Creating Claudin-16 Reporter Assays for Studying Epithelial Ovarian Cancer

Miguel Cuevas*^, Joel Manwaring*^, Joe Wilkerson*^, and Kurt Lehnardt*^, Mary Nelson^, Adam Blaszczak^, and Colleen Hough*

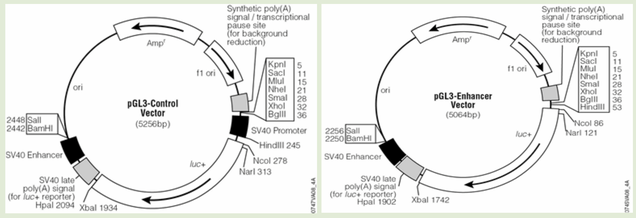
Utah Valley University* and InnovaBio, Salt Lake Community College^

Introduction

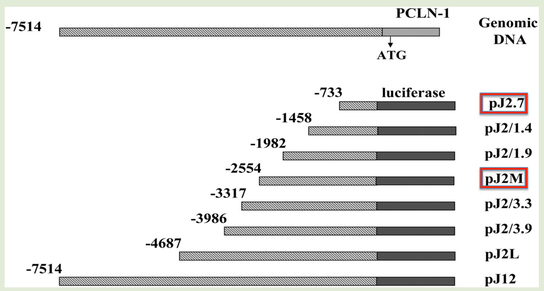
Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States, and the fifth leading cause of cancer death among American women. It is estimated that over 22,000 women were diagnosed with ovarian cancer in the United States in 2012, and approximately 15,500 women succumbed to the disease. This is in part because only 20 percent of cases are diagnosed before the cancer has spread to the peritoneal cavity.

The only diagnostic test currently available is the CA125 tumor antigen blood test. This test is inadequate and not available as a general screening tool; additional diagnostics are therefore required to effectively diagnose this disease.

Materials—Constructs



pGL3 Luciferase Reporter Vectors Technical Manual. Promega corporation, 2007



Efrati, E. et al. 2005 American Journal of Physiology-Renal Physiology

PCLN (Claudin-16) promoter regions were subcloned into the pGL3 enhancer vector upstream of the firefly luciferase gene. Each construct created correlates with a truncated section of the promoter region defined by Efrati and colleagues.

Sources:

1. Efrati E, Arsentiev-Rozenfeld J, Zelikovic I. "The human paracellin-1 gene (hPCLN-1): renal epithelial cell-specific expression and regulation." Am J Physiol Renal Physiol 288: F272–F283, 2005

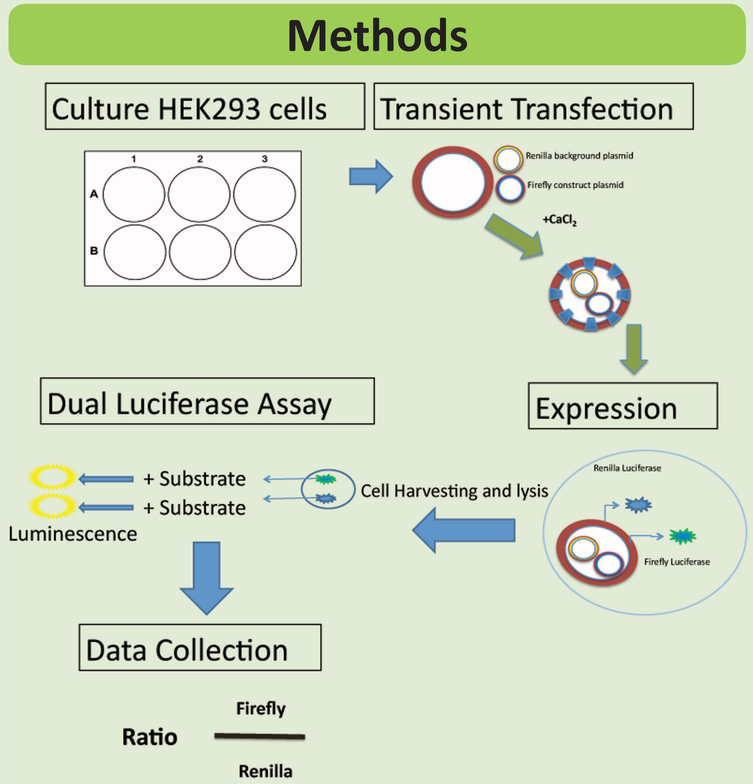
2. Rangel LB, Sherman-Baust CA, Wernyj RP, Schwartz DR, Cho KR, Morin PJ. (2003). "Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by serial analysis of gene expression." Oncogene 22: 7225–7232.

Abstract

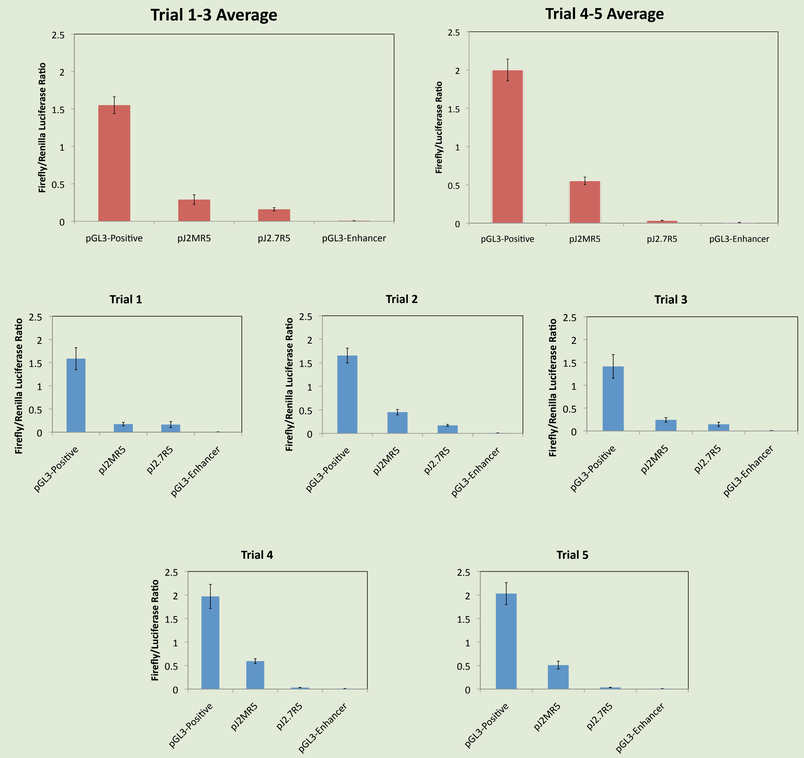
The tight junction protein claudin-16, normally found only in kidney cells, is a good candidate for ovarian cancer diagnostics and targeted therapy, as it is aberrantly expressed in epithelial ovarian tumors (Rangel LB, 2003). To compare the promoter regions that control cldn-16 gene expression in ovarian tumors and human embryonic kidney (HEK) cells, a luciferase reporter system was created.

Various sections of the upstream regulatory region previously identified in kidney cell lines (Efrati et al. 2005) were PCR-amplified and sub-cloned into the pGL3 luciferase reporter vector. A dual luciferase assay was used to assess the expression level from each regulatory element. Promoter activity has already been verified in kidney cell lines that normally express claudin-16. These assays will be repeated on a collection of ovarian cancer and non-cancer cell lines to determine claudin-16 promoter activity. If validated, the constructs may be used in a cell-based assay for identifying claudin-16 expression in ovarian cell lines for testing potential therapeutics.

Methods



Results



Conclusion & Future Direction

HEK-293 cells transiently transfected with claudin-16 promoter constructs showed relative luciferase activities consistent with the results of Efrati and colleagues. Of the two constructs tested, J2MR5 showed the highest expression of firefly luciferase relative to the background of renilla luciferase.

Future studies will focus on testing an increased number of constructs in NIH-3T3 and HEK-293 cell lines, development of a methodology for transient transfection in the OVCAR-3 cell line using non-lipid based transfection systems, and eventually testing the claudin-16 regulatory constructs in OVCAR-3 cells.