Review

Gene therapy for carcinoma of the breast
Therapeutic genetic correction strategies

Patrice S Obermiller, David L Tait* and Jeffrey T Holt

Vanderbilt University Cancer Center, Nashville, Tennessee and *East Carolina University School of Medicine, Greenville, North Carolina, USA

Received: 2 November 1999
Revisions requested: 23 November 1999
Revisions received: 23 November 1999
Accepted: 24 November 1999
Published: 17 December 1999

Breast Cancer Res 2000, **2**:28–31

Abstract

Gene therapy is a therapeutic approach that is designed to correct specific molecular defects that contribute to the cause or progression of cancer. Genes that are mutated or deleted in cancers include the cancer susceptibility genes *p53* and *BRCA1*. Because mutational inactivation of gene function is specific to tumor cells in these settings, cancer gene correction strategies may provide an opportunity for selective targeting without significant toxicity for normal nontumor cells. Both *p53* and *BRCA1* appear to inhibit cancer cells that lack mutations in these genes, suggesting that the so-called gene correction strategies may have broader potential than initially believed. Increasing knowledge of cancer genetics has identified these and other genes as potential targets for gene replacement therapy. Initial patient trials of *p53* and *BRCA1* gene therapy have provided some indications of potential efficacy, but have also identified areas of basic and clinical research that are needed before these approaches may be widely used in patient care.

Keywords: adenovirus, *BRCA1*, cancer gene therapy, *p53*, retrovirus

Introduction

Because cancer is a somatic genetic disease, it should not be surprising that many gene therapy protocols have been designed to treat various forms of cancer. Even hereditary cancer syndromes generally include a required somatic gene alteration. For example, patients with hereditary breast and ovarian cancer due to *BRCA1* mutations are born with a mutation in one *BRCA1* allele, but only develop cancer after mutation or allelic loss of the other *BRCA1* allele. Thus, the complete genetic disorder is only present in the tumor cells that are targeted for somatic gene therapy. This is in contrast to inherited germline diseases, in which the genetic defect is present in all cells.

One strategy for cancer gene therapy is that of genetic correction. Because known gene defects are present exclusively in the cancer cells, genetic correction therapies provide a potential rational therapy that is molecularly targeted to cancer cells, but should not necessarily affect normal nonmalignant cells. A number of genetic correction strategies have been designed to treat cancer, including correction of *p53* and *BRCA1* [1•,2,3]. This review discusses some of the scientific information related to genetic correction strategies and then describes animal models and patient trials designed to initially test this therapeutic approach.

p53 gene therapy

p53 is the most commonly mutated tumor suppressor gene in solid tumors and is also mutated in the germline of patients with the rare hereditary Li-Fraumeni syndrome [4]. The *p53* gene is specifically relevant to the development

and progression of breast cancer, because *p53* is frequently mutated in breast cancer specimens and Li-Fraumeni syndrome patients develop breast cancer as part of their multiple cancer syndrome [4]. Thus, *p53* genetic correction is a rational approach for breast cancer, particularly in those rare patients with breast cancer as part of Li-Fraumeni syndrome. The function of wild-type *p53* is suppression of cell proliferation through a multiprotein regulatory pathway that is focused around the retinoblastoma gene and control of apoptosis [5]. Because *p53* may naturally function as an inhibitor of cell proliferation, it inhibits cell growth in most normal and malignant cells [6,7], with few exceptions [8]. For this reason it may effectively inhibit tumor growth even in cancers that do not have *p53* mutations [6,7]. Preclinical animal studies of adenovirus-based *p53* gene therapy for cancer in both cell culture and animal models [9–11] have demonstrated tumor suppression. Clinical trials of *p53* gene therapy for lung cancer [1•,12•] have been reported, and these studies demonstrate gene transfer of adenoviral-p53, induction of apoptosis, and some indication of therapeutic response. In a phase 1 clinical trial of nonsmall cell lung cancer [12•], 8% of treated patients showed a partial response and 64% of patients showed disease stabilization ranging in duration from 2 to 14 months.

BRCA1 gene therapy

Although the molecular function of *BRCA1* is controversial and may include DNA repair or transcriptional functions [13], overexpression of *BRCA1* into sporadic breast or ovarian cancer cells, which usually show low *BRCA1* expression [14], results in growth inhibition and tumor suppression [15–23]. Growth inhibition by *BRCA1* has itself been controversial, leading some to question the rationale for *BRCA1* gene therapy [24], but subsequent studies have shown growth inhibition or tumor suppression by *BRCA1 in vitro* [16,17,19–23] and *in vivo* [15,17,21,25]. The mechanism of growth inhibition by *BRCA1* is unknown and may involve interactions with *WAF1/CIP1* [16], *p53* [17], *Rb* [23] or induction of apoptosis [18,19].

Although *BRCA1* is only mutated in a small percentage of breast or ovarian cancers, the majority of sporadic breast and ovarian cancers appear to express low levels of *BRCA1* messenger RNA and protein [14,26–30]. This appears to be a consequence of loss of heterozygosity and promoter methylation of the remaining *BRCA1* allele [14,26–35]. This finding is important because it indicates that restoration of normal 'wild-type' *BRCA1* expression levels in many sporadic cancers may inhibit tumors by a 'genetic correction' strategy, wherein the loss of *BRCA1* expression contributes to tumorigenesis. These results constitute the scientific basis for testing *BRCA1* gene therapy in patients with sporadic breast, ovarian, and prostate cancers that lack specific point mutations in the *BRCA1* gene.

Several different *BRCA1* viral vectors have been constructed and tested for efficacy in preclinical xenograft models of breast and ovarian cancer. Initial studies of a *BRCA1* retroviral vector employed a complementary DNA that encoded a splice variant vector that eliminates the first 71 amino acids of the human protein, termed BRCA1sv [2,25,36•]. Studies of both growth inhibition and DNA repair do not identify cellular or molecular differences between *BRCA1sv* and *BRCA1* complementary DNAs [36•,37–40]. Intaperitoneal injection of either BRCA1sv or a full-length BRCA1 retroviral vector into ovarian cancer or breast cancer xenografts in nude mice produces tumor inhibition [2,15,25,36•,37–39]. These studies show that treatment of established SKOV3 or PA-1 ovarian cancer nude mice xenografts with either the full-length or the splice variant *BRCA1* retroviral vector results in tumor suppression. Necropsies showed that PA-1 tumor-bearing mice treated with control media or low-dose LXSN-*BRCA1sv* died with large intra-abdominal tumors and ascites, whereas mice with high-dose LXSN-*BRCA1sv* treatments died of lung metastasis with significantly smaller abdominal tumor. The PA-1 tumor model is an established xenograft model for ovarian cancer [37–39,41,42].

The published phase 1 trial of *BRCA1sv* retroviral gene therapy [2] demonstrated gene transfer and expression of the intraperitoneally injected LXSN-*BRCA1sv* vector. The vector was moderately stable in the peritoneum of these patients, and antibody formation was rare. The phase 2 trial performed in a group of patients with lower tumor burdens [36•], however, demonstrated that tumor size and immune status strongly influence patient response to retroviral vectors, and that vectors packaged in mouse cells are not sufficiently stable to treat patients with small volume intraperitoneal ovarian cancer.

Patient model systems for breast and ovarian cancer

The initial patient trials of *p53* and *BRCA1* gene therapy demonstrated the potential for gene therapy, but also clearly demonstrated both basic and clinical research that is needed before these therapies can be successful in patients. Initial human clinical trials of *BRCA1* retroviral gene therapy taught us that the approach was safe, but that healthy patients developed immune responses towards retroviral vector therapy that decreased vector stability and presumably prevented response. Based on this prior experience, we have redesigned our current human trials to employ a more immune-resistant MFG-based retroviral vector that is packaged in human producer cells. These trials should demonstrate whether this new generation of human cell-produced retroviral vector is more stable in the healthier patients with small volume disease. Because breast and ovarian cancers exhibit significant biologic and genetic similarities, gene therapies may be initially

tested in the setting of ovarian cancer, employing peritoneal injection, and then ultimately tested in a more relevant breast cancer human disease model. Both types of patient model will be described in the following discussion.

The model system of metastatic ovarian cancer growing within a confined anatomic space (often bathed in ascitic fluid) has several advantages in safety and efficacy for studies of gene transfer into solid tumors. The pathology of metastasis into peritoneal-lined spaces often consists of small tumor implants with extravasation of tumor cells into the surrounding space, potentially allowing a reservoir for delivering gene transfer vectors to malignant cells. This pattern of spread is in contrast to other solid tumors such as breast, lung or melanoma that grow as solid three-dimensional masses anatomically located in sites that are inaccessible to currently available vector systems. Ovarian cancer provides a model system in which regional therapy (intraperitoneal infusion) could be curative in a reasonable percentage of cases. We have reported [2,36•] that the uptake and expression of the vectors can be readily assessed in this model system because direct access to the peritoneal cavity is possible through an implantable peritoneal catheter.

Because present gene therapy tools preclude systemic treatment strategies, we are initiating a regional treatment protocol to test the safety and potential efficacy of a human producer cell-derived MFG-*BRCA1* gene therapy by studying the effect of *BRCA1* retroviral gene transfer into breast cancer tumors that have recurred on the chest wall. The study population will consist of women who have failed one course of standard therapy and have biopsy-proved metastatic breast cancer involving the chest wall. Nodular chest wall disease constitutes a particular pattern of metastatic or recurrent breast cancer that is frequently resistant to treatment with standard therapy. We have selected chest wall recurrence of breast cancer for this study because of accessibility of the tumor both for vector administration and for biopsy and biologic analysis. Chest wall nodules will be biopsied and then injected daily for 4 days with MFG-*BRCA1* viral vector. The tumors will then be excisionally biopsied 1–4 weeks later, so that molecular and cellular studies of gene transfer, expression, and immune response can be evaluated. Because these nodules are often multiple, we will inject paired nodules with a placebo retroviral vector MFG in order to test directly whether observed effects are gene related or merely nonspecific effects of retroviral vector injection in humans. Immunologic and molecular studies will be performed on blood samples and tissue samples to evaluate immune response and vector pharmacokinetics, as in the ovarian cancer trial.

Conclusion

Genetic correction strategies are presently being developed and tested in animal models for human malignancies and in early patient trials. The cancer susceptibility genes *p53* and *BRCA1* have been tested in lung cancer and ovarian cancer patients, respectively, and have shown some potential for this antitumor strategy. *p53* gene therapy may be effective even against tumors that lack *p53* mutations, because *p53* may function as a growth inhibitor in a variety of gene transfer settings. The observation that sporadic breast and ovarian cancers show decreased *BRCA1* expression indicates that *BRCA1* gene therapy may be effective even against sporadic breast or ovarian cancer without *BRCA1* gene mutations. Although initial approaches to human gene therapy focused on germline inherited diseases, it has become evident that somatic genetic diseases like cancer represent appropriate targets for somatic gene therapy.

The clinical application of gene correction therapy will require advances in both basic science and clinical research. Key problems at present include the degradation of vector by the immune system and a need for higher levels of gene transduction. Solutions will require the development of improved vectors, improved vector delivery systems, and the fine-tuning of human gene therapy in appropriate models of human cancer.

References

Articles of particular interest have been highlighted as:
- • of special interest
- •• of outstanding interest

1. • Roth JA, Nguyen D, Lawrence DD, *et al*: **Retroviral mediated wild-type p53 gene transfer to tumors of patients with lung cancer.** *Nature Med* 1996, **2**:985–991.

This is an important initial report of the genetic correction strategy for cancer gene therapy.

2. Tait DL, Obermiller PS, Redlin-Frazier S, *et al*: **Phase I trial of BRCA1 retroviral gene therapy for ovarian cancer.** *Clin Cancer Res* 1997, **3**:1959–1968.

3. Roth JA, Cristiano RJ: **Gene therapy for cancer: what have we done and where are we going?** *J Natl Cancer Inst* 1997, **89**:21–39.

4. Malkin D, Li FP, Strong LC, *et al*: **Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms.** *Science* 1990, **250**:1233–1238.

5. Harris CC: **The p53 tumor suppressor gene as a target for new anticancer therapies.** *Adv Oncol* 1998, **14**:3–7.

6. Leach SD, Scatena CD, Keefer CJ, *et al*: **Negative regulation of Wee1 expression and Cdc2 phosphorylation during p53-mediated growth arrest and apoptosis.** *Cancer Res* 1998, **58**:3231–3236

7. Pietenpol JA, Tokino T, Thiagalingam S, *et al*: **Sequence-specific transcriptional activation is essential for growth suppression by p53.** *Proc Natl Acad Sci USA* 1994, **91**:1998–2002.

8. Pietenpol JA, Lengauer C, Jordan J, Kinzler KW, Vogelstein B: **Mammalian cells resistant to tumor suppressor genes.** *Proc Natl Acad Sci USA* 1996, **93**:8390–8394.

9. Song KM, Cowan KH, Sinha BK: ***In vivo* studies of adenovirus-mediated p53 gene therapy for cis-platinum-resistant human ovarian tumor xenografts.** *Oncol Res* 1999, **11**:153–159.

10. Santoso JT, Tang DC, Lane SB, *et al*: **Adenovirus-based p53 gene therapy in ovarian cancer.** *Gynecol Oncol* 1995, **59**:171–178.

11. von Gruenigen VE, Santoso JT, Coleman RL, *et al*: ***In vivo* studies of adenovirus-based p53 gene therapy for ovarian cancer.** *Gynecol Oncol* 1998, **69**:197–204.

12. • Swisher SG, Roth JA, Nemunaitis J, *et al*: **Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer.** *J Natl Cancer Inst* 1999, **91**:763–771.

This is a detailed report of results of treatment of 28 patients with adenovirus-*p53* therapy for lung cancer.

13. Kinzler KW, Vogelstein B: **Gatekeepers and caretakers.** *Nature* 1997, **386**:761–763.

14. Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT: **Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression.** *Nature Genet* 1996, **9**:444–450.

15. Holt JT, Thompson ME, Szabo C, *et al*: **Growth retardation and tumor inhibition by BRCA1.** *Nature Genet* 1996, **12**:298–302.

16. Somasundaram K, Zhang H, Zeng YX, *et al*: **Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1.** *Nature* 1997, **389**:187–190.

17. Zhang H, Somasundaram K, Peng Y, *et al*: **BRCA1 physically associates with p53 and stimulates its transcriptional activity.** *Oncogene* 1998, **16**:1713–1721.

18. Shao N, Chai YL, Shyam E, Reddy P, Rao VN: **Induction of apoptosis by the tumor suppressor protein BRCA1.** *Oncogene* 1996, **13**:1–7.

19. Harkin DP, Bean JM, Miklos D, *et al*: **Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1.** *Cell* 1999, **97**:575–586.

20. Humphrey JS, Salim A, Erdos MR, *et al*: **Human BRCA1 Inhibits growth in yeast: potential use in diagnostic testing.** *Proc Natl Acad Sci USA* 1997, **94**:5820–5825.

21. Burke TF, Cocke KS, Lemke SJ, *et al*: **Identification of a BRCA1-associated kinase with potential biological relevance.** *Oncogene* 1998, **16**:1031–1041.

22. Jensen DE, Proctor M, Marquis ST, *et al*: **BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression.** *Oncogene* 1998, **16**:1097–1112.

23. Aprelikova ON, Fang BS, Meissner EG, *et al*: **BRCA1-associated growth arrest is RB-dependent.** *Proc Natl Acad Sci USA* 1999, **96**: 11866–11871.

24. Anonymous: **No stranger to controversy [editorial].** *Nature Genet* 1997, **17**:247–248.

25. Holt JT, Thompson ME, Szabo C, *et al*: **Growth retardation and tumor inhibition by BRCA1 (correction).** *Nature Genet* 1998, **19**:102.

26. Mancini DN, Rodenhiser DI, Ainsworth PJ, *et al*: **CpG methylation within the 5′ regulatory region of the BRCA1 gene is tumor specific and includes a putative CREB binding site.** *Oncogene* 1998, **9**: 1161–1169.

27. Rice JC, Massey-Brown KS, Futscher BW: **Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells.** *Oncogene* 1998, **17**: 1807–1812

28. Ozcelik H, To MD, Couture J, Bull SB, Andrulis IL: **Preferential allelic expression can lead to reduced expression of BRCA1 in sporadic breast cancers.** *Int J Cancer* 1998, **77**:1–6.

29. Jarvis EM, Kirk JA, Clarke CL: **Loss of nuclear BRCA1 expression in breast cancers is associated with a highly proliferative tumor phenotype.** *Cancer Genet Cytogenet* 1998, **101**:109–115.

30. Wilson CA, Ramos L, Villasenor MR, *et al*: **Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas.** *Nature Genet* 1999, **21**:236–240.

31. Yoshikawa K, Honda K, Inamoto T, *et al*: **Reduction of BRCA1 protein expression in Japanese sporadic breast carcinomasand its frequent loss in BRCA1-associated cases.** *Clin Cancer Res* 1999, **5**:1249–1261.

32. Seery LT, Knowlden JM, Gee JM, *et al*: **BRCA1 expression levels predict distant metastasis of sporadic breast cancers.** *Int J Cancer* 1999, **84**:258–262.

33. Rio PG, Maurizis JC, Peffault de Latour M, Bignon YJ, Bernard-Gallon DJ: **Quantification of BRCA1 protein in sporadic breast carcinoma with or without loss of heterozygosity of the BRCA1 gene.** *Int J Cancer* 1999, **80**:823–826.

34. Lee WY, Jin YT, Chang TW, Lin PW, Su IJ: **Immunolocalization of BRCA1 protein in normal breast tissue and sporadic invasive ductal carcinomas: a correlation with other biological parameters.** *Histopathology* 1999, **34**:106–112.

35. Catteau A, Harris WH, Xu CF, Solomon E: **Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics.** *Oncogene* 1999, **18**:1957–1965.

36. • Tait DL, Hatmaker AR, Redlin-Frazier S, Obermiller PS, Holt JT: **Ovarian cancer BRCA1 gene therapy: phase I & II trial differences in immune response and vector stability.** *Clin Cancer Res* 1999, **5**:1708–1714.

This study compares immune responses to retroviral vector and vector stability in patients with extensive intraperitoneal tumors versus those with small volume residual disease.

37. Tait DL, Jones H, Holt JT: **Current status of gene therapy for ovarian cancer.** *CME J Gynecol Oncol* 2000, in press.

38. Tait DL, Jensen RA, Gralow J, *et al*: **Gene therapy for breast and ovarian cancer with BRCA1.** *Breast Dis* 1998, **10**:89–98.

39. Tait DL, Obermiller PS, Jensen RA, Holt JT: **Ovarian cancer gene therapy.** *Hematol Oncol Clin North Am* 1998, **12**:539–552.

40. Abbott DW, Thompson ME, Robinson-Benion C, Jensen RA, Holt JT: **BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells enhancement of transcription coupled DNA repair.** *J Biol Chem* 1999, **274**:18808–18812.

41. Manetta A, Emma D, Gamboa G, *et al*: **Failure to enhance the in vivo killing of human ovarian carcinoma by sequentialtreatment with dequalinium chloride and tumor necrosis factor.** *Gynecol Oncol* 1993, **50**:38–44.

42. Yaginuma Y, Westphal H: **Abnormal structure and expression of the p53 gene in human ovarian carcinoma cell lines.** *Cancer Res* 1992, **52**:4196–4199.

Authors' affiliations: Jeffrey T Holt and Patrice S Obermiller (Vanderbilt University Cancer Center, Nashville, Tennessee, USA) and David L Tait (Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, East Carolina University School of Medicine, Greenville, North Carolina, USA)

Correspondence: Jeffrey T Holt, Professor of Cell Biology and Pathology, Vanderbilt University Cancer Center, 2220 Pierce Avenue South MRB II 659, Nashville, TN 37232-6838, USA.
Tel: +1 615 936 3114; fax: +1 615 936 1790;
e-mail: jeff.holt@mcmail.vanderbilt.edu