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Metabolic Targeting with a Novel Iron Chelator Drug Candidate Causes Radiosensitization in HNSCC Cells in a 3-D Environment

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BACKGROUND & AIM

Metabolic targeting is a promising strategy to radiosensitize resistant tumors. Thereby, pathophysiologically altered metabolic pathways and characteristics of malignant cells are therapeutically intervened. Recently, the potential of a new drug candidate VLX600 for metabolic cancer targeting was discovered. It was shown in an advanced *in vitro* assay that VLX600 inhibits cellular oxygen consumption (*Zhang, X. et al., Nat. Commun. 5:3295, 2014*). Drug exposure appears to result in an enhanced locoregional oxygen level and reduced hypoxia, respectively, and was thus expected to be accompanied by radiosensitization. This hypothesis was to be proven.

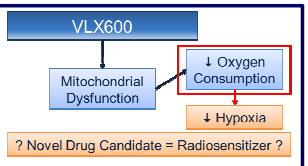

Figure 1: VLX600 potential radiosensitizer?

MATERIALS & METHODS

Cell lines:
- Head and neck squamous cell carcinoma (HNSCC) FaDu & SAS

Cell culturing:
- 3-D: Liquid overlay-method
- Spheroids with clear pimonidazole hypoxic fraction (pHF; Ø 600-650 µm)

Spheroid monitoring:
- Phase-contrast-imaging as function of time
- Determination of spheroid integrity, volume & membrane intact cells

Treatment:
- Mono-treatment = VLX600: 6 µM, 6 h / control: 0.2% DMSO in medium, 6 h
- Combined treatment = VLX600 (6 µM, 6 h) + single dose irradiation (0-45 Gy)

Analyses & Endpoints:
- Spheroid growth curves & membrane-intact cells per spheroid
- Spheroid control probability (SCP) & spheroid control dose 50 (SCD_{50})
- Immunofluorescence detection of pHF (shock frozen spheroids) & γH2AX-foci (paraffin embedded spheroids)

RESULTS

Mono-Treatment

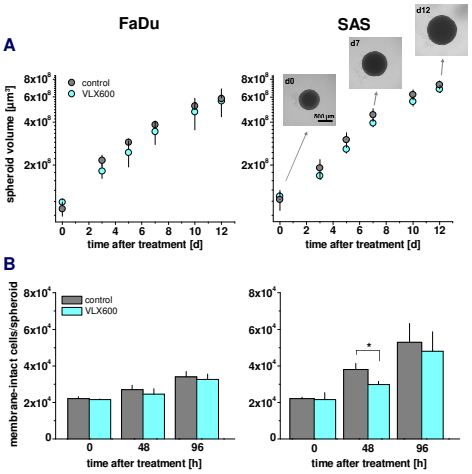

Figure 2: (A) Spheroid volume growth & (B) membrane-intact cells per spheroid (n=3) with an without treatment.

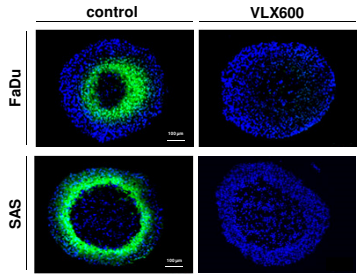

Figure 3: Immunofluorescence detection of pHF in 10 µm median sections of frozen spheroids with an without treatment.. Green: hypoxic cells (pimonidazole); blue: nuclei (DAPI).

Spheroid volume growth & number of membrane-intact cells are not systematically affected by VLX600 at the concentration of interest
BUT a massive reduction of hypoxia is observed

Combined Treatment

SPHEROID CONTROL PROBABILITY

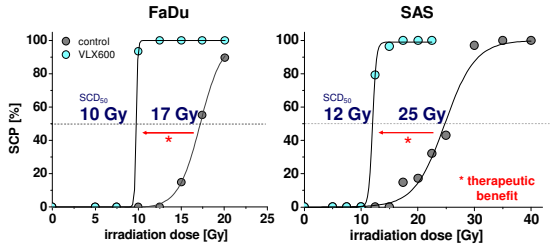

Figure 4: SCD_{50} determination in spheroids after single or combined treatment.

controlled = spheroids show no recovery & regrowth 60 d post treatment
SCP = spheroid control probability proportion of spheroids which are controlled by treatment
SCD_{50} = spheroid control dose 50 irradiation dose leading to 50% of controlled spheroids

VLX600 treatment leads to massive radiosensitization and a therapeutic benefit in both FaDu & SAS spheroids

DNA DOUBLE STRAND BREAKS

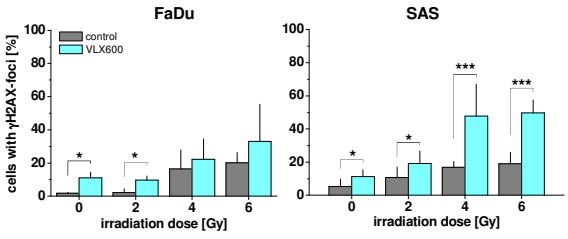

Figure 5: Overall proportion of cells with residual γH2AX-foci (all nuclei/spheroid).

VLX600 treatment causes:
- ➢ more DNA double strand breaks in general

In SAS spheroids (analysis in FaDu spheroids in progress):
- ➢ increased number of foci per nuclear area
- ➢ higher DNA damage in hypoxic regions

!!! VLX600 enhances residual DNA damage after irradiation !!!

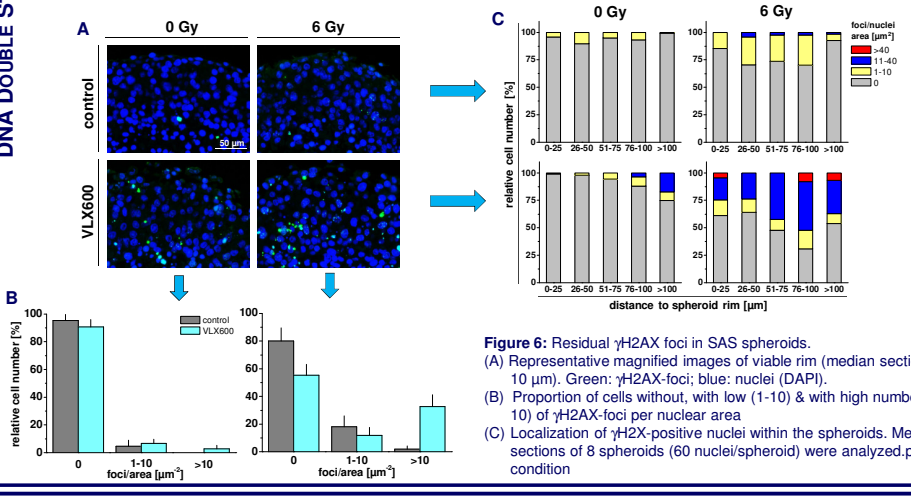

Figure 6: Residual γH2AX foci in SAS spheroids.
(A) Representative magnified images of viable rim (median sections, 10 µm). Green: γH2AX-foci; blue: nuclei (DAPI).
(B) Proportion of cells without, with low (1-10) & with high numbers (> 10) of γH2AX-foci per nuclear area
(C) Localization of γH2X-positive nuclei within the spheroids. Median sections of 8 spheroids (60 nuclei/spheroid) were analyzed.per condition

CONCLUSIONS & OUTLOOK

Exposure to VLX600 causes a remarkable reduction of the hypoxic area in 600-650 µm HNSCC spheroids. Spheroid volume growth and viability of spheroid cells was not essentuially affected by short-term treatment. However, the combination with irradiation resulted in a critical reduction of the SCD_{50} as opposed to irradiation alone. This radiosensitizing effect was confirmed by the analysis of DNA double strand breaks. Our data indicate that the new metabolically active drug VLX600 may enhance the curative potential of radiotherapy.
Mechanistic studies are ongoing and first *in vivo* experiments have been designed.

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