

## Telomerase-Specific Replication-Selective Virotherapy for Human Cancer

Takeshi Kawashima,<sup>1</sup> Shunsuke Kagawa,<sup>1,2</sup>  
Naoya Kobayashi,<sup>1</sup> Yoshiko Shirakiya,<sup>1</sup>  
Tatsuo Umeoka,<sup>1</sup> Fuminori Teraishi,<sup>1</sup>  
Masaki Taki,<sup>1</sup> Satoru Kyo,<sup>3</sup> Noriaki Tanaka,<sup>1</sup> and  
Toshiyoshi Fujiwara<sup>1,2</sup>

<sup>1</sup>Division of Surgical Oncology, Department of Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan; <sup>2</sup>Center for Gene and Cell Therapy, Okayama University Hospital, Okayama, Japan; and <sup>3</sup>Department of Obstetrics and Gynecology, Kanazawa University School of Medicine, Kanazawa, Japan

### ABSTRACT

**Purpose:** Replication-selective tumor-specific viruses present a novel approach for treating neoplastic disease. These vectors are designed to induce virus-mediated lysis of tumor cells after selective viral propagation within the tumor. Telomerase activation is considered to be a critical step in carcinogenesis, and its activity is closely correlated with human telomerase reverse transcriptase (hTERT) expression. We investigated the antitumor effect of the hTERT-specific replication-competent adenovirus on human cancer cells.

**Experimental Design:** We constructed an adenovirus 5 vector [tumor- or telomerase-specific replication-competent adenovirus (TRAD)], in which the hTERT promoter element drives expression of *E1A* and *E1B* genes linked with an internal ribosome entry site, and we examined the selective replication and antitumor effect in human cancer cells *in vitro* and *in vivo*.

**Results:** TRAD induced selective *E1A* and *E1B* expression in human cancer cells, but not in normal cells such as human fibroblasts. TRAD replicated efficiently and induced marked cell killing in a panel of human cancer cell lines, whereas replication as well as cytotoxicity was highly attenuated in normal human fibroblasts lacking telomerase activity. In *nu/nu* mice carrying s.c. human lung tumor xenografts, intratumoral injection of TRAD resulted in a

significant inhibition of tumor growth. No evidence of TRAD was identified in tissues outside of the tumors, despite the presence of TRAD in the circulation. Moreover, TRAD replication in the distant, noninjected tumors was demonstrated.

**Conclusions:** Our results suggest that the hTERT promoter confers competence for selective replication of TRAD in human cancer cells, an outcome that has important implications for the treatment of human cancers.