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Epigenetic, transcriptional and splicing changes in the glioblastoma marker genes *CHI3L1* and *MGMT* during the acquisition of temozolomide resistance

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Background/Aim. CHI3L1 (chitinase 3-like protein 1) is a secretory glycoprotein highly upregulated in glioblastoma (GBM) and is recognized as a molecular marker of tumor mesenchymal subtype. However the gene's short transcriptional isoform (*CHI3L1Δ8*) is poorly studied regarding its biological role in GBM. O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation is considered a prognostic marker for temozolomide (TMZ) resistance in GBM patients, though some conflicting data exist. We aimed to analyze methylation of the *MGMT* promoter and *CHI3L1/CHI3L1Δ8* expression in cells of the panel of U-251 MG (U251) glioblastoma cell lines, sensitive and resistant to TMZ. **Methods.** Transgenic cell lines U251-*GFP,* U251-*CHI3L1* and U251-*CHI3L2* have been generated previously by lentiviral transduction. TMZ-resistant derivates of transgenic and parental U251 (tmzU251, tmzU251-GFP, tmzU251-CHI3L1 and tmzU251-CHI3L2) have been obtained by cells selection with augmenting concentrations of TMZ. Methyl-specific (MS) PCR and MS-sequencing were used for *MGMT* promoter methylation determination. qPCR with isoform-specific primers was carried out for the analysis of *CHI3L1/CHI3L1Δ8* expression. **Results.** Both MS-PCR and MS-sequencing demonstrated an increase in *MGMT* promoter methylation in all the transgenic and the TMZ-resistant cell lines comparing to intact U251. MS-sequencing showed the most significant increase of methylation level in the tmzU251 cell line comparing to the original U251 ($p < 0.001$ by Kolmogorov-Smirnov test). TMZ also leads to an increase in methylation level of the *MGMT* promoter region in all the transgenic cell lines compared to the original U251 ($p < 0.01$). Transgenic cell lines except the U251-CHI3L1 demonstrated reduced expression of both *CHI3L1* isoforms. On the contrary, TMZ long-term treatment leads to an increase of both *CHI3L1* isoforms expression. Surprisingly the most significant rising was revealed in tmzU251-CHI3L2 with the isoforms ratio retention contrary to tmzU251 and tmzU251-GFP cells. Both these sublines showed bias towards *CHI3L1* full isoform expression whereas tmzU251-CHI3L1 possessed balance shifting in favor of short *CHI3L1Δ8* isoform. **Conclusions.** The obtained data indicate involvement of cellular mechanisms other than the reparative activity of MGMT in the evolution and maintenance of the TMZ-resistant phenotype. This is consistent with other studies on the decreasing of the prognostic role of the *MGMT* promoter methylation status for the assessment of therapeutic effect of TMZ in the treatment of glioblastoma. Balance alteration between the two *CHI3L1* transcripts could be a part of the glioblastoma chemoresistance mechanism in response to TMZ treatment. **Grants/Fundings:** The work is supported by the Simons Support Grant 1290589.

Keywords: glioblastoma, temozolomide resistance, CHI3L1, MGMT.