Apoptosis-Inducing Cellular Vehicles for Cancer Gene Therapy

*Endothelial and Neural Progenitors*

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### Summary

Endothelial progenitor cells (EPCs) and neural progenitor cells (NPCs) are promising for cancer therapy because they specifically target tumors. They have the capacity to home to, invade, migrate within, and incorporate into tumor structures. They are easily expanded and can be armed with therapeutic payloads protected within the progenitor cells. Once in the tumor, armed progenitors can be triggered to induce apoptosis in surrounding tumor cells. Pro- and antiapoptotic mechanisms are pivotal to effectively kill tumor cells while simultaneously protecting the cellular vehicles from premature demise. Increasing the ratio of tumor cell apoptosis to progenitor apoptosis will be crucial among other efforts to enhance the efficacy of endothelial and neural progenitor cells to a level sufficient for clinical application.

**Key Words:** Apoptosis; endothelial progenitor cells; neural progenitor cells; cellular vehicle; cancer; gene therapy.

### 1. Cellular Vehicles for Gene Therapy of Tumors

Conventional tumor therapy by surgery, chemotherapy, and radiation often fails or is associated with severe side effects. Improved therapies are therefore needed. This may be achieved by improved targeting, that is, by confining the therapy-induced cytotoxicity to the tumor cells. Recent developments in tumor targeting have led to small molecules, such as receptor tyrosine kinase (TK) inhibitors, aimed at signaling pathways pivotal for tumors, to antibodies targeted at surface molecules, and to targeted gene therapy. However, the degree of targeting is often inefficient: the targets of small molecules may not be as tumor-specific as required and large antibodies may not penetrate the depth of a tumor mass. The success of gene therapy for treatment of cancer, in particular, depends on delivering genes specifically to tumor cells. For this, both viral and nonviral vectors have been used. Despite significant advances, gene therapy approaches using these vectors have been hampered by imprecise tumor targeting leading to unexpected side effects, low-viral titers, rapid degradation by the immune system, nonspecific adhesion, poor transduction efficiency, and low-level transgene expression. Moreover, these vectors have been mostly administered loco-regionally by direct intratumoral injection. Because metastases or inaccessible tumors account for a significant proportion of morbidity and mortality in cancer patients, there is a need for vectors that can be

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administered systemically to target these tumor manifestations. To overcome the limitations of viral vectors, mammalian cells have been proposed as potential gene delivery vehicles. In principle, cellular vehicles offer several advantages. They may be able to home to tumors and mediate tumor cell death while, ideally, being protected against apoptosis induced by their therapeutic payload. If chosen appropriately, they should not evoke an immune response. Furthermore, some cell types intended as vehicles may be readily available, easy to isolate noninvasively, expandable ex vivo, and receptive to genetic manipulation. Not surprisingly, a variety of cellular vehicles have been investigated. In a pioneering clinical study genetically marked autologous tumor-infiltrating lymphocytes, expanded and activated in vitro, were used as vehicles for tumor-specific immunotherapy (1). Other cell types, including fibroblasts (3–6), endothelial cells (ECs) (8), natural killer (NK) cells, dendritic cells, and autologous or allogeneic tumor cells (9) have been evaluated as cellular vehicles for tumor gene therapy. However, modest tumor-specific homing, limited ex vivo expandability, difficult genetic manipulation, or poor tolerance have hindered clinical translation of these vehicles. By virtue of their intrinsic properties, adult and embryonic stem and progenitor cells promise to overcome these problems. This chapter focuses on two well-characterized progenitors, endothelial cell (EPC) and neural progenitor cells (NPC) as potential cellular vehicles to target two of the most therapy-recalcitrant cancer manifestations: metastases and brain tumors.

2. Endothelial Progenitors for Systemic Apoptosis-Inducing Cancer Gene Therapy

2.1. The Physiological Role of Endothelial Progenitors—Vasculogenesis and Angiogenesis

During development, the vasculature is formed by vasculogenesis, that is the in situ differentiation of ECs from their precursors and their subsequent organization into a primary capillary network. Vasculogenesis during development requires EPCs that are derived from a common precursor of both the hematopoietic system and the vascular system called the hemangioblast (10). The crucial role of vascular endothelial growth factor receptor (VEGFR)2 and VEGF in embryonic vasculogenesis is shown by the phenotype of both VEGFR2 and VEGF knock-out mice who die in utero as a result of lack of endothelial and blood cells (11,12). In adult life new vessels are formed during wound healing, the endometrial cycle and the growth of tumors. This postnatal vessel formation has for a long time been thought to occur exclusively by sprouting from existing vessels and was termed angiogenesis. Recent work has shown that adult EPCs are recruited from the bone marrow to form new vessels in adults during both physiological and pathological conditions (13,14). Adult EPCs have been characterized as highly proliferative cells, having properties similar to those of embryonic angioblasts. In response to angiogenic stimuli they are mobilized from the bone marrow and after differentiation into mature ECs they functionally incorporate into vessels. These findings have suggested that vasculogenesis is not restricted to embryogenesis but also plays an important role in adults. The discovery of EPCs has led to the concept that angiogenesis—the remodeling or sprouting of preexisting blood vessels—and vasculogenesis may constitute complementary mechanisms for postnatal neo-vascularization.

To what extent and under which conditions bone marrow-derived endothelial progenitors contribute to new vessel formation still has to be elucidated. Recent preclinical and clinical