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Liposome-plasmid complexes encoding angiostatin and endostatin inhibit breast cancer in nude mice

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Keywords

Liposomes, antiangiogenesis, endostatin, angiostatin

Introduction

Angiogenesis has been shown to be crucial for both tumor growth and metastasis. Preventing angiogenesis with antiangiogenic factors is an obvious therapeutic strategy. Two antiangiogenic factors that have been identified are angiostatin and endostatin. Derived from parent proteins (plasminogen and collagen XVIII respectively) these polypeptides inhibit endothelial cell proliferation while having no obvious toxicity although delivery of these agents is difficult, and usually requires prolonged infusions. An alternative delivery method is gene therapy transfer. Standard gene therapy uses viral vectors; however, there are safety and toxicity issues surrounding the use of viral vectors. This study examined whether liposomes complexed to plasmids encoding angiostatin or endostatin could successfully inhibit angiogenesis and the growth of MDA-MB-435 tumors implanted in the mammary fat pads of nude mice.

Aims

To assess the feasibility of nonviral gene transfer of antiangiogenic compounds by liposome-plasmid complexes, and to determine if there is an anti-tumor effect.

Comments

Targeting tumor angiogenesis has become a major research area. One unanswered question is how to best deliver the antiangiogenic compounds to the tumor. Several groups have already looked at delivering antiangiogenic genes using viral vectors. This interesting paper examines an alternative method of delivering antiangiogenic genes using a nonviral method. Obviously, this method could be used to deliver a range of different genes into tumor cells. The data in this paper are preliminary and further work is required. The low transfection efficiency is cause for concern and, as the authors themselves point out, a number of other studies have found that liposome-plasmid complexes are targeted primarily to the lung, which may reduce the chance of anti-tumor effects elsewhere. It would

have been interesting to see the effect on tumor growth of a liposome-only injection, to put beyond doubt that it was actually the angiostatin or endostatin that affected the tumor growth.

Methods

Murine angiostatin and endostatin cDNAs were constructed and cloned into the PCI vector [Promega, Madison WI] (PCI-Angio and PCI-Endo) and the sequence and orientation verified. *In vitro* analysis was performed to assess whether the constructs expressed the relevant gene products, whether they could transfect cell lines, and (following transfection) whether the respective proteins could be detected. *In vivo*analysis included an angiogenesis assay based upon the insertion of a Matrigel plug [Collaborative Biomedical Products, Bedford MI] into a mouse for a week, followed by its removal and the assessment of hemoglobin content and blood vessel density. Finally, the mammary fat pads of female athymic nude mice were injected with breast cancer cells, and six days later a prepared liposome-plasmid complex was injected either directly into the tumor or intravenously. Multiple injections were given over specific time frames and tumor measurements taken. In the group of mice treated with intratumoral injections there were four treatment groups, each consisting of 10 mice: untreated control; treated with empty vector; treated with liposome-PCI-Angio; and treated with liposome-PCI-Endo. In the group of mice treated with intravenous injections there were three treatment groups, each with 10 mice: untreated control; treated with liposome-PCI; and treated with liposome-PCI-Endo.

Results

In vitro analysis confirmed the expression of both the angiostatin and endostatin gene products from PCI-Angio and PCI-Endo respectively. Transfection of these into Chinese hamster ovarian cells resulted in the production of detectable levels of the proteins in the culture medium. This medium was then added to the Matrigel angiogenesis model, and significant inhibition of neovascularisation by the culture medium was demonstrated. *In vivo*experiments showed that both liposome-PCI-Angio and liposome-PCI-Endo significantly reduced tumor size when injected intratumorally ($p < 0.05$), although transfection efficiency of an intratumoral injection was shown to be only 5%. Compared to the untreated control group, the mice treated with PCI-Angio and PCI-Endo exhibited a reduction in tumor size of 36% and 49%, respectively. Intravenous injections of liposome-PCI-Endo was also found to reduce tumor growth in the nude mice by nearly 40% when compared to either untreated controls or empty vector (PCI) or ($p < 0.05$).

Discussion

These results indicate that plasmids encoding angiostatin and endostatin can be successfully constructed, complexed to liposomes, and when injected into nude mice, cause inhibition of tumor growth. These findings provide a basis for the further development of nonviral delivery of antiangiogenic genes.

References

1. Chen Q-R, Kumar D, Stass SA, Mixson AJ : Liposomes complexed to plasmids encoding angiostatin and endostatin inhibit breast cancer in nude mice. Cancer Res. 1999, 59: 3308-3312.