Combining Hedgehog inhibition with metformin to induce radiosensitization in prostate cancer cells

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Background
Recently, a link between Hedgehog (Hh) signaling and the cellular energy metabolism has been described, more specific at the level of AMP-activated protein kinase (AMPK). Both signaling pathways have been shown to influence the cellular radiation response. Therefore, it seems worthwhile to explore whether the combination of Hh signaling inhibitors and AMPK activators such as metformin could further increase the response to radiotherapy.

Conclusions
Both GANT61 and metformin induced radiosensitization in the 22Rv1 PCa cells. The combination of both agents further enhanced the response to radiotherapy significantly, indicating that this might be a more powerful radiosensitization strategy compared to either agent alone. Further investigations in xenograft mouse models are ongoing and will provide additional evidence for the therapeutic potential of GANT61 and metformin in combination with radiotherapy and will help to elucidate its working mechanism.

Material and methods
Three PCa cell lines (PC3, DU145, 22Rv1) were treated with the SMO inhibitor GDC-0449 or GLI1/2 inhibitor GANT61, with or without metformin. The effects on cell survival and radiation sensitivity were investigated by means of Sulforhodamine B (SRB) assays and colony assays. The effects on gene and protein expression (qRT-PCR/Western blotting) and on apoptosis (flow cytometry, Annexin-V/PI staining) were also examined, both in the absence and presence of ionizing radiation (4Gy).

Results

Hh inhibition at the level of GLI and not at the level of SMO decreases cell viability of PCa cells

GANT61 specifically inhibits Hh target genes in PCa cells

Combination of GANT61 and metformin enhances the effect of IR on cellular survival

Differential effect of GANT61 and metformin on radiosensitivity of PCa cells

Differential effect of GANT61 and metformin on apoptosis in different PCa cell lines

Combination of GANT61 and metformin is very effective in suppressing PCa cell growth

Working hypothesis combined effect GANT61 and metformin on radiosensitivity of PCa cells

Annexin-V-positive/negative cells after 72h GANT61 (1µM) ± metformin (5mM) prior to/during IR (4Gy). Means ± SEM of 3 independent experiments performed in triplicate. * p<0.05 vs. control, # p<0.05 vs. metformin, $<0.05 vs. GANT61

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