구두발표

Eukaryotic expression of recombinant proteoglycans and glycoproteins and their structure and function

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Structural and functional studies of mammalian glycoprotein domains can be facilitated by the isolation and purification of native glycoproteins. Most current procedures for isolation of glycoproteins from tissues require the use of denaturing solvents. An alternative method for the generation of native glycoproteins is the use of a recombinant expression system. Due to the extensive post-translational modifications of mammalian glycoproteins, an expression system capable of complex modifications, especially addition of glycosylations, is essential. We have modified the basic vaccinia/T7 cloning and expression system to facilitate targeted secretion and nondenaturing purification of recombinant glycoproteins. The vaccinia virus/T7 bacteriophage expression system was used to express human decorin in HT-1080 cells by co-infection with vTF7-3, encoding T7 RNA polymerase, and vDCN, encoding the decorin core proteins fused to a polyhistidine-insulin signal sequence fusion-protein cassette. Overexpression using the vaccinia virus/T7 phage system resulted in secretion of approximately 30 mg of decorin/10^9 cells per 24 h which enabled purification and separation of multiple glycoforms under native conditions. Expression of decorin resulted in secretion of two distinct glycoforms: a proteoglycan substituted with a single chondroitin sulfate chain and *N*-linked oligosaccharides and a core protein glycoform substituted with *N*-linked glycans but without a glycosaminoglycan

chain. The role of glycosylation for secretion of decorin was determined by using inhibitors of glycosylation and site-specific deletion mutagenesis combined with over-expression of decorin and decorin mutants in CHO cell lines deficient in specific glycosylation steps. Decorin can be efficiently secreted provided that the core protein is substituted with at least one N-linked oligosaccharide or with at least one chondroitin sulfate chain. However, there is severely impaired secretion of core protein devoid of any glycosylation. A decorin core protein mutant devoid of *N*-linked oligosaccharide attachment sites will not be secreted by CHO cells deficient in xylosyltransferase, or by parental CHO wild type cells if the xylosyltransferase recognition sequence is disrupted. This data suggests that quality control mechanisms sensitive to the absence of *N*-linked oligosaccharides can be abrogated by interaction of the core protein with the glycosaminoglycan synthetic machinery. We proposed a model of regulation of decorin secretion that has several components, including appropriate substitution with *N*-linked oligosaccharides and factors involved in glycosaminoglycan synthesis.

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