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Expression and functional role of the Shigatoxin-receptor GB3/CD77 in porcine cancer models

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Purpose: In various cancer entities like colon, pancreatic and gastric cancer, the glycosphingolipid Gb_3 /CD77 is highly expressed. Our preliminary data indicate that this is also observable for a large animal model for colon cancer. Globotriaosylceramide (Gb_3) is synthesized by glycosylation of potentially proapoptotic ceramide, forming a specific receptor for bacterial Shigatoxin. Gb_3 is usually poorly expressed in adult tissue in human, mainly in kidney and some endothelia. Therefore, the non-toxic B subunit of Shigatoxin (StxB) constitutes a promising tool for diagnostic and therapeutic tumor targeting. However, the pathophysiological role of Gb_3 is largely unknown. In this work, the expression of Gb_3 in osteosarcoma was investigated, by using primary porcine as well as human cell lines. Furthermore, the cellular role of Gb_3 in porcine tumor cells should be elucidated in vitro, with the aim to generate large animal models.

Methods: The biosynthesis of Gb_3 and intracellular uptake of Shigatoxin in primary porcine osteosarcoma cells, and human osteosarcoma cell lines is studied using fluorescence labeled recombinant StxB. Moreover, a CRISPR/Cas9 knockout of the enzyme Gb_3-synthase (A4GALT) was generated to inhibit the last step of Gb_3-synthesis. The ability of these knockout clones to migrate is investigated by transwell migration and wound healing assays.

Results: High expression of Gb_3 was detected in three independent primary osteosarcoma cell lines. Fluorescence labeled StxB was rapidly internalized in live osteosarcoma cells, following the retrograde uptake route to the Golgi apparatus, in accordance with earlier results for colon cancer cells. These results are currently being confirmed in human osteosarcoma cells. The functional role of high Gb_3 in tumor cells is assessed by genetic invalidation of A4GALT, and the cellular consequences will be tested in vitro. As working hypothesis, glycosylceramide metabolism could be altered in tumor cells to remove ceramides which can inhibit cell growth and lead to apoptosis.

Conclusion: Osteosarcoma represents a further cancer entity with high Gb_3 expression, and could be amenable for tumor targeting by radiolabeled StxB, which could allow more efficient resection of metastatic tumors. Further, also targeted tumor therapy could be possible by linking chemotherapeutics to StxB which gets internalized specifically and rapidly in tumor cells.