Commentary

Nanodiamonds and nanoparticles as tumor cell radiosensitizers—promising results but an obscure mechanism of action

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Provenance: This is a Guest Commentary commissioned by Section Editor Hongcheng Zhu, MD, PhD (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Comment on: Grall R, Girard H, Saad L, *et al.* Impairing the radioresistance of cancer cells by hydrogenated nanodiamonds. Biomaterials 2015;61:290-8.

Submitted Nov 18, 2016. Accepted for publication Nov 23, 2016.

doi: 10.21037/atm.2016.12.62

View this article at: http://dx.doi.org/10.21037/atm.2016.12.62

While cancer still represents one of the most serious threats to human health, a boom of nanoparticle applications has been experienced in medicine in the last decade, in the fields of both diagnostics and therapy [reviewed in (1-5)]. Hence, it is not surprising that in order to fight malignant diseases a veritable 'ZOO' of nanoparticles is being investigated (6).

Currently, the most efficient ways to eradicate tumors, or at least limit tumor growth, are radiotherapy and chemotherapy (R&CH). Both these methods are based on damaging the DNA molecules in cancer cells, which are more sensitive to this damage compared to slower dividing normal cells. There are many different types of DNA lesions introduced by radiotherapy and some kinds of chemotherapy, of which double strand breaks (DSBs) [reviewed in (7)] represent the most deleterious lesions and are mostly responsible for the cell-killing effect of these therapeutic approaches. In many cases, such as in head and neck cancers, R&CH are preferred over primary surgery since they are less mutilating for the patients (8). However, both these approaches are burdensome and risky. Radiotherapy, the main focus of this article, results in limited clinical outcomes due to frequently insufficient tumor responses. If radiotherapy fails, salvage surgery on irradiated tissue and tissue healing are complicated by the radiation damage (8). Moreover, incomplete eradication of the tumor supports selection of even more radioresistant tumor cell clones. Further risks of radiotherapy arise from potential patient hypersensitivity to ionizing radiation and the damage caused to normal tissue adjacent to the tumor. While hypersensitivity may produce acute life-threatening side effects, the latter phenomenon may initiate the development of secondary (therapy-induced) cancers. Radiotherapy thus urgently calls for new improvements reducing these limitations.

In principle, several strategies exist for making radiotherapy more efficient and safer: first, γ-rays or X-rays, mostly used in current medicine, could be replaced by other types of ionizing radiation with more suitable physical characteristics in indicated cases [reviewed in (9)]. Accelerated protons (10) and ions (11,12) are generating significant improvements in radiotherapy due to their preferable dose deposition course (Bragg peak) and better tumor targeting. Accelerated ions also offer higher linear energy transfer (LET) and thus higher radiobiological effectiveness (RBE). Due to these characteristics, even some tumors resistant to conventional (photon) radiotherapy can be successfully controlled by accelerated ions. However, while proton therapy is quickly spreading all over the world, ion therapy remains expensive, technically demanding, and is still an experimental method (12). A different approach to improving radiotherapy is to radiosensitize tumor cells (5,13-16) and/or radioprotect normal cells surrounding the tumor (17). This strategy can also be combined with the above-described ion beam irradiation to maximize the final effect of radiotherapy. This combined strategy will be further discussed in this article.

As already mentioned, a plethora of nanoparticles is currently being studied for various medical applications,

including usage as tumor cell radiosensitizers in radiotherapy. Even in the mid-1970s, several studies revealed enhanced radiation damage of chromosomal DNA in patients undergoing iodine angiography; this was accompanied by enhanced lymphocyte death (18). Consequently, the findings were confirmed *in vitro* (19) and the radiosensitizing effects of numerous nanoparticle types have been well described in terms of physics. However, the biological mechanisms of nanoparticle-mediated radiosensitization (N-MR) remain more obscure (16).

One type of promising versatile nanotool in medicine is hydrogenated nanodiamonds (HD), which were proposed as radiosensitizers in the recently published study by Grall *et al*. (15). The size of nanodiamonds can range from 5 to 100 nm and their surfaces can be modified in various ways to achieve a broad scale of specific physical and chemical characteristics [see (15) and literature therein for more details]. This means that nanodiamonds can be applied to a plenitude of possible situations with significantly improved effects; for instance, current imaging/diagnostics, targeted drug delivery, and/or enhance drug/therapy effects.

Physically, HD exhibit a negative electron affinity together with a positive charge in aqueous solutions (15). These characteristics ensure their high reactivity with oxygen species and allow them to emit secondary electrons upon 'activation' by ionizing radiation. On the bases of these effects, it could be expected that HD can radiosensitize (tumor) cells primarily through locally enhancing the nuclear DNA damage caused by ionizing radiation. This is shown in Grall *et al*. (15). Similarly, a greater efficiency of cell killing was also correlated with an increased induction of DNA DSB in irradiated cells preincubated with some kinds of metal nanoparticles (i.e., nanoparticles composed of high-Z atoms) (20-22).

From these results, the mechanism of N-MR seems to be simple: the main target for ionizing radiation is nuclear DNA and nanoparticles escalate the attack of radiation on this molecule. In support of this conclusion, there is a general consensus in the literature on increased DSB induction by irradiated nanoparticles *in vitro* (23). However, many reports failed to demonstrate augmented nanoparticle-mediated DSB damage in irradiated cells, though a significant radiosensitizing effect had occurred (16). Importantly, the discrepancy between results also exists for nanoparticles of the same or very similar physico-chemical parameters [e.g., (23) *vs.* (16)] pointing to fundamental roles of nanoparticle-cell interactions and biological behavior of nanoparticles in the mechanism of N-MR.

Reactive radicals and secondary electrons produced by irradiated nanoparticles are short living and can only travel for a limited range. Therefore, upon irradiation, these damaging agents only concentrate themselves to high levels in tight shells around the ND/nanoparticle clusters. The primary cellular (biological) targets for ND/N-MR could thus be searched for in close proximity to ND/nanoparticle intracellular distribution hotspots. This opens an unresolved paradox: while the main target for ionizing radiation is undoubtedly the nuclear DNA, most reports show that ND/nanoparticles, efficiently amplifying the effect of radiation, remain localized in the cytoplasm without penetrating into the cell nucleus [e.g., (16)].

Though some secondary electrons or radicals produced by ND/nanoparticles can occasionally reach the nucleus and damage the DNA, it is still unclear whether this damage can sufficiently explain the increase in radiation-induced cell death by ND/nanoparticles. Moreover, as already noted, many studies failed to detect additional DNA damage due to nanoparticles present in irradiated cells (16). Therefore, at least for some nanoparticles, an alternative target to the nuclear DNA might exist.

Logically, a potential cytoplasmic target for ND/nanoparticles could be mitochondria since, in addition to the nucleus, they also contain DNA and exert irreplaceable functions in cell metabolism. However, recent reports showed that nanoparticles in the cytoplasm colocalize with lysosomes instead of mitochondria [(16) and citations therein]. Until recently, lysosomes have been only considered as cellular trash liquidators. However, a growing body of evidence suggests that these organelles are also involved in cell signaling pathways that regulate cell survival (24). Therefore, though more experiments are needed, extensive damage to lysosomes can potentially result in altered acido-basic cell homeostasis, release of proteolytic enzymes into the cytoplasm, and/or deregulation of cell signaling (16). All these processes, or their combinations, might in principle mediate the radiosensitizing effect of nanoparticles.

To summarize, the mechanism of N-MR still represents a subject of intensive disputations. The lack of conclusive information reflects differences among the studied cell types and, especially, nanoparticle diversity (material, composition, size, surface modifications, etc.). ND/nanoparticles have huge potential for physical modifications, which in turn can change many aspects of their biological behavior. This is extremely promising for future ND/nanoparticle design and therefore for clinical applications.

For instance, though nanoparticles are preferentially internalized by tumor cells, even passively due to the EPR effect, their tumor targeting and therapeutic effectivity can be further stimulated by nanoparticle association with specific antibodies and/or therapeutic agents. For instance, triplex-forming oligonucleotides (TFO) tagging seems to potentiate transport and accumulation of nanoparticles in proximity/inside the cell nucleus (25,26), which not only allows the nuclear DNA damage to be more efficient (25), but also enables simultaneous silencing of mutated or otherwise altered genes in cancer cells (26), such as *BRCA1* in breast cancer (27,28). Moreover, some ND/nanoparticles, such as hydrogenated ND, described in Grall *et al*. (15), can even be active by themselves; they can release free radicals even in absence of irradiation (15).

On the other hand, the variability of ND/nanoparticles largely complicates research into their effects. Data on direct nanoparticle cytotoxicity differs with each type of nanoparticle, but many types have been shown to be non-toxic or only slightly toxic. Therefore, direct cytotoxicity does not seem to significantly contribute to the nanoparticle-mediated radiosensitizing effect. Results on nuclear DNA involvement in the radiosensitizing mechanism are contradictory and alternative targets for N-MR, such as lysosomes, have been proposed on the basis of intracellular nanoparticle localization. However, future work is necessary to determine the organelle-specific effects of ND/nanoparticles and to further comprehension of the whole topic.

Acknowledgements

Funding: Cited research of the author was supported by the Czech Science Foundation (projects 16-12454S, P302/12/G157), Ministry of Health of the Czech Republic (16-29835A) and MEYS CR (project of the Czech Plenipotentiary 2016, the 3+3 Project for 2016–2018).

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine 2007;2:129-41.
2. Biffi S, Voltan R, Rampazzo E, et al. Applications of nanoparticles in cancer medicine and beyond: optical and multimodal in vivo imaging, tissue targeting and drug delivery. Expert Opin Drug Deliv 2015;12:1837-49.
3. Lin W. Introduction: Nanoparticles in Medicine. Chem Rev 2015;115:10407-9.
4. Luque-Michel E, Imbuluzqueta E, Sebastián V, et al. Clinical advances of nanocarrier-based cancer therapy and diagnostics. Expert Opin Drug Deliv 2017;14:75-92.
5. Babaei M, Ganjalikhani M. The potential effectiveness of nanoparticles as radio sensitizers for radiotherapy. Bioimpacts 2014;4:15-20.
6. Falk M. Nanoscopy and Nanoparticles Hand-in-Hand to Fight Cancer: An Exciting Entrée into the Rising NANOworld. Biophys J 2016;110:872-3.
7. Falk M, Lukasova E, Kozubek S. Higher-order chromatin structure in DSB induction, repair and misrepair. Mutat Res 2010;704:88-100.
8. Falková I, Falk M, Horáková Z, et al. DNA repair in the head and neck cancers and their radiosensitivity – the dilemma of the first therapy. Health and Social Work 2016;11:19-25.
9. Jiang GL. Particle therapy for cancers: a new weapon in radiation therapy. Front Med 2012;6:165-72.
10. Ježková L, Falk M, Falková I, et al. Function of chromatin structure and dynamics in DNA damage, repair and misrepair: γ-rays and protons in action. Appl Radiat Isot 2014;83 Pt B:128-36.
11. Marx V. Cancer treatment: Sharp shooters. Nature 2014;508:133-8.
12. Kamada T, Tsujii H, Blakely EA, et al. Carbon ion radiotherapy in Japan: an assessment of 20 years of clinical experience. Lancet Oncol 2015;16:e93-e100.
13. Kwatra D, Venugopal A, Anant S. Nanoparticles in radiation therapy: a summary of various approaches to enhance radiosensitization in cancer. Transl Cancer Res 2013;2:330-42.
14. Belz JE, Ngwa W, Korideck H, et al. Multifunctional nanoparticles in radiation oncology: an emerging paradigm. In: Harper-Leatherman AS, Solbrig CM. editors. The science and function of nanomaterials: from synthesis to application. Washington, DC: American Chemical Society, 2014:75-106.
15. Grall R, Girard H, Saad L, et al. Impairing the radioresistance of cancer cells by hydrogenated nanodiamonds. Biomaterials 2015;61:290-8.
16. Štefančíková L, Lacombe S, Salado D, et al. Effect of gadolinium-based nanoparticles on nuclear DNA

damage and repair in glioblastoma tumor cells. J Nanobiotechnology 2016;14:63.
17. Hofer M, Falk M, Komůrková D, et al. Two New Faces of Amifostine: Protector from DNA Damage in Normal Cells and Inhibitor of DNA Repair in Cancer Cells. J Med Chem 2016;59:3003-17.
18. Adams FH, Norman A, Mello RS, et al. Effect of radiation and contrast media on chromosomes. Preliminary report. Radiology 1977;124:823-6.
19. Matsudaira H, Ueno AM, Furuno I. Iodine contrast medium sensitizes cultured mammalian cells to X rays but not to gamma rays. Radiat Res 1980;84:144-8.
20. Watson C, Ge J, Cohen J, et al. High-throughput screening platform for engineered nanoparticle-mediated genotoxicity using CometChip technology. ACS Nano 2014;8:2118-33.
21. Mowat P, Mignot A, Rima W, et al. In vitro radiosensitizing effects of ultrasmall gadolinium based particles on tumour cells. J Nanosci Nanotechnol 2011;11:7833-9.
22. Miladi I, Aloy MT, Armandy E, et al. Combining ultrasmall gadolinium-based nanoparticles with photon irradiation overcomes radioresistance of head and neck squamous cell carcinoma. Nanomedicine 2015;11:247-57.
23. Porcel E, Liehn S, Remita H, et al. Platinum nanoparticles: a promising material for future cancer therapy? Nanotechnology 2010;21:85103.
24. Turk B, Turk V. Lysosomes as "suicide bags" in cell death: myth or reality? J Biol Chem 2009;284:21783-7.
25. Moser F, Hildenbrand G, Müller P, et al. Cellular Uptake of Gold Nanoparticles and Their Behavior as Labels for Localization Microscopy. Biophys J 2016;110:947-53.
26. Huo S, Jin S, Ma X, et al. Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry. ACS Nano 2014;8:5852-62.
27. Sevcik J, Falk M, Kleiblova P, et al. The BRCA1 alternative splicing variant Δ14-15 with an in-frame deletion of part of the regulatory serine-containing domain (SCD) impairs the DNA repair capacity in MCF-7 cells. Cell Signal 2012;24:1023-30.
28. Sevcik J, Falk M, Macurek L, et al. Expression of human BRCA1Δ17-19 alternative splicing variant with a truncated BRCT domain in MCF-7 cells results in impaired assembly of DNA repair complexes and aberrant DNA damage response. Cell Signal 2013;25:1186-93.

Cite this article as: Falk M. Nanodiamonds and nanoparticles as tumor cell radiosensitizers—promising results but an obscure mechanism of action. Ann Transl Med 2017;5(1):18. doi: 10.21037/atm.2016.12.62

