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 consequences 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 18-(p-iodophenyl) octadecyl phosphocholine (CLR127), a clinical-grade phospholipid ether analog with selective sequestration in cancer cells, in pediatric and adult human solid tumor cell lines.

We investigated mechanisms responsible for the radiosensitizing properties of CLR127. Western blotting of tumor cell lysates indicated increased accumulation and prolonged persistence of γ-H2AX in irradiated tumor cells pretreated with CLR127 compared to non-treated controls. Expression of select markers of homologous and non-homologous end-joining dsDNA radiation damage repair pathways were markedly decreased and significantly delayed in CLR127 treated tumor cells after exposure to irradiation compared to non-CLR127 treated cells. 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 cells uptake and retain 6- to 10-fold more CLR127 when compared to normal primary cells.

In vitro, the clonogenic 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 regrowth in CLR127 - treated mice compared to controls.

CLR127-BODIPY is selectively taken up by cancer cell lines

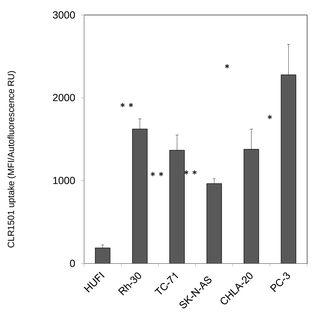

Figure 1. Uptake of CLR127-BODIPY in cancer cells compared to normal cells. Flow cytometry of the uptake of CLR127 fluorescent analog (CLR1501) by normal cells (HUFI, human skin fibroblasts) and cancer cell lines. Averages ± standard error (SE) from three repeats per cell type. *p<0.05, ** p≤0.01 tumor cells versus normal cells.

CLR127 sensitizes cancer cell lines to radiation

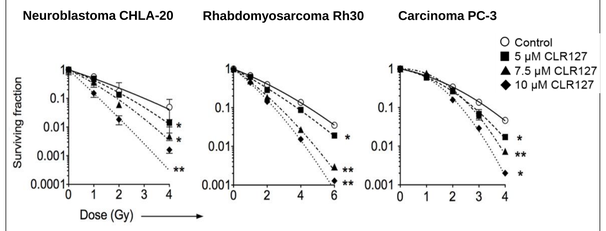

Figure 2. CLR127 enhances radiosensitivity of tumor cells. Clonogenic survival analysis of tumor cells treated with CLR127 or vehicle control. Selected numbers of cells were seeded in six-well plates and treated with 5, 7.5 and 10 μM CLR127 or excipient for 20 hours and subsequently irradiated at escalating doses. After 14 days, plates were stained with crystal violet and colonies were manually counted. *p<0.05, **p<0.001.

CLR127 Augments Radiotherapeutic Effect in Human Tumor Xenografts

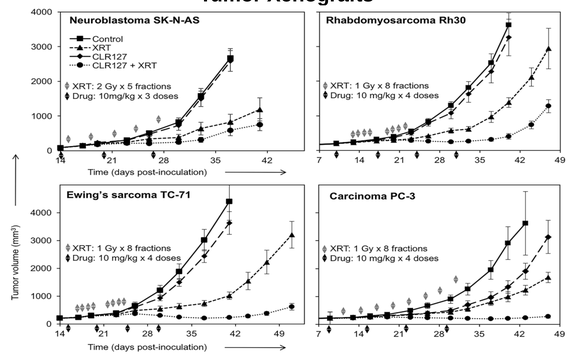

Figure 3. CLR127 increases radiation response in human tumor xenografts. Mice with tumor xenografts from SK-N-AS, PC-3, RH30 and TC-71 cell lines were treated with CLR127 , radiation, or both modalities during the time interval indicated by the black and gray arrows in each figure (n= 10-12 xenografts per group). **p<0.001.

Objective

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

Results

CLR127 and Radiation treatment shows no alteration in ATM phosphorylation

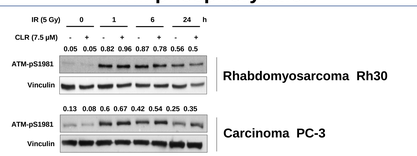

Figure 5: phosphorylation of ATM, An important kinase in the double strand break sensing had no effect of CLR127 and radiation. Cells were treated with or without 7.5 μM CLR127 for 16 hours, exposed to 5 Gy XRT dose, collected at indicated times and subjected to western blotting. directly. The ratios of the band intensities of each protein relative to Vinculin are indicated above the images.

Combining CLR127 and Radiation delays DNA Damage Repair

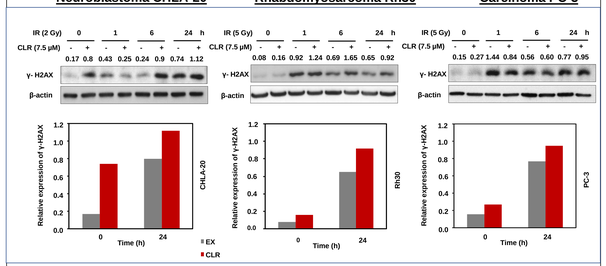

Figure 4. CLR127 pretreatment followed by irradiation induces persistent DNA damage. Cells were cultured with or without 7.5 μM CLR127 for 16 hours, exposed to XRT doses 2 and 5 Gy, collected at indicated times and subjected to western blotting. The ratios of the band intensities of each protein relative to β-actin are indicated above the images. The bar graphs show the relative expression of γ-H2AX before and 24 hours after irradiation.

Combining CLR127 and Radiation prevents BRCA1 signaling

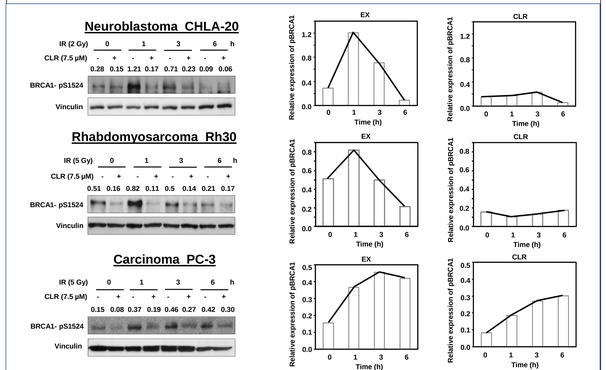

Figure 5: CLR127 (CLR) and radiation downregulates phosphorylation of BRCA1, a protein involved in repairing DNA double strand breaks . Cells were treated with or without 7.5 μM CLR127 for 16 hours, exposed to 2 and 5 Gy XRT doses, collected at indicated times and subjected to western blotting. directly. The ratios of the band intensities of each protein relative to Vinculin are indicated above the images. The bar graphs show the relative expression of pBRCA1.

Combining CLR127 and Radiation downregulates p53 phosphorylation

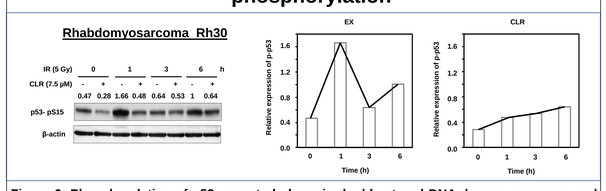

Figure 6: Phosphorylation of p53, a central player in double strand DNA damage response and repair, downregulates after CLR127 (CLR) and radiation treatment . Cells were treated with or without 7.5 μM CLR127 for 16 hours, exposed to 5 Gy XRT dose, collected at indicated times and subjected to Western blotting. The ratios of the band intensities of each protein relative to vinculin are indicated above the images. The bar graphs show the relative expression of p-p53.

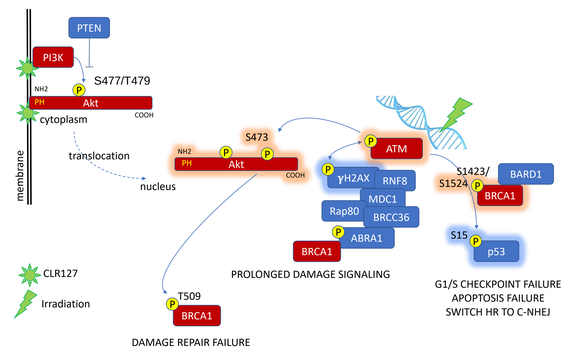

Figure 7. Possible molecular pathway depicting the combining effect of CLR127 and radiation on DNA double strand break sensing and repair.

Conclusions

- In cancer cells treated with CLR127, γH2AX persists at higher levels 24 hours after irradiation. Prolonged dsDNA damage signaling may reflect a failure of dsDNA damage repair.
- CLR127 treatment of cancer cells reduces the level, or slows down the kinetics, of BRCA1 phosphorylation at Ser1524 after irradiation, suggesting that CLR127 interferes with downstream dsDNA damage signaling towards efficient HR, forcing a switch to C-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/S 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/S checkpoint failure and apoptosis failure, altogether resulting in cell crash following irradiation.

Acknowledgements

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