Studies on glycosphingolipids in regenerative medicine

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet, kommer att offentligen försvaras i hörsal Ragnar Sandberg, Medicinaregatan 7A, Göteborg, onsdagen den 28 maj 2014 kl. 13.00

av

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Avhandlingen baseras på följande delarbeten:

I. Barone, A., Benktander, J., Ångström, Aspegren, A., Björquist, P., Teneberg, S., Breimer, M.E. (2013) **Structural complexity of non-acid glycosphingolipids in human embryonic stem cells grown under feeder-free conditions.** *J. Biol. Chem*. 288, 10035-10050

II. Barone, A., Säljö, K., Benktander, J., Blomqvist, M., Månsson, J.-E., Johansson, B. R., Mölne, J., Aspegren, A., Björquist, P., Breimer, M. E., Teneberg, S. **Sialyl-lactotetra: a novel cell surface marker of undifferentiated human stem cells.** *Submitted manuscript.*

III. Barone, A., Benktander, J., Teneberg, S., Breimer, M. E. **Characterization of acid and non-acid glycosphingolipids of porcine heart valves as potential immune targets in biological heart valve grafts.** *Submitted manuscript*

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Regenerative medicine, including stem cell based therapies and xenotransplantation, is a new and developing field that aims to restore normal function in end stage cell/organ failure. However, transfer of cells with a different genetic background, will expose the recipient to non-self cell surface antigenic determinants that may evoke an immune response and subsequent graft damage. Thus, the cell surface antigen expression of cells aimed for transplantation has to be defined.

The first part of this study deals with glycosphingolipids of human embryonic stem cells (hESC). The glycosphingolipids were isolated from a relatively high number of cells ($1x10^9$ cells/cell line) allowing separation of the glycosphingolipids into total non-acid and acid fractions, that could be further separated into sub-fractions. These fractions were structurally characterized by mass spectrometry, proton NMR spectroscopy and binding studies with carbohydrate binding ligands. This allowed identification of several glycosphingolipids not previously described in hESC. In the non-acid glycosphingolipid fractions several novel blood group H, Le^x and Le^y compounds based on neolacto core chains were characterized. The acid glycosphingolipid fractions contained several novel hESC acid glycosphingolipids, like the gangliosides sialyl-globotetraosylceramide and sialyl-lactotetraosylceramide, and the sulfated glycosphingolipids sulfatide, sulf-lactosylceramide and sulf-globopentaosylceramide. The cellular and subcellular distribution of sialyl-lactotetraosylceramide and sulfated glycosphingolipids in hESC and in human induced pluripotent stem cells (hiPSC) was explored by flow cytometry, immunohistochemistry and electron microscopy. A high cell surface expression of sialyl-lactotetra on hESC and hiPSC was demonstrated, whereas the sulfated glycosphingolipids were restricted to intracellular compartments. During differentiation of hiPSC into hepatocyte-like cells a rapid down-regulation of the sialyl-lactotetra epitope was found. Taken together these data demonstrate that the sialyl-lactotetra carbohydrate sequence is a novel marker for undifferentiated human pluripotent stem cells.

Diseased human heart valves are substituted with either mechanical valves or biological heart valves (BHV) produced from porcine and bovine valves or pericardial tissues. The BHV function deteriorates with time partly due to an immunological process. The second part of the study aimed at defining carbohydrate antigens of porcine heart valves with a potential of being immune targets for this process. Here, a number of acid glycosphingolipids as sulfatide and various gangliosides, and non-acid glycosphingolipids, as Galα3-neolactotetraosylceramide were characterized. Interestingly, no gangliosides with the non-Gal xenoantigen NeuGc were found. However, the Galα3 epitope is the major xenoantigen, and thus a possible target for antibody mediated immune reactions to xenogeneic bioprosthetic heart valves in humans.

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