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Effect of genetic background on glycosylation heterogeneity in human antithrombin produced in the mammary gland of transgenic goats

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Abstract

Glycosylation is involved in the correct folding, targeting, bioactivity and clearance of therapeutic glycoproteins. With the development of transgenic animals as expression systems it is important to understand the impact of different genetic backgrounds and lactations on glycosylation. We have evaluated the glycosylation of recombinant antithrombin produced in several transgenic goat lines, from cloned animals and from different types of lactation including induced lactations.

Our results show glycosylation patterns from the protein expressed in animals, derived from the same founder goat, are mostly comparable. Furthermore, the protein expressed in two cloned goats had highly consistent oligosaccharide profiles and similar carbohydrate composition. However, there were significantly different oligosaccharide profiles from the proteins derived from different founder goats. Artificial induction of lactation did not have significant effects on overall carbohydrate structures when compared to natural lactation. The only major difference was that recombinant antithrombin from induced lactations contained a slightly higher ratio of *N*-acetylneuraminic acid to *N*-glycolylneuraminic acid and less amount of oligosaccharides containing *N*-glycolylneuraminic acid.

Abbreviations: HPAEC, high pH anion exchange chromatography; PAD, pulsed amperometric detection; HPLC, high-performance liquid chromatography; MALDI-TOF-MS, matrix-assisted laser desorption/ionization-time of flight-mass spectrometry; rhAT, recombinant human antithrombin; AT, antithrombin; PNGase F, peptide *N*-glycosidase F; CHO, Chinese hamster ovary; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Gal, galactose; Man, mannose; Fuc, fucose; NA2, asialo biantennary complex-type oligosaccharide; NA4, asialo tetra-antennary complex-type oligosaccharide

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The oligosaccharides from all animals were a mixture of high mannose-, hybrid- and complex-type oligosaccharides. Sialic acid was present as α-2,6-linkage and no α-1,3-linked galactose was observed. These results indicate that transgenic animals with closely related genetic backgrounds express recombinant protein with comparable glycosylation.

Keywords: Carbohydrates; Transgenic animals; Antithrombin; Genetic polymorphism; Cloned goats

1. Introduction

Recombinant proteins provide effective therapies for many life-threatening diseases. The use of expression systems that express high levels of protein, such as bacterial, yeast and insect cells, for production of therapeutic protein are limited to small proteins without extensive post-translational modifications. Mammalian cell systems, while producing the appropriate post-translational modifications, are more expensive per gram of protein due to the complex, and therefore sophisticated culture systems required as well as generally reduced protein expression levels. Some of the limitations of mammalian cell culture systems have been overcome by the expression of recombinant proteins in transgenic animals (Meade et al., 1998). Proteins have been produced in mammary glands of various transgenic animals with expression levels suitable for cost effective production at the scale of hundreds of kilograms of protein per year. Although the post-translational modification of proteins produced using transgenic technology has been published (Edmunds et al., 1998; James et al., 1995), the effect of genetic polymorphisms and lactation on these post-translational modifications, especially glycosylation, has not been reported.

Since glycosylation plays an important role in many biological processes including protein stability, serum half-life, immune response, intracellular signaling and cell–cell/cell–extracellular matrix interaction (Varki et al., 1999), it is important to ensure that glycosylation is consistent from batch to batch of recombinant protein and from animal to animal in the case of transgenic production.

This study was designed to elucidate potential variability of glycosylation due to genetic polymorphism and induced versus natural lactation. The carbohydrates on human recombinant antithrombin (rhAT) produced in transgenic goat lines and cloned animals were chosen for this investigation. Human AT is the main inhibitor of thrombosis in blood. It is a serine protease inhibitor involved in the blood coagulation cascade and acts by inhibiting the function of thrombin and factor Xa (Bjork and Olson, 1997; Levy et al., 2001; McCoy et al., 2003). AT binds to and is activated by heparin-like proteoglycans. Its activation results in the inhibition of thrombin. Human AT is a 58-kDa single chain glycoprotein with four N-linked glycosylation sites and contains 10–15% carbohydrate by weight. It has also been expressed as a recombinant protein in the mammary gland of transgenic goats and its protein and carbohydrate structures have been compared to human plasma AT (Edmunds et al., 1998).

The current studies further characterize and compare the N-linked oligosaccharides of rhAT purified from various transgenic goat lines with different protein expression levels, and also from cloned transgenic goats, produced by somatic cell nuclear transfer (Baguisi et al., 1999). The effects of induced and natural lactation on the carbohydrate structures on rhAT from cloned animals were also investigated. This study provides the first report comparing the carbohydrate heterogeneity of recombinant proteins from mammalian clones and heterozygous animals derived from the same and different transgenic founder goats.

2. Materials and methods

2.1. Production of transgenic and cloned animals

Transgenic animals expressing rhAT in their milk were produced as described previously (Edmunds et al., 1998; Meade et al., 1998). The rhAT gene was under the control of the β-casein promoter. For the production of transgenic animals, embryos were collected, microinjected with the rhAT gene and transferred to recipient female goats. Founder (F_0) goats were identified by analyzing genomic DNA from tissue and blood. The founder male or female goats were bred to non-