RADIATION RESEARCH 152, 493–498 (1999)
0033-7587/99 $5.00
© 1999 by Radiation Research Society.
All rights of reproduction in any form reserved.

Sensitizing Human Cervical Cancer Cells *In Vitro* to Ionizing Radiation with Interferon β or γ

L. Grüninger, E. Cottin, Y. X. Li, A. Noël, M. Ozsahin and P. A. Coucke[1]

Laboratory of Radiobiology, Department of Radiation Oncology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland

Grüninger, L., Cottin, E., Li, Y. X., Noël, A., Ozsahin, M. and Coucke, P. A. Sensitizing Human Cervical Cancer Cells *In Vitro* to Ionizing Radiation with Interferon β or γ. *Radiat. Res.* 152, 493–498 (1999).

Human cervical cancer is often associated with human papilloma virus (HPV). HPV products, such as the oncoproteins E6 and E7, are known to disrupt the function of TP53 (formerly known as p53). The protein encoded by the *TP53* gene plays a central role in managing cellular damage. Interferons are known to down-regulate E6/E7 and may therefore restore TP53 function and influence radiation sensitivity. We investigated whether IFNB or IFNG, at various concentrations (2–300 IU/ml) and for a range of durations of exposure (from 48 h before to 8 h after irradiation), were able to modify the radiation response of HeLa, C4-1, Me-180, C33-A and SiHa cells. In parallel to the clonogenic assays, we analyzed the effect on the mRNA that encodes IFNB and E6 by Northern blotting in the same experimental conditions. A significant change in the initial slope of the dose–response curve was observed more consistently with IFNB than with IFNG. No changes in the mRNA or protein level of TP53 and E6 could be detected. Thus other mechanisms of action need to be investigated to explain radiosensitization with recombinant IFNB in cells of human cervical cancer cell lines. © 1999 by Radiation Research Society

INTRODUCTION

Squamous cell carcinoma of the cervix is characterized by the presence of high-risk human papilloma virus integrated in its cellular DNA in 90% of the cases (*1*). The oncoproteins E6 and E7, encoded respectively by HPV 16 and 18, are known to inhibit TP53 (formerly known as p53) and RB (*2–4*). These proteins are of paramount importance in cell cycle regulation and cell proliferation. The tumor suppressor gene *TP53*, the guardian of the genome, encodes a protein that functions as a brake on the cell cycle (*5–9*). E6 can facilitate degradation of TP53, resulting in genomic instability and accumulation of further genetic alteration (*10*). Cells lacking TP53 function are usually considered to be more resistant to chemo- and/or radiotherapy (*11*). Hence a possible therapeutic approach is restoring TP53 function by down-regulation of the expression of the E6 oncoprotein (*12*). Recently, the effect of IFN on viral transcription has been demonstrated in the SiHa cell line, where treatment resulted in an inhibition of colony-forming ability, especially with IFNB and IFNG (*13*). Therefore, we investigated the effect of type I (IFNB) and type II (IFNG) on the radiosensitivity of cells of five different cervical cancer cell lines *in vitro*. The effect of IFN on viral transcription in SiHa cells was shown, and it was conjectured that this might be related to reduced proliferation, but no causal connection was demonstrated (*13*).

[1] Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Cell Cultures

The cells of the human cervical cancer cell lines (HeLa, Me-180, C33-A, SiHa and Caski) were purchased from ATCC (Rockville, MD) and grown in medium as described in the ATCC Catalogue. The C4-1 cells were kindly provided by Peter Beard of ISREC, Institut Suisse pour la Recherche contre le Cancer (Epalinges, Switzerland). The medium were supplemented with 10% fetal calf serum (FCS, Sigma) (Seromed, Fakola AG Switzerland).

The cells were maintained in 25-cm^2 flasks (Corning, Corning, NY) containing 10 ml medium and passaged twice a week. All cells were tested for the presence of mycoplasma with the Myco Test Assay system (Gibco BRL) and were consistently found to be free of contamination.

Interferons

IFNB was kindly supplied by ARES-Serono, Geneva, Switzerland. IFNG was purchased from Boeringher, Ingelheim am Rhein, Germany. Stock solutions of IFN were prepared in 55% phosphate-buffered saline, 25% BSA, and 10% and 20% 12.5% sucrose. IFNG was kept at 4°C and IFNB was kept frozen at −80°C. These IFNs were used at concentrations allowing at least 80% plating efficiency compared to untreated control cells. Interferon was added 48 h before, during or 8 h after irradiation to determine a possible time-dependent modulation of E6 and hence restoration of TP53.

Irradiation

Prior to irradiation, the cells were trypsinized and replated at a density of 2×10^5 cells per flask. After 24 h incubation at 37°C in 5% CO_2 and at a relative humidity of >95%, the cells were exposed to IFNB or IFNG. Untreated flasks were maintained as controls. The cells were incubated with concentrations ranging from 2 to 300 IU/ml for 48 h. The medium was changed every 24 h, and freshly prepared IFN was added at the appropriate concentration. The medium for the control cells was also changed, but IFN was not added.