Modification of the goat mammary gland glycosylation pathway by overexpression of GnT-IVa

María José Leiva Carrasco

Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department, School of Biological Sciences, Universidad de Concepción.

Statement of the Problem: Recombinant glycoprotein expression has been carried out in different expression systems based on the genetic modification. Specifically, recombinant glycoproteins whose biological activity depends on post-translational modifications are produced in cell tissue culture, which increases the manufacturing cost. The production of biotherapeutics in the mammary glands of genetically modified mammals results in an alternative method to overcome the drawback of cell culture expression system. However, the N-glycosylation pattern of complex glycoproteins produced in the mammary epithelia has showed diminished antennae formation and lower sialic acid contents compare to native protein. An alternative to obtain high- quality biopharmaceuticals in milk could be the modification of the N-glycosylation pattern by overexpression of exogenous glycosyltransferases. The purpose of this study is to modify in vivo the glycosylation pattern of recombinant protein expressed in goat mammary gland. Methodology & Theoretical Orientation: Human erythropoietin fused to human IgG Fc (EPO-Fc) was co- expressed with N-acetyl-glucosaminyltransferase-IVa (GnT-IVa) by adenoviral transduction in goat mammary gland. Findings: The modification in vivo of the enzymatic glycosylation machinery in the mammary gland generated an increment in the antennae number. A higher population of tri-antennary structures for the EPO-Fc/GnT-IV variant was obtained by N-glycans mass spectrometry analysis, compared to bi-antennary structures N- linked to EPO-Fc expressed in the same cells. Conclusion & Significance: These results demonstrate, for the first time, that it is possible to modify in vivo the glycosylation pattern of recombinant biopharmaceutical expressed in the goat mammary gland epithelial cells to obtain a glycosylation pattern similar to native glycoproteins.

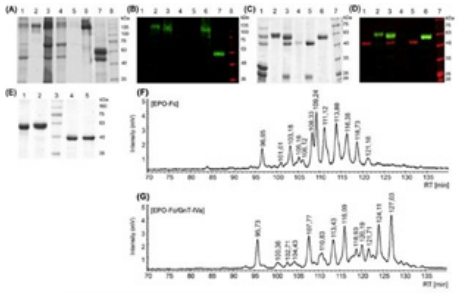

Figure 1: Analysis of EPO-Fc variants produced in the goat mammary gland. SDS-PAGE (A) and western blotting (B) in non-reducing conditions of EPO-Fc/GnTIVa fractions obtained after Protein-A affinity chromatography. Lane 1: goat milk whey (negative control), lane 2: EPO-Fc from SiHa cells (positive control), lanes 3-5: initial sample, unbound proteins and wash fractions respectively, lanes 6, 7: eluted proteins in non-reducing and reducing conditions, lane 8: AccuRuler RGB PLUS prestained protein ladder (Maestrogen, Taiwan). SDS-PAGE (C) and western blotting (D) in reducing conditions of EPO-Fc/GnT-IVa obtained after purification by Blue Sepharose affinity chromatography. Lane 1: goat milk whey (negative control), lane 2: EPO-Fc from SiHa cells (positive control), lanes 3, 4: initial sample and unbound proteins, lane 5: wash fraction, lane 6: eluted fraction. A mouse anti-EPO mAb and a donkey anti-mouse IgG (H + L) Alexa Fluor®790 (green), were used to identify EPO-Fc. A donkey anti-goat IgG (H + L) Alexa Fluor®680 (red) was used to identify the goat IgG. (E) SDS-PAGE of purified EPO-Fc variants, after PNGase F digestion. Lanes 1, 4: EPO-Fc (glycosylated and deglycosylated), lanes 2, 5: EPO-Fc/GnT-IVa (glycosylated and deglycosylated), lane 3: AccuRuler RGB PLUS prestained protein ladder (Maestrogen, Taiwan). Oligosaccharide profiles of 2-AB-labeled N-glycans isolated from EPO-Fc (F) and EPO-Fc/GnT-IVa (G) obtained by normal-phase HPLC.

Biography

María José obtained her Biochemistry degree at the Universidad de Concepcion, Chile in 2015. She has researched glycosylation pattern modification of recombinant biopharmaceuticals expressed in cell culture and in the goat´s milk. Maria Jose has led two projects in the area of production of antibodies for therapeutic use. Currently, she is studying a PHD. in Molecular Biotechnology at the University of Concepcion. Her Ph.D. is entitled "Aberrant glycosylation and its role in colorectal cancer development and progression".