

## Hyperglycosylated hCG

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**INTRODUCTION:** In 1987 we discovered that a variant of hCG with exclusively hexasaccharide O-linked sugar side chains represented the majority of choriocarcinoma patient hCG. A lesser percentage of these sugars was found in normal first trimester pregnancy (Cole LA, *J Clin Endocrinol Metab*, 1987). In 1997 we showed that larger oligosaccharides were also found on N-linked sugar units on choriocarcinoma hCG  $\beta$ -subunit (Elliott MM, et al., *Endocrine*, 1997). We called this molecule with larger sugar side chains hyperglycosylated hCG (H-hCG). Shortly thereafter, a specific monoclonal antibody was raised to this variant. With the establishment of an assay our laboratory and others were able to show that H-hCG was the predominant form of hCG in very early pregnancy (3 weeks following implantation), in choriocarcinoma, and in testicular germ cell malignancies. It was also found that it's the predominant hCG-related molecule produced by JAR and JEG-3 choriocarcinoma cell lines and NTERA testicular germ cell cancer cell lines. Other researchers later confirmed that while H-hCG was produced by invasive cytotrophoblast cells, hCG was made by differentiated syncytiotrophoblast cells. As published, H-hCG has only 3% of the steroidogenic activity of hCG. With an apparent association with invasive states (implantation & cancer) we considered the possibility of a separate role for H-hCG in invasion.

**BIOLOGICAL FUNCTION:** Monolayers of normal placenta cytotrophoblast cells and JEG-3 choriocarcinoma cells were cultured on Matrigel membranes to assess invasion. The addition of H-hCG (4-fold excess over endogenous levels) significantly promoted invasion of membranes ( $66 \pm 13\%$  and  $88 \pm 6\%$ ) vs. control cultures ( $40 \pm 10\%$  and  $48\% \pm 11\%$ ),  $P=0.05$  and  $P=0.005$ . In contrast, hCG did not enhance invasion in either study. Athymic nude mice ( $n=24$ ) received subcutaneous transplants of JEG-3 choriocarcinoma cells. Half received non-specific IgG and half received B152 anti-H-hCG twice weekly. When antibody therapy was started at the time of transplantation it significantly inhibited ( $P=0.007$ ) tumor formation. When therapy was started 2 weeks later, after tumor formation, it significantly blocked ( $P=0.003$ ) tumor growth. These findings are supported by Lei *et al*, who showed that anti-sense cDNA blocks hCG production by JAR choriocarcinoma cells, and tumor formation in nude mice (*Troph Res*, 1999). These results clearly show a separate autocrine function for H-hCG as a direct promoter of trophoblast invasion whether in cancer or at implantation. Antibodies against H-hCG and vaccines against H-hCG derivatives may be invaluable in the treatment of gestational trophoblastic diseases, choriocarcinoma and testicular malignancies.

**CLINICAL APPLICATION OF H-hCG TESTING:** A commercial assay is now available for detecting H-hCG. As a separate molecule from hCG with different functions, it offers improved means for a) pregnancy detection; b) determining pregnancy outcome; c) detecting ectopic pregnancy; d) identifying gestational Down syndrome; e) diagnosis and management of GTD, choriocarcinoma and testicular malignancies.