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Title: EXTRACELLULAR GLUTATHIONE CATABOLISM AS AN ALTERNATIVE CYST(E)INE SOURCE IN CANCER

Abstract:

Glutathione (GSH) is the most abundant antioxidant in the human body and plays important role in both physiology and disease. The role of antioxidants in cancer is fairly complex and while reactive oxygen species (ROS) are involved in tumor initiation and progression, many studies show that ROS-scavenging molecules, such as glutathione (GSH), not only do not impede tumor growth but accelerates it. However, preliminary data from our lab show that genetically ablating tumor-specific GSH production does not affect its growth, suggesting that extracellular sources may be pivotal for the positive effect of GSH on tumor growth. Indeed, GSH is found in surprisingly high concentrations at the tumor interstitial fluid, reaching up to 10 times its plasma concentration. The exact function of extracellular GSH is not known. While it is suggested that it may function as an antioxidant combating extracellular protein and membrane oxidation, there is also strong evidence that suggests that catabolism of GSH may serve as a cysteine source, an indispensable amino acid found in low concentrations in the tumor environment. Gamma-glutamyl-transpeptidase 1 (GGT-1) is a cell surface hydrolase that cleaves γ-glutamyl-compounds such as reduced or oxidized glutathione, yielding free glutamate and the dipeptide cysteinyl-glycine that can be further broken down to produce free cysteine. This study aims to understand the involvement of GGT1 on cysteine acquisition, dissect the players involved in the uptake and utilization of GSH breakdown products, and elucidate the stress-response pathways triggered by cyst(e)ine depletion that might coordinate these processes. We evaluated the response of three different TNBC cell lines to cystine depletion for 96 hours and observed a decrease in cell number that was almost entirely rescued by both 500µM GSH and 500µM cysteinyl-glycine, the product of GGT1-mediated GSH breakdown. We also observed in HCC-1806 cells a decrease in cell proliferation and increased cell death, all reversed by both GSH and cysteinyl-glycine. Next, we pharmacologically targeted GGT1 to evaluate its contribution to the GSH-mediated cell survival in cystine-free conditions. Our results show that HCC-1806 cells grown in high cystine media (208µM) are not affected by the GGT1 inhibitor GGsTop. However, when cystine is replaced by GSH in the media, the presence of GGsTop causes a dose-dependent decrease in cell number, revealing that the ability of GSH to reverse the effect of cystine depletion is dependent on GGT1 activity. This effect is not observed when cystine is replaced by cysteinyl-glycine, which, as expected, bypasses the GGT1 inhibition. Next, we evaluated the effect of GGsTop, CB839, and Auranofin on HCC1806 cells under different media conditions and observed that, again, GGsTop impairs the GSH-mediated rescue of cell number. Also, the cysteine acquisition via GSH catabolism renders the cells resistant to CB839 - an inhibitor of the conversion of glutamine to glutamate - suggesting the use of this alternative pathway instead of the system xCt preserves intracellular glutamate pools making the glutamine-to-glutamate conversion dispensable. To better understand the signaling pathways involved in the cell response to cystine starvation, we conducted qPCR for target genes of the NRF2 and ATF4 pathways. We observed that both NRF2 targets (HMOX, NQO1) and ATF4 targets (ASNS, PHGDH) are activated. However, while GSH and cysteinyl-glycine reverse the activation of the NRF2 targets, it does not reverse the ATF4 targets, revealing an interesting disparity in the deactivation of these two stress responses. Finally, the screening of genomic databases reveals candidate genes for the uptake and intracellular utilization of cysteinyl-glycine. The cysteinyl-glycine transporter SLC15A1 and SLC15A2 and the dipeptidases DPEP1 are found elevated in basal breast tumors, raising the possibility that they may participate in the cysteine acquisition in TNBC. Future studies focused on those candidate genes will help us dissect the players involved in GGT1-mediated GSH catabolism.