Canady Helios Cold Plasma Reduces Soft Tissue Sarcoma Cell Viability by Inducing Apoptosis and Differential Gene Regulation

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Introduction

Soft tissue sarcomas (STS) are a rare and heterogenous type of cancer that arises from connective tissue [1]. Cold atmospheric plasma (CAP) has been studied for the treatment of carcinomas, however, its effects on STS remain relatively uninvestigated. Since current polychemotherapeutic options for STS are severely toxic [2], there exists a need for an effective yet tolerable adjuvant therapy for STS. Our previous studies demonstrated that Canady Cold Helios Plasma (CHCP) effectively reduced cell viability of various types of carcinomas (*e.g.* pancreatic adenocarcinoma, renal adenocarcinoma, esophageal adenocarcinoma, colorectal carcinoma, cholangiocarcinoma, and breast adenocarcinomas) [4-7]. In this study, we investigate the effects of CHCP on several STS cell lines.

Human Cell Line	STS Subtype	Tissue of Origin
HT-1080	Fibrosarcoma	Connective Tissue
SW-982	Synovial sarcoma	Joint; Synovium
RD	Rhabdomyosarcoma	Muscle
94T778	Liposarcoma	Adipose Tissue

Table 1 A list of the four human STS cell lines used in this study.

Cell Viability

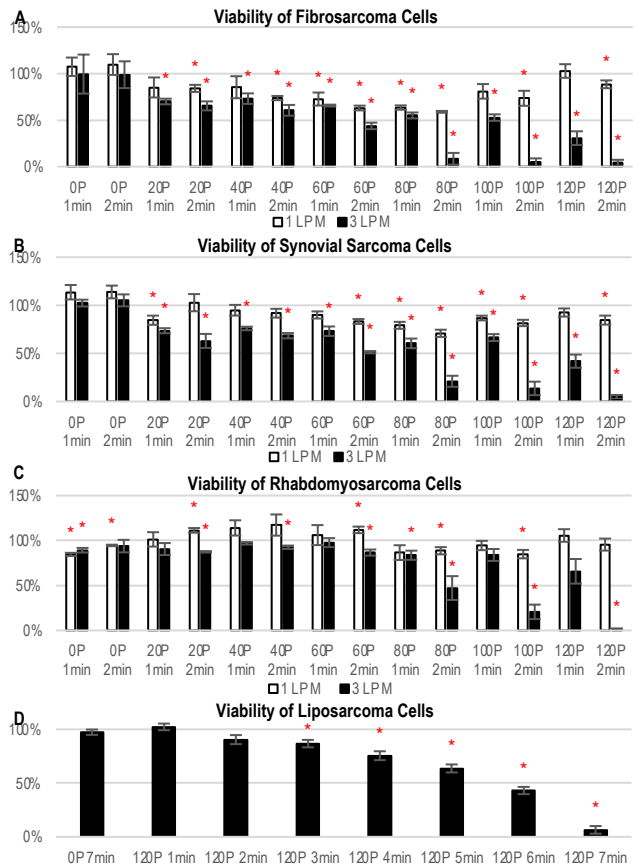

Fig. 1 Viability of CHCP-treated (A) HT-1080, (B) SW-982, (C) RD, and (D) 94T778 cells after 48 hr, compared to mock controls. Cells were seeded at a concentration of 5.0 x 10^3 cells/well into 96-well plates (Fig. 1A, 1B, 1C) or 2.5 x 10^4 into 12-well plates (Fig. 1D) and viability was measured by MTT assay. Viability was reduced in dose-dependent manner (* $p < 0.05$).

Proliferation

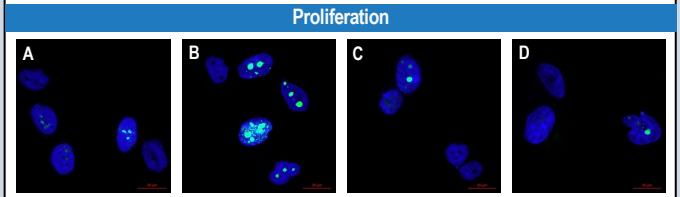

Fig 2. Expression of Ki67 in (A) mock control and CHCP-treated liposarcoma cells after (B) 6, (C) 24, and (D) 48 hr by confocal microscopy. A CHCP treatment dose of 120 P for 5 min was used. Most mock control cells expressed Ki67, indicating normal cancer proliferation (Fig. 2A). CHCP-treated cells showed an initial spike in Ki67 expression (Fig. 2B), followed by a sharp decrease in Ki67 expression and nucleus shrinkage (Fig. 2C and 2D).

Cell Cycle

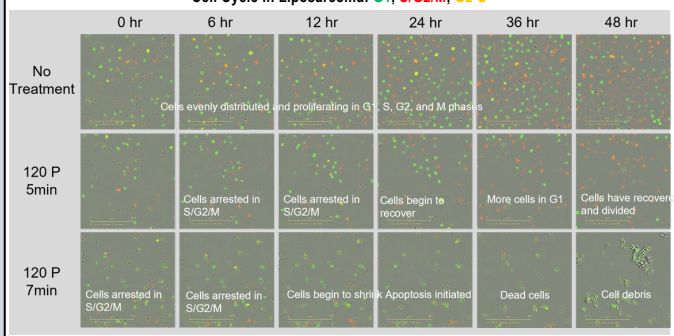

Fig. 3 Distribution of CHCP-treated 94T778 cell population in G1, S/G2/M, or G2-S phases after 0-48 hr. Stable cells were imaged and analyzed with IncuCyte Live Cell Imaging System. Mock cells were evenly distributed among the three cell cycle phases. Cells treated with 120 P 5 min were initially arrested in S/G2/M phase but continued to proliferate 24 hr post-treatment. Cells treated with 120P 7 min arrested in S/G2/M phase but never recovered from apoptosis- cells shrank after 12 hours, became spherical by 24 hours, and were completely dead by 48 hours.

Apoptosis

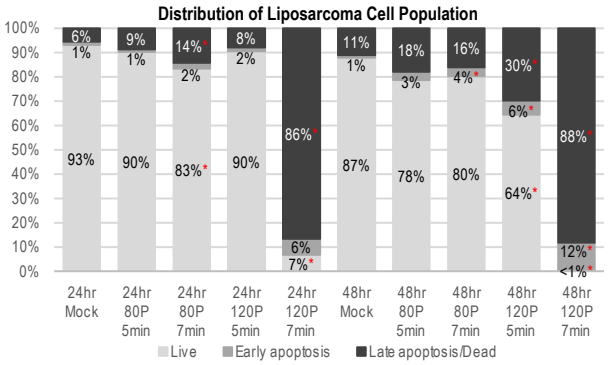

Fig. 4 Distribution of CHCP-treated 94T778 cell population in live, early, or late apoptotic/dead stages after 24 and 48 hr. Cells were stained with FITC Annexin V and Propidium Iodide, and apoptosis was quantified by flow cytometry. Apoptosis was induced in a dose-dependent manner. Statistical significance for CHCP versus mock controls were considered (* $p < 0.05$).

Gene Expression

Methods: Human tumor tissue samples were collected during the FDA-approved phase I clinical trial for CHCP. Pleomorphic sarcoma samples from one patient were treated with CHCP *ex vivo*. Treated and untreated tumor samples were processed for RNA isolation.

Ongoing Experiments: Real-time qPCR is being used to analyze a panel of 111 genes (related to apoptosis, oxidative stress, cell cycle checkpoint, etc.) in treated versus untreated tumor samples. Preliminary screening shows that most of the genes in this panel are downregulated.

Summary and Future Studies

- CHCP reduced the viability of fibrosarcoma, synovial sarcoma, rhabdomyosarcoma, and liposarcoma cells in a dose-dependent manner *in vitro*, consistent with our previous *in vitro* studies on other solid tumor cell types.
- CHCP induced apoptotic cell death of liposarcoma cells in a dose-dependent manner *in vitro*, supporting the results of the viability experiments.
- CHCP caused differential gene expression in *ex vivo* pleomorphic sarcoma samples, demonstrated by a preliminary screening. Results will be presented in a future report.
- Protein studies will be performed to confirm the results of the gene expression experiments and to elucidate CHCP mechanism in STS cell death.

References

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