PROJECT REVIEW

"Novel methods for identification of recombinant glycoprotein hormones"

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Aims of our research are a) methodology development for comprehensive differentiation of endogenous and exogenous hCG, LH and ESA by glycane profiling, b) construction of lectin-carbohydrate interaction database that allows rapid identification of WADA prohibited glycoprotein hormones, and c) development of sample preparation procedure to allow detection of recombinant glycoprotein hormones by normal immuno-assays currently equipped in WADA accredited laboratories. Result verification by second immuno assay is necessary when commercial immuno assay kit is used for doping tests, but the verification is not always easy because hCG is extensively metabolized or degraded in vivo. Misuse of biosimilar EPO having different isoform compositions from reference EPO became known since 2007 and caused sometimes in identification difficulties. A sole human cell derived EPO (Dynepo) was withdrawn from the market in 2007, and currently, commercial glycoprotein hormones are largely manufactured from CHO cell line. Many of other glycoprotein hormones such as hCG and LH from human has been replaced by the recombinant products. It is reported that CHO cells do not normally express sialyl-alpha2-6 transferase. We have analyzed sialyl- alpha2-6-linked hexose of glycoprotein hormones by the reactivity with 45 array of lectins, and found that human origin EPO and hCG has Sialyl- alpha2-6-linked galactose/galactosamine residues but CHO-derived glycoprotein hormones are lacking this moiety. Thus, origins of glycoprotein hormones were successfully identified. In this project, we plan to construct stereo-specific sample preparation procedure using lectin beads/columns, gathering lectin-carbohydrate spectra by analyzing LH, hCG and ESA from human and CHO-cell line, and development of antibody assisted lectin micro array test system to identify recombinant glycoprotein hormones as a definitive conclusion of doping. Our preliminary results supported that high sensitive and robust detection of prohibited recombinant glycoproteins could be accomplished in few steps without any special technical skill.

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Results and Conclusion

Our results of lectin-glycan interaction monitoring confirmed that origin dependent difference of human glycoprotein hormones, and genetically manufactured corresponding glycoprotein preparation arises not from gene expression but from the posttranslational modification process. Synthesis of core peptide is genetically controlled but the addition of *O*-and *N*-Linked glycan to the core peptide depends on the available sialyl transferase (ST) and the substrate carbohydrates in the culture medium. Use of SSA, SNA or TJA-I lectin for isolation of endogenous- and exogenous glycoprotein by capturing Sialylα2-6Gal/GalNac as the tag carbohydrate allows origin dependent separation of ESAs and Gonadotropins. Hormones in isolated recombinant or human fraction are to be assayed by means of normal immunoassays just for detection. By this configuration, both peptide and carbohydrate moieties of the target compounds are to be recognized, thus, highly specific lectin-antibody identification can be achieved.

Because overall results of lectin fractionation coupled with immunoassay shows the origin dependent difference of glycans, the positive finding of glycoproteins in the fraction 1 and 2 can be considered as adverse analytical finding regardless of the hormone concentration. The results represented a possibility to establish high throughput origin specific screening of glycoprotein hormones by lectin column fractionation followed by normal sandwich immuno assay kits or immuno assay instruments.