

Study of essential hypomutated genes in skin melanoma

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Motivation and Aim: Current methods of studying tumors are aimed at finding new proto-oncogenes, tumor suppressors, markers of cancer, etc. Information about the origination of the tumor and its genetic causes may be useful in the diagnostics, but its use for cancer therapy is limited. The searching for the genes essential for cancer cell surviving could help to develop effective cancer therapy.

Methods: For *in vitro* analysis we used melanoma cell line A375. We performed knockdown (KD) by lipofection with siRNAs, assessed the efficiency of KD by RT-qPCR. Cell proliferation status was analyzed by MTT-test and flow cytometry. Induction of apoptosis was measured by Caspase-Glo[®] 3/7 Assay System. Cell migration was assessed by wound-healing assay.

Results: Using the principle named after Abraham Wald our colleagues conducted a bioinformatic analysis of mutations in the genome of the melanoma tumors and identified 91 genes not affected by considerable mutations (1). 12 out of 91 genes were selected for further analysis. We analyzed the expression level of these genes in melanoma cell line A375 by RT-qPCR. For three genes with highest expression level (*UNC45A*, *RHPN2* and *ZNFx1*) we performed knockdown (KD) experiments on the A375 cell line followed by proliferation, apoptosis and migration assays. We achieved KD efficiency for the *UNC45A* gene about 70 %, for *RHPN2* – 55 %, and for *ZNFx1* – 60 %. Caspase activity raised up to 150 %, 125 % and 200 % comparing to control, respectively. Whereas proliferation assay did not reveal significant changes. Data of the migration of the cells is in progress.

Conclusion: Our results demonstrate that knockdown of chosen genes do affect caspase activity of the melanoma cells, but do not cause changes in viability of the cells. Further investigations are needed to fully confirm these results.

References

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