Chapter 9

Summary
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Ovarian cancer is the most lethal cause of death among the gynecological cancers. Conventional therapies of surgery and chemotherapy failed to significantly improve the survival rates for this disease. Therefore novel therapeutic approaches are needed.

Ovarian cancer is a result of accumulation of genetic alterations. This and specific disease features such as confinement of the disease mostly to peritoneal cavity makes gene therapy an attractive and feasible approach. This thesis describes novel gene therapy approaches for the treatment of ovarian cancer. First, Chapter 1 reviews various gene therapy strategies developed for ovarian cancer, such as mutation compensation (including siRNA), molecular chemotherapy, genetic immunopotentiation, and recent approaches of anti-angiogenesis and virotherapy. This chapter also describes various gene delivery vectors such as adenovirus, AAV, retrovirus, and other non-viral vectors utilized for gene therapy of ovarian cancer. Further, strategies for targeted delivery of genes to tumor tissues such as transducatonal targeting and transcriptional targeting are also described in this chapter.

In Chapter 2, the Aim of the thesis is presented. Chapter 3 subsequently describes the development of novel anti-angiogenic gene therapy approach for ovarian carcinoma. Ovarian cancer is critically dependent on angiogenesis for its growth beyond 2 mm³ and metastases. Vascular endothelial growth factor (VEGF) is a major angiogenic factor promoting angiogenesis in ovarian cancer and its overexpression is correlated with increased tumor growth, ascites fluid accumulation, metastases, poor prognosis and shorter survival. On this basis a naturally occurring soluble FLT-1 (sFLT-1) gene which inhibits angiogenesis by antagonizing VEGF is employed for ovarian cancer gene therapy to inhibit tumor angiogenesis and thereby tumor growth. These studies utilized an infectivity enhanced adenoviral vector that contains an RGD motif incorporated in adenovirus capsid for efficient delivery of the sFLT-1 gene to tumor cells. The ability of sFLT-1 to inhibit angiogenesis is demonstrated in vitro by proliferation inhibition studies of human vascular endothelial cells. Subsequent to this functional validation, inhibition of ovarian tumor growth was demonstrated in vivo in a subcutaneous tumor mouse model. Next, prolongation of survival using this gene therapy approach was demonstrated using a more stringent intraperitoneal (i.p.) human ovarian cancer xenograft mouse model. In this model, sFLT-1 gene was delivered by i.p. injection to inhibit the tumor growth loco-regionally. These studies established the inhibition of tumor growth by angiogenesis inhibition using the naturally occurring sFLT-1 molecule delivered via an Ad vector.

In Chapter 4, evaluation of adenovirus-mediated sFLT-1 gene therapy to inhibit metastases of ovarian cancer is endeavored. To inhibit metastases of ovarian cancer, the Ad vector encoding sFLT-1 gene was delivered intravenously in an ovarian xenograft mouse model to achieve high systemic levels of sFLT-1. Although this systemic approach did inhibit tumor growth, it also resulted in hepatic toxicity due to ectopic localization of adenovirus to liver. The high local concentrations of sFLT-1 in liver ultimately resulted in toxicity possibly due to inhibition of VEGF, which is required for survival of sinusoidal endothelial cells in liver. This chapter thus cautions the over expression of therapeutic genes in normal tissues and emphasizes the need for tumor-specific expression of therapeutic genes to circumvent or minimize toxicity to normal tissues. Towards this end, targeted gene delivery approaches are warranted.

One of the strategies for tumor specific gene therapy is transcriptional targeting, which involves the employment of tumor specific promoters to drive the expression of therapeutic gene. Chapter 5 describes the evaluation of tumor/tissue specific promoters (TSPs) for transcriptional targeting of ovarian tumors. In this chapter, the midkine and two cyclooxygenase-2 (cox-2L and cox-2M) promoters were evaluated for specificity of gene expression in ovarian tumors by analyzing the marker gene expression in established ovarian cell lines, primary ovarian tumor cells and normal mesothelial cells. Tumor specificity of these promoters was also shown in vivo in murine mouse models by expression of the marker gene specifically in tumors and very minimal expression in liver and other normal tissues. Among these promoters cox-2M
showed the best “liver off” and “tumor on” profile. The utility of this promoter to reduce toxicity to normal tissues and achieve therapeutic effect was demonstrated by the cytotoxic effect of thymidine kinase gene expression specifically in tumor cells but not in liver and peritoneum using MTT assays and histological evaluation of organs. This selective cytotoxic effect thus showed the mitigation of toxicity by transcriptional targeting strategy using tissue specific promoters.

Although transcriptional targeting can achieve mitigation of toxic effects of therapeutic gene, a therapeutic gene which do not cause toxicity to normal tissues would be most desirable. Therefore an ideal therapeutic gene for in vivo gene therapy of cancer should not display toxicity to normal tissues and should be able to inhibit tumor growth by multiple mechanisms. Such a gene would be extremely useful for effective control of ovarian cancer and for clinical translational potential. In Chapter 6, a novel anti-cancer melanoma-differentiation associated gene-7 (mda-7), which induces apoptosis specifically in cancer cells but not in normal cells such as epithelial, endothelial, fibroblasts was evaluated for ovarian cancer therapy. mda-7 induces apoptosis in tumor cells by multiple apoptosis pathways. In addition, it inhibits tumor growth by multiple mechanisms such as inhibition of tumor angiogenesis and by immunostimulation. In vitro studies in this chapter show that adenovirus-mediated mda-7 (Ad.mda-7) induces apoptosis specifically in human ovarian cancer cells but not in normal mesothelial cells, thus limiting the gene related toxicity. However, the low CAR expression levels on ovarian cancer cells is a major limitation to achieve full potential of this gene. This problem was overcome by retargeting adenovirus encoding mda-7 (Ad.mda-7) to receptors that are overexpressed in ovarian cancer cells, such as CD40 and EGFR. Retargeting of Ad.mda-7 was achieved using bi-specific adapter molecules which bind to both the knob domain of the adenovirus and the respective receptor on the cancer cell. This strategy called as transductional targeting, significantly enhanced the apoptosis induction compared to untargeted Ad.mda-7. Thus these studies established that targeted Ad.mda-7 gene therapy is an attractive therapeutic gene for ovarian cancer gene therapy owing to its growth inhibition by multiple mechanisms and lack of toxicity in normal tissues. Based on these feasibilities mda-7 gene therapy may have a high potential for clinical translation. Therefore, evaluation of mda-7 gene therapy in vivo in murine models is required.

For in vivo evaluation studies of Ad.mda-7, employing bi-specific adapters (two-component system) for transductional targeting may not be suitable due to more complex pharmaco-dynamics and kinetics and lack of sufficient data on their stability in vivo. Further, producing such retargeting molecules is relatively complex and expensive to develop. Therefore one-component systems may be more easily applicable to in vivo pre-clinical studies and for clinical translation. In Chapter 7 derivation and evaluation of single component adenoviruses, which are retargeted to integrins or heparan sulfate-containing receptors over expressed on ovarian cancer is shown. Single component adenoviruses retargeted to either integrins or heparan sulfate containing receptors were developed by incorporation of their targeting ligands namely either “RGD” and “polylysine” or both into the capsid of adenovirus by genetic modification. These genetically modified retargeted adenoviruses encoding mda-7 showed significant enhancement in apoptosis in vitro with the adenoviral vector containing both RGD and polylysine ligands displaying highest level of apoptosis induction. Further, in vivo evaluation of these retargeted viruses showed significant inhibition of tumor growth and prolongation of the survival of mice, compared to unmodified controls. Again, adenovirus containing both targeting ligands was shown to be most effective in inhibiting tumor growth and prolonging the survival of mice with human ovarian cancer xenografts. These studies showed high therapeutic efficacy for effective management of ovarian cancer.

In conclusion, the studies presented in this thesis demonstrate the novel approaches of adenovirus-mediated gene therapy for effective therapy of ovarian carcinoma. Specifically, the studies demonstrated that an anti-angiogenesis gene therapy approach is effective in inhibiting tumor growth and prolonging survival in a loco-regional manner, but cautions the use of systemic gene therapy due to toxicity. Further, strategies of transductional and transcriptional targeted gene therapy for tumor specific killing and circumventing toxicity were demonstrated. Finally, exploitation of the effective therapeutic gene mda-7,
which specifically induces apoptosis in cancer cells but not in normal cells has been demonstrated for ovarian cancer gene therapy. Further, mda-7 gene therapy via genetically modified, ovarian cancer targeted adenovirus demonstrated significant inhibition of tumor growth and prolonged survival of mice. Since mda-7 was shown to have multiple anti-cancer properties such as inhibition of tumor angiogenesis and immunostimulation in addition to apoptosis induction, it has a large advantage in achieving good therapeutic effect compared to genes that inhibit tumor growth by only one mechanism. Further, the greatest advantage of mda-7 is that it does not induce toxicity in normal cells. Based on these feasibilities, mda-7 gene therapy may have very high clinical translational potential and should be exploited for human ovarian cancer gene therapy.