expression of relevant antigens in different organs of possible relevance for the xenotransplantation situation.

S16.4

Biosynthetic Basis of Incompatible A Antigen Expression in Cancer of 0 Individuals: Evidence that the 0 Gene is Responsible for a Transferase Expression

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The expression of incompatible A carbohydrate antigens in some adenocarcinomas may provide an explanation for the generally observed lower incidence of adenocarcinoma among O and B versus A individuals. The chemistry and genetic basis of incompatible A expression is largely unknown. Here we have screened 31 cases of gastric tumors of phenotype 0 for the expression of blood group A gene defined glycosyltransferase by immunohistology on frozen sections using newly developed monoclonal antibodies to the transferases. Three cases were positive and transferase expression was confirmed by enzyme analysis of extracts from the specimens. Blood group A carbohydrate antigens were also identified immunohistologically in these three cases as well as in five other cases. Thin-layer chromatography immunostaining analysis of glycolipid extracts from the three cases did not confirm the chemical presence of A antigen. The ABO genotype of all patients was found to be 00 showing that all carried 0 alleles with a structural defect at nucleotide position 258 leading to a shift in the reading frame. The data suggest that incompatible A antigen expression is a result of transferase expression derived from the ABO genes, and that this must occur by some yet unknown repair mechanism of the 0 allele.

S16.5

ABO Related Glycosylation Phenotypes and Genotypes in Urothelial Cell Lines of Low and High Grade Malignancy

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Three immortalized, urothelial cell lines were characterized with respect to their ABO related carbohydrate phenotype using a panel of monoclonal antibodies directed to a series of carbohydrate epitopes (Lac, sialylated Lac, Le^a, sialylated Le^a, Le^x, sialylated Le^x, H type I and II, Le^y, Le^b, A monofucosylated type I and II, ALe^y, ALe^b, and A type III). The glycosyltransferases forming some of these epitopes (β1-3/4 Galtransferase, α1-2 fucosyltransferase, α1-3 Galtransferase, and α1-3GalNActransferase) were determined by enzyme assays. The ABO gene complex was analyzed by Southern blotting, PCR across the *O*-deletion and across base differences between the A gene and the B gene. The immunocytochemical stainings showed marked differences between the three cell lines, with the high grade (tumorigenic, metastatic) expressing difucosylated structures of both Type I and Type II, and the low grade (non-tumorigenic, non-metastatic) lines expressing monofucosylated Type I and Type II structures. PCR genotyping of the cell lines indicated, that one was OO, one AA, and one A plus a mutated allele. However, even though both of the A cell lines seemed to have the intact gene to produce A transferase, none of them produced significant amounts of the A gene encoded enzyme, nor any A-structures at the cell surface. Absence of A transferase protein was further substantiated by lack of staining in any of the cell lines with monoclonal anti-transferase antibody.

In conclusion, the three urothelial cell lines, reflect *in vivo* findings in humans, and represent a competent system for *in vitro* studies of the different carbohydrate transferase genes, responsible for the carbohydrate structures expressed on the cell surface in bladder tumors.

S16.6

ABO(H) Blood Group Antigens Found in Human Plasma Glycoproteins

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ABO(H) blood group antigens are complex oligosaccharides widely distributed from blood cells to various tissues and body fluids, as glycolipids or glycoproteins. Presence of these antigens in plasma has been demonstrated earlier, but plasma substances responsible for blood group antigens has never been identified on the molecular basis except for some glycosphingolipids. Recently, ABO(H) blood group structures have been found in Asn-linked sugar chains of human plasma von Willebrand factor (vWF)[1,2] and coagulation factor VIII (FVIII)[3]. We surveyed plasma glycoproteins carrying ABO(H) blood group antigens by Western blotting analysis and ELISA using blood group-specific monoclonal antibodies (MoAbs) and lectin. Two major proteins in plasma showing apparent M_r of about 180 K and 270 K by SDS-PAGE under reducing conditions were immunoprecipitated with blood group specific MoAbs consistent with each blood group donor. Direct sequence analysis of those proteins showed the *N*-terminal sequences of α_2-macroglobulin (α_2M) and vWF, respectively. α_2M and vWF prepared from each blood group plasma by immunoprecipitation showed the blood group antigenicity. Upon incubation of each protein with endoglycosidase F, both α_2M and vWF lost almost all reactivity with anti-blood group reagents. Secretor status had no significant effect on the reactivities. FVIII and vWF in FVIII concentrates showed ABO(H) blood group antigenicity. These results indicate that at least three plasma glycoproteins, vWF, α_2M and FVIII, possess Asn-linked ABO(H) blood group antigens in normal individuals with respective ABO phenotype. It seems to be important to consider the recovery and the turn over rate of plasma glycoproteins with blood group antigens in preparation of plasma materials, and also in the therapeutic use.

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