New and Notable

Nanoscopy and Nanoparticles Hand-in-Hand to Fight Cancer: An Exciting Entrée into the Rising NANOworld

Martin Falk[1,*]
[1]Department of Cell Biology and Radiobiology, Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic

Two words with the prefix "NANO"—nanoparticles and nanoscopy—appear in the title of Moser's article published in this issue of the *Biophysical Journal* (1), although the latter one remains hidden behind the term "localization microscopy". What is so exciting about this title (and Moser's article) is that it describes how two nanotechnologies are used to fight cancer and further amend each other. The article thus unequivocally demonstrates that both biological sciences and medical research have definitely left the micro-dimensions and dives head-first into the rising NANOworld.

Nanoparticles might represent new versatile weapons in the war on cancer. Among numerous applications, gold (GNPs) and other metal nanoparticles (composed of high-*Z* atoms) were proposed as selective tumor cell radiosensitizers (2). In animal experiments, GNPs were preferentially sequestered by tumors and, upon irradiation, locally enhanced the dose by emitting showers of Auger electrons. Metal nanoparticles thus promise to increase the radiotherapy efficiency while reducing its side effects. Although the radioactivation of nanoparticles is well described in terms of physics, practical development of these new nanodrugs depends on our better understanding of the fate of nanoparticles in cells.

Submitted December 22, 2015, and accepted for publication January 11, 2016.

*Correspondence: mfalk@seznam.cz

Editor: Paul Wiseman.

0006-3495/16/02/0872/2

In their work, Moser et al. (1) study intracellular uptake, distribution, and persistence of GNPs differing in diameter by means of spectral position determination microscopy (SPDM) with previously unprecedented resolution. This represents a breakthrough innovation over earlier studies. Although modern applications of classical optical microscopy (like 4D live-cell fluorescence confocal microscopy, FRAP, FLIM, etc.) are among the most fruitful methods of biological research in the past decade and brought about many important discoveries of the principal features of cell architecture and function (e.g., Falk et al. (3)), the restriction of optical resolution determined by Abbe criterion to ~0.2 micrometers could hardly be circumvented. Already developed optical single-molecule localization techniques have allowed us to determine intracellular nanoparticle location (in low-density limits where single fluorochrome molecule localization is possible), but not to quantify more densely arranged particle clusters, which is necessary for making more precise structural and mechanistic interpretations. A number of electron microscopy studies recently appeared taking advantage of electron-beam contrast qualities of gold nanoparticles to analyze their uptake by cells under physiological conditions (4,5). However, usability of electron microscopy for intact/living cell applications is rather exceptional. Microscopy limitations thus largely complicate identification of intracellular targets and processes influenced by GNPs and other nanoparticles.

The situation has dramatically changed with the discovery of superresolution methods like localization microscopy, which SPDM variant has been employed in Moser's work (1). Recognizing this milestone, superresolution microscopy has been published in *Nature Methods* (6) as the Method of the Year for 2008 and in 2014, Eric Betzig, William Moerner, and Stefan Hell were awarded the Nobel Prize in Chemistry for converting the microscope into the nanoscope. Although several other ways for circumventing the Abbe criterion have also been proposed and transferred into practice, SPDM—originally published in Esa et al. (7) and substantially elaborated by teams of Christoph Cremer and Michael Hausmann (both coauthoring the article) (reviewed in Cremer et al. (8))—offers an important advantage over many of the other techniques. While ensuring effective optical resolution of ~10 nm, the method allows working with numerous conventional dyes and is naturally compatible with spatial (3D) cell fixation and even living cell observation. The trick of SPDM consists in optical isolation of individual fluorochrome molecules/nanoparticles and their position determination with nanometer precision via their time-dependent blinking. Thus, for instance, spatial arrangements of individual nanoparticles or nanostructures of organelles, membranes, or chromatin can be studied under truly physiological conditions of the cell.

Despite tremendous progress, SPDM is still in its infancy. Similar to standard fluorescence microscopy, one of substantial problems with SPDM may appear with permanent bleaching of conventional blinking dyes. While originally aiming to improve cancer radiotherapy by exploring intracellular behavior of GNPs, Moser et al. (1) also recognized their stable blinking. Because the effect followed from plasmon resonance and not from reversible photobleaching as for dyes, the blinking lasted for a sufficiently long time to preclude current problems with conventional fluorochromes and facilitate usage of GNPs as preferable tags for various labeling strategies associated with SPDM. Importantly, spectral fluorescence of GNPs depended on their size and environment; this opens doors to simultaneous nanoimaging of multiple

http://dx.doi.org/10.1016/j.bpj.2016.01.005

cellular targets and promises broad experimental possibilities.

In considering future perspectives of SPDM, Moser et al. (1) performed their observations on spatially (3D) fixed cells in two colors. Although multicolor and 3D SPDM applications have been already reported (frequently by M. Hausmann and C. Cremer coauthoring Moser's work) (9), practical mastering of this technique still represents a challenge contrasting with its simple principle. Thousands of images must be registered and analyzed for each color and each optical section to obtain the desired resolution. A future dream thus remains 3D time-lapse (4D) SPDM in living cells, where whole image mega-sets have to be taken continuously with time intervals of minutes, seconds, or perhaps milliseconds. New nonbleachable blinking labels, as discussed, might avoid the problem with permanent bleaching of intensively and repeatedly illuminated samples. Future attempts must also discover a method, equivalent to GFP-tagging of proteins, to label specific DNA loci in vivo. Combinatorial oligonucleotide fluorescence in situ hybridization (COMBO FISH), proposed by M. Hausmann (for review, see Burger et al. (10)), might point the direction to this Holy Grail of cell biology (11).

With the exciting SPDM technology at hand, Moser et al. (1) quantify cell internalization of experimentally more (25 nm) and less (10 nm) therapeutically efficient gold nanoparticles, for the first time simultaneously under physiological conditions and in terms of absolute numbers. This attempt is important to understand the biological mechanism of nanoparticle-mediated radiosensitization, quantitative and dynamic modeling of this effect, and therapy planning in future. For instance, it is not clear on the basis of literature review and our unpublished data whether the radiosensitization always relies on enhancing the DNA damage or the cytoplasmic events (like mitochondria or lysosomes disruption) might be involved. Evidently, SPDMs have strong potential to substantially participate in answering this and other fundamental questions associated with nanoparticle research: In addition to providing superresolution under physiological conditions, the method also enables spectroscopic resolution of nanoparticles of different sizes, as nanoparticles optically blink at a size-dependent wavelength (demonstrated for GNPs in Moser's work). Finally, Stuhlmüller and Hausmann (11) recently proposed new technology of how to deliver and retain therapeutically more efficient bigger gold nanoparticles in cells; GNPs were tagged with modified DNA oligonucleotides and conveyed into the cells by transfection. In Moser's work (1), the authors demonstrate, using SPDM, that this strategy allows enhanced and stable cellular internalization of these nanoparticles.

REFERENCES

1. Moser, F., G. Hildenbrand, …, M. Hausmann. 2015. Cellular uptake of gold nanoparticles and their environment-dependent behavior as labels for localization microscopy. *Biophys. J.* 110:947–953.
2. Retif, P., S. Pinel, …, M. Barberi-Heyob. 2015. Nanoparticles for radiation therapy enhancement: the key parameters. *Theranostics.* 5:1030–1044.
3. Falk, M., E. Lukasova, and S. Kozubek. 2010. Higher-order chromatin structure in DSB induction, repair and misrepair. *Mutat. Res.* 704:88–100.
4. Peckys, D. B., and N. de Jonge. 2011. Visualizing gold nanoparticle uptake in live cells with liquid scanning transmission electron microscopy. *Nano Lett.* 11:1733–1738.
5. Peckys, D. B., and N. de Jonge. 2014. Gold nanoparticle uptake in whole cells in liquid examined by environmental scanning electron microscopy. *Microsc. Microanal.* 20:189–197.
6. Editorial. 2009. Method of the Year 2008. *Nature Methods.* 6:1.
7. Esa, A., P. Edelmann, …, C. Cremer. 2000. Three-dimensional spectral precision distance microscopy of chromatin nanostructures after triple-colour DNA labelling: a study of the BCR region on chromosome 22 and the Philadelphia chromosome. *J. Microsc.* 199:96–105.
8. Cremer, C., R. Kaufmann, …, M. Hausmann. 2011. Superresolution imaging of biological nanostructures by spectral precision distance microscopy. *Biotechnol. J.* 6:1037–1051.
9. Kaufmann, R., P. Müller, …, C. Cremer. 2011. Analysis of Her2/neu membrane protein clusters in different types of breast cancer cells using localization microscopy. *J. Microsc.* 242:46–54.
10. Burger, N., A. Biswas, …, M. R. Veldwijk. 2014. A method for the efficient cellular uptake and retention of small modified gold nanoparticles for the radiosensitization of cells. *Nanomedicine (Lond.).* 10:1365–1373.
11. Stuhlmüller, M., and M. Hausmann. 2015. Selection of COMBO-FISH probes for multi-purpose applications. *J. Theor. Comput. Sci.* 2:131–132.