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# β1 Integrin and JNK Targeting in Glioblastoma multiforme: Radiochemosensitization and Inhibition of Invasion

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**Background:** The poor prognosis associated with Glioblastoma multiforme (GBM) largely arises from the radiochemotherapy resistance of GBM cells and their aggressive infiltration and destruction of the healthy brain [1, 2]. β1 integrin and c-Jun N terminal kinase (JNK) are potential therapeutic targets in GBM owing to their regulation of radioprotective pro-survival and pro-invasive signaling hubs in GBM stem-like cells and the tumor bulk [3, 4]. Here, we evaluate the effect of simultaneous targeting of β1 integrin and JNK in combination with radiochemotherapy on clonogenic survival and invasion of GBM stem-like cells and GBM cell lines in vitro and in an orthotopic GBM mouse model.

**Hypothesis:** Dual targeting of β1 Integrin und JNK reduces radio-chemoresistance and invasion of GBM cells.

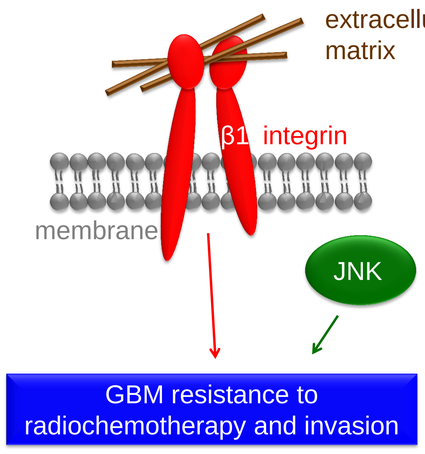


**Fig. 1: β1 integrin and JNK signaling function in radiochemoresistance and invasion of GBM cells.**

## References:

[1] Weller & Wick, 2014, Nat Rev Neurol
[2] Vehlow & Cordes, 2013, Biochim Biophys Acta
[3] Kitanaka et al., 2013, Genes Cancer
[4] Eke, Stoch, Kästner, Vehlow et al., 2012, IJROBP

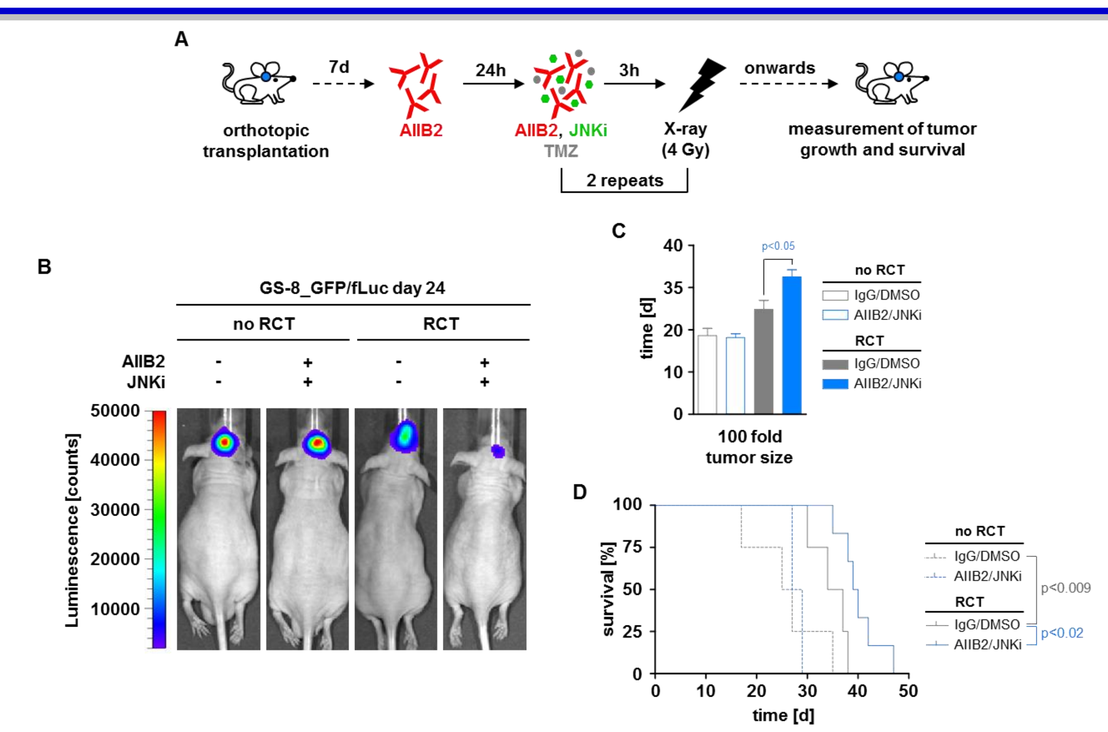


**Fig. 4: Simultaneous β1 integrin/JNK inhibition enhances radiochemotherapy response of GBM stem-like cells in vivo. (A)** Treatment scheme of nude mice orthotopically transplanted with GS-8_GFP/fLuc cells. **(B)** Luminescence IVIS imaging of GS-8_GFP/fLuc mice 24 days after inhibiting β1 integrin and JNK without or in combination with radiochemotherapy (RCT). Representative images are shown. **(C)** Time to reach 100 fold tumor size of GS-8_GFP/fLuc mice after the indicated treatment. **(D)** Percentage of survival of GS-8_GFP/fLuc mice treated as indicated.

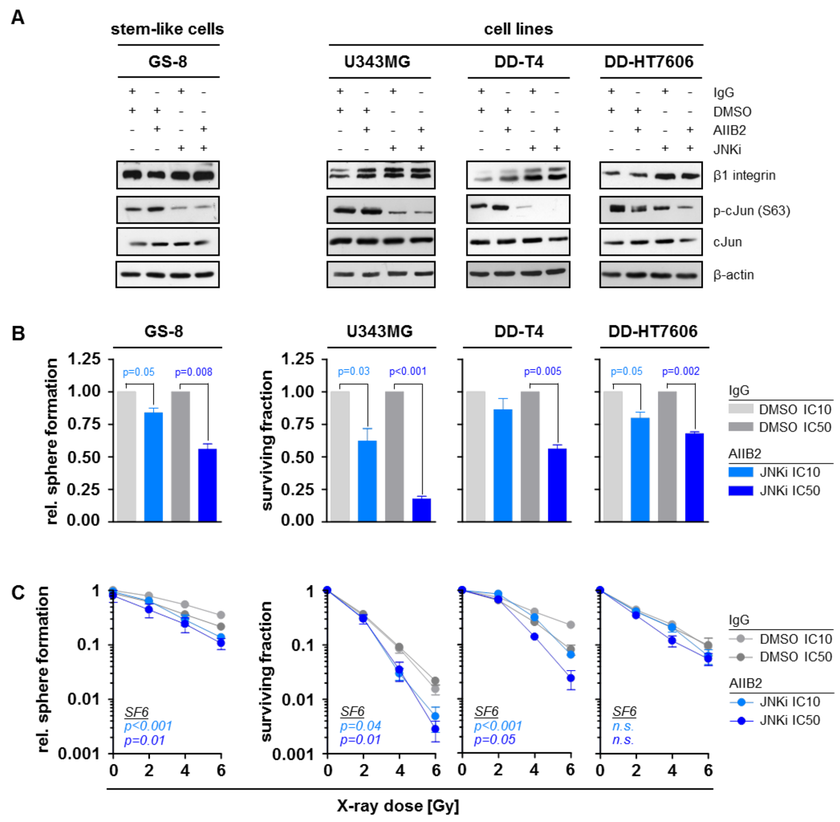


**Fig. 2: Simultaneous β1 integrin/JNK inhibition reduces radioresistance of GBM stem-like cells and GBM cell lines. (A)** Analysis of protein expression after single or simultaneous inhibition of β1 integrin (using 10 µg/ml AIIB2 or IgG control) and JNK (using IC10 of SP600125 or DMSO control) in GBM stem-like cells (GS-8) and GBM cell lines (U343MG, DD-T4, DD-HT7606). **(B)** Quantification of relative sphere formation and clonogenic surviving fraction of non-irradiated GBM cells upon inhibition of β1 integrin and JNK. **(C)** Quantification of relative sphere formation and clonogenic surviving fraction of irradiated (2, 4, 6 Gy X-ray) GBM cells upon inhibition of β1 integrin and JNK. Results show mean ± SD (n = 3; t-test).

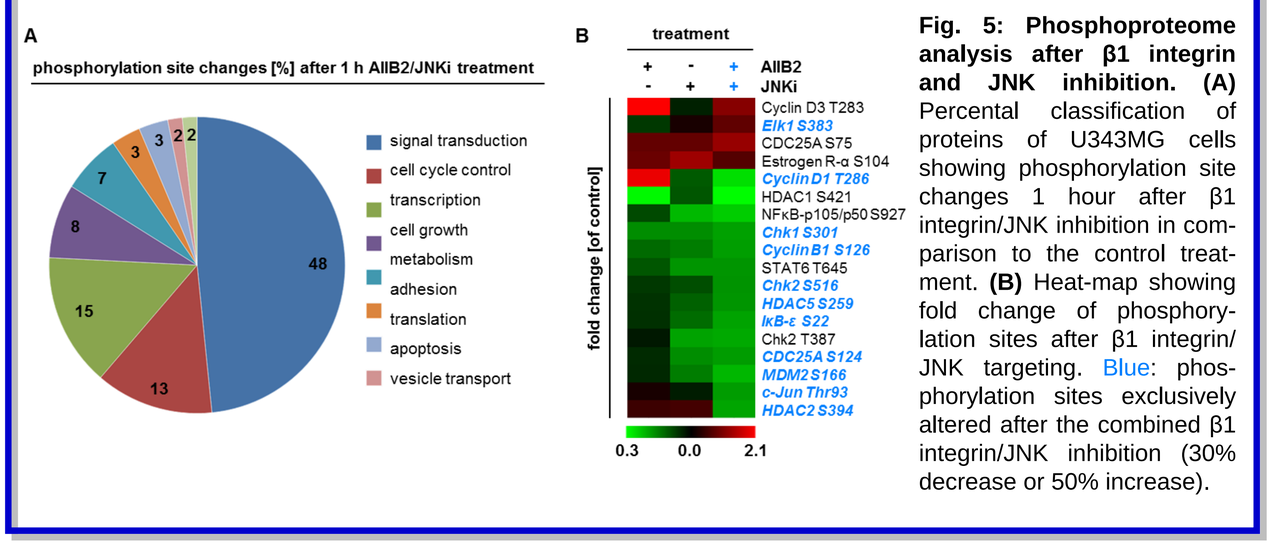


**Fig. 5: Phosphoproteome analysis after β1 integrin and JNK inhibition. (A)** Percental classification of proteins of U343MG cells showing phosphorylation site changes 1 hour after β1 integrin/JNK inhibition in comparison to the control treatment. **(B)** Heat-map showing fold change of phosphorylation sites after β1 integrin/JNK targeting. Blue: phosphorylation sites exclusively altered after the combined β1 integrin/JNK inhibition (30% decrease or 50% increase).

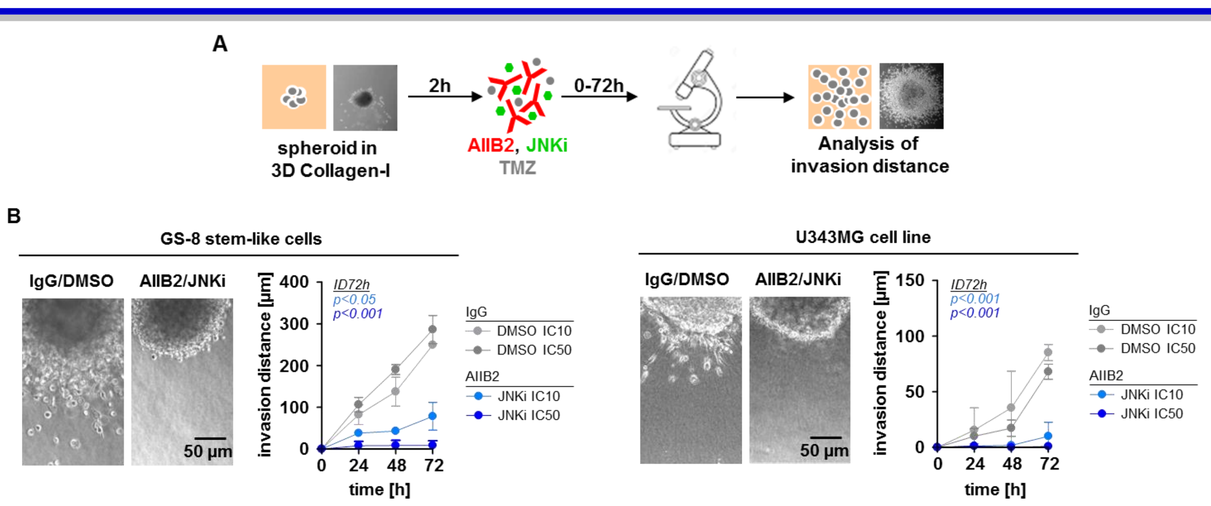


**Fig. 3: Dual β1 integrin and JNK inhibition inhibits GBM cell invasion and secretion of matrix metalloproteinases. (A)** Treatment scheme. **(B)** Analysis of the invasion distance of GBM cells upon co-inhibition of β1 integrin and JNK in a 3D collagen type 1 matrix. Results show mean ± SD (n = 3, t-test). Representative microscopic images are shown. **(C)** Quantification of MMP2 and MMP9 secretion into the supernatant of U343MG cells upon inhibition of β1 integrin, JNK or both. Results show mean ± SD (n = 3; t-test; *p<0.05, **p<0.001).

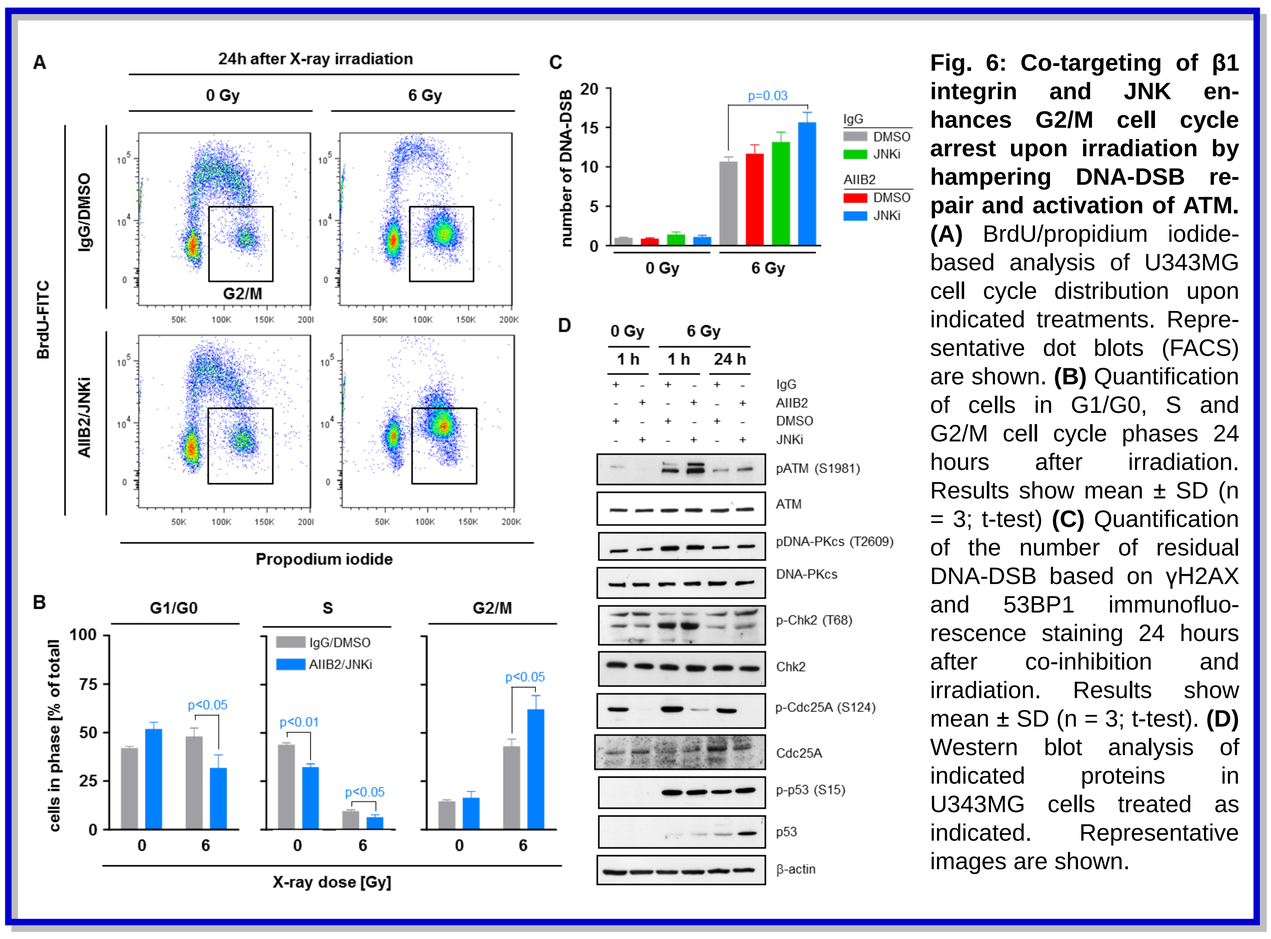


**Fig. 6: Co-targeting of β1 integrin and JNK enhances G2/M cell cycle arrest upon irradiation by hampering DNA-DSB repair and activation of ATM. (A)** BrdU/propidium iodide-based analysis of U343MG cell cycle distribution upon indicated treatments. Representative dot blots (FACS) are shown. **(B)** Quantification of cells in G1/G0, S and G2/M cell cycle phases 24 hours after irradiation. Results show mean ± SD (n = 3; t-test) **(C)** Quantification of the number of residual DNA-DSB based on γH2AX and 53BP1 immunofluorescence staining 24 hours after co-inhibition and irradiation. Results show mean ± SD (n = 3; t-test). **(D)** Western blot analysis of indicated proteins in U343MG cells treated as indicated. Representative images are shown.

## Conclusions:

- Here, we suggest co-targeting of β1 integrin and JNK as a promising approach to overcome radiochemoresistance and invasion of GBM.
- Currently, ongoing studies are aimed at identifying possible bypass mechanisms stimulated upon dual targeting of β1 integrin and JNK.

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