

1.0 OBJECTIVES

- 1.1 To determine the biological effects at the molecular level of intratumoral administration of Ad-p53 by comparing human brain tumor specimens treated with replication-deficient adenovirus vector containing wild-type p53 cDNA gene (Ad-p53) for the expression and distribution of exogenous p53 protein, the occurrence of apoptosis, and the induction of an immune response.
- 1.2 To determine the maximum tolerated dose of replication-deficient adenovirus vector containing wild-type p53 cDNA gene (Ad-p53) administered by intratumoral injection in patients with recurrent malignant gliomas.
- 1.3 To determine the qualitative and quantitative toxicity of Ad-p53 administered by intratumoral injection.

2.0 BACKGROUND AND RATIONALE

2.1 Malignant glioma survival and therapy

Malignant glioma (anaplastic astrocytoma, glioblastoma multiforme, anaplastic oligodendroglioma) is the most common primary brain tumor and is a major cause of morbidity and mortality. A recent survey estimated an annual incidence of 17,000 primary intracranial neoplasms or 8.2 per 100,000 population, more than half of which are malignant gliomas (Walker et al., 1995). This incidence appears to be increasing, particularly in older age groups (Mao et al., 1991). The case:fatality ratio is high with more than 10,000 Americans dying from these tumors each year. Surgical resection, conventional radiation therapy, and conventional chemotherapy result in a median survival of 51 weeks, with a 24 month survival rate of only 15% (Walker et al., 1980). These dismal results necessitate new and aggressive clinical approaches.

Because cancer is the result of the acquisition and accumulation of mutations in genes that regulate cellular growth and differentiation (i.e. proto-oncogenes and tumor suppressor genes), therapeutic strategies based on reversing or altering these molecular events represent a novel clinical approach that may impact on the survival of patients with gliomas. One option is the replacement of tumor suppressor genes that are frequently deleted or mutated in gliomas and that subserve critical functions related to cell division and cell death. The p53 gene meets both these criteria.

2.2 The p53 Tumor Suppressor Gene in Gliomas

The p53 gene, located on chromosome 17p, is the most frequently mutated gene in human cancers. James et al. (1988) showed that 17p was lost in up to 70% of

malignant gliomas using RFLP analysis. Nigro et al. (1989) showed that 80% of brain tumors tested harbored p53 mutations. Frankel et al. (1992) analyzed 40 glioblastomas and anaplastic astrocytomas and found p53 mutations in 40%. These mutations tended to be coupled with 17p deletions, resulting in complete inactivation of both p53 alleles, as would be expected for a tumor suppressor gene involved in malignant transformation. Von Deimling et al. (1992) demonstrated that mutations in the p53 gene occurred in each stage of astrocytoma progression, suggesting that alteration of p53 may be an early event initiating astrocytoma formation. P53 protein aberrations may also occur in the absence of p53 gene mutations via post translational protein inactivation by viral proteins (e.g., HBV, E1A) or proto-oncogenes (e.g. mdm-2) (Lang et al., 1994). Taken together these data demonstrate that alterations in p53 aberrations are frequent in human gliomas and are probably important in their formation and progression.

2.3 Function of p53

The p53 gene encodes a 393-amino-acid phosphoprotein that mediates cell growth. The wild-type p53 gene product functions as a transcription factor that induces either cell cycle arrest or apoptosis, depending upon the cell type tested. P53-dependent G1 arrest is mediated through p21 inhibition of cyclin-dependent kinases, whereas p53-dependent apoptosis results from an independent signal transduction pathway probably mediated through bax. The potential to induce apoptosis makes p53 particularly attractive as a therapeutic agent in cancer (Velculescu and El-Deiry, 1996).

Transfection of plasmids containing wild-type p53 gene has been shown to induce apoptosis in myeloid leukemic cell lines (Yonish-Rouach, et al., 1991), human colon cell lines (Shaw, et al., 1992), and in a Burkitt lymphoma cell line (Ramqvist, et al., 1993) Mercer et al. (1990) demonstrated in human glioma cell lines that introduction and expression of exogenous wild-type p53 gene suppresses the malignant glioma phenotype.

2.4 Adenovirus mediated p53 gene transfer against other cancers

Replication-deficient adenovirus has been used as a vector for transferring wild-type p53 gene into human cancer cells (Zhang, et al., 1994). Adenovirus mediated p53 gene transfer uses a replication deficient type 5 adenovirus in which the E1 region is replaced with a CMV promoter and wild-type cDNA of the p53 gene. In vitro and in vivo laboratory studies have demonstrated that adenovirus mediated p53 gene delivery suppresses growth in human cancer cell lines in which p53 gene mutations are common, including lung (Nguyen et al., 1996), colon (Spitz et al., 1996), head and neck (Clayman et al., 1995), cervical (Hamada et al., 1996), and prostate (Eastman et al., 1995) cancers.