Diverse sensitivity of melanoma cells originated from various parts of the tumor to dacarbazine treatment

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Melanoma is an aggressive disease with the highest death rate within cutaneous malignancies. One of the reasons for it is the tumor heterogeneity and the formation of subclones with different sensitivity to anti-cancer therapy.

This study was aimed to evaluate the response of melanoma cells from different tumor sites to dacarbazine treatment. Two fragments of primary melanoma from 35-years-old female patient were isolated and cultured: from the central and peripheral part of the tumor. The cells were treated with different concentrations of dacarbazine – 250 mg/l, 500 mg/l, 750 mg/l and 1000 mg/l – and then cell viability/proliferation was estimated using MTT-test after 24 hours of incubation. It was shown that cells from central and marginal sites had similar sensitivity to the treatment and demonstrate 50% loss of viability at the concentration of dacarbazine 885.0 $\mu\text{g/mL}$ and 792.5 $\mu\text{g/mL}$ respectively (according to linear approximation) (Fig. 1.A).

Further we evaluated the effect of 1000 $\mu\text{g/mL}$ of dacarbazine on cell apoptosis, migration and invasion after 24 hours. Dacarbazine treatment induced 30% decrease of alive melanoma cells originated from the central part of the tumor but among melanoma cells from marginal zone 5.4% only were alive (Fig. 1.B). 62.2% cells from the center of tumor were observed at the early apoptosis stage while 54.5% cells from marginal zone were at later apoptosis/necrosis.

Migration and invasive capacities were increased in melanoma cells originated from central part of the tumor after 24 hours incubation with 1000 $\mu\text{g/mL}$ of dacarbazine while melanoma cells from peripheral part diminished their ability to migration and invasion after dacarbazine incubation (Fig. 1.C,D). Our study demonstrates different sensitivity to dacarbazine of melanoma cells depending on tumor site that could be due to heterogenic pattern of tumor cells and need to be considered for melanoma treatment administration.

Fig. 1. The influence of dacarbazine treatment on melanoma cells originated from central and peripheral part of the tumor. Data presented as mean $\pm$ standard error of mean.

(A) Change in viability/proliferation of melanoma cells from depending on the concentration of dacarbazine (MTT-test). Dotted lines indicate 50% inhibitory concentration of dacarbazine (IC₅₀). (B) The effect of 1000 mg/L dacarbazine on melanoma cells apoptosis. */**/***/ - significant differences in the percent of alive (*), early apoptotic (**) and late apoptotic/necrotic (***) cells after dacarbazine treatment ($p < 0.05$). (C) Migration and invasion assay results after 100 mg/L dacarbazine treatment of melanoma cells. * - significant as compared with negative control ($p < 0.05$). (D) Microscopy of migrated cells on polycarbonate membrane.

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