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Sphingolipid biosynthesis and inflammatory signaling in asthma (605.21)

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Abstract

Objective – SNPs located within human chromosomal region 17q21 are strongly associated with the incidence of inherited asthma and have been correlated by others to elevated transcript levels of nearby genes, including ORMDL3. Studies in yeast have identified the yeast ORMDL3 orthologs, as negative regulators of the serine palmitoyl-CoA transferase (SPT) enzyme complex. This study investigates the role of mammalian ORMDL3. Methods and Results – To further understand the function ORMDL3, a stable doxycycline-inducible human embryonic kidney (HEK) cell line was developed that overproduces ORMDL3 up to 40-fold compared to control cells. SPT activity in ORMDL3 overexpressing cells was reduced to 40–60% compared to that of control cells. Sphingolipidomic analysis revealed that all sphingolipid species, were reduced in these cells, consistent with significant inhibition of SPT activity. Conversely, combined RNAi-mediated knockdown of all ORMDL isoforms resulted in higher SPT activity and increased sphingolipids. However, knockdown of any single ORMDL isoform did not alter SPT activity, suggesting that they serve a redundant function in inhibiting SPT. Additionally, individual knockdown of the two, different, small non-catalytic subunits of SPT, SPTSSA and SPTSSB, did not alter ORMDL3-mediated inhibition of SPT. Furthermore, to attempt to determine the consequence of increased ORMDL3 expression in asthma, LPS–induced TLR4 endocytosis was measured in RAW macrophages overproducing ORMDL3. There was a very modest increase in the fraction of cells displaying cell surface TLR4 in the ORMDL3 overexpressing macrophages as compared to control cells. This result may due to inefficient transfection of the cell population. Conclusions – Taken together, these findings suggest that, although Ormdl3 demonstrably impacts sphingolipid biosynthesis at the level of SPT, overproduction ORMDL3 does not dramatically affect at least one arm of the innate immune response.