FLOW CYTOMETRIC ANALYSES OF TNFα INFLUENCE ON BIOLOGY OF MELANOMA AND COLORECTAL CARCINOMA CELLS

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Pleiotropic cytokine tumor necrosis factor alpha (TNFα) has both anti-tumorigenic and pro-tumorigenic activity. Overexpression of TNFα in tumor cells results in reduced tumorigenic potential *in vivo*. In order to study the direct effects of TNFα on tumor cell biology and on cancer stem cell- like subpopulation (CSCs) *in vitro*, we engineered cells of two different origins continually overexpressing human TNFα gene: epithelial colorectal adenocarcinoma line HT29 and malignant melanoma line A375 of neural crest origin.
Using flow cytometry, quantitative RT-PCR and spheroid formation assay, we evaluated CSCs subpopulation and activity of aldehyde dehydrogenase (ALDH). Mitochondrial status and autophagy were also determined by flow cytometry.
Under the TNFα overexpression, we documented significantly lowered mitochondrial mass, lowered ATP production, and changes in mitochondrial morphology in both cell lines. The expression of main CSCs surface markers and stemness related genes *in vitro* remained unchanged. However, we could demonstrate a decrease of ALDH activity (up to 50%), which is linked to the stemness phenotype. The 3D cultivation of spheroids, which are believed to be enriched by CSCs, revealed no significant changes. But the TNFα overexpression resulted in increased autophagy on day 3, followed by senescence induction on day 6 in melanoma cells A375hTNFα.
Our *in vitro* study provides concise report of cellular processes initiated in malignant cells upon the TNFα overexpression. Despite its high overexpression in engineered cells, the subpopulation of CSCs responsible for tumor growth initiation remained unaffected and probably is not directly linked with the loss of tumorigenic potential. Our results point out the pleiotropic nature of TNFα and its diverse effect on cancer cells of different origin.

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