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Mechanisms of action of 2-Deoxy-D-Glucose in Cancer Cells and Evaluation of Novel Approaches of Application

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Altered glucose metabolism, namely a high activity of the glycolytic pathway over oxidative phosphorylation is a common feature of virtually all malignant tumors. The low amount of energy per glucose molecule gained by this pathway results in a high glucose dependence of cancer cells and consequently a high glucose uptake. The universality of this cancer hallmark makes it particularly attractive as a target for therapeutic approaches. 2DG is a substance capable of blocking glycolytic pathway due to its structural similarity to glucose, leaving a cancer cell without its major energy source and thereby leading to energy starvation, at least in theory. In the practical application, however, 2DG itself is not able to completely eradicate cancer at maximally tolerable concentrations, but can only cause strong suppression of tumor growth. Therefore, and to develop novel cancer therapies targeting the universal hallmark of aerobic glycolysis, it is required to develop methods of either sensitizing cancer cells to low doses of 2DG, or delivering high doses of 2DG directly to the tumor and thereby minimizing its side effects.

In the present thesis, sensitization of cancer cells to low doses of 2DG was achieved using combination with Rocaglamide and FL3, flavaglines, which possess anti-cancer properties. Applied in combination, 2DG and Rocaglamide were shown *in vitro* to achieve a stronger growth inhibitory effect than each of drugs applied alone, confirmed by independent methods and a variety of cancer cell lines of different origin. 2DG was shown to substantially reduce the half maximal inhibitory concentration of Rocaglamide and FL3 alongside with protecting normal PBMCs from potential cytotoxic effects of the tested flavaglines. Moreover, the combination was also tested *in vivo* in frame of an exploratory animal experiment and was shown to significantly inhibit tumor outgrowth. Future experiments should encompass testing the combination on a larger set of animals using an animal model, suitable for addressing of not only anti-cancer efficacy, but also possible side effects and toxicity of the combination.

In order to explore the possibility of targeted 2DG delivery directly to the tumor site, the drug was coupled to superparamagnetic iron oxide nanoparticles. It was demonstrated that coupling was successful and led to formation of well-shaped particles with a small size range, optimal for potential delivery *in vivo*. Moreover, 2DG in its coupled form maintained its growth inhibitory efficacy on tumor cells: 2DG-coupled nanoparticles significantly decreased cancer cell viability *in vitro*, confirming the general feasibility of loading 2DG on nanocarriers, at the same time maintaining its growth suppressing properties. The distribution of obtained 2DG-

coupled nanoparticles in the living organism and confirmation of their targeted delivery to site of interest shall be addressed in future experiments in a suitable animal model.

The absence of the hydroxyl group at the second carbon atom makes 2DG similar not only glucose, but also to its C-2 epimer, mannose, which is one of the most relevant monosaccharides participating in N-linked protein glycosylation. Therefore, 2DG can also interact with protein glycosylation. Previous data reported inhibition of N-linked glycosylation, mostly addressing the effect of high doses of 2DG. In contrast to these studies, in the present thesis, 2DG at low doses was shown for the first time to induce substantial hypermannosylation of a broad spectrum of cellular glycoproteins, including those of the membrane fraction, by increasing the incorporation of mannose into glycoproteins. The significant boost in glycosylation was observed already after first hours of 2DG treatment and had a long-lasting character, which was confirmed further by increased protein mannosylation of tumor specimens obtained from mice treated with 2DG. Induction of hyperglycosylation in cellular proteins may open the path towards novel strategies of making tumor cells more immunogenic, because 2DG is preferentially uptaken by cancer cells, in which it has the potential to induce novel glycoantigens that can be recognized by the immune system. This observation 2DG raised the question whether additional immune-modulatory effects were caused by 2DG, which might interfere with the potential enhancement of immunogenicity of tumors by 2DG-induced hyperglycosylation. Therefore, the influence of 2DG on classical antigen-presenting pathways, including MHC class I molecules, was also examined in this thesis. The data showed downregulation of intracellular MHC class I antigen levels, but maintenance of the functional MHC class I membrane expression upon 2DG, suggesting that MHC class I molecules in 2DG-treated cells are more efficiently transferred to the cell surface membrane.

Taken together, these findings underscore the relevance of metabolic alterations in cancer cells as possible targets for cancer therapy and provide novel approaches for increasing cancer cell sensitivity towards drugs targeting the energy metabolism of cancer cells. The results of the present thesis allow considering 2DG not only as a glucose antimetabolite, but also a strong enhancer of protein glycosylation, which in turn may possibly contribute to increased immunogenicity of cancer cells.