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AUTOPHAGIC PUNCTUM

Blocking glycosphingolipid production alters autophagy in osteoclasts and improves myeloma bone disease

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ABSTRACT

Glycosphingolipids (GSLs) are key constituents of membrane bilayers playing a role in structural integrity, cell signalling in microdomains, endosomes and lysosomes, and cell death pathways. Conversion of ceramide into GSLs is controlled by GCS (glucosylceramide synthase) and inhibitors of this enzyme for the treatment of lipid storage disorders and specific cancers. With a diverse range of functions attributed to GSLs, the ability of the GSC inhibitor, eliglustat, to reduce myeloma bone disease was investigated. In pre-clinical models of multiple myeloma, osteoclast-driven bone loss was reduced by eliglustat in a mechanistically separate manner to zoledronic acid, a bisphosphonate that prevents osteoclast-mediated bone destruction. Autophagic degradation of TNF receptor-associated factor 3 (TRAF3), a key step for osteoclast differentiation, was inhibited by eliglustat as evidenced by TRAF3 lysosomal and cytoplasmic accumulation. By altering GSL composition, eliglustat prevented lysosomal degradation whilst exogenous addition of missing GSLs rescued TRAF3 degradation to restore osteoclast formation in bone marrow cells from myeloma patients. This work highlights the clinical potential of eliglustat as a therapy for myeloma bone disease. Furthermore, using eliglustat as a lysosomal inhibitor in osteoclasts may widen its therapeutic uses to other bone disorders such as bone metastasis, osteoporosis and inflammatory bone loss.

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Autophagy; glycosphingolipid; TRAF3; osteoclast; multiple myeloma

Glycosphingolipids (GSLs) are ubiquitous components of membrane bilayers that perform key structural functions ensuring membrane integrity and the organisation of microdomains. In addition to regulating biophysical properties, they participate in many biological processes, including mitochondrial function, proliferation, migration, cell signalling, endoplasmic reticulum (ER) stress, and autophagy.

De novo synthesis of GSLs begins in the ER and leads to ceramide production, a key component for sphingolipid metabolism. Subsequently the enzyme UDP-glucose ceramide glucosyltransferase (gene *UGCG*; protein known as glucosylceramide synthase (GCS)) catalyses the transfer of glucose from UDP-glucose to ceramide to produce glucosylceramide (GlcCer). Inhibition of GSLs synthesis at this step has been shown to reduce disease activity in lipid storage disorders, various cancers and inflammation, outlining the importance of GSLs in cellular processes. In Gaucher's disease, a rare, genetic lysosomal disorder with lipid dysfunction and accumulation, the drug eliglustat is used as a GCS inhibitor to lower the amount of GSLs in lysosomes. There are also abnormalities in bone modelling and reduced bone density in Gaucher disease, indicating that excess bioactive GSLs affect the balance between osteoblast and osteoclast function.

Multiple myeloma is a haematological cancer caused by abnormal plasma cell expansion in the bone marrow, which is accompanied by bone loss. We have shown that eliglustat is able to reduce the associated bone destruction in this disease by reducing excessive osteoclast formation and activity in murine models of myeloma. The mode of inhibition is distinct from that of the most used therapeutic for bone loss in myeloma, the bisphosphonate zoledronic acid. Based on the potent inhibitory effect of eliglustat on osteoclast observed *in vivo*, studies were conducted to ascertain the mechanism of action. RAW264.7 macrophages were used as osteoclast precursors and we revealed that their membrane lipid structures were more fluid after eliglustat administration, which can be achieved by reducing cholesterol or increasing the levels of unsaturated lipids in the cellular membranes. GSLs, in combination with cholesterol, sphingomyelin, and GPI-anchored proteins and membrane-associated signal transduction proteins molecules, form GSL-enriched microdomains. These microdomains coordinate signal transduction and they are essential for osteoclast differentiation. Additionally, TNF receptor-associated factor 6 (TRAF6)-mediated receptor activator of NF-κB ligand (RANKL; TNFSF11) signalling accompanied by the degradation of TRAF3 needs to occur for osteoclast formation. In our study, eliglustat did not disrupt canonical nuclear factor kappa B (NF-κB) signaling as TRAF6 was unaffected. However, there was an accumulation of TRAF3 indicating that eliglustat blocks TRAF3 lysosomal degradation that is elicited by receptor activator of NF-κB (RANK; TNFRSF11A) activation. Thus, the alterations in lipid structure induced by eliglustat may affect the formation and/or fusion of intracellular vesicles.

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Since GSLs, such as GD3 and GM2, play an essential role in autophagy, the requirement for autophagy in TRAF3 degradation was investigated. An accumulation of single-membrane autolysosomes was observed in RAW264.7 cells treated with eliglustat, indicating that eliglustat prevents degradation within autolysosomes and thereby maintains TRAF3 level in line with our findings, the GSL inhibitor D-PDMP similarly acts as a lysosomal/autophagy inhibitor in neuroblastoma cells. Furthermore, in autophagy-deficient A549 cells, elevated TRAF3 levels suppress tumorigenicity, suggesting that eliglustat could have anti-tumour effects or may be useful to treat diseases with activation of the non-canonical NF-κB pathway. Our findings pave the way to further investigate whether as a lysosomal inhibitor, eliglustat can be used in combination with other compounds to treat diseases beyond bone, including refractory myeloma and melanoma.

Profiling specific GSL composition changes in response to eliglustat was undertaken using LTQ-ESI-MS glycosphingolipidomics and showed a significant reduction in specific types of LacCer and GlcCer. Exogenous addition of GSLs reversed the osteoclast inhibition effect caused by eliglustat in both the murine cell line and, importantly, in osteoclasts derived from patients with myeloma. In addition to the gangliosides GD3 and GM2, known to be involved in autophagy, our study further identified that LacCer(s) and GlcCer(s), the precursor molecules of all gangliosides, promote autophagy-dependent osteoclast differentiation. Hence, by removing specific GSLs from osteoclasts, eliglustat inhibits the process of autophagy and maintains TRAF3 protein levels thus inhibiting osteoclast formation.

While only GD3 and GM2 were previously shown to have a clear role in the initiation, maturation, and fusion stages of the autophagy process, how the rest of the GSLs contribute was unclear. Our results show that GSL inhibition with eliglustat prevents degradation in the autolysosome interior suggesting a broader role for GSL throughout autophagy (Figure 1). For example, depletion of GSL in cardiomyocytes adversely affects cellular trafficking by reducing endolysosomal retrograde transport and autophagy. Additionally, whether GSL inhibitors specifically effect lysosomal features, such as pH, lysosomal membrane protein composition, and functional components involved in cargo turnover like proteases of the cathepsin family, remains not elucidated. GCS inhibition has been shown to augment lysosomal membrane permeabilization suggesting that depletion of relevant GSL destabilises the lysosome. Furthermore, GCS inhibition combined with lysosomal inhibition can impair tumour growth and prolong murine survival time in xenograft tumour models. Thirdly, the role of GSL in clathrin-independent endocytosis warrants further investigation. Pathogens, including viruses, bacteria, and fungi, can enter host cells by binding to GSL-containing microdomains. Meanwhile, pathogens that are phagocytosed utilise a variety of molecular strategies to avoid elimination, such as blocking the fusion of phagosomes with lysosomes or preventing acidification of phagolysosomes, by manipulating signalling via microdomains or altering GSL metabolism. Thus, there

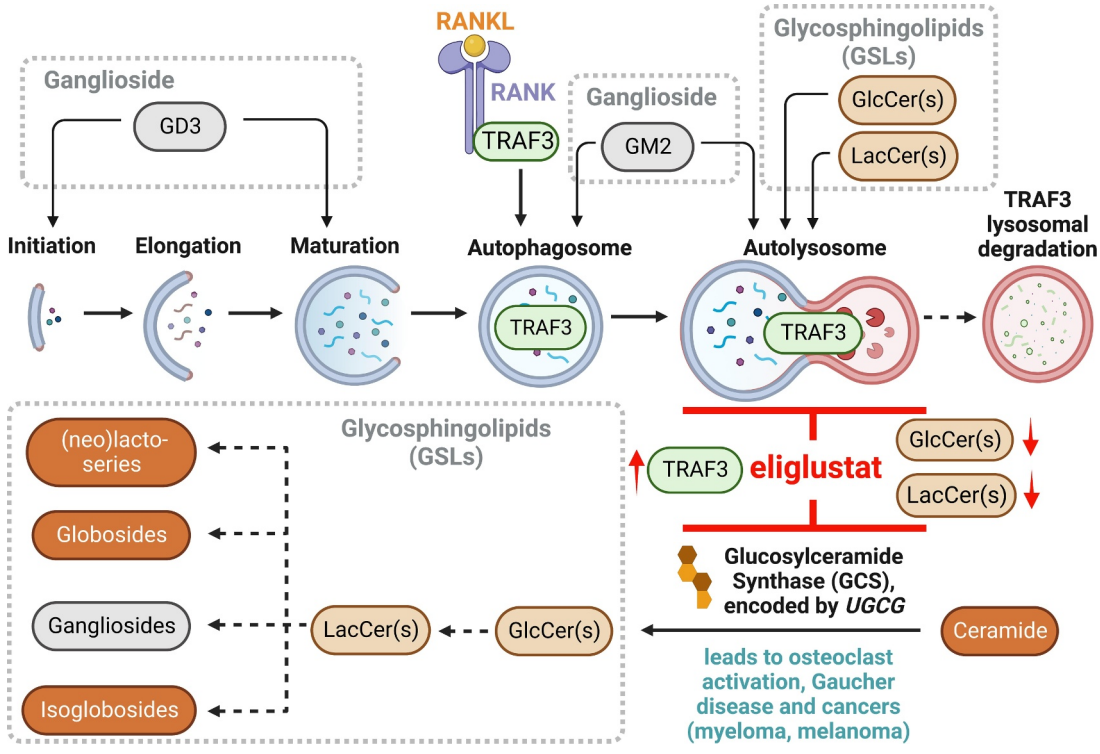

Figure 1: Illustration of the autophagy process including initiation, elongation, maturation, autophagosome fusion and degradation. Binding of RANKL to its receptor, RANK, activates TRAF3 degradation via the autophagy/lysosome system, which can be disrupted by eliglustat due to the prevention of lysosome degradation. Eliglustat blocks GSLs formation from ceramide by inhibiting GCS (encoded by *UGCG*), to reduce GlcCer, LacCer, gangliosides and other GSLs ([neo]lacto-series, globosides, isoglobosides). Specifically, gangliosides GD3 and GM2 support autophagy during initiation and maturation. In our study, we concluded that GlcCer and LacCer are essential components for lysosome degradation. Hence, blocking GlcCer and LacCer formation by eliglustat disrupts autophagy, and halts osteoclast formation and activation, in addition to its known roles in the treatment of Gaucher disease. Created with BioRender.com.

are several avenues for further investigation to gain a complete understanding of the role of GSL in the lysosomal, endosomal and autophagic processes.

In conclusion, we have demonstrated the potential for the translational use of eliglustat in myeloma bone disease. GSL inhibition in diseases that involve bone loss, such as in breast cancer and prostate cancer metastasis, post-menopausal osteoporosis, and arthritis, may lead to an improvement in the bone mass and quality. Thus, eliglustat, a clinically approved drug for the Gaucher's disease, has the potential to be repurposed for patients who suffer from various bone loss diseases.[1]

Abbreviations

ER	Endoplasmic reticulum
GCS	Glucosylceramide synthase
GlcCer	Glucosylceramide
GSLs	Glycosphingolipids
NF-κB	Nuclear factor kappa B
RANK	Receptor activator of NF-κB
RANKL	Receptor activator of NF-κB ligand
TRAF3	TNF receptor-associated factor 3
TRAF6	TNF receptor-associated factor 6
UGCG	UDP-glucose ceramide glucosyltransferase

Disclosure statement

H.L, A.K.S. and N.J.H declare no competing interests.

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Reference

1. Houfu Leng, Hanlin Zhang, Linsen Li, et al. Modulating glycosphingolipid metabolism and autophagy improves outcomes in pre-clinical models of myeloma bone disease. Nature Communications. 2022 Dec 22;13(1):7868. doi: 10.1038/s41467-022-35358-3