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Editorial

Antiangiogenic gene therapy

It is now well established that tumour growth and metastasis are dependent upon the tumours' ability to recruit a functional blood supply through the process known as angiogenesis. Indeed, the angiogenic phenotype of the tumour correlates negatively to prognosis,[1,2] and that tumours that have not acquired their own blood supply cannot grow to more than 2–3 mm^3.[3] Thus for the first time there is a universal *proviso* for all cancers, for which an effective therapy can be designed to target. With this recognition came the discovery of molecules that promote and inhibit angiogenesis. Antiangiogenic therapies devised so far target different steps of the angiogenic process, ranging from the inhibition of expression of angiogenic molecules, overexpression of antiangiogenic molecules, to direct targeting of tumour endothelial cells using endogenous angiogenic inhibitors or artificially constructed targeting ligands. These studies have revealed a number of salient features about the destructive targeting of the tumour vasculature. Therapies aimed at destroying the tumour vasculature can lead to rapid regression of existing tumours[4–6] due to enhanced tumour cell apoptosis. Furthermore, unlike existing chemotherapy, antiangiogenic therapy does not lead to drug resistance.[7] Last but not least is the therapy's unprecedented ability to regress a tumour and maintain it in the regressed state, a phenomenon known as 'tumour dormancy'.[7]

Gene therapy has been advocated as a means of delivering antiangiogenic factors to the tumour vasculature. There are a number of arguments favouring this mode of delivery. First, antiangiogenic therapy requires prolonged administration of therapeutic proteins to maintain tumour suppression. Compared with alternative strategies requiring systemic administration of antiangiogenic recombinant proteins, monoclonal antibodies and other drugs, gene therapy theoretically allows a one-off administration of an easily produced vector, resulting in persistent production of therapeutic levels of proteins. Second, selective expression of antiangiogenic agents at sites of active tumour growth can achieve high local concentrations of proteins coupled with low systemic levels of proteins. This is important because antiangiogenic agents can affect angiogenesis in normal physiology, and some angiogenic factors are required for the maintenance of certain tissues.[8]

Since the first report of antiangiogenic gene therapy in 1994 using a dominant negative mutant of the VEGF receptor,[9] antiangiogenic gene therapy has established itself in the laboratory as an effective and potent way to suppress tumour growth and spread. To date, antiangiogenic therapy has focused on three main targets. The first of these has used gene therapy targeted to tumour cells to suppress the expression of angiogenic factors such as VEGF. Tumour cells directly transfected with VEGF antisense,[10,11] or indirectly infected with viruses carrying this same antisense,[12,13] can both result in partial inhibition of tumour growth mediated through reduced neoangiogenesis. Due to this strategy's inherent high risk for the development of drug resistance, it is unlikely to find significant clinical use on its own. Tumour cells that express alternate but potent angiogenic factors such as bFGF and thymidine phosphorylase will be selected for, resulting in resistance.

Another strategy that has been pursued aims at transferring genes that disrupt specific signalling pathways used by angiogenic factors. ExTek and sFLT-1, both dominant negative functioning extracellular domains of the endothelial-specific tyrosine kinase receptor tie-2 and the VEGF receptor flt-1, respectively, have been reported to inhibit tumour growth and metastasis after gene transfer.[14,15] This approach targets the angiogenic vasculature directly, and does not require transfer of genes to each tumour cell. In fact, the tumour inhibiting effects in these two studies were mediated through systemic and paracrine expression of the decoy receptors, respectively, and in the latter case, high levels of circulating ExTek were detected transiently following systemic adenoviral delivery. These results prove that disruption of receptors for factors required for angiogenesis can result in potent inhibition of tumour growth.

Whereas both the above strategies target angiogenic signals that drive tumour angiogenesis, there have also been reports of the direct transfer of genes encoding potent antiangiogenic molecules. The past 5 years have seen an expanding list of endogenous angiogenesis inhibitors, such as thrombospondin-1, platelet factor 4, angiostatin and endostatin. This group of molecules acts directly on endothelial cells to cause selective apoptosis of stimulated and proliferating endothelial cells.[16,17] They can achieve remarkable tumour regression and dormancy[7] without significant side-effects.[18] Several groups have therefore investigated the delivery of these inhibitors through gene transfer to achieve long lasting suppression of tumour growth.

The past 2 years have seen several reports of *in vivo* gene transfer of angiogenesis inhibitors, with concomitant tumour suppressive effects. Tanaka and colleagues[19,20] have shown that intratumoral adenovirus-mediated transfer of either platelet-factor 4 or angiostatin inhibits the ability of a highly angiogenic glioblastoma

cell line to grow in the relatively avascular renal subcapsular space, unlike the wild-type cell line. Furthermore, animal survival was prolonged in an intracerebral xenograft model. Post-mortem investigations demonstrated that these treated tumours are poorly vascularised with significantly elevated apoptotic rates.

The ability of antiangiogenic gene therapy to contain an expanding tumour mass may not be as important as its ability to curtail metastasis, the dissemination that is ultimately fatal in most cancers. Two studies have utilised intravenous injection of gene vectors to achieve more generalised and systemic expression of the angiogenesis inhibitors ExTek, thrombospondin-1 and angiostatin.[14,21] Delivery of these vectors resulted in significant inhibition of the establishment and growth of metastases. A different approach taken by Blezinger and colleagues[22] utilised gene delivery to muscle to produce high levels of endostatin systemically. Both tumour growth and metastases were inhibited after gene transfer.

Vector development is still a major obstacle in gene therapy. Present vectors are either inefficient at gene transduction, or are unable to maintain prolonged expression of transgene. Adenoviruses are the most promising vector today, however, they are rapidly sequestered by the liver, transduce endothelium poorly[23] and expression is rapidly curtailed by effective immune responses, resulting in rapidly decreasing levels of expression over a short period of time. Readministration of the vector is hindered by a strong neutralising response. Strategies to develop non-antigenic vectors and vectors with altered tropism may one day allow the targeted delivery of these vectors to sites of tumour tissue, at the same time maintaining high local levels of expression of angiogenic inhibitors.

Outside of tumours, angiogenesis also takes place in normal physiological processes such as pregnancy and wound healing. Systemic administration of some antiangiogenic factors may therefore be detrimental to these processes. On the other hand, localised gene transfer will fail to control disseminated multi-focal metastases. Furthermore, antiangiogenic treatment needs to be prolonged, but does not necessarily need to be lifelong. Thus a mechanism allowing gene expression to be switched off could protect the patient from long-term side-effects of such treatments. A solution to these problems is to have a conditional promoter localise gene expression to areas of tumour angiogenesis. Expression of the antiangiogenic gene can be placed under the control of a promoter that is switched on only in areas of tumour growth. For example, a feature of the tumour microenvironment is the presence of regions of acute hypoxia. Our laboratory has developed transgenes driven by the hypoxic response elements to render gene expression conditional upon hypoxia. Expression of the transgene in such systems would target expression to tumours, and for only as long as the stimulus is present, providing a localised supply of antiangiogenic factors.

Perhaps one of the more disconcerting results that has arisen is that unlike the dramatic regression seen after direct injection of angiogenic inhibitors, antiangiogenic gene delivery so far results in incomplete suppression of tumour growth and metastasis, and no tumour regression. There are several explanations for this. First, the vectors used may be unable to sustain high level expression of the transgene.[13,14,22] Second, many of these studies have relied on the highly angiogenic glioblastoma cell line C6 to test the efficacy of different vectors. Such cell lines tip the angiogenesis balance heavily towards pro-angiogenesis, demanding equally potent antiangiogenic signals to counteract these signals. Third, a recent report by Bergers and colleagues[24] has demonstrated differential sensitivity of tumours at varying stages of carcinogenesis to different angiogenesis inhibitors. It is therefore conceivable that tumour escape represents either changes in the profile of angiogenic factor expression by tumours, or a switch in sensitivity of the tumour vasculature to different agents. These results implicate the use of a combination of antiangiogenic agents in therapy, in a manner similar to current chemotherapeutic protocols. Studies into such combinations are underway.

In conclusion, gene therapy has now been adopted for the delivery of antiangiogenic factors to suppress tumour growth. It represents a more cost-effective and clinically more convenient way of delivering steady-state levels of angiogenesis inhibitors to the tumour. Many reports have now established that delivery of a variety of antiangiogenic genes can suppress tumour growth and metastasis. However, the problems that plague gene therapy, especially the development of systemically administerable vectors that allow long-term expression of the transgenes, need to be resolved before antiangiogenic gene therapy can become a clinical reality. Angiogenesis remains a universal and attractive target for cancer therapy with excellent prospects, and for this reason, we can expect much effort to be invested in antiangiogenic gene therapy in the next few years.

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