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Glutamine-Independent Survival Mechanisms in Colorectal Cancer Cells: An Integrative RNA-seq, ATAC-seq, and miRNA-seq Study

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Cancer cells have a higher proliferation rate compared to normal cells. To support this rapid growth, they primarily depend on glutamine, an essential nutrient that fuels their energy production through the TCA cycle. While most cancer cells cannot survive without glutamine, some adapt through epigenetic regulation. Notably, in colorectal cancer characterized by its heterogeneity, the presence of PIK3CA mutations allows for the reprogramming of glutamine metabolism. We generated a colorectal cancer cell line resistant to glutamine deficiency (Gln^-) by sustaining HCT15, a colorectal cancer cell line with PIK3CA mutations, for approximately two months in a glutamine-deprived environment. Afterward, we conducted RNA sequencing (RNA-seq) to identify specific targets that exhibited differential expression in Gln^- cells compared to parental cells (WT). Additionally, we performed ATAC sequencing (ATAC-seq) and microRNA sequencing (miRNA-seq) to investigate the epigenetic regulation of these targets. Focusing on the evasion of cell death programs in the absence of glutamine, we observed a significant difference in the expression of ferroptosis-related genes. Furthermore, among the genes that exhibited significant changes in expression, we identified the enzyme GLS2, responsible for converting glutamine to glutamate, by focusing on genes with common relevance to both ferroptosis and glutamine metabolism. Specifically, based on the results of ATAC-seq, we found that in Gln^- cells, the GLS2 promoter region was relatively closed in comparison to WT cells. This relative closure of the GLS2 promoter region implies a reduced binding of transcription factors to this site, which could potentially account for the observed decrease in GLS2 mRNA expression. Additionally, through miRNA-seq, we confirmed a significant increase in

the levels of miR-561-3p and miR-4796-5p, which are expected to bind to GLS2 mRNA, in Gln^- cells. We anticipate our research findings to facilitate the development of new drugs by understanding the survival strategies of colorectal cancer cells in glutamine-depleted environments.