Activation of Glucosylceramide synthesis by Src family tyrosine kinase.

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【Introduction】Glucosylceramide (GlcCer), a member of sphingolipids, is synthesized from Ceramide by Glucosylceramide synthase (GCS). GlcCer and its metabolites are involved in cancer cell adhesion, migration, and metastasis. It is well studied that the control of GCS protein level by transcriptional regulation. However, the regulation of GCS enzymatic activity is not revealed. Our laboratory previously reported that the tyrosine kinase Src regulated Ceramide metabolites. So, we focus on the effects of Src-mediated tyrosine phosphorylation to GCS enzymic activity

【Results】To analyze the involvement of Src in GCS activity, we used v-Src, active mutant of Src, inducible expression cell lines. First, we quantitated GlcCer synthesis from Ceramide using Nitrobenzoxadiazole (NBD)-labeled Ceramide. v-Src expression increased the synthesis of GlcCer, and it was inhibited by SU6656, Src inhibitor. Moreover, v-Src expression did not affect the protein level of GCS. Next, we analyzed the tyrosine phosphorylation of GCS by v-Src. GCS Tyr-132 was phosphorylated by v-Src expression, and replacement of the GCS Tyr-132 with phenylalanine decreased the GCS enzymic activity. These results suggest that v-Src-mediated tyrosine phosphorylation increased GCS activity, and phosphorylation of GCS Tyr-132 is involved in GCS enzymic activity. Further studies are needed to reveal the biological function of regulation of GCS enzymic activity by phosphorylation.