Molecular Mechanisms Mediating the Synergistic Antiproliferative Effect of Erlotinib and NSC23766 on Glioblastoma Cell Lines

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Background

Glioblastoma is the most frequent brain tumor of glial origin in adults. Despite the best available standard of care, patients with this disease face a life expectancy of only approximately 15 months after diagnosis. Since the epidermal growth factor receptor (HER1/EGFR) is one of the most commonly dysregulated oncogenes in glioblastoma, HER1/EGFR-targeted agents such as erlotinib were developed. However, application of erlotinib in the clinical setting has failed. We previously identified several candidate genes for resistance of human glioblastoma cell lines towards erlotinib. Based on this panel of genes, we found that combined inhibition of HER1/EGFR by erlotinib and of RAC1 by NSC23766 yielded a synergistic antiproliferative effect on established and primary glioblastoma cell lines. The current study, aimed at the identification of the cellular and molecular mechanisms underlying this effect.

Methods

Staining for annexin/PI or carboxyfluorescein succinimidyl ester was performed prior to flow cytometric analysis in order to determine the induction of apoptosis, necrosis or cytostasis in U87 and A172 glioblastoma cell lines. Moreover, expression of Ki67 was determined by immunofluorescence and microscopic analysis. Induction of senescence was examined by staining for beta-galactosidase, and the expression of cell cycle proteins was analysed by Western blot. All analyses were performed after 144 h of continuous exposure to erlotinib, NSC23766 or both at the respective inhibitory concentration 50.

Results

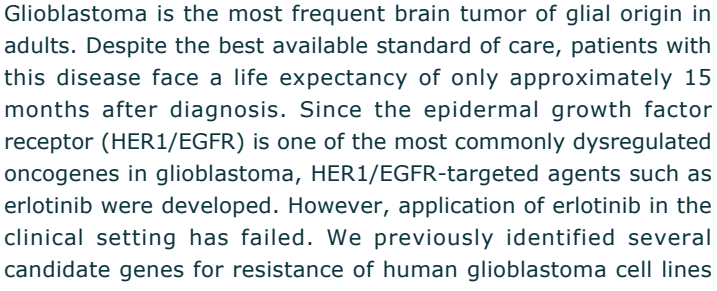

Figure 1: Combined treatment with erlotinib and NSC23766 results in a synergistic antiproliferative effect. Cellular proliferation of three established and two primary cell lines treated for 144 h with both erlotinib and NSC23766 or either agent alone was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay and cell count analysis. Treatment with both agents at their respective IC50 resulted in a synergistic antiproliferative effect in all five glioblastoma cell lines that were examined. The data displayed represent the mean of three independent experiments.

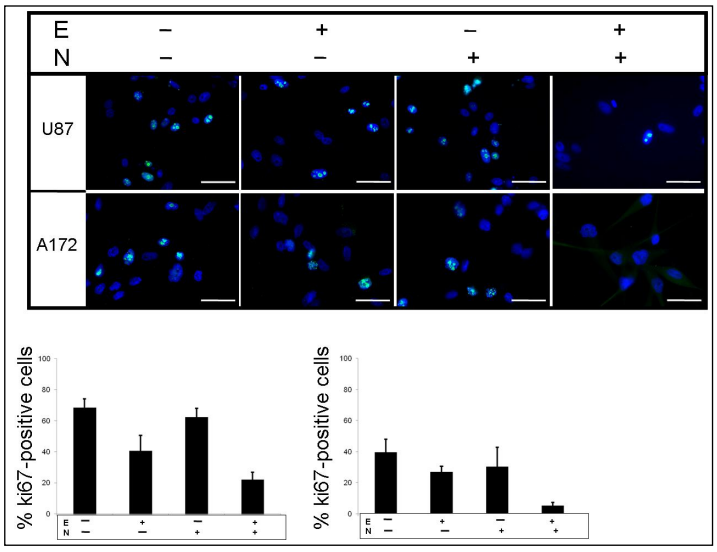

Figure 2: Combined treatment with erlotinib and NSC23766 inhibits the expression of Ki67. Cells were seeded onto cell culture slides and treated with erlotinib, NSC23766 or both agents for 144 h prior to staining for Ki67. Representative microscopic images are shown for three separate experiments done in triplicate (magnification, x40).

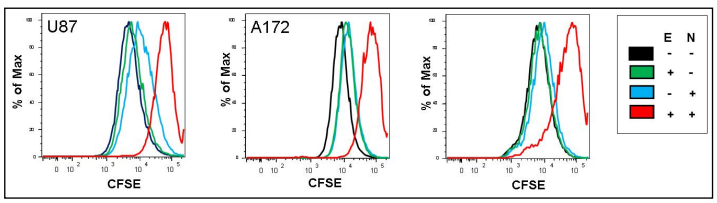

Figure 3: NSC23766 [N] enhances the cytostatic effect of erlotinib [E]. CFSE-labeled U87, A172 and T98 cells treated with both erlotinib and NSC23766, either agent alone (IC50) or the corresponding solvents for 144 h were analysed by flow cytometry. The fluorescence intensity of CFSE was found to be highest for cells treated with both agents when compared to single-agent treatment. These data suggest that NSC23766 enhances the antiproliferative effect exerted by erlotinib on established human glioblastoma cell lines by inhibiting cellular division. The data depicted for each cell line is representative for three independent experiments.

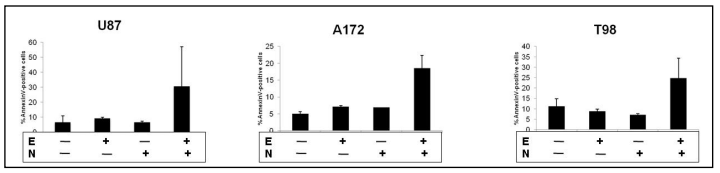

Figure 4: NSC23766 [N] enhances the pro-apoptotic effects of erlotinib [E]. Apoptosis of U87 A172 and T98 cells treated with NSC23766 and erlotinib, either agent alone (IC50) or the corresponding solvents for 144 h was assessed using annexin V/PI staining and flow cytometry. The fractions of annexin V-positive cells were significantly increased upon treatment with both agents when compared to single-agent treatment.

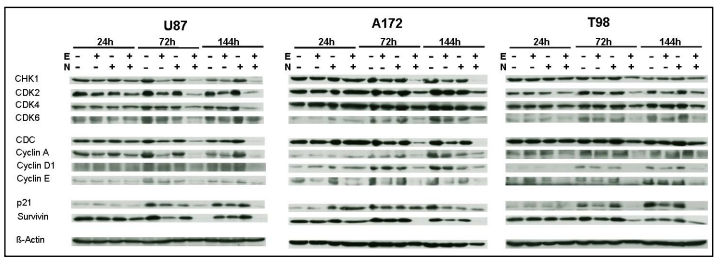

Figure 5: Western blots demonstrating that treatment with erlotinib [E] and NSC23766 [N] inhibits the expression of proteins regulating cell cycle. U87, A172 and T98 cells were treated for 144 h with erlotinib, NSC23766, both agents or solvents under serum starvation. Whole-cell extracts were examined by Western blot for CHK1, CDK2, CDK4, CDK6, CDC, Cyclin A, Cyclin D1, Cyclin E, p21 and survivin protein levels. Actin Western blot analysis was performed to confirm equal protein loading. The results shown are representative for at least two independent experiments.

Conclusions

In our previous work, we identified that the combined treatment with erlotinib and the RAC1 inhibitor NSC23766 yielded a synergistic antiproliferative effect in established and primary glioblastoma cells. Our current study suggests that this effect is due to an enhanced reduction of the number of cell divisions. This fact is supported by a significantly decreased Ki67 expression when compared to single agent treatments. On the molecular level, concomitant treatment with both agents resulted in a pronounced downregulation of cyclin D1, cyclin-dependent kinases 2, 4 and 6, as well as of survivin. Taken together, our data suggest that the combination treatment with erlotinib and a RAC1 inhibitor may represent a promising novel therapeutic approach in glioblastoma.