HEAD AND NECK CANCER: RESPONSE TO p53-BASED THERAPEUTICS

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Abstract: Limited options are available for patients with advanced stage head and neck cancer. The p53 gene is known as the "guardian of the genome." Mutations of the p53 gene predispose to carcinogenesis. The p53 mutations are common in head and neck cancer. Replacement of p53 gene function in preclinical models demonstrates cancer regression and improved survival. Clinical data with an adenoviral based p53 gene delivery product (Advexin) supports safety and clinical response after direct intratumoral injection. We summarize p53-related therapeutics in this review. © 2010 Wiley Periodicals, Inc. *Head Neck* **33:** 131–134, 2011

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Is the door opening for gene therapy utilization in squamous cell cancer of the head and neck region? Treatment options for recurrent head and neck squamous cell carcinoma (HNSCC) are limited. Morbidity and mortality are largely related to local recurrence. Treatment of early-stage head and neck cancer involves surgery and/or radiation therapy. The choice of therapy often relates to concerns over functional outcome rather than efficacy because both forms of local treatment result in 75% to 90% 5-year survival rates in patients with stage 1 and stage 2 disease.[1] Unfortunately, >60% of patients with HNSCC present with locally advanced disease.[2] Patients with advanced-stage disease typically require a 'combined modality' approach utilizing radiation, chemotherapy, and/or surgery. Although functional, the outcome (and therefore the choice of modality) varies by site, survival outcomes are poor, and treatment uniformly morbid. A total of 35% to 55% of patients with advanced-stage head and neck cancer remain disease-free 3 years after standard treatment. However, locoregional recurrence develops in 30% to 40% of patients and distant metastases occur in 20% to 30% of patients.[3] Local regional recurrence is again usually treated with a combination of surgery, radiation, and/or chemotherapy, and metastatic disease is treated with chemotherapy (generally platinum- or taxane-based regimens). Studies that use reirradiation combined with chemotherapy demonstrate 2-year survival rates ranging from 5% to 45%.[4] Therefore, recurrent head and neck cancer and resistance to conventional therapy are frequent clinical problems. Due to these dismal results, a better understanding of molecular biology has led to the development of gene therapy for refractory or recurrent head and neck cancer. Therapies that target the p53 gene using an adenovirus as a vehicle to transport the gene into target cells are of particular interest.

Viral vector–mediated gene transfer has been used as part of a new experimental strategy to treat HNSCC with advanced disease and locoregional occurrence. This method replaces a portion of the virus' genes with the desired genetic sequence. The virus is then injected into the tumor and allowed to infect different cell types, thereby spreading the desired genetic sequence among the tumor cells. This is an attractive treatment because HNSCC tumors are often accessible for direct injection of gene therapy. Unlike retroviral vectors, which are composed of RNA instead of DNA, adenoviral DNA is not integrated into the host genome and thus does not effect germ lineage transmission. Only cells that are infected by the virus will express the desired genetic sequence. It has been known for many years that modified adenoviruses can be used to deliver gene therapy to head and neck cancer cells.[5] Also, it has been observed that HNSCCs are among the many human cancers that frequently exhibit inactivation of the tumor suppressor gene p53.[6–11] This inactivation can occur by several cellular mechanisms: (1) mutation of the p53 gene[12]; (2) over-expression of the primary negative regulator of p53[13]; (3) inactivation of the inhibitor of the negative regulator[14,15]; and (4) interference with p53 posttranslational modifications which may be necessary for the gene to function (eg, phosphorylation).[16]

Adenoviruses provide higher gene transfer efficiencies because they infect and transduce both dividing and nondividing cells. Work with tumor-specific replicating adenoviruses, which have been engineered

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to have enhanced replication capacity in p53 dysfunctional cells (eg, ONYX-015) and do not involve gene delivery, have shown promise as local regional therapeutic modalities in HNSCC and have been tested for safety as a systemic oncolytic agent.[17–19] ONYX-015, in particular, is approved for management of early-stage HNSCC by the Chinese regulatory system in China.

The p53 gene is crucial to the regulation of the cell cycle and control of apoptosis (cell death).[20–22] The general purpose of p53 seems to be maintenance of the genetic integrity of the cell and induction of apoptosis when DNA damage is too great to guarantee a normal progeny cell. It directly or indirectly affects: (1) cell cycle regulation; (2) cellular apoptosis; and (3) DNA repair.

Abnormalities in p53 cell cycle regulation and apoptotic pathways are among the most common and fundamental molecular mechanisms of cancer pathogenesis and treatment resistance.[23,24] The p53 pathway is disrupted in >50% of human tumors. Overexpression of the p53 protein is associated with poor patient outcome and poor tumor response to therapy. Multiple studies have found that p53 is mutated in 40% or more of HNSCC.[25–27] These observations formed the rationale for developing p53 cancer therapy.[28–31]

Ad-p53 is a replication defective adenoviral vector ad5CMV-p53 which contains the cytomegalovirus (CMV) promoter, wild-type human p53 cDNA, and an SV40 polyadenylation signal in a minigene cassette inserted into the E1-deleted region of modified adenovirus-5 (Figure 1).[32,33] Ad-p53 is an adenoviral vector that contains a functional p53 gene. This treatment, in combination with radiation and/or chemotherapy, results in dramatic apoptosis in p53-deficient cancer cell lines.[34,35] Extensive animal studies reveal significant dose-related efficacy and safety after local intratumoral injection with Ad-p53 in HNSCC models.

Phase I trials have generally shown that repeated intratumoral injections of Ad-p53 vector are well tolerated, result in expression of normal p53 protein, and are associated with antitumor activity. Generally, side effects were mild and involved local inflammatory responses and transient fever with occasional mild flu-like symptoms.

Patients with recurrent or refractory HNSCC who had either surgically resectable lesions or unresectable lesions were studied as separate entities. Results concluded that repeated intratumoral injections of up to 10^{11} plaque forming units (PFU) were safe and well tolerated. Expression of the p53 protein was again identifiable despite evidence of the body's immune response to the injected virus. Perioperative and postoperative Ad-p53 injection had no reported negative effects on surgical morbidity and/or wound healing. Evidence of activity based on tumor regression after injection of Ad-p53 was observed. In the unresectable patient group, 2 of 17 patients achieved a partial response to Ad-p53 intratumoral injection, whereas 6 others achieved stable disease for 1 to 3.5 months, and 9 had progressive disease.[30] Patients ($n = 15$) with recurrent disease were in the surgical resection group and thus underwent surgery. Of these 15 patients who were injected with Ad-p53, 4 (27%) remain free of disease with a median follow-up time of 18 months, which is a greater than expected survival in recurrent resectable HNSCC disease in patients. Results of phase I trial justified further phase II investigation in HNSCC.

Several phase II trials of Ad-p53 (including 173 patients) have been conducted in unresectable recurrent HNSCC.[36] Different schedules of viral administration were tested. The studies were designed to assess for activity and side effects. In study T201, doses ranged per injection from 5×10^{10} viral particles (vp) to 2.5×10^{12} vp; median dose was 1.2×10^{11} vp per injection. Two cohorts of patients were treated, with 1 receiving injection on days 1, 2, and 3 of a 28-day cycle and another receiving injection on days 1, 3, 5, 8, 10, and 12 of a 28-day cycle. A maximum of 12 cycles were administered. Out of 106 evaluable patients treated, 6 had objective response (complete response or partial response). This was not different between patients treated with high-dose and low-dose regimens. If minor responses (≥25% decrease in bi-dimensional measurements) are analyzed, 28.6% of high-dose patients achieved a minor response or better compared with 13.4% of lower-dose patients. Some responses were extremely long lasting (≥1.4 years) and correlation with improvement in performance status was demonstrated. Evidence was presented suggesting a possible synergy between Ad-p53 and recent exposure to chemotherapy. Another phase II study included a similar patient population, but evaluated a dosage that was 50 times less than the T201 study. This study had 58 evaluable patients. In the high-dose group, 20% of patients demonstrated a durable (>3 month) stable disease, compared with 14% of patients in the low-dose group that demonstrated a durable stable disease. Similarly, a median survival of 6.0 months versus 3.5 months and mortality rate of 150 days of 60% versus 40% for the high- and low-dose populations were observed. The results indicate a dose-dependent effect. Both studies conclude that treatment with Ad-p53 is safe and demonstrates evidence of durable activity in patients with HNSCC.

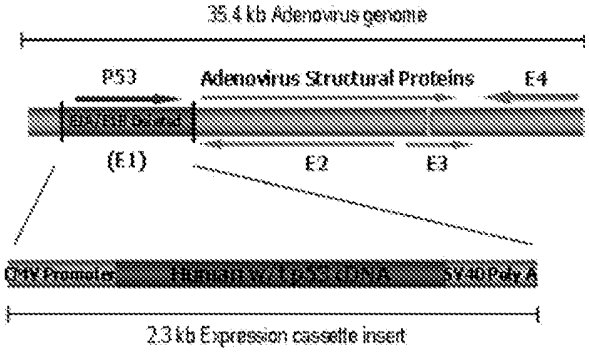

FIGURE 1. Key components of Advexin demonstrating the adenovirus genome structure and position of the transgene wtp53.

Interestingly, responses occurred regardless of the endogenous p53 status of the patient, indicating that patients with wild-type p53 can respond to Ad-p53 therapy.

There are a number of mechanisms through which antitumor activity of Ad-p53 might occur. The first is through the increased amount of p53 protein found after Ad-p53 transduction that alters the ratio of p53 protein to inhibitors of its function. Immune responses generated after vector administration may also contribute to effects in tumors of either genotype.[37–40] These encouraging phase I and phase II trial results supported the development of 2 phase III trials, 1 of which has recently been completed comparing Ad-p53 to methotrexate in advanced recurrent HNSCC. Researchers in this trial analyzed the results for their correlation with p53 biomarkers.[36] They found that the vast majority of responders to Ad-p53 therapy had wild-type p53 in which p53 was inactivated by upregulation of the p53 inhibitors mdm-2 or mdm-4 or had low expression of mutated p53. Patients with this favorable p53 profile were associated with a significant increase in survival compared with patients with an unfavorable p53 profile (7.2 months vs 2.7 months, $p < .0001$). In contrast, the vast majority of methotrexate responders (87%) had high expression of mutated p53. Overall, without accounting for sensitivity based on molecular signaling of p53, there was no significant difference between patients treated with Ad-p53 and methotrexate. Based on these preliminary results, the phase III trial supported the data from phase I and II trials and demonstrated a lower toxicity profile to Ad-p53 therapy as compared to methotrexate.

Potential application of Ad-p53 as a local regional therapeutic in the management of HNSCC awaits further analysis of the phase III trial. Utilization will involve patients with recurrent HNSCC who are unresectable and require palliative management. Application may also broaden as further trials are performed to adjust therapy applied locally at the time of surgery in high recurrence risk patients. Local regional application of Ad-p53 may also involve combination with radiation therapy.

Technology involving genomics and proteomics (an individual's unique protein expression) has improved our ability to detect molecular targets in individual tumor specimens.[41–44] Through this type of developing technology, it should be possible to engineer "designer" drugs expressing genes specifically targeted to a particular individual's unique tumor genomic and proteomic properties. Results of Ad-p53 may lead toward an expansion of such a strategy.

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