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Metalloporphyrins: Radioprotector and Radiosensitizer

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ABSTRACT

Cancer has become the leading cause of human death in the world, due to its uncontrolled and rapid proliferation properties. Radio and chemotherapy are used in the treatment of almost all types of cancer. They mostly act by increasing the production of reactive oxygen specious (ROS) and free radicals. ROS levels are believed to be higher in cancer cells compared to their normal counterparts this is suggested to be related to cancer cell growth, angiogenesis, and metastasis. Although the cure rate for many types of cancer would be increased by radiation dose escalation, balancing the potential for cure against the risk for normal tissue injury is a complex endeavor. Because normal tissue toxicity during radiotherapy and pathological conditions that include overproduction of unstable oxygen species, efforts are ongoing to develop new radioprotective drugs. Antioxidants is an example of co treatment which has potential to protect normal tissues against radiation-induced damage with increasing IR-induced damage for cancer. Since the discovery of superoxide dimutases (SODs), a family of metalloproteins, it has become clear that these enzymes provide an essential defense against the superoxide radical. Application of native SOD has been limited due to short half-lives, lack of cellular uptake and hypersensitivity. Metal-containing SOD mimetics have now emerged as being especially promising, they have been used for treatment of different kinds of diseases, as cancer. It is believed to protect cells from radiation damage by removing free radicals produced by irradiation. The objective of this review is to summarize the most current metalloporphyrin as an example of SODm in treatment of cancer and in combination with other radio and chemotherapy.

***Keywords:** SOD; Metalloporphyrin; Cancer; ROS.*

INTRODUCTION

Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. Cancer cells have four characteristics that distinguish them from normal cells, uncontrolled proliferation, dedifferentiation and loss of function, invasiveness and metastasis[1].

There are three types of treatment for lung cancer: chemical, radiation, and surgical treatments, alone or in combination depending on the stage at which the cancer is diagnosed as well as the biologic nature of the individual case. Most anticancer drugs acting as antiproliferative causing damage of DNA or function by elevation of ROS production and causing irreversible oxidative damage with the initiation of apoptosis[2,3].

Increased ROS could be exploited for therapeutic targeting of tumor tissue [4]. Because of heightened basal level of ROS, cancer cells may be more susceptible to further oxidative stress than normal cells because their endogenous antioxidant systems can be overwhelmed [5]. Diverse chemotherapeutic agents have been developed to kill tumor cells by amplifying oxidant stress, such as agents that directly generate ROS (elevating ROS levels) or ones that inhibit antioxidant enzymes (decreasing ROS scavenging potential) [6-8]. Radiotherapy can force/localize almost all radiation exclusively on tumors once precisely positioned, thus reducing possible toxicity to the surrounding normal tissues[9]. However, monotherapy of cancer often confronted with challenges and has limited success, as the tumor cells usually develop resistance to the agents used and tumors are usually genetically diverse [10, 11]. In addition, intrinsic radioresistance of cancer cells usually leads to recurrence or metastasis [12, 13]. Although increasing the irradiation intensity could improve the therapeutic effect, but there are difficulties in delivering high radiotherapy doses to the tumor due to potential toxicity of normal cells that are in the vicinity of the tumor being treated with radiation [14]. Using antioxidants in treatment abrogating ROS-signaling and suppressing tumor growth. Some studies suggested that antioxidant supplementation could sensitize the cancer cells to chemo- or radiotherapy, and at the same time, reduce the side effects of radiotherapy by protecting the normal cells. Although several radioprotectors and radiosensitizers have been tested, only one compound, amifostine, has been FDA approved as a radioprotector [15, 16]. Thus, it is important to investigate agents that can enhance tumor response to radiation while protecting the surrounding normal (non-cancerous) cells, this is makes a further improvement of radiotherapy for cancer challenging.

Superoxide dismutase (SOD) are the first line defense against $O_2^{\bullet-}$ in cells and tissues [17,18], It is believed that they may protect cells from radiation damage by removing free radicals produced by irradiation. In the presence of SOD, superoxide is dismutated to H_2O_2 and O2. H_2O_2 is then subsequently eliminated by catalase and glutathione peroxidase via water and oxygen [19, 20]. Three SODs have been identified in rodents and humans (SOD1–3) they are metalloproteins enzymes which include the manganese (Mn) enzyme SOD2, MnSOD is a nuclear-encoded and mitochondria-matrix-localized homotetrameric enzyme [21-23]. The copper-dependent (CuZnSOD) enzyme (SOD1) a cytoplasmic homodimer [22] that is localized in the cytoplasm, cystol, nucleus, and mitochondria intermembrane space [22, 24].

Many studies show a reduction in MnSOD expression in various types of cancer compared to normal tissues suggesting MnSOD acts as tumor suppressor. Conversely, other studies report an elevation in MnSOD expression in cancer and its association with cancer aggressiveness, growth, survival, and metastatic potential [22], implying that MnSOD supports progression of tumors to a more aggressive stage acting as an oncogene. SOD_2 may act as a tumor suppressor during the initial onset/proliferative stage of tumor initiation. During early stages of cancer, when MnSOD levels are low, MnSOD overexpression may suppress cancer growth through various mechanisms because of greater hydrogen peroxide flux [25], yet once the tumor progresses to a more aggressive and invasive phenotype, SOD_2 levels appear to positively correlate and contribute to enhanced metastatic behavior of cancer cells. At late stages of cancer progression, when cancer cells experience persistent oxidative stress [26-28], increased MnSOD expression may benefit cancer cells by the stimulation of metastasis [29-32].

It has been suggested that MnSOD, is a primary antioxidant enzyme, may function as a tumor suppressor for the following reasons: (a) many types of cultured tumor cells have low MnSOD activity compared to their normal counterparts [33-35]. (b) Mutations in the MnSOD gene and its regulatory sequence have been reported in several types of human cancer [36].(c) In addition, it has shown that overexpression of MnSOD reduces tumorigenicity and metastatic ability in a large number of experimental tumors in vitro and in vivo [33, 37-39]. Manipulation of native SOD enzyme for therapeutic purposes has been problematic and does not show efficacy due to its short half-life and large molecular weight (do not penetrate the blood–brain barrier) [40, 41]. Several low-molecular-mass SOD mimetics have been developed to overcome some of these limitations. Three groups of SOD mimetics have been developed to date and used in different models of oxidative stress injuries. These mimetics, including manganese (II) penta-azamacrocyclic complexes [42, 43], manganese (III) (salen) complexes [44-46], and manganese porphyrins [45, 47]. Metalloporphyrins are the popular classes of SODm, which consist of a carbon-based porphyrin ring and redox metal core. Mn-based metalloporphyrins complexes exhibit among the highest SODm activity [69]. Three lead manganese porphyrins compounds have been developed: Mn (III) meso-tetrakis (N-ethylpyridinium-2-yl) porphyrin (MnTE-2-PyP5+), Mn(III)meso-tetrakis(N-n-hexylpyridinium-2-yl)porphyrin (MnTnHex-2-PyP5+), Mn(III)meso-tetrakis(N-n-butoxyethylpyridinium-2-yl)porphyrin (MnTnBuOE-2-PyP5+). MnTE-2-PyP5+ was the first compound developed. Because of its hydrophilicity, a 5,000-fold more lipophilic analogue was developed with lengthened hexyl alkylpyridyl chains, MnTnHex-2- PyP5+.

Table 1. Properties of Mn (II)-based selective superoxide dismutase mimetics (e.g. M40403, GC4419)

- Manganese-containing biscyclohexylpyridine
- Catalytic activity equivalent to that of the native enzyme
- Penetrates cells and wide organ distribution
- Selective for superoxide (no interaction with other biologically relevant molecules, for example, nitric oxide, hydrogen peroxide, peroxynitrite)
- Stable in vivo: no loss of manganese and excreted intact
- Not deactivated by peroxynitrite
- Suitable pharmacological tool to dissect the role of superoxide in physiopathological conditions
- Pharmacologically efficacious in numerous animal models of disease

Its higher mitochondrial distribution and transfer across the blood–brain barrier (BBB) have been demonstrate [48,49]. Insertion of oxygen atoms into its hydrophobic chains resulted in the synthesis of MnTnBuOE-2-PyP5+, which showed less toxicity than MnTnHex-2-PyP5+ while maintaining high lipophilicity and redox-related performance [50]. The manganese (II) penta-azamacrocyclic complexes as M40403 and its enantiomer GC4419 had advantages over other SODm: equal catalytic activity to the native SOD, selectivity for dismutation superoxide and stable in vivo [51, 52] (**Table 1**).

The major objectives of this study are to summarize metalloporphyrin antioxidant effects on tumor response to radiation as well as its effect on normal cells.

Four powerful MnP-based SOD mimics were identified and explored in vitro and in vivo, starting from non-active and non-substituted SOD analog MnT-4-PyP+ [47], by adding methyl group forming plan compound MnTM-4-PyP5+ but due to its positive charge it binds to nucleic acid made it losing SOD activity [45]. Modification was done by replacing methyl with ethyl which enhanced lipophilicity and suppress association with nucleic acid producing MnTE-2-PyP5+, which had been studied the most for its safety [53]. Improvement in the bioavailability of Mn porphyrins by lengthening of the alkyl groups resulted in an increase in the lipophilicity. Several alkylpyridyl analogs were synthesized, of which hexyl porphyrin, MnTnHex-2-PyP5+, which has markedly increased ability to cross the blood/brain barrier and accumulate into mitochondria and has the optimally balanced bioavailability and toxicity [54]. The introduction of oxygen atoms into alkyl chains of MnTnHex-2-PyP5+ gave rise to a less toxic butoxyethyl analog, MnTnBuOE-2-PyP5+ [50, 55, 56], polar alkoxyalkyl chains suppressed the toxicity and reduced the liver accumulation while slightly reducing its accumulation within the brain, relative to MnTnHex-2-PyP5+.

MnTE-2-PyP5+

Mn (III) tetrakis (N-methyl-2-pyridyl) was the first discovered MnP, by going through the literature MnTE-2-PyP5+ showed antitumor effect by itself and in combination with radiotherapy which increasing killing effect of IR on cancer cells. Injection of breast tumor xenograft with 2mg/kg or 15 mg/kg of MnTE-2-PyP5+ subcutaneously as a single dose daily for 13 days[57], reduced many angiogenesis markers, including hypoxia-inducible factor 1a (HIF-1a) and vascular endothelial growth factor (VEGF), which are important for tumor growth. The cytotoxic effect of MnTE-2-PyP5+ was increased in other study, when it is combined with both IR and ascorbate in treatment of breast cancer [58]. In accord, pretreatment of PC3, DU145 and LNCaP prostate cancer cells with MnTE-2-PyP5+ using 1,10, or 30 μM then exposed them to 2 and 5Gy of IR causing reduction of cancer growth [59]. On the other hand, normal rat lungs were prolonged protected from IR-induced injury by the administration of MnTE-2-PyP5+ [60, 61]. Another study was confirming the protection effect of MnTE-2-PyP5+ after exposing rat rectum to 20-30Gy by the enhancement of the injury [62]. Oberley-Deegan et al. showed remarkable radioprotection of erectile function, prostate, and testes by MnTE-2-PyP5+ in a model where the low pelvic region of rat was irradiated [63-66].

MnTnHex-2-PyP+

Our second example of metalloporphyrin, which is more lipophilic as mentioned before. In vivo study was showing radio sensitization of glioblastoma xenograft mice after injection with of 1.6mg/kg of MnTnHex-2-PyP+ 24hr before IR, while IR was given 1Gy for 3 days, tumor volume was decreased significantly [67]. Treatment of 4T1 and B16 cells with 10 μM of MnTnHex-2-PyP+ combined with 4Gy IR resulted in a significant reduction in the viability and clonogenic cell survivability of both cell lines. Also, MnTnHex-2-PyP+ exacerbate apoptosis induced by IR and upregulated proapoptotic markers as BAX and Bim, showing high radio sensitization of the cancer cells [68]. In the same study, they confirmed the in vitro results by performing an in vivo xenograft experiment mice was implanted with cells and treated with 2mg/kg/day for 3 days then exposed to 5Gy IR daily for 3 days in combination with MnTnHex-2-PyP+, tumor growth was ameliorated compared to IR alone. Moreover, the induction of the survival signals by IR were reverted by the addition of MnTnHex-2-PyP+ showing reduction in phosphorylated p38, AKT, ERK and JNK. Chemosensetization was proved also after treatment of human mammary cells (MCF7 and MDA-MB-231) with 5 µM of MnTnHex-2-PyP+ (which was not toxic dose) combined with 0.1 µM doxorubicin, showing a significant elevation in ROS levels, reduction in cell migration with reduction in MDA-MB-231 cell invasion[69]. Protection normal tissue also is so important to be studied after proving its radio and chemo sensitization of cancer cells. In literature studies were detecting the protection effect of MnTnHex-2-PyP+. An animal experiment was showing powerful pulmonary radioprotectant in a rat study at a low dose of 0.05 mg/kg/day for 2 weeks, starting 2 h after whole thorax radiation [70].

MnTnBuOE-2-PyP+

In subsequent study, MnTnBuOE-2-PyP+ demonstrated glioblastoma tumor radio sensitizing effect in vitro [71]. Author showed chemo sensitization of glioblastoma xenograft to temozolimide or cisplatin after intraperitoneal injection of 5mg/kg/day of MnTnBuOE-2-PyP+ for 5 days, in addition, sensitized glioblastomas to either 10Gy RT ± temozolomide in flank tumor models [72]. Prostate cancer cells when treated with MnTnBuOE-2-PyP+, growth was inhibited significantly at the same time implanted cells to the mice`s prostate then exposing it to 2Gy IR daily for 5 days combined with 3 times injection of MnTnBuOE-2-PyP+ in the week, the tumor volume was decreased more than monotherapy [64]. Another study was showing different effect on normal vs. cancer colorectal fibroblasts, by treating them with IR 1Gy and 0.5µM MnTnBuOE-2-PyP+ the results were in accordance with the previous studies, inhibition of the viability and clonogenicity after monotherapy. In addition, the combination exacerbates IR-killing effect of cancer cell. On the other hand, combination reverted the IR-effect on normal cells which means radioprotection. The study was also continued by the addition of 5-flurouacil with IR and MnTnBuOE-2-PyP+, a significant decrease in tumor growth was observed compared to monotherapies[73]. Moreover, radioprotection of normal cells including mucositis, xerostomia, and fibrosis, and augmented the antitumor effect of radiation at the same time using different doses of IR in pre-clinical head and neck cancer model [74]. On the same line, MnTnBuOE-2-PyP+ had radioprotection to normal brain by protection of neurogenesis the effect was evaluated after 3 to 4 months after single 10 or 8Gy RT to the brain, respectively. MnTnBuOE-2-PyP+ was given subcutaneous twice daily for a month at 1.5 mg/kg, starting 1 week before 8Gy RT and continuing once daily for another month at 0.5 mg/kg [71]. MnTnBuOE-2-PyP+ ameliorated RT-induced loss of axons and motor efficiency after 3months by testing neurocognitive activity. The follow-up study, MnP was given sc twice weekly for a week before 10 Gy RT at 3 mg/kg, and continued twice weekly for 4 months at 0.5 mg/kg [72], cisplatin was injected intraperitoneal once at 6 mg/kg 24 h before RT, and temozolomide was administered ip at 5 mg/kg for 5 days starting at 24 h before RT. Protection of neurogenesis was seen when MnTnBuOE-2-PyP5+ was given sc a week before and a week after 5Gy cranial RT. In another mouse study, mice received 9Gy RT to oral cavity and salivary glands and 6 mg/kg cisplatin via ip injection. MnP dosing started at 24 h before RT at 0.2, 0.6, and 2 mg/kg, and continued three times per week at 0.1, 0.3, and 1 mg/kg, respectively. Stimulated saliva production and salivary gland fibrosis were quantified post-RT. MnP protected normal tissue against RT-induced damage at early and late time points. Cisplatin did not interfere with MnP induced radioprotection and MnP did not interfere with RT/ cisplatin-mediated tumor growth [75]. In a cisplatin experiment, MnP sc injections started at 24 h before RT/cisplatin at 0.6mg/kg and continued at 0.3mg/kg three times per week for 5 weeks. MnP lessened cisplatin-induced mouth ulceration, bleeding, and moist desquamation in the irradiated areas, and protected against RT/cisplatin-induced weight loss.

GC4419

As an example of Mn (II) pentaazamacrocycle complex: is a__new high potency SOD mimetic characterized by high cell permeability, high stability and high selectivity towards $O_2^{\bullet-}$. Combination therapy with GC4419 has shown great promise in cancer treatment for the reduction of IR-induced side effects. Reduction of oral mucositis incidence and severity was observed in phase IIb clinical trials, and a phase III trials is now underway [23, 24]. Therefore, it is of great importance to characterize the differential effects of GC4419 on cancer cells and normal cells as well as its effects in selective sensitization of cancer cells to IR and the underlying mechanisms of this process. In our previous study, we measured the processes of IR-induced radical generation, and then the subsequent $O_2^{\bullet-}$ generation that occurs, in normal and lung cancer cells, as well as the effect of GC4419 on these processes. With EPR spin-trapping, we observe that GC4419 does not alter IR-mediated hydroxyl radical (·OH) generation; however, GC4419 effectively quenches the elevated levels of $O_2^{\bullet-}$ generation detected in cancer cells before and after IR. GC4419 was showing anti-proliferative effect after treatment of lung cancer cells (A549 and H1299) for 48hr (5, 10 and 20 µM) for 48hr as monotherapy in a dose dependent manner without any effect on the normal lung cells (Beas 2b). Co-treatment of GC4419 and IR (2 and 8Gy) ameliorated IR-killing effect of cancer cells, while it protected normal cells from IR harmful effect significantly. In accordance with our results, a recent study on lung cancer xenograft model showed that GC4419 mitigated IR-induced lung fibrosis and enhanced the response of cancer cells to radiation. Moreover, in combination with pharmacological ascorbate, GC4419 had a synergistic effect on irradiation-induced cancer cell killing [76]. Furthermore, the observed protective effect of GC4419 on Beas 2b is in line with a cohort study in patients with oral cavity or oropharyngeal cancer in which GC4419 reduced the frequency and duration of irradiation-induced oral mucositis [77]. Also, we performed further studies to investigate characterize by which GC4419 enhanced cancer cell death. IR-mediated tumor growth inhibition is linked to its ability to cleave DNA and induce apoptosis. At 48 hours post IR, it was observed that GC4419 (10µM) increased DNA fragmentation in both the cancer cells lines by 20 – 40 % compared to IR alone, while decreasing this in the control cells by over 50%. Again, GC4419 was seen to confer protection of the normal cells from IR while sensitizing the cancer cells to injury. The increased DNA fragmentation is consistent with apoptotic cell death. In addition, caspase 3 activity was increased after treatment of cancer cells with 10 uM GC4419 and 2Gy, with reduction in its activity in normal cells compared to IR. Thus, GC4419 is a promising therapeutic for cancer treatment by enhancing the efficacy of IR in cancer cell killing while decreasing IR-induced toxicity to normal tissues.

CONCLUSION

In conclusion, usage of antioxidant in treatment of cancer in combination with radio and chemotherapy is showing good impact by increasing IR-killing effect in cancer with protection of the normal tissue adjacent to cancer tissues, one of the effective examples is GC4419 which under clinical investigations stage III.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Rang, H. P.; Dale, M. M.; Ritter, J.; Flower, R. J.; Henderson, G. *Rang and Dale Pharmacology*. 7th edition. Edinburgh ; New York : Elsevier/Churchill Livingstone, **2012**.
2. Kong, Q.; K.O. Lillehei. Antioxidant inhibitors for cancer therapy. *Med. Hypotheses* **1998**, *51* (5), 405-409.
3. Wondrak, G.T. Redox-directed cancer therapeutics: molecular mechanisms and opportunities. *Antioxid. Redox. Signal.* **2009**, *11* (12), 3013-3069.
4. Liu, J.; Wang, Z. Increased Oxidative Stress as a Selective Anticancer Therapy. *Oxid. Med. Cell Longev.*, **2015**, *2015*, 294303.
5. Trachootham, D., Lu, W.; M.A. Ogasawara, MA.; Nilsa, RD.; P. Huang, P. Redox regulation of cell survival. *Antioxid. Redox. Signal*, **2008**, *10* (8), 1343-1374.
6. Schumacker, P.T. Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer Cell* **2006**. *10* (3), 175-176.
7. Jing, Y., J. Dai, Chalmers-Redman, R.M.; Tatton, W.G.; Waxman, S. Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. *Blood* **1999**, *94* (6), 2102-2111.
8. Miyajima, A., J. Nakashima, K. Yoshioka, M. Tachibana, H. Tazaki, and M. Murai. Role of reactive oxygen species in cis-dichlorodiammineplatinum-induced cytotoxicity on bladder cancer cells. *Br. J. Cancer* **1997**. *76* (2) 206-210.
9. O'Sullivan, B., A.M. Griffin, C.I. Dickie, M.B. Sharpe, P.W. Chung, C.N. Catton, P.C. Ferguson, J.S. Wunder, B.M. Deheshi, L.M. White, R.A. Kandel, D.A. Jaffray, R.S. Bell. Phase 2 study of preoperative image-guided intensity-modulated radiation therapy to reduce wound and combined modality morbidities in lower extremity soft tissue sarcoma. *Cancer* **2013**, *119* (10), 1878-1884.
10. Kaiser, J. Combining targeted drugs to stop resistant tumors. *Science*, **2011**. *331*(6024), 1542-1545.
11. Li, J., S. Bonifati, G. Hristov, T. Marttila, S. Valmary-Degano, S. Stanzel, M. Schnolzer, C. Mougin, M. Aprahamian, S.P. Grekova, Z. Raykov, J. Rommelaere, and A. Marchini. Synergistic combination of valproic acid and oncolytic parvovirus H-1PV as a potential therapy against cervical and pancreatic carcinomas. *EMBO Mol. Med.*, **2013**, *5* (10), 1537-1555.
12. Marie-Egyptienne, D.T., I. Lohse, and R.P. Hill. Cancer stem cells, the epithelial to mesenchymal transition (EMT) and radioresistance: potential role of hypoxia. *Cancer Lett*, **2013**, *341* (1), 63-72.
13. Chang, L., P. Graham, J. Hao, J. Bucci, D. Malouf, D. Gillatt, and Y. Li. Proteomics discovery of radioresistant cancer biomarkers for radiotherapy. *Cancer Lett*, **2015**. *369* (2), 289-297.
14. Cannon, D.M., M.P. Mehta, J.B. Adkison, D. Khuntia, A.M. Traynor, W.A. Tome, R.J. Chappell, R. Tolakanahalli, P. Mohindra, S.M. Bentzen, and

G.M. Cannon. Dose-limiting toxicity after hypofractionated dose-escalated radiotherapy in non-small-cell lung cancer. *J. Clin. Oncol.* **2013**. *31* (34), 4343-4348.

15. Andreassen, C.N., C. Grau, and J.C. Lindegaard. Chemical radioprotection: a critical review of amifostine as a cytoprotector in radiotherapy. *Semin. Radiat. Oncol.*, **2003**. *13* (1), 62-72.
16. Kuruba, V. and P. Gollapalli. Natural radioprotectors and their impact on cancer drug discovery. Radiat. Oncol. J., 2018. 36 (4), 265-275.
17. Huang, T.T., Y. Zou, and R. Corniola. Oxidative stress and adult neurogenesis--effects of radiation and superoxide dismutase deficiency. *Semin. Cell Dev. Biol.*, **2012**. *23* (7), 738-744.
18. Kam, W.W.; R.B. Banati. Effects of ionizing radiation on mitochondria. Free Radic Biol Med, **2013**. 65, 607-619.
19. Quinlan, T., S. Spivack, and B.T. Mossman, Regulation of antioxidant enzymes in lung after oxidant injury. *Environ. Health Perspect.*, **1994**. *102* Suppl 2, 79-87.
20. Tsan, M.F., Superoxide dismutase and pulmonary oxygen toxicity. *Proc. Soc. Exp. Biol. Med.*, **1997**. *214* (2), 107-113.
21. McCord, J.M. and I. Fridovich, Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.*, **1969**. *244* (22), 6049-6055.
22. Holley, A.K., L. Miao, D.K. St Clair, and W.H. St Clair, Redox-modulated phenomena and radiation therapy: the central role of superoxide dismutases. *Antioxid. Redox. Signal*, **2014**. *20* (10), 1567-1589.
23. Fukai, T. and M. Ushio-Fukai, Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid. Redox. Signal*, **2011**. 15(6), 1583-1606.
24. Field, L.S., Y. Furukawa, T.V. O'Halloran, and V.C. Culotta, Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. *J. Biol. Chem.*, **2003**. *278* (30), 28052-28059.
25. Li, S., T. Yan, J.Q. Yang, T.D. Oberley, and L.W. Oberley, The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res*, **2000**. 60(14), 3927-3939.
26. Biaglow, J.E. and R.A. Miller, The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. *Cancer Biol Ther*, 2005. 4 (1), 6-13.
27. Powis, G. and D.L. Kirkpatrick, Thioredoxin signaling as a target for cancer therapy. *Curr. Opin. Pharmacol*, **2007**. 7 (4), 392-397.
28. Pani, G., T. Galeotti, and P. Chiarugi, Metastasis: cancer cell's escape from oxidative stress. *Cancer Metastasis Rev.*, **2010**. *29* (2), 351-378.
29. Connor, K.M., N. Hempel, K.K. Nelson, G. Dabiri, A. Gamarra, J. Belarmino, L. Van De Water, B.M. Mian, and J.A. Melendez, Manganese superoxide dismutase enhances the invasive and migratory activity of tumor cells. *Cancer Res.*, **2007**. *67* (21), 10260-10677.
30. Hempel, N., P.M. Carrico, and J.A. Melendez, Manganese superoxide dismutase (Sod2) and redox-control of signaling events that drive metastasis. *Anticancer Agents Med. Chem.*, **2011**. *11* (2), 191-201.
31. Holley, A.K., K.K. Kiningham, D.R. Spitz, D.P. Edwards, J.T. Jenkins, and M.R. Moore, Progestin stimulation of manganese superoxide dismutase and invasive properties in T47D human breast cancer cells. *J. Steroid Biochem. Mol. Biol*, **2009**. *117* (1-3), 23-30.
32. Nelson, K.K., A.C. Ranganathan, J. Mansouri, A.M. Rodriguez, K.M. Providence, J.L. Rutter, K. Pumiglia, J.A. Bennett, and J.A. Melendez, Elevated sod2 activity augments matrix metalloproteinase expression: evidence for the involvement of endogenous hydrogen peroxide in regulating metastasis. *Clin. Cancer Res*, **2003**. *9* (1), 424-32.
33. Oberley, L.W. and G.R. Buettner, Role of superoxide dismutase in cancer: a review. *Cancer Res*, **1979**. *39* (4), 1141-1149.
34. St Clair, D.K. and L.W. Oberley, Manganese superoxide dismutase expression in human cancer cells: a possible role of mRNA processing. *Free Radic. Res. Commun.*, **1991**. 12-13 Pt 2, 771-778.
35. Sun, Y., L.W. Oberley, T.D. Oberley, J.H. Elwell, and E. Sierra-Rivera, Lowered antioxidant enzymes in spontaneously transformed embryonic mouse liver cells in culture. *Carcinogenesis*, **1993**. *14* (7), 1457-1463.
36. Xu, Y., A. Krishnan, X.S. Wan, H. Majima, C.C. Yeh, G. Ludewig, E.J. Kasarskis, and D.K. St Clair, Mutations in the promoter reveal a cause for the reduced expression of the human manganese superoxide dismutase gene in cancer cells. *Oncogene*, **1999**. *18* (1), 93-102.
37. Zhong, W., L.W. Oberley, T.D. Oberley, and D.K. St Clair, Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene*, **1997**. *14* (4), 481-490.
38. Church, S.L., J.W. Grant, L.A. Ridnour, L.W. Oberley, P.E. Swanson, P.S. Meltzer, and J.M. Trent, Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc. Natl. Acad. Sci. USA*, **1993**. *90* (7), 3113-3117.

39. Urano, M., M. Kuroda, R. Reynolds, T.D. Oberley, and D.K. St Clair, Expression of manganese superoxide dismutase reduces tumor control radiation dose: gene-radiotherapy. *Cancer Res.*, **1995**. *55* (12), 2490-2493.
40. Somack, R., M.G. Saifer, and L.D. Williams, Preparation of long-acting superoxide dismutase using high molecular weight polyethylene glycol (41,000-72,000 daltons). *Free Radic. Res. Commun.*, **1991**. 12-13 Pt 2, 553-62.
41. DiGregorio, K.A., E.V. Cilento, and R.C. Lantz, A kinetic model of superoxide production from single pulmonary alveolar macrophages. *Am. J. Physiol.*, **1989**. *256* (2 Pt 1), C405-12.
42. Riley, D.P., Manganese macrocyclic ligand complexes as mimics of superoxide dismutase. *J. Am. Chem. Soc.*, **1994**, *116*, 387.
43. Salvemini, D., Z.Q. Wang, J.L. Zweier, A. Samouilov, H. Macarthur, T.P. Misko, M.G. Currie, S. Cuzzocrea, J.A. Sikorski, and D.P. Riley, A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science*, **1999**. *286* (5438), 304-306.
44. Baker, K., C.B. Marcus, K. Huffman, H. Kruk, B. Malfroy, and S.R. Doctrow, Synthetic combined superoxide dismutase/catalase mimetics are protective as a delayed treatment in a rat stroke model: a key role for reactive oxygen species in ischemic brain injury. *J. Pharmacol. Exp. Ther.*, **1998**. *284* (1), 215-221.
45. Batinic-Haberle, I., L. Benov, I. Spasojevic, and I. Fridovich, The ortho effect makes manganese(III) meso-tetrakis(N-methylpyridinium-2-yl)porphyrin a powerful and potentially useful superoxide dismutase mimic. *J. Biol. Chem.*, **1998**. *273* (38), 24521-2458.
46. Melov, S., J. Ravenscroft, S. Malik, M.S. Gill, D.W. Walker, P.E. Clayton, D.C. Wallace, B. Malfroy, S.R. Doctrow, and G.J. Lithgow, Extension of life-span with superoxide dismutase/catalase mimetics. *Science*, **2000**. *289* (5484), 1567-1569.
47. Spasojevic I , B.-H.I., Manganese(III) complexes with porphyrins and related compounds as catalytic scavengers of superoxide. *Inorg. Chim. Acta.*, **2002**. *317*, 230.
48. Aitken, J.B., E.L. Shearer, N.M. Giles, B. Lai, S. Vogt, J.S. Reboucas, I. Batinic-Haberle, P.A. Lay, and G.I. Giles, Intracellular targeting and pharmacological activity of the superoxide dismutase mimics MnTE-2-PyP5+ and MnTnHex-2-PyP5+ regulated by their porphyrin ring substituents. *Inorg .Chem.*, **2013**. *52* (8), 4121-413.
49. Weitner, T., I. Kos, H. Sheng, A. Tovmasyan, J.S. Reboucas, P. Fan, D.S. Warner, Z. Vujaskovic, I. Batinic-Haberle, and I. Spasojevic, Comprehensive pharmacokinetic studies and oral bioavailability of two Mn porphyrin-based SOD mimics, MnTE-2-PyP5+ and MnTnHex-2-PyP5+. *Free Radic. Biol. Med.*, **2013**. *58*, 73-80.
50. Rajic, Z., A. Tovmasyan, I. Spasojevic, H. Sheng, M. Lu, A.M. Li, E.B. Gralla, D.S. Warner, L. Benov, and I. Batinic-Haberle, A new SOD mimic, Mn(III) ortho N-butoxyethylpyridylporphyrin, combines superb potency and lipophilicity with low toxicity. *Free Radic Biol. Med.*, **2012**. *52* (9), 1828-1834.
51. Batinic-Haberle, I., J.S. Reboucas, and I. Spasojevic, Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential. *Antioxid. Redox Signal*, **2010**. *13* (6), 877-918.
52. Muscoli, C., S. Cuzzocrea, D.P. Riley, J.L. Zweier, C. Thiemermann, Z.Q. Wang, and D. Salvemini, On the selectivity of superoxide dismutase mimetics and its importance in pharmacological studies. *Br. J. Pharmacol.*, **2003**. *140* (3), 445-460.
53. Gad, S.C., D.W. Sullivan, Jr., J.D. Crapo, and C.B. Spainhour, A nonclinical safety assessment of MnTE-2-PyP, a manganese porphyrin. *Int. J. Toxicol*, **2013**. *32* (4), 274-287.
54. Batinic´-Haberle I, S.I., Stevens RD, Hambright P, and a.F. I., Manganese(III) meso-tetrakis(ortho-Nalkylpyridyl) porphyrins. Synthesis, characterization, and catalysis of O2_- dismutation. *J. Chem. Soc. Dalton Trans*, **2002**, *13*, 2689–2696.
55. Gad, S.C., D.W. Sullivan, Jr., I. Spasojevic, C.V. Mujer, C.B. Spainhour, and J.D. Crapo, Nonclinical Safety and Toxicokinetics of MnTnBuOE-2-PyP5+ (BMX-001). *Int. J. Toxicol.*, **2016**. *35* (4), 438-453.
56. Leu, D., I. Spasojevic, H. Nguyen, B. Deng, A. Tovmasyan, T. Weitner, R.S. Sampaio, I. Batinic-Haberle, and T.T. Huang, CNS bioavailability and radiation protection of normal hippocampal neurogenesis by a lipophilic Mn porphyrin-based superoxide dismutase mimic, MnTnBuOE-2-PyP(5). *Redox. Biol.*, **2017**. *12*, 864-871.
57. Rabbani, Z.N., I. Spasojevic, X. Zhang, B.J. Moeller, S. Haberle, J. Vasquez-Vivar, M.W. Dewhirst, Z. Vujaskovic, and I. Batinic-Haberle, Antiangiogenic action of redox-modulating Mn(III) meso-tetrakis(N-ethylpyridinium-2-yl)porphyrin, MnTE-2-PyP(5+), via suppression of oxidative stress in a mouse model of breast tumor. *Free Radic. Biol. Med.*, **2009**. *47* (7), 992-1004.
58. Tovmasyan, A., R.S. Sampaio, M.K. Boss, J.C. Bueno-Janice, B.H. Bader, M. Thomas, J.S. Reboucas, M. Orr, J.D. Chandler, Y.M. Go, D.P. Jones, T.N. Venkatraman, S. Haberle, N. Kyui, C.D. Lascola, M.W. Dewhirst, I. Spasojevic, L. Benov, and I. Batinic-Haberle, Anticancer therapeutic potential of Mn porphyrin/ascorbate system. *Free Radic. Biol. Med.*, **2015**. *89*, 1231-1247.
59. Tong, Q., M.R. Weaver, E.A. Kosmacek, B.P. O'Connor, L. Harmacek, S. Venkataraman, R.E.

Oberley-Deegan, MnTE-2-PyP reduces prostate cancer growth and metastasis by suppressing p300 activity and p300/HIF-1/CREB binding to the promoter region of the PAI-1 gene. *Free Radic. Biol. Med*, **2016**. *94*, 185-194.

60. Vujaskovic, Z., I. Batinic-Haberle, Z.N. Rabbani, Q.F. Feng, S.K. Kang, I. Spasojevic, T.V. Samulski, I. Fridovich, M.W. Dewhirst, and M.S. Anscher, A small molecular weight catalytic metalloporphyrin antioxidant with superoxide dismutase (SOD) mimetic properties protects lungs from radiation-induced injury. *Free Radic. Biol. Med.*, **2002**. *33* (6), 857-863.
61. Gauter-Fleckenstein, B., K. Fleckenstein, K. Owzar, C. Jiang, I. Batinic-Haberle, and Z. Vujaskovic, Comparison of two Mn porphyrin-based mimics of superoxide dismutase in pulmonary radioprotection. *Free Radic. Biol. Med.*, **2008**. *44* (6), 982-989.
62. Archambeau, J.O., A. Tovmasyan, R.D. Pearlstein, J.D. Crapo, and I. Batinic-Haberle, Superoxide dismutase mimic, MnTE-2-PyP(5+) ameliorates acute and chronic proctitis following focal proton irradiation of the rat rectum. *Redox Biol.*, **2013**. 1, 599-607.
63. Chatterjee, A., E.A. Kosmacek, and R.E. Oberley-Deegan, MnTE-2-PyP Treatment, or NOX4 Inhibition, Protects against Radiation-Induced Damage in Mouse Primary Prostate Fibroblasts by Inhibiting the TGF-Beta 1 Signaling Pathway. *Radiat. Res*, **2017**. *187* (3), 367-381.
64. Chatterjee, A., Y. Zhu, Q. Tong, E.A. Kosmacek, E.Z. Lichter, and R.E. Oberley-Deegan, The Addition of Manganese Porphyrins during Radiation Inhibits Prostate Cancer Growth and Simultaneously Protects Normal Prostate Tissue from Radiation Damage. *Antioxidants* (Basel), **2018**. 7 (1).
65. Oberley-Deegan, R.E., J.J. Steffan, K.O. Rove, K.M. Pate, M.W. Weaver, I. Spasojevic, B. Frederick, D. Raben, R.B. Meacham, J.D. Crapo, and H.K. Koul, The antioxidant, MnTE-2-PyP, prevents side-effects incurred by prostate cancer irradiation. *PLoS One*, **2012**. *7* (9), e44178.
66. Shrishrimal, S., E.A. Kosmacek, A. Chatterjee, M.J. Tyson, and R.E. Oberley-Deegan, The SOD Mimic, MnTE-2-PyP, Protects from Chronic Fibrosis and Inflammation in Irradiated Normal Pelvic Tissues. *Antioxidants* (Basel), **2017**, *6* (4).
67. Batinic-Haberle, I., A. Tovmasyan, E.R. Roberts, Z. Vujaskovic, K.W. Leong, and I. Spasojevic, SOD therapeutics: latest insights into their structure-activity relationships and impact on the cellular redox-based signaling pathways. *Antioxid Redox Signal*, **2014**. *20* (15), 2372-415.
68. Shin, S.W., C. Choi, G.H. Lee, A. Son, S.H. Kim, H.C. Park, I. Batinic-Haberle, and W. Park, Mechanism of the Antitumor and Radiosensitizing Effects of a Manganese Porphyrin, MnHex-2-PyP. *Antioxid .Redox Signal*, **2017**. 27 (14), 1067-1082.
69. Florido, A., N. Saraiva, S. Cerqueira, N. Almeida, M. Parsons, I. Batinic-Haberle, J.P. Miranda, J.G. Costa, G. Carrara, M. Castro, N.G. Oliveira, and A.S. Fernandes, The manganese(III) porphyrin MnTnHex-2-PyP(5+) modulates intracellular ROS and breast cancer cell migration: Impact on doxorubicin-treated cells. *Redox Bio.l*, **2019**. 20, 367-378.
70. Gauter-Fleckenstein, B., J.S. Reboucas, K. Fleckenstein, A. Tovmasyan, K. Owzar, C. Jiang, I. Batinic-Haberle, and Z. Vujaskovic, Robust rat pulmonary radioprotection by a lipophilic Mn N-alkylpyridylporphyrin, MnTnHex-2-PyP(5+). *Redox Biol*, **2014**. 2, 400-410.
71. Weitzel, D.H., A. Tovmasyan, K.A. Ashcraft, Z. Rajic, T. Weitner, C. Liu, W. Li, A.F. Buckley, M.R. Prasad, K.H. Young, R.M. Rodriguiz, W.C. Wetsel, K.B. Peters, I. Spasojevic, J.E. Herndon, 2nd, I. Batinic-Haberle, and M.W. Dewhirst, Radioprotection of the brain white matter by Mn(III) n-Butoxyethylpyridylporphyrin-based superoxide dismutase mimic MnTnBuOE-2-PyP5+. *Mol Cancer Ther.*, **2015**. 14 (1), 70-79.
72. Weitzel, D.H., A. Tovmasyan, K.A. Ashcraft, A. Boico, S.R. Birer, K. Roy Choudhury, J. Herndon, 2nd, R.M. Rodriguiz, W.C. Wetsel, K.B. Peters, I. Spasojevic, I. Batinic-Haberle, and M.W. Dewhirst, Neurobehavioral radiation mitigation to standard brain cancer therapy regimens by Mn(III) n-butoxyethylpyridylporphyrin-based redox modifier. *Environ. Mo.l Mutagen*, **2016**. 57 (5), 372-381.
73. Kosmacek, E.A., A. Chatterjee, Q. Tong, C. Lin, and R.E. Oberley-Deegan, MnTnBuOE-2-PyP protects normal colorectal fibroblasts from radiation damage and simultaneously enhances radio/chemotherapeutic killing of colorectal cancer cells. *Oncotarget*, **2016**. 7 (23), 34532-34545.
74. Ashcraft, K.A., M.K. Boss, A. Tovmasyan, K. Roy Choudhury, A.N. Fontanella, K.H. Young, G.M. Palmer, S.R. Birer, C.D. Landon, W. Park, S.K. Das, T. Weitner, H. Sheng, D.S. Warner, D.M. Brizel, I. Spasojevic, I. Batinic-Haberle, and M.W. Dewhirst, Novel Manganese-Porphyrin Superoxide Dismutase-Mimetic Widens the Therapeutic Margin in a Preclinical Head and Neck Cancer Model. *Int. J. Radiat. Oncol. Biol. Phys.*, **2015**. *93* (4), 892-900.
75. Birer, S.R., C.T. Lee, K.R. Choudhury, K.H. Young, I. Spasojevic, I. Batinic-Haberle, J.D. Crapo, M.W. Dewhirst, and K.A. Ashcraft, Inhibition of the Continuum of Radiation-Induced Normal Tissue Injury by a Redox-Active Mn Porphyrin. *Radiat Res*, **2017**. *188* (1), 94-104.

76. Heer, C.D., A.B. Davis, D.B. Riffe, B.A. Wagner, K.C. Falls, B.G. Allen, G.R. Buettner, R.A. Beardsley, D.P. Riley, and D.R. Spitz, Superoxide Dismutase Mimetic GC4419 Enhances the Oxidation of Pharmacological Ascorbate and Its Anticancer Effects in an H(2)O(2)-Dependent Manner. *Antioxidants* (Basel), **2018**. *7* (1).
77. Anderson, C.M., S.T. Sonis, C.M. Lee, D. Adkins, B.G. Allen, W. Sun, S.S. Agarwala, M.L. Venigalla, Y. Chen, W. Zhen, D.R. Mould, J.T. Holmlund, J.M. Brill, and J.M. Buatti, Phase 1b/2a Trial of the Superoxide Dismutase Mimetic GC4419 to Reduce Chemoradiotherapy-Induced Oral Mucositis in Patients With Oral Cavity or Oropharyngeal Carcinoma. *Int J Radiat Oncol Biol Phys*, **2018**. 100 (2), 427-435.