[Frontiers in Bioscience 13, 1887-1891, January 1, 2008]

Vector-producing tumor-tracking multipotent mesenchymal stromal cells for suicide cancer gene therapy

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1. ABSTRACT

Suicide cancer gene therapy with retroviral vector-producing cells was in the way of an adjuvant to the surgical resection of recurrent glioblastoma, although any benefit appeared to be marginal. It is likely that this therapeutic approach may have better outcomes if the vectors and transgenes are delivered more efficiently to the tumor cells. We have shown previously that tumor cells engineered by adenovirus-retrovirus hybrid vectors to produce retroviral progeny destroy satellite tumor cells. Whether the systemic delivery of vector-producing cells can effectively treat aggressive tumors remains to be determined. Effective retroviral vector delivery vehicles may be multipotent mesenchymal stromal cells (MSCs), which have been shown to home to tumor cells *in vivo* and deliver cancer-killing gene or immune products with minimal host rejection. Therefore, it may be possible to transduce tumors with recombinant progeny vectors delivered by MSCs. This may be particularly suitable for treating diffuse cancers like glioblastoma multiforme. While this strategy remains to be tested in various orthotopic or metastatic tumor models, it has the potential to greatly improve the outcome of suicide gene therapy.

2. SUICIDE CANCER GENE THERAPY USING VECTOR-PRODUCING CELLS

A gene therapy against glioma has been developed in which a cell line that continuously secretes a retroviral vector is implanted into brain tumors (1). The vector, which expresses the herpes simplex thymidine kinase (HSV-*tk*) transgene, "infects" the local tumor cells, which then become susceptible to tumoricidal metabolites generated by HSV-*tk*-mediated activation of the prodrug ganciclovir (GCV). The therapeutic value of this technique appeared to be enhanced by a bystander effect wherein the transduced tumor cells communicate the apoptosis signal to neighboring cells. However, while this cancer gene therapy system was shown to have some clinical benefit, its efficacy was limited due to the poor efficiency of gene transfer (2). To improve the therapeutic potential of this system, it is necessary to enhance (a) the efficiency of therapeutic gene delivery *in vivo* and (b) the stability of the vector-producing cells.

With regard to therapeutic gene delivery *in vivo*, we have previously described a hybrid vector system where adenoviral vectors are used to deliver retroviral vector andpackaging proteins into cells (3). This system benefits

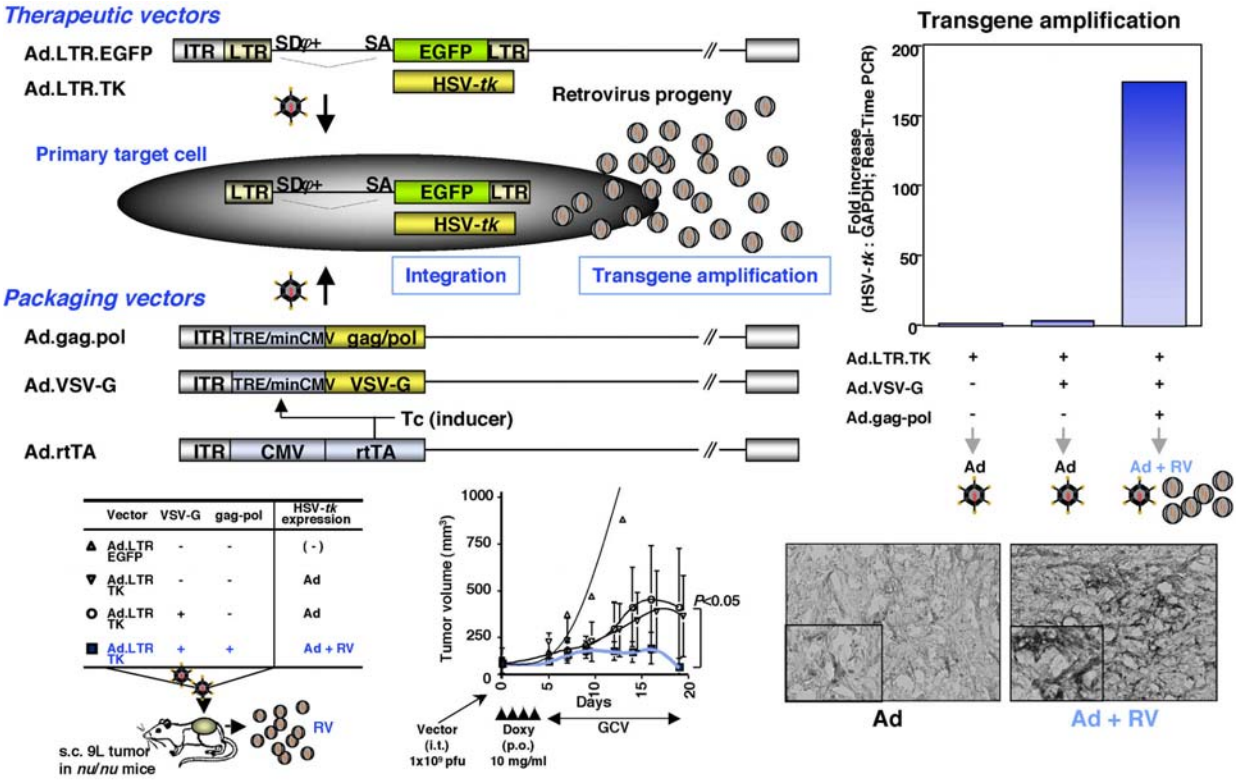

Figure 1. Two adeno-retroviral hybrid vectors containing a retroviral vector genome (Ad.LTR.EGFP or Ad.LTR.TK) were constructed. Co-transduction of rat 9L glioma cells with an adeno-retroviral hybrid vector together with vectors expressing retrovirus packaging proteins (Ad.gag.pol, Ad.VSV-G) as well as an inducer (Ad.rtTA) increased the transduction efficiency. Injection of established subcutaneous 9L tumors on athymic mice with a combination of AVC2.GCTK and packaging vectors followed by GCV treatment resulted in complete regression by 50% of the tumors at day 22, while no tumor regression was observed in control animals. Furthermore, the relative copy number of the HSV-tk gene in tumors treated with the adeno-retroviral vectors was significantly higher than in control tumors. In situ hybridization analysis also suggested dispersion of the HSV-tk product across a wider area of the tumor than in control tumors, which indicates the spread of the in situ-generated retroviruses.

from the efficient gene transfer characteristics of adenoviral vectors as well as the stable and long-term gene expression that is typical of retroviral vectors. We have shown that direct transduction of primary target tumor cells with hybrid adeno-retroviral vectors results in their transient production of recombinant retrovirus particles that then subsequently transduce neighboring tumor cells (3). Moreover, when we transduced established subcutaneous 9L tumors on athymic mice *in situ* with adenovirus vectors that express transcomplementing genes encoding retroviral proteins and retroviral vector RNAs, upon GCV treatment, 50% of the tumors showed complete regression at day 22, while no tumor regression was observed in control animals (Figure 1). This strategy can now be developed further by using cells with tumor-tracking properties as the vector-producing cells, thereby targeting the therapeutic gene to the tumor cells *in vivo*.

3. MULTIPOTENT MESENCHYMAL STROMAL CELLS (MSCs) AS A PLATFORM FOR VECTOR PRODUCTION *IN SITU*

We propose here an improved *in situ* vector production strategy where cells bearing tumor-tracking properties efficiently produce retrovirus- or other virus-based progeny vectors (Figure 2). Candidate tumor-tracking vector-producing cells are adult stem cells. In particular, the fibroblast-like plastic-adherent cells isolated from bone marrow and other sources that are now widely known as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs) (4), may be useful as they have been shown to have tumor-seeking properties (5). While the mechanism that induces MSCs to preferentially engraft themselves in tumors remains poorly undertsood, this phenomenon may be mediated by the cytokines released by the tumor or inflammatory tissue. These include hepatocyte growth factor (HGF) (6), vascular endothelial cell growth factor (VEGF) (7), transforming growth factor (TGF) (7), fibroblast growth factor (FGF) (6), platelet-derived growth factor (PDGF) (8), monocyte chemoattractant protein-1 (MCP-1) (9), and IL-8 (9). Moreover, chemokine C-X-C motif receptor 4 (CXCR4), which is present on the surface of an MSC subset, is known to mediate not only the specific migration of MSCs to bone marrow (10), it also governs the migration and homing of a variety of cell types in the developing brain, including neuronal and glial precursors. The only known chemokine that binds with CXCR4 is stromal-cell derived factor-1 (SDF-1). While CXCR4 itself is a major chemokinereceptor on glioma cells and promotes their

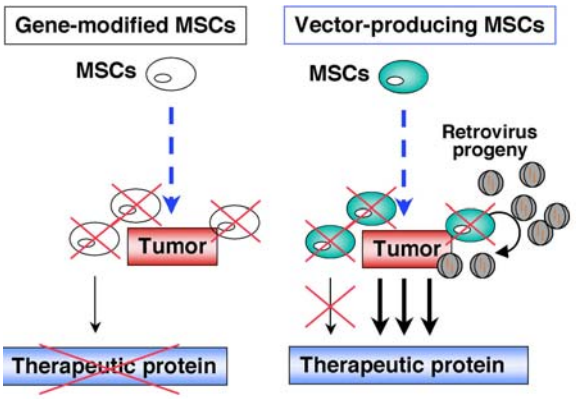

Figure 2. Left panel; Gene-modified MSCs. Although gene-modified MSCs have tumor-seeking properties, the local expression of the therapeutic protein is dependent on the continuing presence of the MSCs. Right panel; Vector-producing MSCs. The retrovirus progeny produced by the MSCs can transduce tumor cells *in situ*, which extends the expression of the therapeutic protein, even when the MSCs die off.

survival (11), high-grade gliomas have recently been found to secrete significant levels of SDF-1 (12). SDF-1 alpha stimulates human glioblastoma cell growth by activating both extracellular signal-regulated kinases 1/2 and Akt. Therefore, CXCR4 expression by MSCs may help them to home to gliomas. When MSCs are infused in mice, they are rapidly and efficiently arrested in the microvasculature (13). Furthermore, these cells are not immunogenic and escape recognition by alloreactive T cells and natural killer cells (14). It also appears that the engraftment of MSCs into the tumor helps them to maintain their stem cell properties *in vivo*. Thus, it appears that MSCs may efficiently engraft human gliomas after intravascular or local delivery and can be used as *in situ* therapeutic vector producers.

Other candidate tumor-tracking cells that may be used as vector-producing cells include endothelial progenitor cells (EPCs). EPCs have been isolated from peripheral blood CD34, Flk-1, or AC133 antigen-positive cells, which are believed to include a hematopoietic stem cell population, and have been shown to incorporate into neovascularization foci (15). Moreover, VEGF promotes adult vasculogenesis by enhancing EPC recruitment and vessel formation at sites of tumor neovascularization (16). Magnetic resonance imaging (MRI) of magnetically labeled endothelial progenitor cells also demonstrated that they traffick to sites of tumor angiogenesis (17).

4. GENETIC MANIPULATION OF MSCs TO FACILITATE THEIR PRODUCTION OF PROGENY VECTORS

MSCs exhibit senescence-associated growth arrest and phenotypic changes during long-term *in vitro* culture. However, overexpression of human telomerase reverse transcriptase (hTERT) in MSCs reconstitutes their telomerase activity and extends their life span (18). Telomerization of MSCs by hTERT overexpression also maintains the stem cell phenotype of MSCs and thus may be useful for generating the numbers of stable MSCs needed for cell differentiation studies and tissue engineering protocols.

To produce therapeutic vectors, the MSCs must be efficiently transduced with viral components. Virus-based transduction techniques have been shown to achieve high gene transduction and transgene expression in many cellular models, and attempts have been made to transduce MSCs with various virus-based vectors such as oncogenic retrovirus- or lentivirus-based vectors. However, the use of integrating viral vectors has several disadvantages, particularly with regard to their safety risks. Many non-viral methods also have limited utility as they are rather inefficient with most primary cells. However, nucleofection, which is a non-viral electroporation-based gene transfer technique, has been shown to be an efficient non-viral transfection technique for MSCs, which then may be used as cellular vehicles for the delivery of biological agents (19). Thus, the Nucleofector technology may be promising as an alternative tool for efficiently transfecting MSCs so that they produce progeny virus.

5. FUTURE DIRECTIONS

Here we propose that current suicide cancer gene therapy strategies may be improved by using vector-producing tumor-tracking MSCs. This strategy is likely to generate *in situ* the vector numbers needed for the killing of solid tumors. We also showed that it may be feasible to produce large-scale preparations of vector-producing cells by transient transduction of MSCs by hybrid adenovirus-based vector infection. It has been shown that the hybrid adenovirus-based vectors that express retroviral proteins can efficiently transduce cells, which then produce progeny vectors (3). However, an impediment for this aim is that MSCs lack the Coxsackie adenovirus receptor (CAR) (20). To overcome this problem, it may be necessary to use a chimeric Ad35 fiber-containing Ad5 vector (21) or a fiber-modified Ad5 vector bearing an RGD-motif peptide in the HI loop of the fiber knob domain (22). Alternatively, it may be possible to use an adaptor molecule that bridges the gap between the viruses and MSCs. Supporting the latter possibility is that we have previously developed a CAR-SCF fusion protein that improves the transduction efficiency of the adenovirus vector with c-kit positive cells (23). Similar CAR-ligand adaptor molecules may be useful for enhancing MSC transduction with the adenovirus vector.

To improve the tumor-targeting properties of the vector-producing cell, how MSCs naturally seek out tumors should be investigated in more detail. In addition, the localization, stability, and vector-producing capacity of gene-manipulated MSCs should be adequately analyzed *in vivo*. Tracking the localization of the MSCs may also help diagnose the recurrence of the disease. Such tracking may be performed by using a molecular imaging technique with MRI. To this end, it has been shown that MSCs labeled with fluorophore particles (IFPs) provide MRI contrast *in vivo* (24). Thus, this type of technology would enable us to

closely study MSC retention, engraftment, and migration in the clinic.

Although previous studies have illuminated the exciting possibilities of suicide cancer gene therapy, in most cases the therapies that were used delivered rather limited clinical benefits. For the sake of safety as well as improving the therapeutic effect of suicide cancer gene therapy, it is important that the suicide gene-expressing vector is accurately delivered to the tumor. This may be achieved by using MSCs to initiate virus production near tumor cells *in situ*. These viruses then transduce the tumor cells, which themselves produce virus progeny, thereby amplifying the transgene expression of the tumor. While the therapeutic benefit of this strategy remains to be tested in various orthotopic or metastatic tumor models, it may be promising for detecting and eradicating evasive tumors *in vivo*.

6. REFERENCES

1. Ram, Z., Culver, K.W., Oshiro, E.M., Viola, J.J., DeVroom, H.L. *et al*: Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat Med* 3, 1354-1361 (1997)
2. Rainov, N.G: A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum Gene Ther* 11, 2389-2401 (2000)
3. Okada, T., Caplen, N.J., Ramsey, W.J., Onodera, M., Shimazaki, K. *et al*: *In situ* generation of pseudotyped retroviral progeny by adenovirus-mediated transduction of tumor cells enhances the killing effect of HSV-tk suicide gene therapy *in vitro* and *in vivo*. *J Gene Med* 6, 288-299 (2004)
4. Horwitz, E.M., Le Blanc, K., Dominici, M., Mueller, I., Slaper-Cortenbach, I., Marini, F.C., Deans, R.J., Krause, D.S. and Keating, A: Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7, 393-395 (2005)
5. Nakamizo, A., Marini, F., Amano, T., Khan, A., Studeny, M. *et al*: Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 65, 3307-3318 (2005)
6. Yamamoto, S., Wakimoto, H., Aoyagi, M., Hirakawa, K. and Hamada, H: Modulation of motility and proliferation of glioma cells by hepatocyte growth factor. *Jpn J Cancer Res* 88, 564-577 (1997)
7. Tille, J.C. and Pepper, M.S: Mesenchymal cells potentiate vascular endothelial growth factor-induced angiogenesis *in vitro*. *Exp Cell Res* 280, 179-191 (2002)
8. Hellstrom, M., Kalen, M., Lindahl, P., Abramsson, A. and Betsholtz, C: Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 126, 3047-3055 (1999)
9. Wang, L., Li, Y., Chen, X., Chen, J., Gautam, S.C., Xu, Y. and Chopp, M: MCP-1, MIP-1, IL-8 and ischemic cerebral tissue enhance human bone marrow stromal cell migration in interface culture. *Hematology* **7**, 113-117 (2002)
10. Wynn, R.F., Hart, C.A., Corradi-Perini, C., O'Neill, L., Evans, C.A., Wraith, J.E., Fairbairn, L.J. and Bellantuono, I: A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* 104, 2643-2645 (2004)
11. Zhou, Y., Larsen, P.H., Hao, C. and Yong, V.W. CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. *J Biol Chem* 277, 49481-49487 (2002)
12. Barbero, S., Bonavia, R., Bajetto, A., Porcile, C., Pirani, P., Ravetti, J.L., Zona, G.L., Spaziante, R., Florio, T. and Schettini, G: Stromal cell-derived factor 1alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. *Cancer Res* 63, 1969-1974 (2003)
13. Bentzon, J.F., Stenderup, K., Hansen, F.D., Schroder, H.D., Abdallah, B.M., Jensen, T.G. and Kassem, M: Tissue distribution and engraftment of human mesenchymal stem cells immortalized by human telomerase reverse transcriptase gene. *Biochem Biophys Res Commun* 330, 633-640 (2005)
14. Le Blanc, K., Rasmusson, I., Sundberg, B., Gotherstrom, C., Hassan, M., Uzunel, M. and Ringden, O: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363, 1439-1441 (2004)
15. Asahara, T. and Kawamoto, A: Endothelial progenitor cells for postnatal vasculogenesis. *Am J Physiol Cell Physiol* 287, C572-579 (2004)
16. Li, B., Sharpe, E.E., Maupin, A.B., Teleron, A.A., Pyle, A.L., Carmeliet, P. and Young, P.P: VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *Faseb J* 20, 1495-1497 (2006)
17. Arbab, A.S., Pandit, S.D., Anderson, S.A., Yocum, G.T., Bur, M., Frenkel, V., Khuu, H.M., Read, E.J. and Frank, J.A: Magnetic resonance imaging and confocal microscopy studies of magnetically labeled endothelial progenitor cells trafficking to sites of tumor angiogenesis. *Stem Cells* 24, 671-678 (2006)
18. Simonsen, J.L., Rosada, C., Serakinci, N., Justesen, J., Stenderup, K., Rattan, S.I., Jensen, T.G. and Kassem, M: Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat Biotechnol* 20, 592-596 (2002)
19. Aluigi, M., Fogli, M., Curti, A., Isidori, A., Gruppioni, E. *et al*: Nucleofection is an efficient nonviral transfection technique for human bone marrow-derived mesenchymal stem cells. *Stem Cells* 24, 454-461 (2006)
20. Hung, S.C., Lu, C.Y., Shyue, S.K., Liu, H.C. and Ho, L.L:. Lineage differentiation-associated loss of adenoviral susceptibility and Coxsackie-adenovirus receptor expression in human mesenchymal stem cells. *Stem Cells* 22, 1321-1329 (2004)
21. Olmsted-Davis, E.A., Gugala, Z., Gannon, F.H., Yotnda, P., McAlhany, R.E., Lindsey, R.W. and Davis, A.R: Use of a chimeric adenovirus vector enhances BMP2 production and bone formation. *Hum Gene Ther* 13, 1337-1347 (2002)
22. Tsuda, H., Wada, T., Ito, Y., Uchida, H., Dehari, H., Nakamura, K., Sasaki, K., Kobune, M., Yamashita, T. and

Hamada, H: Efficient BMP2 gene transfer and bone formation of mesenchymal stem cells by a fiber-mutant adenoviral vector. *Mol Ther* 7, 354-365 (2003)
23. Ito, A., Okada, T., Mizuguchi, H., Hayakawa, T., Mizukami, H., Kume, A., Takatoku, M., Komatsu, N., Hanazono, Y. and Ozawa, K: A soluble CAR-SCF fusion protein improves adenoviral vector-mediated gene transfer to c-Kit-positive hematopoietic cells. *J. Gene Med.* 5, 929-940 (2003)
24. Hill, J.M., Dick, A.J., Raman, V.K., Thompson, R.B., Yu, Z.X. *et al*: Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. *Circulation* 108, 1009-1014 (2003)

Abbreviations: MSCs: mesenchymal stromal cells; GCV: ganciclovir; EPCs endothelial progenitor cells

Key Words: Cancer gene therapy, Multipotent mesenchymal stromal cells, Vector, Review

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