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Glutamine metabolism as a potential target for prostate cancer radiosensitization

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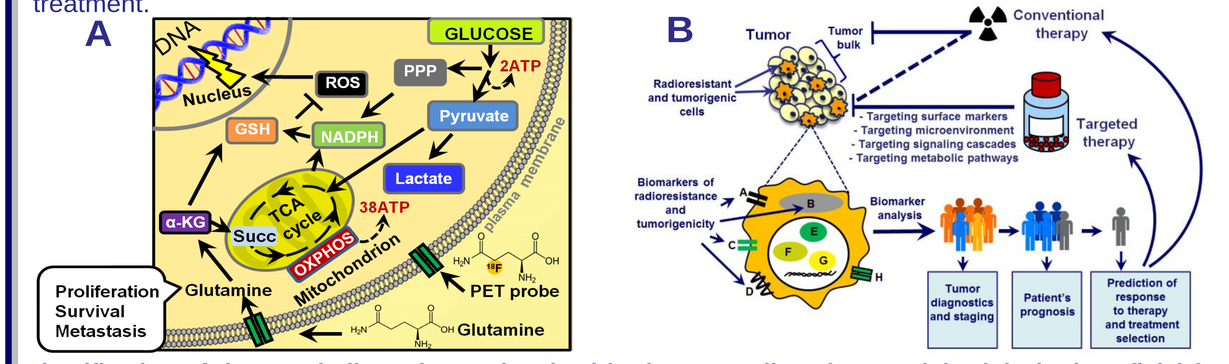
Introduction

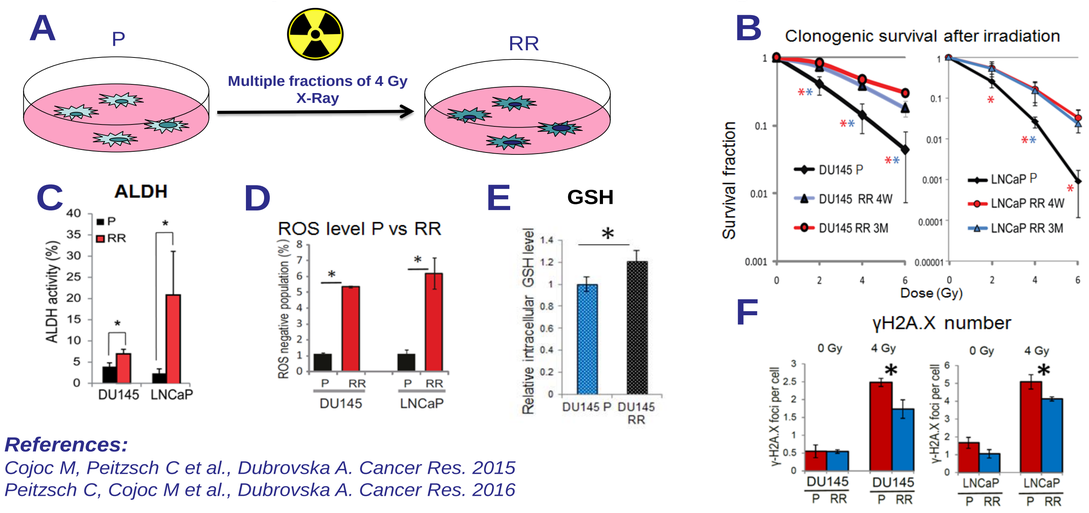
Radiotherapy is one of the mainstays of curative treatment for many types of cancer including prostate tumor. Most of the prostate cancer patients at early stage of disease may be cured with surgery or radiotherapy alone or in combination with androgen deprivation. Nevertheless, local tumor control is often impeded by tumor radioresistance. One hallmark of cancer cells is a major reprogramming of cellular energy metabolism in order to maintain continuous cell growth and proliferation. A significant proportion of the biosynthetic needs may be covered by the metabolism of glutamine as an important donor of nitrogen and carbon for the growth-promoting pathways. The role of glutamine metabolism for prostate cancer development and radioresistance remains unclear, and its deciphering can be employed for the development of new biomarkers and potential therapeutic targets for individualized treatment.



Identification of the metabolic pathways involved in therapy radioresistance (A) might be beneficial for cancer treatment (B). *PPP - pentose phosphate pathway, TCA - tricarboxylic acid cycle, OXPHOS - oxidative phosphorylation; ROS - reactive oxygen species; GSH – glutathione; α-KG – alpha ketoglutarate; NADPH - Nicotinamide adenine dinucleotide phosphate; PET - positron emission tomography; Succ – succinate; ATP – adenosine triphosphate.*

1 Development of the isogenic radiosensitive/radioresistant cell lines model and its characterization

1.1. RR cells possess an enhanced defence against oxidative stress



References:
Cojoc M, Peitzsch C et al., Dubrovska A. Cancer Res. 2015
Peitzsch C, Cojoc M et al., Dubrovska A. Cancer Res. 2016

Figure 1. A*) Radioresistant (RR) isogenic cell lines were created by multiple irradiation of parental (P) prostate cancer (PrCa) cells with 4Gy of X-Ray; **B**) Radioresistance of created sublines was confirmed by colony-formation assay; **C**) Radioresistant cell lines demonstrate higher ALDH (aldehyde dehydrogenase) activity; **D**) Radioresistant cell lines have lower level of intracellular ROS (reactive oxygen species); **E**) Intracellular amount of GSH (glutathione – ROS scavenger) is higher in radioresistant cell lines; **F**) The number of residual γH2A.X foci after 4 Gy irradiation is lower in radioresistant cell lines.*

1.2. Glutamine metabolism is upregulated in radioresistant prostate cancer cells

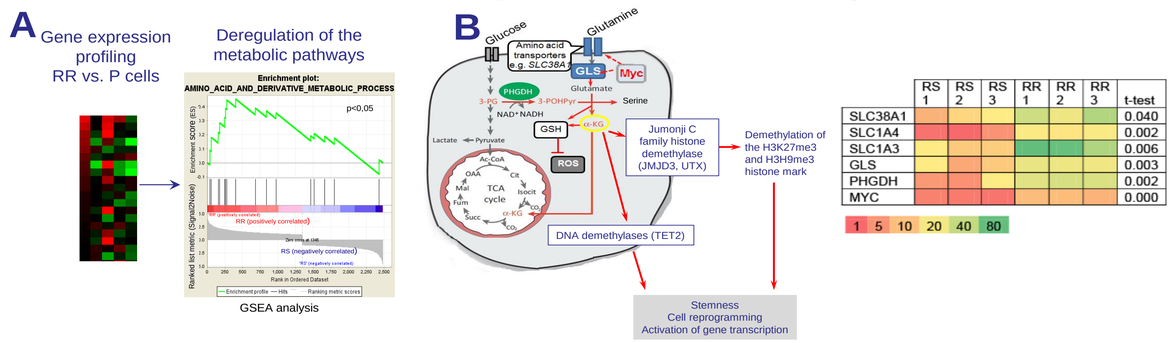


Figure 2. A*) GSEA analysis revealed that gene set upregulated in DU145 RR cells has a strong association with amino acid metabolic processes; **B**) Glutamine (Gln) is important for energy production by tricarboxylic acid (TCA) cycle, biosynthesis and cell protection against oxidative stress via GSH production and regulation of cell reprogramming (picture from Mullen and De Berardinis, 2012, modified). Comparative gene expression profiling revealed several genes involved in Gln metabolism which are significantly upregulated in DU145 RR cells.*

2 The effect of glutamine starvation on PrCa cells viability and radioresistance

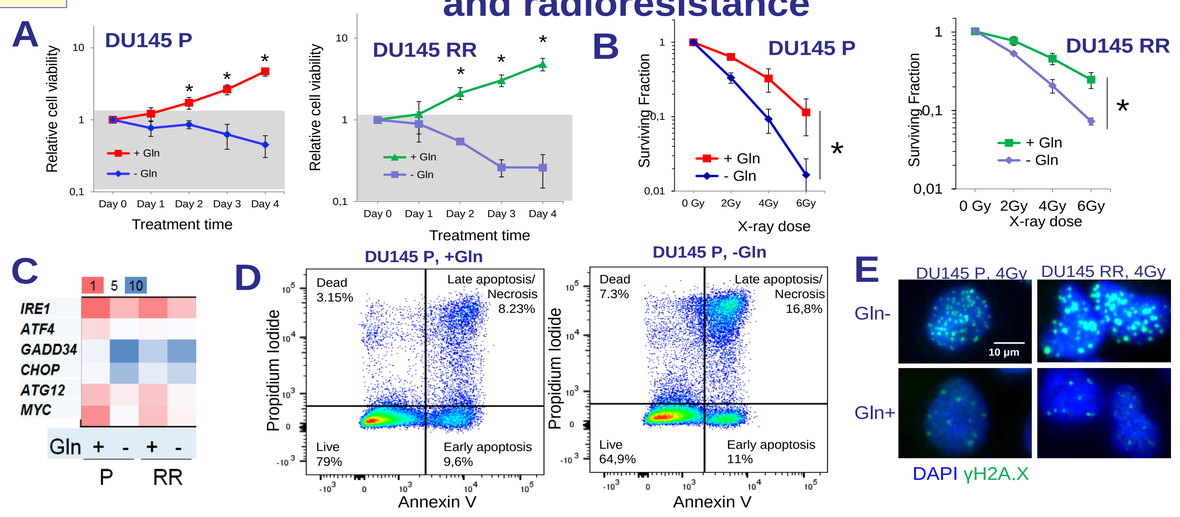


Figure 3. A*) Glutamine starvation (Gln-) leads to a decrease in cell viability; **B**) Glutamine withdrawal causes PrCa cells radiosensitization; n≥3; *, p < 0.05; **C**) Global gene expression profiling revealed that Gln starvation leads to the induction of the endoplasmic reticulum (ER) stress; **D**) Glutamine deprivation results in the induction of cell apoptosis and necrosis; n≥3; **E**) Residual γ-H2AX foci in DU145 P and DU145 RR cells 24 hours after 4 Gy irradiation. Cells were cultured in Gln+ or Gln- medium 48h prior fixation; n≥3.*

3 Targeting of the proteins involved in glutamine metabolism results in prostate cancer cell radiosensitization

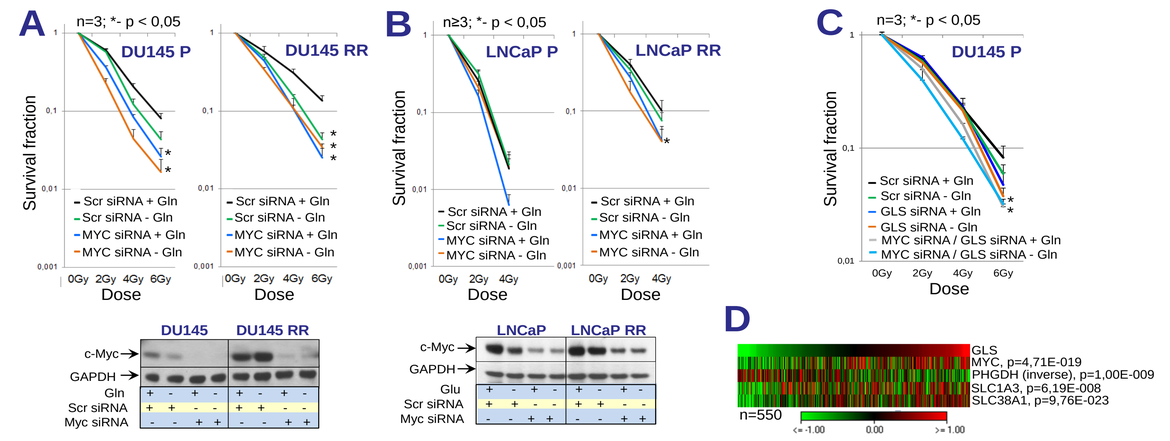


Figure 4. A, B*) siRNA-mediated knockdown of c-MYC expression or (**C**) GLS expression leads to an increase in cell radiosensitivity. siRNA-mediated knockdown of c-MYC and GLS was validated by Western blot analysis. The cells were treated with mix of three siRNA sequences; **D**) Co-regulation of the expression of MYC, PHGDH, SLC38A1 and SLC1A3 genes with GLS expression in TCGA prostate cancer dataset (N= 550).*

4 Gln contributes to the α-ketoglutarate production, which is involved in the GSH production and regulation of cell reprogramming

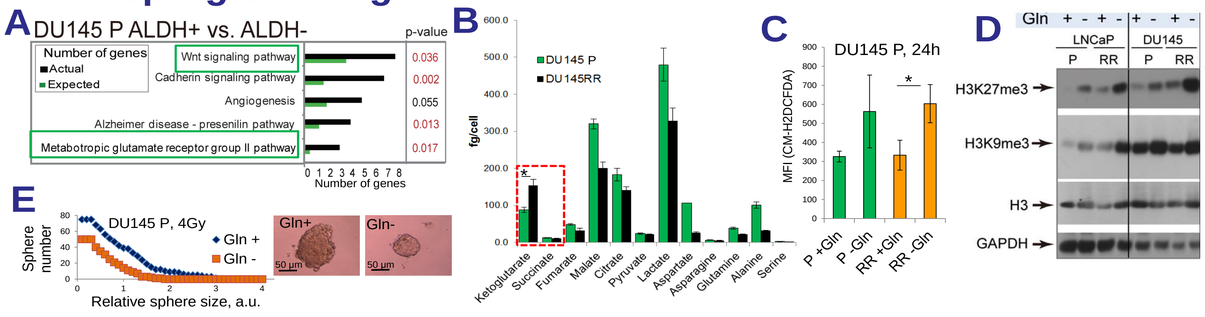


Figure 5 A*) Pathway enrichment analysis of the gene expression data for DU145 ALDH+ and ALDH- cells using PANTHER pathway tool; **B**) Mass spectrometry analysis revealed an increased level of α-ketoglutarate / succinate ratio in radioresistant cells suggesting that glutamine use for TCA is suppressed in DU145 RR cells; n=3; *, p < 0.05; **C**) Glutamine starvation (Gln-) leads to the accumulation of intracellular ROS measured by using CM-H2DCFDA reagent which becomes fluorescent in the presence of ROS; n≥3; MFI - mean fluorescence intensity *, p < 0.05; **D**) Glutamine deprivation results in global changes of histone 3 methylation, n≥3; **E**) Glutamine starvation inhibits sphere-forming properties (sphere size and number) after cell irradiation.*

5 Gln metabolism as potential target for prostate cancer radiosensitization and marker of tumor radioresistance

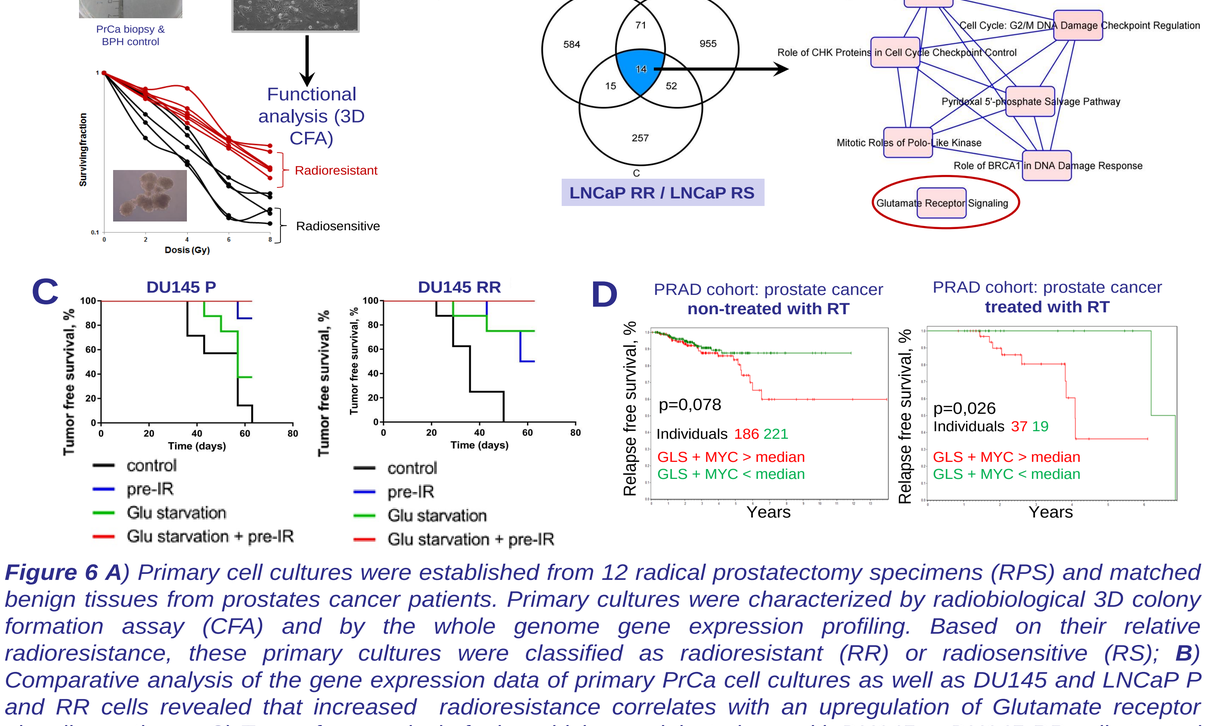


Figure 6 A*) Primary cell cultures were established from 12 radical prostatectomy specimens (RPS) and matched benign tissues from prostates cancer patients. Primary cultures were characterized by radiobiological 3D colony formation assay (CFA) and by the whole genome gene expression profiling. Based on their relative radioresistance, these primary cultures were classified as radioresistant (RR) or radiosensitive (RS); **B**) Comparative analysis of the gene expression data of primary PrCa cell cultures as well as DU145 and LNCaP P and RR cells revealed that increased radioresistance correlates with an upregulation of Glutamate receptor signaling pathway; **C**) Tumor free survival of mice which were injected s.c. with DU145 or DU145-RR cells treated as indicated; n=8 for each experimental group; **D**) Expression level of the key regulators of Gln metabolisms, MYC and GLS genes, is indicative for relapse free survival in prostate cancer patients treated with radiotherapy. Results are based on the analysis of the TCGA gene expression dataset.*

Conclusion:

Our findings suggest that glutamine metabolism contributes to prostate tumor cell proliferation, cancer stem cell properties, tumorigenicity, oxidative stress, radioresistance and epigenetic changes. The combination of irradiation with inhibition of glutamine metabolism may increase the cytotoxic effects of irradiation in prostate tumor cells. Expression of the proteins involved in glutamine metabolism can be used to predict clinical outcome of prostate cancer patients. The intracellular mechanisms of the differential tumor cell sensitivity to glutamine supplementation are in the focus of ongoing study.

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