Principal Investigator: Toshisuke Kawasaki
Grant Title: Novel marker antibodies recognizing carbohydrate structures on human iPS/ES cells

Abstract

1. AIM : Characterization of novel marker antibodies recognizing hiPS and hES cells, R-10G and R-17F. Most of the marker antibodies to hiPS /hES cells are carbohydrate-recognizing antibodies, which include stage specific embryonic antigen (SSEA)-3 and SSEA-4, and tumor rejection antigen (TRA)-1-60 and TRA-1-81. However, these antibodies also recognize human embryonal carcinoma (hEC) cells. Accordingly, we generated monoclonal antibodies, R-10G (1) and R-17F (2), which bind strongly to hiPS/hES cells but exhibit little or no binding to hEC cells.

2. RESULTS : R-10G epitope is characterized as a type of keratan sulfate and its core protein has been identified as a glycoprotein, podocalyxin. In contrast, the R-17F epitope has been identified as a glycolipid, lacto-*N*-fucopentaose I (LNFP I), Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, for the most part. However, the present study indicates that a cross-reactive R-17F epitope, Fucα1-2Galβ1-3GlcNAc (H type 1 triaose) may be expressed on podocalyxin and other glycoproteins as well (3). The latter epitope is expressed on *N*-glycans and the former probably on *O*-glycans. Interestingly, R-17F, when added to hiPS/ES cell suspensions, exhibits potent dose-dependent cytotoxicity. This cell death is not caused by apoptosis but by necrosis and could be mediated by cross-linking of these different types of R-17F epitopes by the antibody. An ELISA test with a series of *N*-acetyllactosamine tetrasaccharides indicated that the minimum epitope structure of R-10G is Galβ1-4GlcNAc(6S)β1-3Galβ1-4GlcNAc(6S) β1 (type 2-type 2, 2S) and that of TRA-1-60 is Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1 (type 1-type 2, 0S), being in agreement with a previous report (Natunen S et al. 2011). In addition, it was shown for the first time that TRA-1-60/81 recognizes also sulfated one, Galβ1-3GlcNAc(6S)β1-3Galβ1-4GlcNAc(6S) β1 (type1-type 2, 2S), as well as non-sulfated one. Furthermore, keratanase II was shown to degrade not only type 2-type 2 glycans but also type 1-type 2 glycans slightly, when GlcNAc residues were sulfated at C6 (4). These observations may be interesting in considering controversies about whether the type 1-type 2 *N*-acetyllactosamine epitopes recognized by TRA-1-60/81 on hiPS/ES cells are modifications of keratan sulfate or mucin-type *O*-glycans.
(1) Kawabe, K., et al., Glycobiology, 23, 322–336 (2013), (2) Matsumoto, S., et al., J Biol Chem, 290, 20071–20085 (2015), (3) Nakao, H., et al., Glycoconj J DOI 10.1007/s10719-016-9710-2 (2016), (4) Nakao, H., et al., Glycoconj J DOI 10.1007/s10719-017-9765-8 (2017)