OPINION REVIEW

1813 Stem cell-derived biofactors fight against coronavirus infection

REVIEW

1826 Application of mesenchymal stem cells derived from human pluripotent stem cells in regenerative medicine
Liu TM

1845 Strategies to improve regenerative potential of mesenchymal stem cells
Choudhery MS

1863 Dental mesenchymal stromal/stem cells in different microenvironments—implications in regenerative therapy
Okić-Đorđević I, Obradović H, Kukolj T, Petrović A, Mojsilović S, Bugarski D, Jauković A

1881 Regulating the fate of stem cells for regenerating the intervertebral disc degeneration
Ekram S, Khalid S, Salim A, Khan I

ORIGINAL ARTICLE

Basic Study

1905 Bone marrow mesenchymal stem cell therapy regulates gut microbiota to improve post-stroke neurological function recovery in rats
Zhao LN, Ma SW, Xiao J, Yang LJ, Xu SX, Zhao L

1918 SmartFlare™ is a reliable method for assessing mRNA expression in single neural stem cells
Diana A, Setzu MD, Kokaia Z, Nat R, Maxia C, Murtas D

1928 Urolithin a alleviates oxidative stress-induced senescence in nucleus pulposus-derived mesenchymal stem cells through SIRT1/PGC-1α pathway
ABOUT COVER

Editorial Board Member of World Journal of Stem Cells, Jyoti Anand Kode, MSc, PhD, Scientific Officer ‘G’, Kode Lab, Tumor Immunology and Immunotherapy Group; Anti-Cancer Drug Screening Facility, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai 410210, Maharashtra, India. jkode@actrec.gov.in

AIMS AND SCOPE

The primary aim of World Journal of Stem Cells (WJSC, World J Stem Cells) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. WJSC publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, etc.

INDEXING/ABSTRACTING

The WJSC is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, Scopus, PubMed, and PubMed Central. The 2021 Edition of Journal Citation Reports® cites the 2020 impact factor (IF) for WJSC as 5.326; IF without journal self cites: 5.035; 5-year IF: 4.956; Journal Citation Indicator: 0.55; Ranking: 14 among 29 journals in cell and tissue engineering; Quartile category: Q2; Ranking: 72 among 195 journals in cell biology; and Quartile category: Q2. The WJSC’s CiteScore for 2020 is 3.1 and Scopus CiteScore rank 2020: Histology is 31/60; Genetics is 205/325; Genetics (clinical) is 64/87; Molecular Biology is 285/382; Cell Biology is 208/279.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Hua-Ge Yu; Production Department Director: Xu Guo; Editorial Office Director: Ze-Mao Gong.

NAME OF JOURNAL

World Journal of Stem Cells

ISSN

ISSN 1948-0210 (online)

LAUNCH DATE

December 31, 2009

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Shengwen Calvin Li, FRSM, FRSB, Carlo Ventura

EDITORIAL BOARD MEMBERS


PUBLICATION DATE

December 26, 2021

COPYRIGHT

© 2021 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS

https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS

https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT

https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE

https://www.wjgnet.com/bpg/gerInfo/242

STEPS FOR SUBMITTING MANUSCRIPTS

https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION

https://www.f6publishing.com

© 2021 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com
Basic Study

**SmartFlare™ is a reliable method for assessing mRNA expression in single neural stem cells**

Andrea Diana, Maria Dolores Setzu, Zaal Kokaia, Roxana Nat, Cristina Maxia, Daniela Murtas

**ORCID number:** Andrea Diana 0000-0002-7247-6994; Maria Dolores Setzu 0000-0002-6934-4042; Zaal Kokaia 0000-0003-2296-2449; Roxana Nat 0000-0002-6543-2336; Cristina Maxia 0000-0002-3490-1793; Daniela Murtas 0000-0001-1357-2492.

**Author contributions:** Maxia C and Murtas D share senior authorship; Diana A, Setzu MD, Maxia C, and Murtas D contributed to the conception and design of the study, data interpretation, and funding acquisition; Diana A, Kokaia Z, and Nat R contributed to methodology and data acquisition and analysis; Diana A wrote the original draft of the article; Diana A, Maxia C and Murtas D wrote, reviewed and edited the paper, and contributed to project administration and supervision; all authors read and approved the final version of the manuscript.

**Institutional review board statement:** The study was reviewed and approved by the Lund/Malmö Ethical Committee of the Lund University, Sweden (ethical permit number No. Dnr 6.1.8-2887/2017).

**Conflict-of-interest statement:** The authors declare that they have no conflict of interest.

**Data sharing statement:** No

**Abstract**

**BACKGROUND**

One of the most challenging tasks of modern biology concerns the real-time tracking and quantification of mRNA expression in living cells. On this matter, a novel platform called SmartFlare™ has taken advantage of fluorophore-linked nanoconstructs for targeting RNA transcripts. Although fluorescence emission does not account for the spatial mRNA distribution, NanoFlare technology has grown a range of theranostic applications starting from detecting biomarkers related to diseases, such as cancer, neurodegenerative pathologies or embryonic developmental disorders.

**AIM**

To investigate the potential of SmartFlare™ in determining time-dependent mRNA expression of prominin 1 (CD133) and octamer-binding transcription factor 4 (OCT4) in single living cells through differentiation.

**METHODS**

Brain fragments from the striatum of aborted human fetuses aged 8 wk postconception were processed to obtain neurospheres. For the *in vitro* differentiation, neurospheres were gently dissociated with Accutase solution. Single cells were resuspended in a basic medium enriched with fetal bovine serum, plated on poly-L-lysine-coated glass coverslips, and grown in a lapse of time from 1 to 4 wk. Live cell mRNA detection was performed using SmartFlare™ probes (CD133, Oct4, Actin, and Scramble). All the samples were incubated at 37 °C for 24 h. For nuclear staining, Hoechst 33342 was added. SmartFlare™ CD133- and OCT4-
specific fluorescence signal was assessed using a semiquantitative visual approach, taking into account the fluorescence intensity and the number of labeled cells.

**RESULTS**

In agreement with previous PCR experiments, a unique expression trend was observed for CD133 and OCT4 genes until 7 d *in vitro* (DIV). Fluorescence resulted in a mixture of diffuse cytoplasmic and spotted-like pattern, also detectable in the contacting neural branches. From 15 to 30 DIV, only few cells showed a scattered fluorescent pattern, in line with the differentiation progression and coherent with mRNA downregulation of these stemness-related genes.

**CONCLUSION**

SmartFlare™ appears to be a reliable, easy-to-handle tool for investigating CD133 and OCT4 expression in a neural stem cell model, preserving cell biological properties in anticipation of downstream experiments.

**Key Words:** mRNA detection; SmartFlare™; NanoFlare; Live staining; Nanotechnology; Neural stem cell genes.

©The Author(s) 2021. Published by Baishideng Publishing Group Inc. All rights reserved.
nanoparticles with natural receptor-mediated endocytosis to uptake the same nanoconstructs. In particular, target RNA-specific complementary single stranded RNA (capture strand) is hybridized with a complementary “reporter” sequence bound to a fluorophore (Cy3 or Cy5) at its 5’-end that, for viscosity to the central gold particle, is permanently quenched. Only upon pairing with the target RNA sequence, the reporter strand can be released and consequently gain the feature to flare with fluorescent emission at the proper wavelength and intensity, consistent with the expression level of the target RNA. Since the introduction of the SmartFlare™ concept [2-4], this molecular procedure has been successfully exploited for the identification and assessment of both tumor and immune cell subsets[5-8]. Interestingly, the SmartFlare™ technique could provide a wide spectrum of research applications, as identifying RNAs into mammalian conceptuses at different developmental stages has already been used as a proper model[9]. Indeed, SmartFlare™ allows the detection of RNAs specific for hereditary diseases, sex determination, performance and conformation traits in early embryonic stages[1,10-13], and the expression of pluripotency genes in embryonic stem cells and induced pluripotent stem cells (iPSCs) of murine, porcine and human origin[14]. Nevertheless, the ultimate confirmation of these experiments still relies on detecting the same transcripts by qRT-PCR.

To answer to some developmental issues related to the expression of the transcription factor Octamer-Binding Transcription Factor 4 (OCT4), involved in the differentiation process of human neurospheres in a time-dependent fashion[15,16], the mRNA pattern of OCT4 at single-cell level was analyzed from 5 to 30 d in vitro (DIV) using specific SmartFlare™ probes to assess a possible downregulation strictly linked to cellular maturation from stem/progenitor to neural phenotype. In parallel, a SmartFlare™ probe for Prominin 1 (CD133), encoding for a transmembrane glycoprotein widely recognized as a marker of neural progenitor cells, was tested[17,18].

Our findings suggest that SmartFlare™ technology is a straightforward tool for discriminating gene transcripts specifically related to some neural stem cell markers.

MATERIALS AND METHODS

Forebrain tissues were obtained from aborted human fetuses aged 8 wk postconception (Lund and Malmö University Hospitals) in accordance with guidelines approved by the Lund/Malmö Ethical Committee (ethical permit No. Dnr 6.1.8-2887/2017). Brain fragments from the striatum were subjected to microdissection under a stereomicroscope (Leica, Germany), incubated for 30 min in an expansion medium at 37 °C, and then mechanically dissociated in order to obtain a single-cell suspension. Expansion medium DMEM/F-12 (1:1; Invitrogen, Life Technologies, United States), 2.92 g/100 mL L-glutamine, 23.8 mg/100 mL HEPEs, 7.5% NaHCO₃, 0.6% glucose, and 2% heparin (all from Sigma-Aldrich, United States) contained B27 supplement (1%; Invitrogen), human Leukemia Inhibitory Factor (LIF; 10 ng/mL; Sigma-Aldrich), Epidermal Growth Factor (EGF; R&D Systems, United States), and Fibroblast Growth Factor (20 ng/mL and 10 ng/mL, respectively; R&D Systems, United States). Live cells were thereafter counted by the Trypan Blue dye exclusion method before plating in culture flasks at the fixed density of 50,000 cells/mL, at 37 °C in a humidified atmosphere with 5% CO₂. After several weeks, neurospheres were developed and supplied by the Laboratory of Stem Cells and Restorative Neurology (Lund). To determine the capacity of cells to form secondary spheres, single neurospheres were first passaged and then plated for 1 wk. The newly shaped neurospheres were enzymatically dissociated with Accutase solution (Sigma-Aldrich) when at least 70% of them were below 100 μm in radial size or, if smaller, when before their inner core faded to dark, indicating an activated oxidative process and subsequent cell death.

For in vitro differentiation, pelleted neurospheres were incubated with Accutase solution for gentle dissociation for 10 min at room temperature (RT), followed by DMEM/F-12 addition for halting the enzymatic activity. After centrifugation, single cells were resuspended in 500 μL basic medium (without growth factors and heparin) containing 1% fetal bovine serum (FBS; differentiation medium) and plated on poly-L-lysine-coated glass coverslips (5000-10000 cells/cm²)[16,19]. During the differentiation period (1-4 wk), the specific medium was refreshed every third day. Live-cell mRNA detection was performed using SmartFlare™ probes, according to the manufacturer’s protocol (Merck Millipore, Temecula, CA, United States). Briefly, all the used probes were rehydrated by 50 μL of sterile nuclease-free double-distilled water to each vial and kept in the dark until needed. Immediately before the use, the
stock solutions were diluted 1:20 in sterile phosphate-buffered saline. Four μL of the same solutions were added to 200 μL of the medium for each tested probe. For each experiment, performed in triplicate, two control samples were run in parallel: a negative one made of a scramble construct that, therefore, does not recognize any cellular sequence and used to quantify the unspecific background (Scramble SmartFlare™ Probe); a positive control (uptake SmartFlare™ Probe) that permanently emits fluorescence supplying the information that the SmartFlare™ particles are uptaken by the target cell type. The following reagents were used: CD133 Hu-Cy3 SmartFlare™ RNA Probe (SF-958), Oct4 Hu-Cy3 SmartFlare™ RNA Probe (SF-438), Actin-Cy3 SmartFlare™ RNA Probe (SF-145), Scramble-Cy3 SmartFlare™ RNA Probe (SF-103), and uptake-Cy3 SmartFlare™ RNA Probe (SF-114), all provided by Merck Millipore. All samples were incubated at 37 °C in a humidified atmosphere with 5% CO₂ for 24 h, since in previous experiments the suggested 16 h incubation was evaluated not sufficient for the complete probe internalization. For nuclear staining, 10 μg/mL Hoechst 33342 (Invitrogen) was added 5 min before evaluation. Observations were made using an inverted microscope (IX 71; Olympus, Tokyo, Japan) with a x40 planapochromatic objective (PlanApo series; Olympus), taking care to grab all images with the same exposure time and filter set. Images (12-bit) were taken with a cooled monochrome CCD camera (Moticam Pro285D, Motic, China) with a 1360 × 1024 pixel chip. Image processing and analysis were performed using the Image-Pro Plus software (Media Cybernetics, United States).

SmartFlare™ CD133- and OCT4-specific fluorescence signal was assessed using a semiquantitative visual approach by three observers in a blinded fashion. This evaluation took into account both the fluorescence intensity and the number of labeled cells.

**RESULTS**

CD133 and OCT4 gene expression was analyzed by SmartFlare™ technology in dissociated human neurospheres upon differentiation commitment, accomplished by switching to growth factor withdrawn media along one-month time frame (from 3 to 30 DIV), with 3 DIV as the minimum time needed by cells both to adhere to the substrate and to grow cytoplasmic area and processes. At 3 DIV, after incubation with specific SmartFlare™ probes, the morphological expression pattern for CD133 and OCT4 mRNAs (Figure 1A and B) was consistent with the Actin-positive cells (Figure 1C). Remarkably, when Hoechst-stained cells were not massively clustered but discernible as single elements, it was possible to evaluate that all cells displayed a diffuse but strong fluorescent signal, sometimes visible as converging single dots filling the thin cytoplasmic processes too. Similarly, the fluorescence of the CD133 reporter probe was as intense as that of Oct4. The Actin housekeeping probe was clearly internalized as a fluorescent patch distributed from the perinuclear area to the peripheral branches, where it appeared as a granular content connecting distant cells (Figure 1C). Fluorescence detection in those living cells was considered a specific marker for mRNAs presence when compared to scramble experiments (Figure 1D), where any background was undetectable in most cells.

At 7 DIV, microscopic images exhibited a clear fluorescence both with CD133 and Oct4 probes (Figure 2A and B). Although the robust arborization network was still detected, in visible branches of very few cells it was observed the presence of fluorescent dots, representative of the molecular beacon-associated mRNAs. The reliability of the results was confirmed by the positive and negative controls (Figure 2C and D).

Cells grown for 15 DIV presented a marked decrease in the SmartFlare™ fluorescence signal, as it was limited to less than half of the analyzed cells, irrespective of the CD133 or Oct4 probe incubation (Figure 3A and B). In addition, the mRNA-like presence was confined to the cytoplasmic domain and always in the shape of tiny and few grains.

Finally, after 30 DIV, even fewer positive cells with specific signal were noticed and again the only morphological feature consisted of single dot-like elements, both in CD133 and Oct4 probe-treated cells (Figure 4A and B). Accordingly, in the last two experiments (15 and 30 DIV), Actin (Figure 3C and 4C, respectively) and Scramble (Figure 3D and 4D, respectively) signals were representative of the specificity of the resulting fluorescence.
**DISCUSSION**

In this study, we carried out a simple and noninvasive RNA-based approach to monitor intracellular gene expression in living cells by fluorescent SmartFlare™ probes. In detail, this study focused on human neurospheres as neural stem cell reservoir, as this is a well-established model to study the progression of differentiation events giving rise to both neuronal and glial lineages. This is a very interesting topic to address, since it involves OCT4, one of the key genes implicated in encoding transcription factors prone to convert somatic cells into iPSCs and, therefore, necessary for the commitment of embryological events[20]. The rationale behind the present investigation dates back to a previous study, where the immunohistochemical presence of Oct4 protein was observed in neural stem cells during the first week of differentiation but disappeared after 4 wk. Coherently, in the same research, RT-PCR experiments supported OCT4 mRNA downregulation, as illustrated by the blurred bands of the electrophoretic assay[16]. Therefore, the advantage of SmartFlare™ probe uptake has emerged for challenging the quantification of mRNA gradient in specific and individual cells. Moreover, the same technique could be useful for identifying neural stem/progenitor cells eventually sorted for further characterization, avoiding any minimal alteration of morpho-functional and biochemical properties. With regard to Oct4, there are some further but possibly misinterpreting studies describing cytoplasmic staining due to splicing variants that make it critical to distinguish transcriptional products[21-24]. For this reason, this study conceived the experimental design of choosing the cell surface antigen CD133 as an alternative positive marker of neural stem cells[25]. The localization of OCT4 mRNA within cells has already been addressed by some researchers[26] using molecular beacon transfection in differentiated human mesencephalic-derived neurospheres. However, after dissociation, adherent differentiated monolayers resulted lacking OCT4 expression. Interestingly, monolayered cells grown from neurospheres revealed the complete absence of mRNA expression just before the first week of differentiation, as further confirmed by immunocytochemistry. Indeed, the initial enthusiasm of the scientific community was damped by some studies reporting “A total lack of correlation between fluorescence intensities of SmartFlare probes and the level of corresponding RNAs assessed by RT-qPCR”[27]. Recent data might explain the resulting different amounts of mRNA
Figure 2 SmartFlare™ detection in 7 d in vitro neural stem cells. A and B: SmartFlare™ CD133 and Oct4 probes fluorescence was less intense than the signal detected in 3 d in vitro neural stem cells, but still present in almost all cells; C: SmartFlare™ probe for Actin showed a robust and overall localization of red fluorescence as indicative of positive mRNA expression; D: Scramble probe-treated cells showed a very faint unspecific red background. Nuclei were stained by Hoechst dye. Scale bar: 50 μm.

detected by SmartFlare™ and qRT-PCR, due to cytoplasmic stress granules where mRNA can be sequestered and made unavailable to be processed for translation[28]. Mason et al[29] argued about the SmartFlare™ probes sequestration by the lysosomal machinery. However, by specific matching Lysosome-associated Membrane Protein 1 (LAMP-1) SmartFlare™, these authors found a very low overlap (mean Manders’ coefficient 0.26), concluding that the unspecific SmartFlare™ fluorescence localized in lysosomes could be negligible compared to cytoplasmic staining. Our findings agree with the heterogeneity of SmartFlare™ expression, either diffuse cytoplasmic or spotted from the perinuclear site to peripheral processes (dendrites and axons). Moreover, ultrastructural evidence of gold nanoparticles, encapsulated within endosomal/lysosomal compartments, does not explain the spotted fluorescent pattern, unless enzyme digestion would degrade and remove the nanostructure links, ultimately quenching the fluorescence signal. So far, there is still no experimental evidence for that degradative machinery, and, on the other hand, it cannot be ruled out whether there are some alternative routes either passively or actively driven by cells.

By means of a qualitative analysis, the strength of the SmartFlare™ technology would not be affected by the decrease of the fluorescence intensity as a reflection of a reduced lysosomal activity, which occurs during cell differentiation[30]. Actually, as shown by our results, it is unlikely to detect all the cells in the same stage of replication or differentiation within single timepoints.

Although FISH is a well-established and reliable qualitative molecular method, the advantages of SmartFlare™ technology could reside in the opportunity of analyzing unfixed single living cells, retaining their viability, morpho-functional and biochemical properties and allowing downstream experiments[31]. In particular, this approach could help to detect and count stem/progenitor living cells, expressing markers of stemness, in terms of differential expression of the relative mRNAs, as well as microRNAs, which could find application in the profiling of tumor cell heterogeneity [32,33]. Moreover, from an empirical perspective, the SmartFlare™ could be a quicker, easier and less expensive method than techniques involving RNA isolation. Thus, in agreement with the findings by Mason et al[29], our results might validate the SmartFlare™ technology as a reliable and easy-to-handle tool, at least in the qualitative analysis framework, although, in some cases, as usually happens, the possibility of an
Figure 3 SmartFlare™ detection in 15 d in vitro neural stem cells. A and B: SmartFlare™ CD133 and Oct4 probes showed a dramatic fluorescence downregulation that was limited to small cytoplasmic granules in less than half of the observed cells; C: SmartFlare™ probe for Actin showed a robust and overall localization of red fluorescence as indicative of positive mRNA expression; D: Scramble probe-treated cells were almost completely lacking in unspecific red background. Nuclei were stained by Hoechst dye. Scale bar: 50 μm.

Figure 4 SmartFlare™ detection in 30 d in vitro neural stem cells. A and B: SmartFlare™ CD133 and Oct4 probes showed few cells expressing a tiny granular pattern in the cytoplasmic domain; C: SmartFlare™ probe for Actin showed an abundant red fluorescence in all observed cells; D: Scramble probe-treated cells were almost completely lacking in unspecific red background. Nuclei were stained by Hoechst dye. Scale bar: 50 μm.
artifact detection may arise.

In the prospect of controversial negative results, it should be considered that FBS supplementation in the culture medium could dramatically play a crucial role in the interpretation of target mRNA detection by SmartFlare™ technology, in terms of cytoplasmic distribution and localization. This methodological issue could partially explain the documentation failure by many research groups[19].

Despite the above-described unsolved criticism, some recent data on molecules and cells involved in immunological and inflammation response against cancer have renewed the interest in an innovative and effective platform to investigate some mRNA functions[34-36]. Besides, it cannot be denied that SmartFlare™ probe detection is not indicative of the real localization of single mRNA molecules. Nevertheless, those NanoFlare probes have paved the way to inspire a novel theranostic wave arising some new sticky-flares for in situ monitoring of human telomerase RNA[37], adopting photoactivation to detect mRNA in specific cells[38].

CONCLUSION

In conclusion, this new age of NanoFlare compounds has opened up or, at least, broadened biomedical applications, paying attention to preserving the physiological integrity of cellular systems with an excellent grade of selectivity and specificity[39].

ARTICLE HIGHLIGHTS

Research background
Although mRNA analysis is still conventionally achieved by fluorescence in situ hybridization and qRT-PCR, there is a strong need for real-time monitoring of specific RNA transcripts in living cells, both for a qualitative and quantitative assessment. Within this context, SmartFlare™ technology is a reliable tool for evaluating the presence and upregulation/downregulation of mRNAs in individual living cells.

In addition, this nanotechnology offers the advantages of retaining cell viability, morpho-functional and biochemical properties and allowing downstream experiments.

Research motivation
SmartFlare™ technology is a devoted and straightforward method for the spatiotemporal investigation of the in situ mRNA expression in living cells.

Research objectives
To study the dynamics of differentiation-related RNA transcripts in human neural stem cells.

Research methods
The presence of CD133 and OCT4 mRNA-linked nanoprobes in neurosphere-derived cells (from 3 to 30 DIV) was investigated by SmartFlare™ as a reliable insight into neural stem cell differentiation.

Research results
Until 7 DIV, all the cells displayed a strong SmartFlare™ fluorescent signal indicative of CD133 and OCT4 mRNA expression, as single dots encompassing both the cytoplasmic domain and the related processes. Upon 15 DIV, cells showed a marked decrease in the fluorescence, both for CD133 and Oct4 probes. In cells grown for 30 DIV, the CD133 and Oct4 probe uptake was very scant but still consisted of single dot-like elements, representative of a downregulation of the same genes.

Research conclusions
Our findings propose the SmartFlare™ technology as a reliable and straightforward tool in the context of a qualitative expression analysis applied to a broad panel of mRNAs in single living stem cells.

Research perspectives
The NanoFlare technology, such as SmartFlare™, could enhance the scenario of
biomedical applications in the field of marker identification mirroring both normal and pathological conditions, with the advantage of ensuring the physiological integrity of cellular systems.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Emanuela Monni (Laboratory of Stem Cells & Restorative Neurology, Lund Stem Cell Center, Lund University, Sweden), for kindly supplying the neurospheres from the human forebrain tissues.

REFERENCES


Ilieva M, Dufva M. SOX2 and OCT4 mRNA-expressing cells, detected by molecular beacons, localize to the center of neurospheres during differentiation. *PloS One* 2013; 8: e73669 [PMID: 24013403 DOI: 10.1371/journal.pone.0073669]

Czarnack M, Bereta J. SmartFlares fail to reflect their target transcripts levels. *Sci Rep* 2017; 7: 11682 [PMID: 28916792 DOI: 10.1038/s41598-017-11067-6]


