

Silencing of human chorionic gonadotropin beta subunit expression induce apoptosis in cervical cancer cells

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Introduction: The ectopic production of hCG and its subunits by patients with nontrophoblastic cancers has been reported by many authors lately. We recently showed that the expression of *hCGβ* is characteristic feature of individual cancer cells in patients with nontrophoblastic gynecological cancers. Guided by the observations that structure of hCG resembles the structure of known growth factors and stimulation by hCGβ of tumor cell lines *in vitro* led to an increase in cell number we reasoned that the reduction of the hormone level would result in decrease of cell viability. We used silencing gene strategy to knock down the expression of *hCGβ* in cultured cervix carcinoma cells.

Methodology: In our study we exploited two techniques to block the expression of *hCGβ* in a cervix carcinoma cell line. We investigated a novel mRNA silencing methodology based on a modified U1 snRNA as well as small interfering RNA strategy to reduce mRNA output of a target gene. The first 10 nucleotides of the human U1 snRNA gene, which normally binds to the 5'ss in pre-mRNA were replaced by a sequence complementary to a 10-nt segment in the terminal exon of the *hCGβ* mRNA. Evaluation of the *hCGβ* expression was performed by the real-time PCR and immunohistochemistry. Apoptotic cells were detected by changes in nucleus morphology and flow cytometry.

Results: The results of the study demonstrated that binding of modified U1 snRNAs to 10 nucleotide regions within the terminal exon of human chorionic gonadotropin beta subunit gene blocked the hormone's expression in HeLa cells. Reduction of *hCGβ* levels resulted in a significantly increased apoptosis rate with cells transfected with modified anti-*hCGβ* U1 snRNAs. The transfection of cervical cancer cells with siRNA was also associated with the knocking down *hCGβ* expression and apoptotic death of affected cells. Thus, the inhibition of the beta subunit of human chorionic gonadotropin expression leads to induction of programmed cell death.

Conclusions: These data suggest that human chorionic gonadotropin beta subunit is a factor require for tumor growth.

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