Expression of Germ Cell Nuclear Factor (GCNF) by Ovarian Cancer Cell Lines

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Introduction:
Ovarian cancer is the fifth most common cancer (neoplasm) among women and the third most common gynecological cancer behind endometrial and cervical cancer [1]. Epithelial ovarian cancer (EOC) represents ~90% of all ovarian cancers. Recently, we have observed the novel expression of the nuclear receptor, the germ cell nuclear factor (GCNF) in several ovarian cancer cell lines. This is noteworthy because of the normally limited expression of this gene. While expressed by several cells of different germ layers during embryogenesis, GCNF is reportedly relegated to the germ line in adults [2]. GCNF exhibits transcriptional repressor activity and several target genes have been identified [3]. Germ cell nuclear factor (GCNF/NCNF/RTR, NR6A1) is an orphan member of the nuclear receptor superfamily that exhibits repressive transcriptional activity and is reportedly relegated to the germ line in adult animals. Recently it was found that GCNF mRNA and protein is expressed in four of the four ovarian cancer cell lines tested: two adenocarcinomas, OVCAR-3 and TOV-112; one clear-cell carcinoma, ES-2; and one teratocarcinoma, PA-1. In addition, GCNF mRNA was highly expressed in mink lung epithelial cells (MLEC), a rapidly proliferating, non-transformed epithelial cell line as well as in several pre-adult tissues of the hamster. Taken together, these findings suggest a relationship between GCNF and cell proliferation. GCNF gene silencing was employed to investigate this relationship in ovarian cancer cells maintained in vitro using the siRNA synthesized commercially by Santa Cruz Biotechnology. The cell counts were analysed post-transfectionally which showed a decrease in the cell proliferation to about 53% and 30% in ES-2 and TOV-112 cells, respectively. Interestingly, the degree of inhibition reflected the relative growth rates of the two cell types. Consistent with the observed decline in cell proliferation, GCNF siRNA treatment decreased the cellular content of GCNF mRNA by 40% in TOV-112 cells. These preliminary results implicate GCNF as a heretofore unrecognized and potentially important growth regulator in ovarian cancer cells.

Materials and methods:
Passaging & RNA isolation:
All the ovarian cancer lines were cultured in 25 cm² flasks in medium (F12: DMEM, 1:1) supplemented with the required levels of fetal calf serum (20% for OVCAR3 cells and 10% for all other cell lines). Following this, the monolayers were processed for total RNA using the UltraSpec RNA isolation reagent.

RT/PCR:
DNA was quantitated by UV spectrometry at 260/280 nm. Aliquots of total RNA (5 µg) were subjected to reverse transcriptase protocol using a Promega Reverse Transcription system, random hexamers and oligo-dT primers. A standard aliquot (10 µl) of RT was combined with the PCR core kit and 2.5 µl of GCNF primers (the following primers were designed after comparing the human and mouse sequences using the BLAST search algorithm: 5’GAACAACGAACCTGTCTCAT-3’ and 3’ GTTAGTGACCTGC TGGTA-5’). Primer addition was followed by 35 cycles within the Amplitron II Thermolyne.

The PCR products were then separated out on a 2% agarose gel containing ethidium bromide. The resulting bands from this gel were visualized by UV spectrum and photographed using a Kodak photodocumentation system.
DNA sequencing:

The DNA was extracted using a Qiagen kit and sent to the University of Kansas for sequencing. The sequence exhibited significant homology with the Human GCNF following a BLAST search.

Determination of GCNF protein expression by western blotting:

GCNF protein was also monitored by immunoblot analysis. GCNF primary antibodies were provided by Dr. Austin Cooney (Baylor College of Medicine). GCNF bands were then detected using Luminol chemiluminescent reagent, which reacted with the HRP-conjugated secondary antibody (Santa Cruz Biotechnology).

GCNF gene knock down (silencing) to determine the effects of GCNF on Growth:

Armed with the siRNA probe (Synthesized by Santa Cruz Biotechnology) and using procedures outlined by Santa Cruz Biotechnology, ES-2 and TOV-112 cells were transfected with GCNF siRNA as well as with the control siRNA. Fifty-eight hours post-transfection, cell count levels were determined using the Coulter Counter and the cellular RNA content was analyzed by RT/PCR respectively. Relative to untreated and control siRNA-treatments, GCNF siRNA-treatment decreased the monolayer cell content to 53% and 30% for ES-2 and TOV-112 cells, respectively. Consistent with the observed decline in cell proliferation, GCNF siRNA treatment decreased the cellular content of GCNF mRNA by 40% in TOV-112 cells. This technique will be applied to the other two ovarian cancer cell lines in order to confirm its consistency.

Experiment to determine the effects of Growth suppression on GCNF expression:

The non-transformed mink lung epithelial cell line (MLEC) expresses GCNF. Importantly, MLEC are both contact inhibited and exquisitely sensitive to TGF-β1, which markedly inhibits proliferation. MLEC was cultured in the presence and absence of TGF-β1 (1-5ng) which showed a marked decrease in the GCNF mRNA expression which indicates the relationship between Growth and GCNF.

Conclusion:

This project may establish GCNF as a functionally important regulator of both normal and neoplastic cells. GCNF expression observed in actual tumor samples, not just in established cancer lines, will provide support for this role. We also wish to determine the ligand for GCNF and its many unraveled target genes to define the mechanism of GCNF regulation, which would reveal many important questions regarding GCNF activity and growth.

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References: