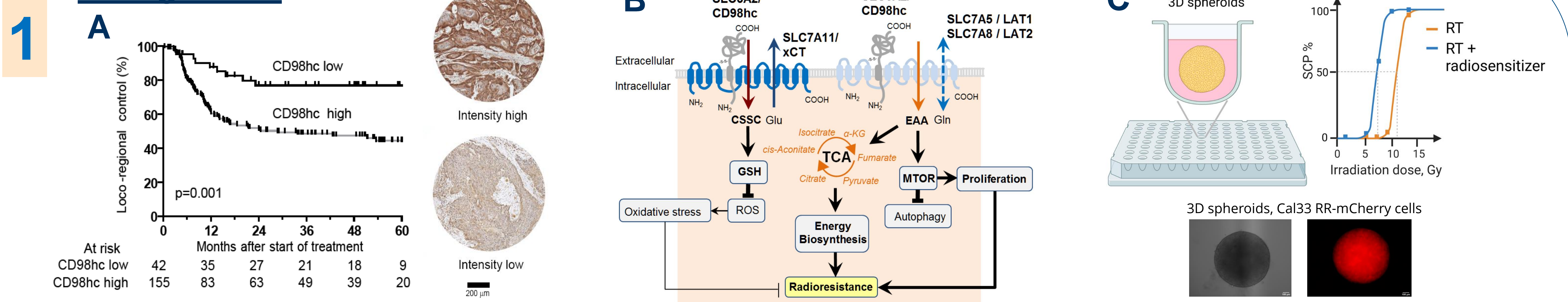


Validation of the role of CD98hc-associated amino acid transporters in the regulation of radiosensitivity in HNSCC 3D models

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Background:



Amino acid transporter molecules *SLC3A2/CD98hc* (solute carrier family 3 member 2) and *SLC7A5/LAT1* (solute carrier family 7 member 5) regulate tumor sensitivity to radiotherapy. (A) Low levels of CD98hc protein expression significantly correlate with good clinical prognosis in patients with locally advanced head and neck squamous cell carcinoma (HNSCC) treated with primary radiochemotherapy (B). High expression levels of CD98hc result in the activation of tumor pro-survival signaling pathways, energy biosynthesis and DNA repair (C). A schematic representation of 3D spheroid assay to analyze the growth of HNSCC cells in response to the knockdown of target genes or chemical inhibition after irradiation.

2 **The aim of the project:** is to establish, characterize, and apply an HNSCC spheroid assay that allows to evaluate (and modify) of CD98hc-related amino acid transporter-dependent radioresistance in a 3D multicellular context with and without the impact of therapeutically-relevant tumor hypoxia.

3 **Work plan:**

WP 1. Establishing a stable knockdown or knockout of CD98hc, LAT1, LAT2 and xCT expression in HNSCC cells;

WP 2. Analysis of the growth kinetics of the 3D cultures (spheroids) of HNSCC cells with or without a stable knockdown / knockout of target genes;

WP 3. Assessment of the metabolic features of the spheroids using Seahorse technology (e.g. oxygen consumption rates (OCR), extracellular acidification rates (ECAR), OxPhos and glycolytic function);

WP 4. Evaluation of the radiotherapeutically-relevant hypoxia in the spheroids by pimonidazole accumulation and immunostaining;

WP 5. Analysis of the radioresponse of the knockdown/knockout vs. control cell spheroids using long-term spheroid control probability (SCP) assays and γ -H2A.X analysis;

WP 6. Assessment of 1-3 commercially available chemical inhibitors of CD98hc-related amino acid transport for their radiosensitizing potential and metabolic effects using the approaches from WP 3 and WP 5;

WP 7. Finalization and evaluation of the data for the publication and Doctoral thesis.

4 **Timetable:**

Month	1-2	3-4	5-6	7-8	9-10	11-12
WP 1						
WP 2						
WP 3						
WP 4						
WP 5						
WP 6						
WP 7						

5 **References:**

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