above cell lines were evaluated by Cell Counting Kit-8 (CCK-8) test and colony forming unit (CFU) assay. In addition, the effect of NTP-combined radiation on proliferation, cell cycle, apoptosis and DNA damage in normal and cancer cells were also examined.

Results

These assays showed that different cell lines presented various sensitivity to radiotherapy, as SW480, SW620 and HT-29, were more vulnerable than HCT116, Caco-2 and LOVO. Interestingly, our results found that HCT116 and LOVO were more sensitive to NTP treatment than SW480, SW620 and HT-29. And the combination treatment of radiotherapy and NTP showed similar pattern as NTP treatment alone, but with stronger inhibition effect on cell viability. The CFU assay revealed that the survival rate was significantly reduced by combination treatment. Moreover, a higher rate of G2/M phase cell cycle arrest was found in combination group than radiotherapy and NTP treatment alone. Additionally, our experiments also showed that the expression of apoptotic-related genes and proteins increased significantly after pretreatment NTP before radiotherapy.

Conclusion

Taken together, the pretreatment of NTP before radiotherapy is a promising method to increase the sensitivity of colorectal cancer cells to radiotherapy. Although more in vitro and in vivo experiments are needed to consolidate our findings, this study highlighted the potential use of NTP to cancer treatment with combination of radiotherapy.

Digital Poster: Radiobiology of particles and heavy ions

PO-1920 Inhibition of Wnt signalling by XAV939 enhances radiosensitivity in human cervical cancer HeLa cells

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Purpose or Objective

Cervical cancer is the second most common malignant tumour threatening women's health. In recent years, heavy-ion beam therapy is becoming a newly emerging therapeutic mean of cancer; however, radio-resistance and radiation-induced damage constitute the main obstacles for curative treatment of cervical cancer.

Materials and Methods

Human cervical carcinoma cell line HeLa was obtained from the First Hospital of Lanzhou University. All cells were maintained in Dulbecco's modified Eagle medium (DMEM; HyCloneTM, Logan, UT) containing 10% (v/v) heat-inactivated foetal bovine serum (FBS; HyCloneTM, Logan, UT, USA) and were cultured in a humidified incubator of 5% CO2 at 37 C. HeLa cells were plated on 35-mm plastic dishes and irradiated with 12C6þ beam at room temperature. Heavy ion beam was provided by the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China), with energy of 80 MeV/u, LET at the centre of the spread-out Bragg peak of 50 keV/mm, and dose rate of 4 Gy/min.

Results

Therefore, to identify the radiosensitizers is essential. Here, we investigated the effects of Wnt signalling pathway on the response of 12C6þ radiation in HeLa cells. XAV939, an inhibitor of Wnt signalling pathway, was added two hours before 12C6þ radiation.12C6þ radiation inhibited the viability of HeLa cells in a time-dependent manner, and inhibiting Wnt signalling using XAV939 significantly intensified this stress. Meanwhile, 12C6þ radiation induced a significant increased cell apoptosis, G2/M phase arrest, and the number of c-H2AX foci. Supplementation with XAV939 significantly increased the effects induced by 12C6þ radiation alone. Combining XAV939 with 12C6þ irradiation, the expression of apoptotic genes (p53, Bax, Bcl-2) was significantly increased, while the expression of Wnt-related genes (Wnt3a, Wnt5a, b-catenin, cyclin D1 and c-Myc) was significantly decreased

Conclusion

Overall, these findings suggested that blockage of the Wnt/b-catenin pathway effectively sensitizes HeLa cells to 12C6þ irradiation, and it may be a potential therapeutic approach in terms of increasing the clinical efficacy of 12C6þ beams.

Digital Poster: Radiation-induced signalling pathways

PO-1921 Radiosensitizing effect of Trabectidin on human soft tissue sarcoma cells

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Purpose or Objective

Soft tissue sarcomas (STS) are aggressive tumors with limited effective therapeutic options. Trabectedin is indicated for the treatment of adult patients with advanced STS. The aim of the study is to evaluate in vitro if trabectedin could enhance radiotherapy by increasing cell radiosensitivity and biological aggressiveness on human cell lines of fibrosarcoma (FS), leiomyosarcoma (LMS), liposarcoma (LPS) and rhabdomyosarcoma (RS)

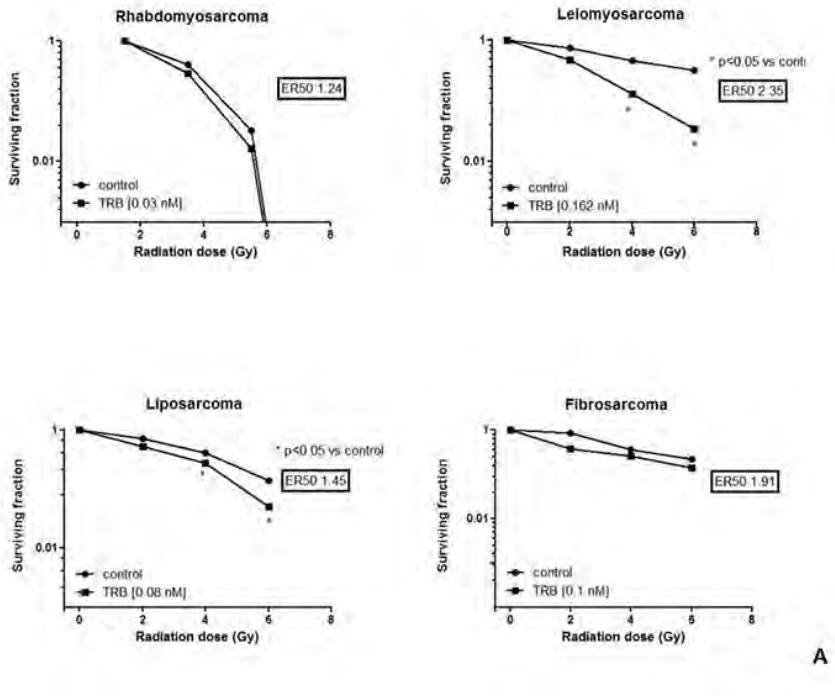
Materials and Methods

For each human FS (HS 93.T), LMS (HS5.T), LPS (SW872), and RS cell line (RD), IC50 was determined by colorimetric assay (MTS). Surviving Fraction (SF) was assessed at Clonogenic assay (CGA) on cells following irradiation (IR) to a dose of 2, 4 or 6 Gy, with or without trabectedin 24 h before IR to calculate radiosensitization enhancement ratio at 50 % survival (ER50). Matrigel invasion assay was performed in 4 groups: IR 4 Gy; IR 4 Gy + trabectedin; trabectedin; control. Repartition to radiosensitive G2/ M phase, cell cycle analyses was assessed with flow cytometry. Induction of DNA strand break and DNA repair have been observed by detecting H2AX serine 139 phosphorylation and quantifying γ-H2AX foci at 30 minutes, 6 h and 24 h after IR.

Results

IC50 was 0,9654 nM; 0,6836 nM;1,296 nM;0,8549 nM for RS,LPS,LMS and FS respectively. Significant reduction of SF in LMS

and LPS cell lines was observed following IR+trabectedin as compared to IR alone, resulting in ER50 of 2.35 and 1.45, respectively. (Fig.A)



All STS cell lines showed a significantly reduced invasiness following trabectedin alone or trabectedin+IR compared to control and trabectedin+IR compared to IR alone. Trabectedin+IR compared to control, resulted in an increasing, similar or reduced G2/M phase cell fraction of cells in LPS, FS and RS/LMS, respectively. In all STS cell lines, trabectedine+IR induced a significantly higher occurrence of γ-H2AX foci compared to control, trabectedin and IR alone. Reduction in the fluorescence intensity associated to the number of foci over 24 hour was significantly lower in the combined treatment arm.

Conclusion

IC50 of Trabectedin for different STS cell line was calculated. Trabectedin+IR induced significant SF reduction in LMS and LPS cell lines and decreased cell invasiveness in all cell lines compared to IR alone. Variable effects on cell cycle were observed according to STS subtype. Trabectedin + IR resulted in increased γ-H2AX foci formation and impaired damage repair.

Digital Poster: Tumour microenvironment

PO-1922 Classification of prostate cancer by hypoxia-related histological features in patient samples

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Purpose or Objective

Tumor hypoxia is an adverse factor in prostate cancer. A better understanding of the spatial mechanisms leading to this feature is needed to develop improved treatment strategies. We utilized digital pathology of tumor biopsies to explore spatial relationships between hypoxia, cell proliferation and distance to blood vessels in individual prostate cancer patients.

Materials and Methods

Totally 119 tumor biopsies from 76 patients who received the hypoxia marker pimonidazole prior to prostatectomy were included. Consecutive sections of 5 µm thickness were stained for pimonidazole, the blood vessel marker CD31, and the proliferation marker Ki67. Cell nuclei were visualized by hematoxylin counter-stain. Images of all sections were acquired at a resolution of 0.46 µm/pixel and co-registered for each biopsy. Digital pathology was used to construct parameter maps for hypoxia, proliferation and vessel distance in tumor parenchyma. Parameter fractions were quantified in regions of 0.16´0.16 mm^2 (350x350 pixels), and spatial relationships between these fractions were assessed in each biopsy by Pearson correlation analysis.Gene expression profiles of the biopsies were generated by Illumina bead arrays. Gene set enrichment analysis (GSEA) was performed based on the expression data, using the hallmark gene sets in the Molecular Signatures Database. Gleason grade of each biopsy was determined by a pathologist.

Results

The biopsies could be divided into two groups based on the correlation between hypoxic fraction and vessel distance fraction; one group (n=44) with a positive, significant correlation ($p<0.05$, $R>0$) and another group (n=75) with a negative ($P<0.05$, $R<0$) or non-significant correlation. Biopsies from the same patient generally belonged to the same group. The