Glutaminolysis as a potential target for prostate cancer radiosensitization


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 impaired by tumor radiosensitivity. 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 radiosensitivity remains unclear, and its deciphering can be employed for the development of new biomarkers and potential therapeutic targets for individualized treatment.

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

1.1. RR cells possess an enhanced defence against oxidative stress

Figure 1 A) Radiosensitive (RR) isogenic cell lines were created by multiple irradiation of parental (P) prostate cancer (PCa) cells with 4Gy of X-Ray. B) Radiosensitivity of created sublines was confirmed by colony-formation assay. C) Radiosensitive cell lines demonstrate higher ALDH (aldehyde dehydrogenase) activity. D) Radiosensitive cell lines have lower level of intracellular ROS (reactive oxygen species); E) Intracellular amount of GSH glutathione - ROS scavenger) is higher in radiosensitive cell lines. F) The number of residual V245 X cells after 4 Gy irradiation is lower in radiosensitive cell lines.

1.2. Glutamine metabolism is upregulated in radiosensitive 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 in prostate cancer. C) Glutaminolysis as a potential target for prostate cancer radiosensitization.

The effect of glutamine starvation on PrCa cells viability and radiosensitivity

Figure 3 A) Glutamine starvation (Gln-) leads to a decrease in cell viability; B) Glutamine withdrawal causes PrCa cells radiosensitization; 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; E) Residual TCA245 Gln+ and DU145 RR cells 24 hours after 4 Gy irradiation. Cells were cultured in Gln- or Gln+ medium 48h prior fixation: n=33.

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) G5S expression leads to an increase in cell radiosensitivity. siRNA-mediated knockdown of c-MYC and G5S 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 G5S expression in TCGA prostate cancer dataset (N=55).

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

Figure 5 A) GSH is a well-characterized major component of redox balance in cells. GSH is synthesized from the amino acid cysteine via the classical GSH synthetic pathway (GCLC/GCLM) and the alternative GSH synthetic pathway (GCS/GSSH). B) Glutamine is a source of N5-carboxymethyl-L-glutamate (N5CMG), which is a substrate for the GSH de novo synthesis.

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

Figure 6 A) Primary cell cultures were established from 12 radical prostatectomy specimens (RPS) and matched benign tissue. B) Human prostate cancer cells were characterized metabolon-wide MS. C) Radiosensitization of DU145 and UNCC-P cells revealed that increased radiosensitivity correlates with an upregulation of glutaminase receptor signaling pathway. D) The level of Gln, Glutamine and G5S expression in primary cell cultures was analyzed by flow cytometry (G5S) and by whole genome expression profiling. E) Primary cell cultures were classified as radiosensitive (RR) or radiosensitive (RS); F) Expression of the key regulators of Gln metabolism, G5S and G5S, were validated by western blot analysis.

Conclusion: Our findings suggest that glutaminolysis metabolism contributes to prostate tumor cell proliferation, cancer stem cell properties, tumorigenesis, oxidative stress, radiosensitivity and epigenetic changes. The combination of irradiation with inhibition of glutaminolysis metabolism may increase the cytotoxic effects of irradiation in prostate tumor cells. Expression of the proteins involved in glutaminolysis 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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