P27KIP1: PROGNOSTIC VALUE AND NEW TARGET FOR THERAPEUTIC INTERVENTION IN EWING'S SARCOMA FAMILY TUMORS

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Introduction
In over 85% of Ewing's sarcoma family tumors (ETs), including peripheral neuroectodermal tumor (PNET), a specific translocation t(11;22) is found, which causes generation of EWS-Fli1 chimeric gene. This chimeric gene product, the EWS-Fli1 protein, functions as an aberrant transcription factor which might be involved in oncogenesis of ETs. Previously, we have reported that the antisense oligonucleotides against EWS-Fli1 inhibited the Ewing's sarcoma cell proliferation and caused G0/G1 arrest in the cell cycle, we also recently showed that down-regulation of EWS-Fli1 resulted in up-regulation of p27kip1 (p27) in both mRNA and protein levels. p27 is a negative regulator of G1 phase progression. Clinically, the inverse relationship between p27 expression and aggressive tumor progression or a poor prognosis has been reported in a variety of human malignancies. In vitro and in vivo experiments, over-expression of p27 using replication-deficient adenovirus resulted in apoptosis in human breast and lung cancer cell lines, or diminished malignant potential in brain tumor cells. In this study, we examined whether staining for p27 would reliably predict the prognosis of patients with ETs and investigated the effects of ectopic over-expression of p27 using a replication-deficient recombinant adenovirus in several human ETs cells.

Materials and methods
Immunohistochemical detection of p27: Surgical specimens from 17 untreated patients with ETs were routinely fixed in formalin and embedded in paraffin. Immunostaining was performed by the standard biotin-streptavidin immunoperoxidase. The p27 labeling Index (%) was defined as the percentage of tumor cells displaying nuclear immunoreactivity and was calculated by counting p27 positive tumor cells in 500 tumor cells. p27 expression was graded as negative : less than or equal to 10%, and positive : greater than 10% of tumor cells stained. The survival of patients was estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated by the generalized Wilcoxon test. PNET cell line SK-N-MC was infected with two replication-deficient adenoviral vectors; one expresses human p27 (Ax-p27), and the other LacZ gene (Ax-LacZ) at m.o.i. 10. The number of viable cells was counted every 24 h for 7 days by hemocytometer and cell lysate were prepared and subjected to Western blot analysis.

Result
Immunohistochemically, patients with p27 negative tumors survived a significantly shorter time than those with p27 positive tumors (Five-year event free survival, 14.1 % and 80.0%, respectively, P<0.01) (Fig.1). Over-expression of p27 was detected in SK-N-MC cells infected with Ax-p27 even 4 days after infection. However, expression of p27 protein in cells infected with Ax-LacZ was not induced. Growth curve indicated that the culture of SK-N-MC treated with Ax-p27 displayed marked growth inhibition when compared with untreated and Ax-LacZ-treated cells (Fig.2).

Discussion
p27 inhibits cyclin D-CDK4, cyclin E-CDK2 and cyclin A-CDK2 complexes, and regulates progression of the cell cycle from G1 to S phase. Loss of p27 expression as assessed by immunohistochemistry is prognostic indicator for various malignant tumors. In this study, we present the first evidence of the prognostic value of p27 in ETs. The patients with p27 negative tumors would survive very short time. So patients with p27 negative ETs should receive intensive therapy. We were motivated by the assumption that EWS-Fli1 may cause down regulation of p27 by unknown mechanism, whereby mitotically quiescent cells can be recruited into proliferative populations, and the decrease in p27 may lead to oncogenic transformation. If this hypothesis can be sustained, transduction of p27 gene to ETs would be very reasonable as gene therapy. As expected, significant growth suppression of SK-N-MC was observed by the forced expression of p27. In conclusion, our data suggested that p27 gene transfer may be a novel and promising strategy for ETs therapy.

Fig.1 Five-year event free survival of patients according to the expression of p27 (+P<0.01).

Fig.2 Effects of infection with Ax-p27 or Ax-LacZ on cell growth. Untreated cells (open square), cells infected with Ax-p27 (open circle) and cells infected with Ax-LacZ (open triangle) are shown.