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# Targeting BUB3 in combination with paclitaxel inhibits proliferation of glioblastoma cells by enhancing cellular senescence

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## Resumo

**Introduction:** Glioblastoma (GBM) is the most aggressive and lethal tumor type from the central nervous system (CNS), and exhibits an impressive aneuploidy with notable tumor heterogeneity [1,2]. The standard treatment of GBM comprises surgery, radiotherapy and concomitant and adjuvant chemotherapy, but no effective treatment exists to date [3,4]. In addition, GBM recurrence remains a clinical bottleneck. Thus, there is a strong interest in understanding the molecular mechanism underlying GBM pathogenesis, with a concomitant strategy towards the development of new therapeutic drugs and biomarkers. **Objectives:** To unveil new GBM chemotherapeutic targets and new therapeutic strategies, exploring the role of the mitotic protein BUB3 [5] in GBM and assessing the potential of BUB3 knockdown and paclitaxel co-treatment as a potential therapeutic strategy. **Material and Methods:** BUB3 expression was determined by quantitative real-time PCR in two glioblastoma cell lines, U251 and U373. BUB3 knockdown was performed using small interfering RNAs (siRNAs). To assess the effect of BUB3 inhibition, either alone or in combination with paclitaxel, we analyzed (i) the cell viability by the MTT cytotoxic assay, (ii) the apoptosis induction by TUNEL assay and measurement of caspase 3 activity and (iii) the cell morphology by immunofluorescence assays. The effect of Bub3 knockdown and paclitaxel co-treatment on cell proliferation was analyzed in a long-term colony-forming assay and time-lapse microscopy was performed to follow cell fate. To assess cellular senescence induction, we performed a beta-galactosidase assay. **Results:** BUB3 is upregulated in GBM. BUB3 knockdown significantly inhibited proliferation of glioblastoma cells, and enhanced the antiproliferative activity of paclitaxel on these cells, through potentiation of multipolar spindles and SAC weakening. Interestingly, we showed that BUB3 downregulation exerts its antiproliferative activity mainly through induction of premature cellular senescence and, to a lesser extent, through apoptosis. Senescence phenotype, but not apoptosis, was highly potentiated in BUB3-depleted glioblastoma cells treated with clinically relevant doses of paclitaxel. **Conclusions:** BUB3 inhibition combined with paclitaxel is suggested as a potentially effective strategy for the treatment of GBM. We propose BUB3 as a novel target and biomarker for GBM.

**Keywords:** glioblastoma; BUB3; paclitaxel; mitosis; senescence.

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