The Phospholipid Ether Analog CLR127 Delays Radiation-induced dsDNA Damage Repair in Pediatric and Adult Solid Tumors

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Summary

Exposure of normal tissues to radiation is a major limitation in cancer radiotherapy, with significant concomitant particularly in the pediatric setting. Increasing the selective anticancer effect of radiation on malignant cells utilizing cancer-targeted drugs could provide an option to lessen adverse radiation effects on healthy tissue. We investigated the radiosensitizing properties of 19-(p-isopropyl)octadecylphosphocholine (CLR127), a clinical-grade phospholipid ether analog with selective sequestration in cancer cells, in pediatic and adult human solid tumor lines. We investigated mechanisms responsible for the radiosensitizing properties of CLR127. Western blotting of tumor cell lysates indicated increased accumulation and prolonged persistence of 53BP1 in irradiated tumor cells pretreated with CLR127 compared to non-treated controls. Expression of select markers of homologous recombination (HR) and non-homologous end joining (NHEJ) DNA damage repair pathways were markedly decreased and significantly delayed in CLR127 treated tumor cells after exposure to irradiation compared to non-CLR127 treated controls. CLR127 treatment also markedly decreased the activation of p53 after irradiation in p53-functional rhabdomyosarcoma cells compared to cells treated with radiation alone.

Background

Preliminary data in our laboratory using a fluorescently-labeled analog indicated that human cancer cell uptake and retain 6-10 fold more CLR127 when compared to normal primary cells. In vitro, the clongenic survival of tumor cells after irradiation was significantly decreased when cells were pretreated with CLR127. This radiosensitizing effect was detected also in vivo, in xenograft-bearing mice treated with CLR127 during fractionated radiation, resulting in decreased tumor volumes and markedly delayed tumor growth in CLR127 -treated mice compared to controls.

CLR127-2BODIPY is selectively taken up by cancer cell lines

Objective

We investigated mechanisms responsible for the radiosensitizing properties of CLR127 by assessing the expression of homologous and non-homologous end-joining DNA damage repair pathways.

Results

CLR127 sensitizes cancer cell lines to radiation

We found that CLR127 sensitizes cancer cells to radiation as shown in Figure 1. Figure 2 shows that CLR127 enhances radiosensitivity of tumor cells. Our data (Figure 3) indicated that CLR127 increases radiation response in human tumor xenografts. Figure 4 shows that combing CLR127 and radiation delays DNA damage repair.

Conclusions

• In cancer cells treated with CLR127, p16INK4A persists at higher levels 24 hours after irradiation. Prolonged dsDNA damage signaling may fail to reflect a change of dsDNA damage repair.
• CLR127 treatment of cancer cells reduces the level, or slows down the kinetics of, BARD1 phosphorylation at Ser1524 after irradiation, suggesting that CLR127 interferes with downstream dsDNA damage signaling towards efficient HR, forcing a switch to NHEJ.
• CLR127 treatment of cancer cells with active p53 protein results in a decreased or delayed phosphorylation of p53 after irradiation, suggesting that CLR127 may induce G1 arrest checkpoint failure following irradiation.
• CLR127 treatment of cancer cells does not affect the level of activation of the damage sensors ATM, ATR and DNA-PK, suggesting that the CLR127 effect is limited to downstream signaling.
• Previous data obtained by our laboratory indicated that in neuroblastoma, CLR127 decreases the level of Akt phosphorylation at Ser 473, a site specific for DNA damage signaling.
• Collectively, our data suggest that CLR127-mediated radiosensitization of cancer cells may be based on prevention or delay of dsDNA damage downstream signaling in multiple pathways, possibly orienting the cells towards dsDNA damage repair failure, switch HR to C-NHEJ, G1 checkpoint failure and apoptosis failure, altogether resulting in cell death following irradiation.

Acknowledgements

This work was supported in part by NIH R21CA198392-01, NHLIHD R01HL145267 (to JW), Carbone Cancer Center, NIH P50 DE26787 (JW Head and Nee SPORE), Midwest Athletes against Childhood Cancer Foundation, Hyundai Hope on Wheels Foundation, and SU2C-St. Baldricks Pediatric Dream Team Translational Research Grant SU2C AACR-DT1113 (to M.O.). CLR127 and analogs were kindly provided by Cellectar Biosciences, Madison, WI.