P53 gene therapy in the treatment of human cancer

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Gene therapy is the re-introduction of the expression of certain defective genes that cause human diseases. Gene therapy has been developed to treat a variety of genetic diseases since the 1990s, and this technique has also been applied in the treatment of human cancer. Germ line gene therapy refers to inserting target genes into gametes, while somatic gene therapy inserts target genes into somatic cells of patients. The insertion of gene into cells require a “vector” system, which is usually a viral particle that helps to deliver designated genes into target cells. Retroviruses, adenoviruses, adeno-associated viruses have been used as a vector to deliver gene therapy. Retroviruses integrate into host cell genome and has been used to treat X-linked severe combined immunodeficiency disease (SCID). It has shown some initial success but further development was limited by the risk of insertional mutagenesis causing malignancy such as leukemia. Adenovirus does not have this problem since it does not insert its gene into the host chromosome but its use was limited after one treatment-related death in 1999. Moreover, the inserted gene introduced by adenoviruses does not replicate during cell division and hence repeated administration is required. Adeno-associated virus represents a promising viral vector

system in delivering gene therapy as it is a small virus that does not trigger a strong immunological reaction and also does not integrate into the host genome. Other viral systems such as lentivirus and envelop protein pseudotyping viral vectors have also been used. Non-viral methods for gene therapy include electroporation of naked DNA, “gene guns”, oligonucleotides, lipoplexes, polyplexes, hybrid methods, as well as dendrimers. Despite some early success of gene therapy, further development of this technique will first need to overcome a few limitations such as the short-lived nature of its efficacy, immune response and immune clearance of viruses, development of better vector systems, as well as ethical concerns.

Tumor suppressor genes (TSGs) encode proteins that play an important role in regulating cell cycle and cell growth, as well as inducing cell apoptosis (programmed cell death). Down-regulation or malfunction of these genes due to chromosomal loss, epigenetic silencing, or gene mutation are major causes of the development of human cancer. Some well-known TSGs are TP53, PTEN and the retinoblastoma (Rb) gene, although there are many more TSGs within the human genome and many are waiting to be discovered. The TP53 gene was discovered in 1979 and cloned in 1984. It is located at the short arm of chromosome 17 and encodes a 53 KDa protein with 393 amino acids and seven domains. There are two major polymorphisms of the P53

protein in humans which is due to SNP on codon 72. P53 is one of the most well-known TSGs which has multiple anti-cancer functions including regulating cell apoptosis, inducing genetic stability, as well as inhibition of angiogenesis. It is an important regulatory protein involved in multiple major molecular pathways that control cell growth and oncogenesis. Germ line mutation in the TP53 gene is associated with the development of a cancer syndrome (Li-Fraumeni disease). Mutation of P53 is very common in many different types of cancer, such as lung cancer and head and neck cancers. P53 mutation in cancer often correlates with poorer response to treatment as well as less favorable clinical outcome. Intact P53 protein function is essential in maintaining genomic stability and preventing mutability.

Gene therapy with P53 entered pre-clinical studies in the 1990s with encouraging pre-clinical data. In animal model, introduction of the wild type P53 gene into cancer cells with P53 mutation resulted in expression of the transduced gene and its encoded protein, and tumor regression was observed. Better response to chemotherapy treatment was also noted after P53 gene insertion (Nguyen et al. J Thor Cardiovasc Surg 1996;112:1372-1377, Fujiwara et al. Cancer Research 1994; 54: 2287-2229). Early trial of P53 gene therapy in treating human cancer employed a

retroviral system carrying the wild type P53 gene which was directly injected to patients' tumors. There was no major treatment-related toxicity and about 30% of patients achieved tumor response (Roth et al. Nat Med 1996;2:985-991). This formed a strong basis for further clinical studies in administering P53 gene therapy in different types of human cancers.

Clinical trials using adenovirus-mediated (serotype 5 replication-defective) wild type P53 gene therapy was initiated nearly a decade ago. The wild type gene was carried by vector containing CMV promotor and polyadenylation signal. In Nemunatis et al.'s study (Journal of Clinical Oncology 2000;18:609-622) , patients with advanced lung cancer and tumor P53 dysfunction demonstrated by immunohistochemistry of gene sequencing were recruited. Patients received cisplatin chemotherapy as well as direct injection of adeno-P53 into target tumor lesions every four weeks for six cycles. Their results showed that P53 mRNA was detected by vector P53-specific RT-PCR in tumor cells after vector injection in 43% of patients. One-third of patients developed transient fever and treatment was generally well-tolerated. Nearly 10% of patients achieved significant tumor response as assessed by CT scan, while 70% of patients' tumors remained stable. 70% of patients who received injection of adeno-P53 into tumors endobronchially achieved significant relief in tumor obstruction, at least

transiently. When assessed by TUNEL apoptosis assay, 80% of patients had an increase in apoptotic cells within their tumors. In another study (Swisher et al. Clin Cancer Res 2003; 9:93-101), patients with localized lung cancer were given concomitant radiotherapy with intralesional adenovirus-P53 therapy. Mild fever and chills were noted in majority of patients. More than 60% of patients achieved tumor response and genes downstream of P53 were noted to be expressed in tumor cells after treatment.

In preclinical models, adeno-P53 transfection into EBV-positive nasopharyngeal cancer (NPC) cell lines led to cytotoxicity and cell apoptosis and significant increase in P53 protein (Li et al. Cancer research 2002;62:171-178). In a randomized controlled trial (Pan et al. Journal of Clinical Oncology 2009;27:799-804), patients with NPC were randomized to receive radiotherapy alone or radiotherapy combined with weekly intralesional injection of recombinant adenovirus-P53 (Gendicine). Complete response rate, patients' survival were both found to be statistically superior in the group with combination therapy. RT-PCR and immunohistochemistry showed upregulation of P53-regulated target genes as well as down-regulation of VEGF in tumor cells after treatment. Treatment was well-tolerated except transient mild fever. Adenovirus-P53 has also been used in treating other types of locally advanced head

and neck cancers by intralesional injection (Clayman et al. Journal of Clinical Oncology 1998;16:2221-2232). P53 protein expression was detected in tumor samples after treatment and close to half of all patients achieved disease response or disease control. Interestingly, successful adenovirus-P53 gene transduction was also noted in bladder cancer when treatment was administered via intravesical route (Kuball et al. Journal of Clinical Oncology 2002;20: 957-965).

In conclusion, adenovirus-P53 gene therapy has shown promising data in early clinical trials in treating different types of human cancer, with successful gene transduction into tumor cell leading to P53 mRNA and protein expression, up-regulation of P53-downstream genes, as well as tumor shrinkage in a proportion of patients. Treatment is well tolerated generally with only mild, transient immune-mediated toxicity. Further larger scale clinical studies in P53 gene therapy as a modality of cancer treatment are warranted.