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Cellular glycosphingolipid imbalance modulates EMT in cancer cells

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Abstract

Sphingolipids are key components of the plasma membrane and are regulators of complex biological processes often altered in cancer cells. In human tumors, genes of key enzymes that regulate levels of glucosylceramide (GlcCer) and lactosylceramide (LacCer) are often amplified. However, it is unknown why these traits are positively selected in transformed cells. In this work, we used CRISPR-Cas9 to knockout two key enzymes amplified in tumors in HeLa and H1703 tumor-derived cell-lines. As expected, the knockout lines had dramatic accumulation of GlcCer and LacCer. However, unexpectedly, they showed significantly decreased in-vitro wound-healing capacity and an almost complete loss of in-vitro extracellular matrix invasion. Based on these results, we probed for protein markers of the epithelial-to-mesenchymal transition (EMT). Data showed a significant increase of the levels of E-cadherin and a decrease of N-cadherin, suggesting that knockout cells acquired a more epithelial-like phenotype. Knockout lines also had significant changes in SNAIL levels, an important regulator of E-cadherin expression and EMT marker. As SNAIL can be regulated by growth factor receptors such as EGFR, we probed for global changes in growth factor receptor tyrosine kinase (RTK) activation. Results showed that, compared to their otherwise isogenic wild-type counterparts, knockout lines had broad changes in growth factor RTK activation patterns. The knockout cells also had significant changes in their responses to cytotoxic chemotherapeutic agents. Our work suggests that increased expression of key glycosphingolipid regulating enzymes in transformed cells are critical to promote malignant phenotypes by impacting EMT, sustained activity of growth factor RTKs, and responses to therapy.

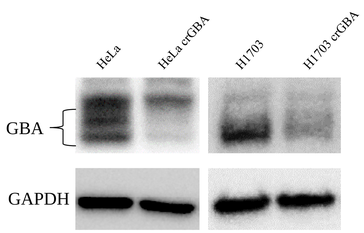
Introduction

Alterations in lipid metabolism are a hallmark of cancer. Dysregulation of lipid metabolism is associated with some of the most common cancers in the United States – such as breast, lung cancers, and prostate cancer. Presently, the scientific community is learning more and more about the role of sphingolipids in the progression of cancers. It has previously been established that sphingolipids are involved in cell growth, cell death, response to therapies, and chemoresistance. The specific mechanisms in which they can modulate these processes, however, remains elusive.

Lipidomic analysis on six different cancer lines indicates that GlcCer is significantly accumulated, without changes in *de novo* biosynthesis. Using genomic mapping to better understand the accumulation revealed *GBA* (enzyme responsible for GlcCer degradation) as one of the most highly altered, often amplified, enzymes in cancers – suggesting that this enzyme is non-functional in cancer or has additional roles beyond what is currently understood.

Methodology

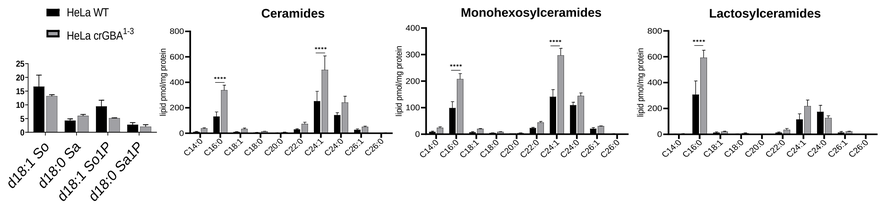
GBA was deleted using CRISPR-Cas9 in HeLa and H1703 cell lines. Cells were grown in EMEM and RMPI, respectively. Experiments used to probe *GBA* function include: lipidomic assessment, migration (scratch) assays, western blot, invasion assays, cytotoxicity assays, signaling assays for growth factor receptors, and microscopy.



Results

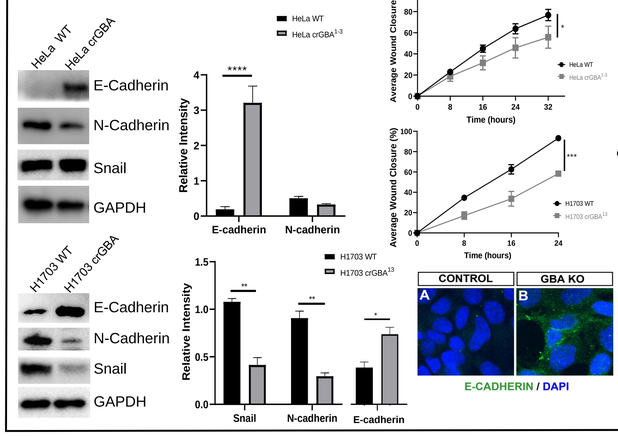
GBA deletion resulted in broad sphingolipid alterations without changes in *de novo* biosynthesis:

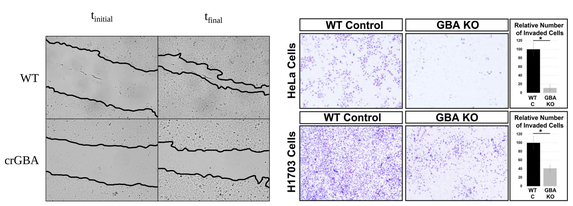
crGBA cells accumulated ceramides, GlcCer, and LacCer – indicating that GBA plays an active role in the cell. The lack of changes in *de novo* biosynthesis support that GBA is a functional enzyme in cancer that likely modulates lipid composition in the cells.



GBA deletion resulted in changes of EMT markers, with alterations being evident in defective crGBA cell migration and invasion:

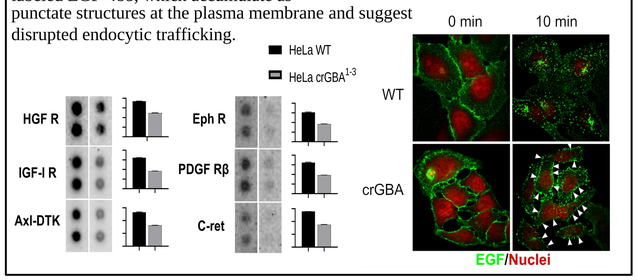
Major markers like E-cadherin, N-cadherin, and SNAIL (a pro-EMT transcription factor) were significantly altered in crGBA cells, suggesting a reverse shift into MET. The lipidomic and western blot data revealed that crGBA cells have highly altered cell membrane compositions. Membrane structure is crucial for a cell's ability to navigate its environment. Invasion and scratch assays (for migration) revealed that this alteration is detrimental to the ability of GBA knockout cells to move through a Matrigel (invasion) and wound close (migration) over time. It is important to note that there is no difference in growth rate over time between each wild-type cell and its respective knockout.





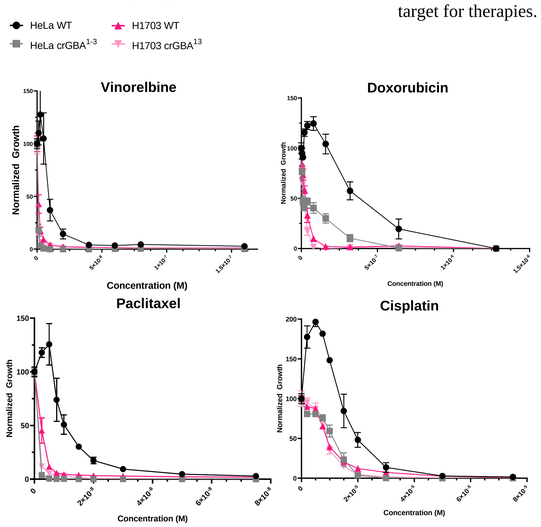
Loss of GBA resulted in global changes in phosphorylation patterns of receptor tyrosine kinases (RTKs) and defective internalization of EGF:

The vast differences in crGBA cell RTK expression supports alterations seen in SNAIL, whose expression is induced by RTKs. E-cadherin is a known modulator of RTK activation, and the broad increases in E may act as suppressors for various RTKs. The combination of these results, in conjunction with lipidomics, suggests that GBA knockouts likely have extensive remodeling of the plasma membrane that contributes to the phosphorylation patterns of unrelated RTKs. Additionally, changes in the plasma membrane may account for the slower internalization of fluorescently-labeled EGF-488, which accumulate as punctate structures at the plasma membrane and suggest disrupted endocytic trafficking.

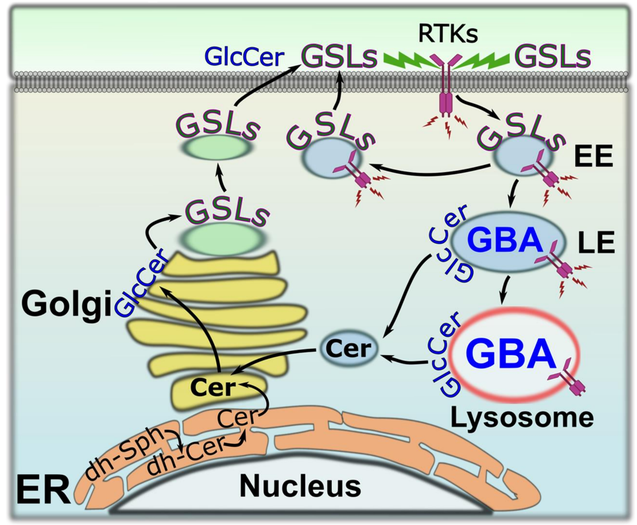


GBA deletion resulted in increased sensitivity to chemotherapeutic drugs Vinorelbine, Doxorubicin, Paclitaxel, and Cisplatin:

crGBA cells, both HeLa and H1703, demonstrated significantly increased sensitivity to major cancer chemotherapeutic drugs. Mass cell death occurred at many of the lowest drug concentrations, which are much lower than the normal drug administration in human patients. The response of these cells to drug suggests that GBA inhibition could prove a meaningful target for therapies.



Conclusion



GBA modulates EMT by influencing endocytic trafficking & activation of receptor tyrosine kinases.

In light of the data collected, we are proposing that GBA functions at the intersection of the late endosome and lysosome, rather than being strictly confined to the lysosome. This means that GBA would be able to affect EMT by influencing endocytic trafficking and activation of receptor tyrosine kinases. GBA regenerates ceramides needed for the synthesis of glycosphingolipids (GSLs), which are important for maintaining EMT status and supporting the activity of RTKs in the membrane.

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