

Detection of hyperglycosylated hCG by surface plasmon resonance

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Introduction: The glycosylation pattern of hCG reflects the physiological or pathophysiological state of the cell in which it was produced, and recognition of an aberrant pattern could prove useful in predicting an early pregnancy loss or a Down syndrome fetus, and in diagnosing and monitoring trophoblastic disease. The goal of this study was to develop an assay using a capture antibody and subsequent lectin probing to discriminate glycoforms of hCG and its derivatives produced under different conditions. An assay was developed using surface plasmon resonance (SPR) to garner quantitative lectin-binding results to antibody-captured hCG or its derivatives. **Methods:** The monoclonal antibody, B108, which binds an epitope common to the hCG heterodimer, its free beta subunit and the beta-core fragment, was proteolytically cleaved with ficin to remove the carbohydrate-containing F_c portion, and then partially reduced to two separate F(ab') chains. The resultant fragments were linked through their free-thiol groups to a sensor chip, obviating the need for purification of the fragments from residual IgG while removing the intrinsic glycosylation that could interfere with interpretation of lectin binding to captured hormone. This treatment reduced binding of a serial panel of lectins flowing over the chip to F(ab') fragments approximately 90% compared to their binding to B108 IgG. These fragments were later used to capture equal amounts of immunoreactive hCG and its derivatives secreted by three choriocarcinoma cell lines, JAR, JEG and BeWo, and from pooled pregnancy urine onto the sensor chip, followed by probing with a series of five lectins. Similarly, the binding levels of eight lectins on immunoreactive hCG molecules captured from the urine of individual pregnant women were compared to that captured from urine samples of patients with complete hydatiform mole, germ cell tumors, or choriocarcinoma. **Results:** Both the choriocarcinoma cell media and urine samples from patients with trophoblastic disease exhibited less binding to lectins recognizing terminal mannose (*Galanthus nivalis* agglutinin) and $\alpha(2-3)$ linked sialic acid (*Maackia amurensis* lectin –II). Interestingly, media from the cell lines demonstrated highly elevated levels of binding to *Sambucus nigra* agglutinin, which recognizes $\alpha(2-6)$ linked sialic acid, but urine from the trophoblastic disease patients did not. **Conclusions:** An SPR assay was designed based on quantitative differences in lectin binding that discriminates glycoforms of hCG and its derivatives produced during normal pregnancy from that produced by choriocarcinoma and other diseased trophoblastic cells. Supported by the UGA Res. Foundation, the GA Res. Alliance, Oncose, Inc. and NIH DK33973.