale bars, 10 μm. (Images courtesy of D. Biard.)

Ready-to-use SilenciX cell lines

To create SilenciX cell lines, we used a replicative pEBV-derived plasmid, which after transfection of human cell lines yields a few episomal plasmid copies per cell. The plasmids remain as independent replicons in human transfected cells and express shRNAs for a long time, leading to stable gene silencing (>500 days)[1]. The use of this vector circumvents the shortcomings resulting from the overexpression of siRNA, such as saturation of the endogenous RNA-induced silencing complex (RISC) machinery, and avoids random integrations into the genome. We used a combination of RNAi technology with pEBV-derived vectors to create a set of stable knockdown SilenciX human cell lines covering various fields of biology and human diseases.

The design of shRNA sequences follows the initial RNAi paradigms and has been optimized based on the current state of the art of RNAi biochemistry, and it is now a comprehensive proprietary tool. Any shRNA can be designed to any gene of interest to be compatible with the vector for a straightforward ligation. After nonviral transfection, the transfectants are amplified by antibiotic selection before analysis by quantitative PCR. A control cell line is established with an shRNA encoding an irrelevant sequence.

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APPLICATION NOTES

Evidence for protein knockdown

After transfecting HeLa cells with a plasmid encoding an shRNA targeting a gene involved in nonhomologous end-joining repair, *PRKDC* (also known as *DNA-PKcs*), Denis Biard, the inventor of the SilenciX technology, described in his paper[1] the establishment of stably transfected cells and their maintenance in culture for over a year. Immunocytochemical staining of DNA-PKcs demonstrated the silencing efficacy (**Fig. 1**). He validated the silencing up to 500 days after transfection by western blot and/or immunofluorescence as well as with functional assays[1].

We also evaluated the loss of function associated with the loss of the targeted protein in HeLa cells. The silencing of genes involved in nucleotide excision repair, such as *XPA* and *XPC*, led to a dramatic decrease in DNA repair capacity after ultraviolet C irradiation. More than 50 genes belonging to the main pre- and postreplicative DNA repair pathway[1] and to cell cycle and signaling pathways were targeted (**Table 1**). We then established stable HeLa silenced cells for all targeted genes and validated the silencing by immunocytochemical staining or western blot. Long-term silencing of specific DNA repair genes led to the expected phenotypic modifications and functional effects[1].

tebu-bio further demonstrated the proof of concept of the SilenciX technology in Caco-2 cells by knocking down the gene *ABCB1*, also known as multidrug resistance 1 (*MDR-1*), which encodes the P-glycoprotein (P-gp). We established stable populations of *MDR-1* SilenciX Caco-2 cells, and studied the expression of P-gp in parental Caco-2 cells, control Caco-2 SilenciX cells and *MDR-1* Caco-2 SilenciX cells by western-blot analyses 42 days after and 6 months after transfection. P-gp expression was undetectable in MDR-1 Caco-2 SilenciX cells (**Fig. 2** and data not shown).

RNA knockdown and gene profiling

We analyzed the expression of a panel of genes by real-time PCR using the RT2 Profiler PCR array from SuperArray Bioscience Corporation. We extracted RNA from both control and silenced SilenciX cell lines to run single-target real-time PCR and/or gene profiling analyses. These demonstrated a 98% silencing efficiency in the XPC SilenciX HeLa cell line (data not shown but available upon request to the author). The profiling studies showed that SilenciX technology allows mimicry of human disease states. The suppression of gene expression over a long period of time may help to construct a model of study reflecting the reality of illness-gene deficiency.

Table 1 | Silenced genes in HeLa SilenciX cells

Pathways	Targets
Nucleotide excision repair	*XPA, XPC, hHR23A (RAD23A), hHR23B (RAD23B)*
Nonhomologous end-joining pathway	*DNA-PKcs (PRKDC), XRCC4, ligase IV (LIG4)*
Base excision repair	*Ogg1 (OGG1), XRCC1, ligase III (LIG3), PARP1, PARP2*
Signaling sensors and transducers	*BRCA1, BRCA2, Rad50 (RAD50), NBS1 (NBN), MRE11 (MRE11A), KIN17 (KIN)*
Cell signaling, cell cycle	*P53 (TRP53), ATM, BLM*

Full catalog list is available at http://www.tebu-bio.com.

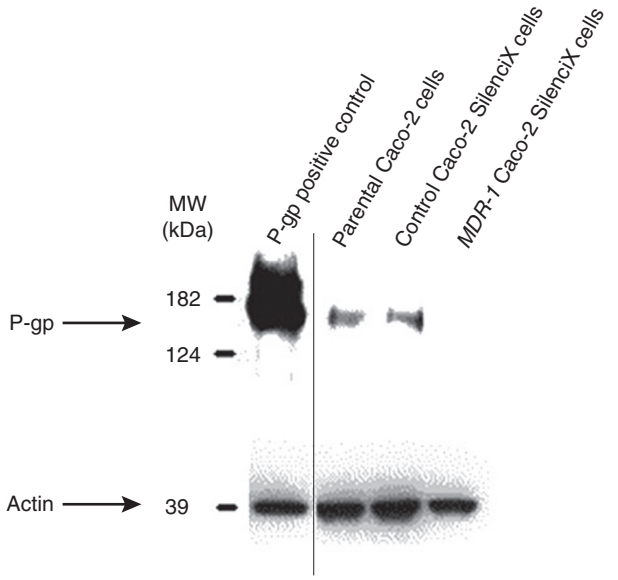

Figure 2 | Western-blot validation of *MDR-1* Caco-2 SilenciX cells 42 days after transfection.

Conclusion

tebu-bio SilenciX technology has applications in drug discovery, functional genomics and screening. The SilenciX cell lines constitute a ready-to-use tool for pathway and signaling studies, target identification and target validation. Access to SilenciX cell lines aids assay development because it makes possible the silencing of any unwanted basal enzymes or any proteins with long half-lives. Furthermore, the stability of the SilenciX knockdown allows comparative studies in the long term, minimizing intra- and inter-experiment variability. It enables the production of large batches of cells with robust consistency, which is a prerequisite for high-throughput screening and absorption, distribution, metabolism, excretion and toxicity (ADMET) studies.

SilenciX is a trademark of tebu-bio. Further information is available on the tebu-bio website (http://www.tebu-bio.com).

ACKNOWLEDGMENTS

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1. Biard, D. Untangling the relationships between DNA repair pathways by silencing more than 20 DNA repair genes in human stable clones. *Nucleic Acids Res.* **35**, 3535–3550 (2007).