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Thymic Tissue Metabolomics and Bioengineering of an Artificial Thymic Carcinoma 3D Cell Culture for in vitro 3D/2D Drug Screening and the Investigation of Toxicity Employing a Novel Nuclear Magnetic Resonance Technique

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Thymomas and thymic carcinomas (TC) are malignant thymic epithelial tumors (TETs) that mostly lack targetable mutations. Metabolic signatures of TETs have not yet been studied and may offer new therapeutic options. Metabolic profiles in snap-frozen thymomas (n=12) and TCs (n=3) were determined by high resolution magic angle spinning 1H nuclear magnetic resonance (HRMAS-1H NMR) spectroscopy. Metabolite-based prediction of active KEGG metabolic pathways was achieved with MetPA. In relation to metabolite-based metabolic pathways, gene expression signatures of TETs (n=115) in “The Cancer Genome Atlas” (TCGA) dataset were investigated using gene set enrichment analysis tools. Overall, thirty-seven metabolites were quantified in TETs, including acetylcholine that was not previously detected in other non-endocrine cancers. Metabolite-based cluster analysis distinguished indolent (WHO types A, AB, B1) and aggressive (B2, B3) thymomas and TCs. Six KEGG metabolic pathways were predicted as activated, including proline/arginine, glycolysis and glutathione pathways, and these pathways were generally enriched transcriptionally in the independent TCGA dataset. Shared high lactic acid and glutamine levels and associated gene expression signatures hint to a strong “Warburg effect”, glutaminolysis and redox homeostasis as potential vulnerabilities in aggressive TETs. Since a recent study observed a vulnerability of neoplastic thymic epithelial cells grown in 2-dimensional (2D) cultures towards Bortezomib, the impact of this proteasome inhibitor on the metabolic profiles of Ty82 and 1889c TC cell lines was analyzed by NMR, revealing a decrease of glutamate and glutathione levels. This suggests an inhibition of glutaminase (GLS) and glutamate-cysteine ligase (GCLC) and hints to a role of oxidative stress as mechanism of action of Bortezomib, as low glutathione levels foster Bortezomib-induced apoptosis. In both TC cell lines, pyruvate levels increased with Bortezomib doses, while lactate concentrations decreased, suggesting inhibition of lactate dehydrogenase A (LDHA). Employing a new spatially selective NMR pulse technique that was developed in the framework of this thesis for in vitro metabolic profiling of 3D cell cultures (spheroids) under viable conditions, the impact of Bortezomib on metabolite profiles could be measured repeatedly in the same spheroids during 24h, revealing a decrease of glutamate and glutathione levels as in 2 D cultures. However, in contrast to the 2D cell culture findings, pyruvate could not be detected at all in 3D cultures/spheroids, while lactate levels were increasing with time, suggesting a high expression of functional LDHA in spheroids. Finally, spheroids of TC cell lines showed relative resistance to Bortezomib when compared with the respetve cell lines grown in 2D cultures. In conclusion, these results underline the need to establish in vitro models that reflect the in vivo tumor setting as closely as possible and cast some light on the impact of Bortezomib on the metabolism of TC cell lines depending on the 2D versus 3D cell culture conditions.