

Combination Interleukin-12 Intraperitoneal Gene Therapy with Chemotherapy for Treatment of Disseminated Ovarian Cancer

Jason G. Fewell, Jennifer S. Rice, Majed M. Matar, Gregory Slobodkin, Danny H. Lewis, Khurshed Anwer
Biology and Pharmacology, Expression Genetics, Inc., Huntsville, AL

ABSTRACT [4778]

Ovarian cancer is the most deadly of all gynecological malignancies. Despite improvements in chemotherapy treatment, the overall 5-year survival rate is only 40%. Recognition of the limitations of conventional chemotherapy has prompted the search for new therapeutics. Here we describe a novel approach for treating ovarian cancer wherein the host immune system is enhanced by local (intraperitoneal) administration of Interleukin-12 (IL-12) gene formulated with a polymeric delivery system, PPC, comprising a low molecular weight branched polyethylenimine covalently linked with functional groups of polyethylene glycol and cholesterol. IL-12 has proved to be one of the most active cytokines in the induction of potent anti-cancer immunity mediated through the induction of local and systemic immune responses. Specifically, IL-12 administration is associated with T-lymphocyte and natural killer (NK) cell proliferation, activation of cytotoxic T-lymphocytes and secretion of interferon-gamma (IFN- γ). Plasmid DNA encoding for murine IL-12 was complexed with PPC to form nanoparticles (mean diameter of ~80 nm) and injected IP into C57BL/6 mice with experimentally induced ovarian cancer. These mice had been previously inoculated (IP) with up to 5.0×10^6 cells of a murine ovarian carcinoma cell line (ID8) producing an aggressive disseminated cancer. Results indicate that four weekly treatments delayed ascites accumulation and significantly prolonged survival in a dose dependent manner. Following administration, protein levels of IL-12 and IFN- γ were detected in both ascites fluid and serum by ELISA. Additionally, protein expression kinetics were determined from ascites fluid of tumor bearing animals and it was shown that the total protein levels of IL-12 peaked 1 day after treatment (731 pg) and IFN- γ 3 days after treatment (2,780 pg) and fell to near baseline levels over the course of one week. Protein expression was again induced following additional injections of mL-12/PPC. Standard front line chemotherapy treatments for ovarian cancer include paclitaxel and platinum based regimens. We evaluated the efficacy of a treatment regimen of mL-12/PPC in combination with Taxol® and Paraplatin® administration. The results indicate that combination treatments significantly improved survival over either mL-12/PPC alone or chemotherapy alone. It was also noted that a high dose chemotherapy regimen resulted in an increase incidence of mortality not related to tumor progression whereas a low dose chemotherapy regimen used in combination with mL-12/PPC showed improved efficacy with a much lower incidence of treatment related toxicity. These results demonstrate the utility of treating disseminated ovarian cancer via IL-12 gene therapy and that additive therapeutic effects can be produced by combining IL-12 treatment with standard chemotherapies.

INTRODUCTION

Ovarian cancer is the fifth most common form of cancer in women with an estimated prevalence of 20,180 women in the USA alone. Ovarian cancer of the epithelial cells, accounts for approximately 85-90% of all ovarian cancers and is the most lethal of gynecological malignancies, ranking fifth in cancer deaths among women (American Cancer Society, 2006).

Immunotherapy strategies that involve cancer treatment by stimulation of body's immune system to fight against cancer have gained acceptance during the last decade. While traditional chemotherapy regimens limit the fast growing ovarian cancer cells, immunotherapy enhances the local and systemic immune response against the cancer cells. Interleukin-12 (IL-12) has proven to be one of the most active cytokines in the induction of potent anti-cancer immunity (Robertson and Ritz, 1998). Interleukin-12 is associated with immunomodulatory properties including T-lymphocyte and natural killer (NK) cell proliferation and cytotoxic activation and secretion of interferon-gamma (IFN- γ) subsequently leading to tumor inhibition. Interleukin-12 also potentiates T helper cell differentiation and development of cytotoxic CD8+ T cell responses against viruses and tumor cells. Despite initial aggressive testing in the clinic, use of recombinant IL-12 therapy has not advanced to an approved therapy due primarily to systemic toxicity concerns, which has led researchers to explore alternative means of IL-12 delivery (Imboden et al., 2003; Kang et al., 2001).

Here we present data showing the utility of using a non-viral gene therapy approach for delivering IL-12 intraperitoneally for the treatment of disseminated ovarian cancer. The IL-12 gene is formulated with a novel polymeric delivery system forming nanocomplexes. Use as a monotherapy as well as in combination with paclitaxel and platinum based chemotherapies is evaluated.

MATERIALS

The novel lipopolymer PPC is composed of a low molecular weight branched polyethylenimine (PEI) covalently linked with the functional groups methoxy polyethylene glycol (PEG) and cholesterol. Synthesis details can be found in Fewell et al. (2005).

The plasmid encoding for murine IL-12 contains both the p35 and p40 genes each under control of separate CMV promoter/enhancer. Each gene is followed by the SV40 late polyadenylation signal sequence. The plasmid expresses the ampicillin resistance gene in order to select for positive bacteria in ampicillin containing medium and was prepared by using an endotoxin free Qiagen QIA-prep protocol following manufacturers instructions.

The ID8 cell line is derived from spontaneous in vitro malignant transformation of ovarian surface epithelial cells of the C57BL/6 mouse. This cell line has been found to be highly metastatic in vivo. The ID8 cell line was established using a fluorescent protein (Zhang et al., 2002). This cell line was a kind gift from Dr. Sung Wan Kim from the University of Utah.

Taxol® and Paraplatin® were kindly provided by Dr. Marshall Shreder and Clint Gregory from the Comprehensive Cancer Center (Huntsville, AL).

RESULTS

PPC, a novel polymer, has been optimized to produce stable nanocomplexes with DNA leading to high transfection levels in solid tumors (Figure 1). The ratio of ~2.1:1 (PEG-PEI-CHOL) was empirically determined to be optimal and used for further in-vivo studies.

Intraperitoneal injection of murine Interleukin-12 (mIL-12) plasmid formulated with PPC produces high levels of mIL-12 in the ascites of ID8 (ovarian carcinoma) tumor bearing mice that persist for several days. Expression of mIL-12 can be re-introduced with weekly injections (Figure 2).

Treating late-stage ID8 tumor bearing mice with 4 weekly doses of mL-12/PPC led to a dose dependent delay in ascites accumulation. Additionally, all treatment doses improved survival with the highest doses increasing median survival time 54% over untreated controls (Figure 3).

Combining mIL-12/PPC administration with standard chemotherapies (Taxol, Paraplatin) produced improved treatment efficacy compared to either monotherapy alone. Use of a high dose chemotherapy regimen similarly produced improved efficacy but led to a very high rate of treatment related toxicity (Figure 4).

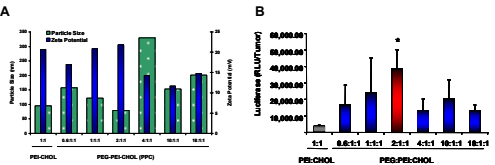


Figure 1.

PPC polymer was synthesized at various PEG to PEI ratios in order to optimize physico-chemical properties (A) and transfection efficiency (B). Particle size and zeta potential was determined by dynamic light scattering. Optimization of PEG to PEI ratios for intratumoral delivery was performed by intratumoral injection into murine mammary carcinomas on the flanks of Balb/c mice. 24 hours after injection, tumors were harvested and analyzed for luciferase activity using a commercially available assay. Values are expressed as mean \pm SD. The symbol (*) indicates $P < 0.05$ vs. PEI-CHOL.

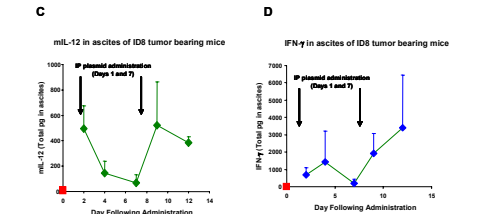
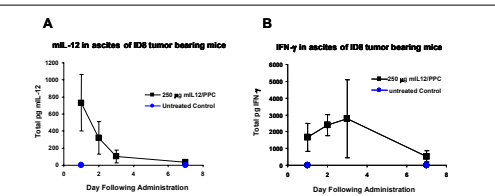


Figure 2.

Expression levels of mL-12 (Panel A,C) and mIFN- γ (Panel B,D) in mouse ascites fluid following a single plasmid administration (A,B) or two weekly treatments (C,D) using mL-12/PPC delivered IP. The levels of IL-12 and IFN- γ were determined using commercially available ELISAs. Values are expressed as mean \pm SD and normalized to total amount of ascites fluid.

