Therapeutic potential of carbon dots from *Lycium barbarum* in radiation-induced bone injury

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Comment on: Guo Z, Wang Z, Liu Y, *et al.* Carbon Dots from Lycium barbarum Attenuate Radiation-Induced Bone Injury by Inhibiting Senescence via METTL3/Clip3 in an m6A-Dependent Manner. ACS Appl Mater Interfaces 2023;15:20726-41.

Keywords: Carbon dots (CDs); osteoradionecrosis (ORN); osseointegration; RNA; senescence

Submitted Jan 21, 2024. Accepted for publication May 16, 2024. Published online May 30, 2024.
doi: 10.21037/atm-24-10
View this article at: https://dx.doi.org/10.21037/atm-24-10

Foreword

It would be a challenge to place this paper into one specific category; it encompasses a lot for one article, by providing a glimpse into a variety of topics, all interconnected but diverse. Even having a quite large supplemental material still requires from a reader—who might be a bioengineer or biochemistry major—to dig into the carbon dots (CDs) and RNA methylomics, and from a clinical scientist—to refresh their molecular knowledge (and insight into the herbal medicine!). We consider this to be a plus for any scientific publication to both enlighten the reader in the new findings as well as to make them wish to learn/read more. What is certain, this report is a must-read for a bone-centered biologist. Here we review this demanding article having mostly this type of audience in mind.

Problem

Radiotherapy alone or in combination with chemotherapy has been proven successful in cancer patients. As survival rates rise, the collateral consequences of radiation therapy become prominent; these include bone deterioration which leads to a wide range of skeletal abnormalities, including osteoradionecrosis (ORN) and pathological (low energy) fractures (1,2). Significant patient morbidity and increased mortality can thus arise from cancer treatment-related radiation and radiation-induced ORN (2,3). Conservative management of ORN includes local irrigation, systemic antibiotics, and hyperbaric oxygen therapy (3,4). ORN is akin to osteonecrosis of the jaw because of bisphosphonate treatment. For the advanced stage of ORN, the radical excision of the jaw with repair using a microvascular osteomyocutaneous free flap is crucial (5). Nevertheless, up to 43% of surgical procedures result in complications (6). Specifically for the jaws and dental implants, radiation therapy induces ORN and therefore significantly reduces the longevity of implants (7,8). Bottom line, at present there is no effective treatment for radiation-induced bone injury.

Proposed solution

In a research article, Guo and colleagues (9) report an important translational study on the potential therapeutic effect of CDs synthesized from *Lycium barbarum* against radiation-induced bone injury in a rat model of ORN. The group of the co-authors are all affiliated with the Department of Oromaxillofacial, Head & Neck Oncology and College of Stomatology, which explains its focus on mainly jaw bones. The main translational outcomes of this study are that CDs alleviated radiation-induced bone injury and stimulated osteogenesis surrounding titanium implants (9).

Nanoparticles

CDs are innovative nanoparticles with stable physicochemical properties and good biocompatibility that are receiving a lot of attention in biomedical applications (10,11). CDs have been shown to increase osteogenic activity (12). Shao and colleagues reported both tracking and the osteogenic potential of citric acid-based CDs in rat bone marrow mesenchymal stem cells (BMSCs). The authors demonstrated that the presence of CDs effectively facilitated osteogenic differentiation of the BMSCs via ROS-mediated MAPK pathway (13). Jin and colleagues reported ascorbic acid CDs which promote osteoblasts differentiation by activation of endoplasmic reticulum stress and PERK-eIF2α-ATF4 pathway by inducing calcium leakage in the endoplasmic reticulum of osteoblasts (14). Wan and colleagues reported dexamethasone CDs that can stimulate the bone immune microenvironment and further accelerate the differentiation of BMSCs to facilitate bone tissue healing (15). We conducted one study in our Musculoskeletal Genetics Laboratory where zebrafish were treated with nitrogen-doped CDs functionalized with hydroxyapatite nanoparticles (NCDs-HA) for up to 35 days after jaw resection surgery (16). Our findings demonstrated that NCDs-HA nanoparticles induced MC3T3-E1 osteoblast line differentiation and proliferation; ensuing micro-CT (μCT) analyses affirmed the ability of NCDs-HA nanoparticles to accelerate regeneration in a zebrafish jawbone (16). In their fundamental work, Guo and colleagues (9) went further; they explored the mechanism behind such remarkable benefits of the CDs.

Their CDs were synthesized from *Lycium barbarum*, a fruit (berry) used in traditional Chinese medicine for thousands of years. Among the effects of *L. barbarum* are antioxidant, antiaging, anti-inflammatory, and immunoregulatory effects. Guo *et al.* (9) synthesized CDs from *L. barbarum* using a hydrothermal strategy. Analysis using high-resolution transmission electron microscopy (HR TEM) showed that these CDs were well dispersed with an average diameter of 3.5 and 0.32 nm lattice spacing. The absolute fluorescence quantum yield of these CDs was up to 67%. The functional groups and the chemical structure of CDs were also validated by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectrometry.

Therapeutic effect

Rat model of ORN was established by mandibular first molar extraction after radiotherapy and the CDs were delivered by local injection (9). We remember that the study was performed by the oral and maxillofacial surgery experts, therefore osseointegration of dental titanium implants installed in irradiated bone was tested and—together with osteogenesis—shown to be promoted by CDs. The authors demonstrated that CDs exhibited excellent biocompatibility and promoted osteogenic differentiation of BMSCs more than adipogenic differentiation.

Following that, titanium implants were placed in the tibia of rats to see if CDs might stimulate osseointegration in irradiated bone. These *in vivo* experiments revealed that CDs enhanced trabecular bone formation around titanium implants of irradiated tibia, confirmed by μCT and histology examination. Furthermore, cell apoptosis analysis, *β*-galactosidase staining, quantitative polymerase chain reaction, and western blots provided evidence that CDs could attenuate the radiation-induced damage by lowering cellular senescence.

Radiation-induced senescence

Senescent cells appear to have dual roles in physiologic tissue homeostasis/repair as well as pathology. The beneficial role of senescent cells is recognized in embryonic skeletal development; senescent cells also possess an essential role in optimal wound healing (17). While there is substantial interest in the beneficial roles of senescent cells, much of cellular senescence research has been aimed at their roles in pathologies and emphasized their contributions to pathoetiology of various organs and tissues, including bone diseases (18-21).

A bone-aging expert would be familiar with the senescence-associated secretory phenotype (SASP) concept, which entered the field relatively recently (20); the clearance of senescent cells *in vivo* is proposed to be beneficial for reducing age-related bone pathology. Beyond many cellular (phenotypic) changes and metabolic alterations, the SASP phenomenon is marked by epigenetic rearrangements. Blocking the secretome of senescent cells and even trying to eliminate these cells is thus a valid therapeutical target to alleviate the radiation-induced bone injury.

And here comes into use the stated effect of *L. barbarum* fruit—anti-aging and anti-senescent agent.

Towards mechanism of action

Mechanistically, CDs cause one of the most frequent

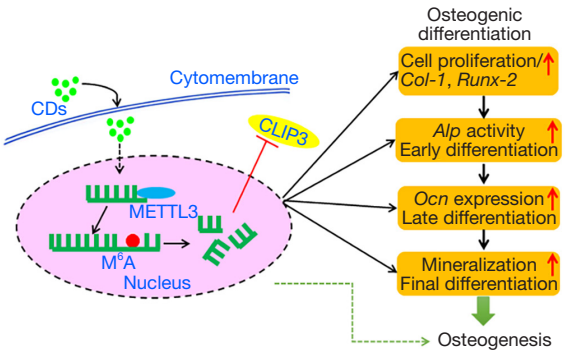

Figure 1 Schematic illustrating the therapeutic action of CDs in promoting osteogenic differentiation of irradiated BMSCs by regulation of METTL3/Clip3 in an m6A-dependent manner. *Col-1*, collagen 1; *Runx-2*, Runt-related transcription factor 2; *Alp*, alkaline phosphatase; *Ocn*, osteocalcin; CD, carbon dot; BMSC, bone marrow mesenchymal stem cell.

chemical modifications—methylations—occurring in mRNAs, by the addition of $-CH_3$ group in the N6-position of adenosine; the product is then called N6-methyladenosine (m^6A). As a fundamental mRNA modification, m^6A participates in various pathological processes (22). The dynamic m^6A level is known to be regulated by RNA modification enzymes, methyltransferases (also known as "writers"), and demethylases (so-called "erasers"); one of these enzymes is methyltransferase-like 3 (METTL3).

METTL3 emerges as a central regulator of BMSCs aging. Thus, Wu *et al.* [2018] had shown that *Mettl3* overexpression in mesenchymal stem cells (MSCs) protects the bone marrow niche in mice (23). Wu *et al.* [2020] reported that METTL3 inhibited premature aging of human MSCs of the kidney thanks to the regulatory role of METTL3 in age-related processes (24). Reduced METTL3 levels in prematurely senescent human MSCs parallel a decreased m^6A RNA methylation (25). The protective role of this enzyme is demonstrated by accelerated senescence in METTL3-knockout MSCs and the reversal of senescence upon METTL3 overexpression. Decreased METTL3 expression significantly downregulates m^6A RNA methylation levels in endometrium cells (22).

In Guo *et al.*'s work, the authors extracted the mRNA of BMSCs irradiated/non-treated and irradiated/treated with CDs for transcriptome-wide RNA sequencing and methylated RNA immunoprecipitation sequencing. They detected increased m^6A levels located in the 3-untranslated regions of mRNA coding for a protein called CAP-GLY domain containing linker protein 3 (CLIP3). Putative m^6A sites of *Clip3* mRNA were predicted by observed sequence-based m^6A modification sites. Subsequently, the increased enzyme METTL3 caused increased m^6A modification, which leads to enhanced degradation of *Clip3* mRNA and downregulated *Clip3* expression (see schematic presentation in *Figure 1*).

This study thus showed that CDs most probably elevate m^6A levels in irradiated MSCs by boosting METTL3 expression. As noted by the authors, the specific mechanisms underlying the process of how CDs promote METTL3 remain unclear. The authors (9) propose that the functional group located at the surface of CDs plays a role. As CDs from *L. barbarum* are rich in $-CH_3$, –OH, –NH, and other functional groups, the authors suggested that there could be an electrostatic attraction between positively charged CDs and protein (or subunit of) since most proteins possess a negative potential *in vivo*. They speculate that the interaction of functional groups of CDs and proteins may lead to a conformational change in *Clip3* mRNA.

Conclusions and significance

Taken together, CDs produced from *L. barbarum* alleviate bone injury inflicted by radiation therapy, by inhibiting cellular senescence via regulation of m^6A modification of *Clip3*. This important preclinical research therefore indicates that CDs mediate radiation-induced bone injury via the downregulation of *Clip3* expression (9).

Appraisal of the above findings reported by Guo *et al.* regarding the alleviation of radiation-induced bone injury should be considered with its merits and limitations (9). There might be alternative mechanisms for the CDs to alleviate bone injury. As Guo *et al.* note, "*the effect of CDs on other cell types might also participate in the therapeutical process; however, the major role was [in] BMSCs*". Also, there were suggestions for the effect of METTL3 on m^6A modification of other genes mRNA, such as *SIRT1* (22) or *MIS12* (24), therefore involving other signaling pathways rather than being downstream of the *Clip3*.

This pioneering study has provided an in-depth comprehension of the impact of CDs on radiation-induced bone injury. Such developments in the field of herbal CDs may provide promising prospects for the clinical management of diseases involving the METTL3/senescence pathway, extending beyond the realm of radiation-induced bone injury. Various research groups have reported and

created therapy agents and drug carriers in *in vitro* and *in vivo* models, however, the data is yet insufficient for clinical use. Guo *et al.* offered an approach for the management of radiation-induced bone injury, which is hardly achieved by other reported CDs. Clearly, more research studies are required to elucidate the therapeutic potential of CDs against radiation-induced bone injury and beyond, e.g., for drug-induced osteonecrosis of the jaw. There is a need for clinical trials, and the long-term toxicological consequences have yet to be discovered. Moreover, the pharmacokinetics and biodistribution of CDs are challenging because of their physiochemical characteristics, surface chemistry, and morphology. To acquire trust in the clinical applications of CDs in the management of radiation-induced bone injury, researchers must conduct repeated and long-term studies. Although such studies will necessitate significant additional effort, we are optimistic that these objectives can be met.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Annals of Translational Medicine*. The article has undergone external peer review.

Peer Review File: Available at https://atm.amegroups.com/article/view/10.21037/atm-24-10/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-24-10/coif). The authors have no conflicts of interest to declare

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Khajuria DK, Karasik D. Therapeutic potential of carbon dots from *Lycium barbarum* in radiation-induced bone injury. Ann Transl Med 2024;12(3):56. doi: 10.21037/atm-24-10