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Comparative study of DNA damage and repair in head and neck cancer after radiation treatment

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Abstract

We compared DNA damage and the efficacy of its repair after genotoxic treatment with γ-radiation of lymphocytes and tissue cells isolated from patients with squamous cell carcinoma of head and neck (HNSCC) and healthy donors. Thirty-seven subjects with HNSCC and 35 healthy donors were enrolled in the study. The extent of DNA damage including oxidative lesions and efficiency of the repair were examined by alkaline comet assay. HNSCC cancer cells were more sensitive to genotoxic treatment and displayed impaired DNA repair. In particular, lesions caused by γ-radiation were repaired less effectively in metastasis of HNSCC than in healthy controls. The differences in radiation sensitivity of cancer and control cells suggested that DNA repair might be critical for HNSCC treatment. We conclude that γ-radiation might be considered as an effective therapeutic strategy for head and neck cancers, including patients in advanced stage of the disease with clear evidence of metastasis.

Keywords: Head and neck cancer; Cancer treatment; Radiotherapy; Oxidative damage; DNA repair; Comet assay

1. Introduction

Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. The major causative agents include smoking and alcohol consumption. Overall the survival rate has improved, but remains low, especially in developing countries (Koskinen, 2006). Cancers localized in this area are especially stressful because they cause serious physical ailments. They can impede or interefere with breathing, feeding and speech.

The choice of treatment depends on the exact localization and clinical stage of neoplasm. Routine treatments are surgery and radiotherapy either alone or in combination. In early-stage disease (T1-2, N0-1), the use of both these methods leads to health restoration in 60–90% of cases. However, in advanced stages the results are worse. The percentage of local adversity exceeds 60% and rises in conjunction with increase in the stage and the risk of distant metastasis (Lawrance et al., 2003). The standard practice is to offer postoperative adjuvant radiation therapy to patients with a high risk of recurrence of HNSCC and death, the hope being that the overall survival will improve (Lundahl et al., 1998).

Radiotherapy is usually chosen on its own in very early stages of HNSCC. It protects organs, unlike surgery, which seems to be the distinct advantage of this method. On the other hand, this type of cancer therapy of head and neck almost inevitably causes pain in part of the upper respiratory and digestive tracts, making swallowing difficult. The decision to use a radiotherapy treatment is made after histopathological

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examination of tumor biopsies (Pszon et al., 2005). In routine radiotherapy, the conventional dose is 1.8–2.0 Gy a day for 5 days, with a weekly total average of 66–72 Gy (Lawrance et al., 2003).

Tumor and healthy tissue responses to genotoxic treatment depend on differences in their DNA repair capacity. In order to estimate DNA repair efficiency after cell treatment with gamma radiation, we compared peripheral blood lymphocytes from patients with HNSCC, cells isolated from tissue biopsies of patients with HNSCC, and cells from tissue biopsies of HNSCC metastases. Lymphocytes from healthy donors and cells from healthy tissue biopsies were used as controls. HTB-43 larynx cancer cells were employed as a control for squamous carcinoma. Treatment beam energies were Cobalt 60 in 28 patients, with the doses of 5, 15, 25, 35 Gy. Comet assay was used to examine DNA damage and repair effectiveness in cells after radiation treatment.

2. Materials and methods

2.1. Patients

Thirty-seven patients with HNSCC were enrolled in the study (19 men and 18 women, mean age 56 ± 7 years), most patients having been diagnosed with larynx cancer. Among the patients, 16 subjects had metastasis (neck, tongue, salivary glands, almond). Cells from 35 subjects without cancers (18 men and 17 women, mean age 52 ± 11 years) were used as controls. The tissue samples and lymphocytes were collected from the patients before they had received any chemotherapy or radiation therapy for their primary disease. The diagnosis of healthy and tumor tissue was made after histopathological examination of patient biopsies. Normal tissues from patient biopsies, made during standard medical examination in order to exclude cancer possibilities, were used as controls. These subjects without cancers were also used as blood donors. Moreover, controls were selected based on family history, in order to exclude familiar predisposition to cancer development. Prior to examination, the patients and control subjects did not receive other medicaments, such as antibiotics or steroids. There were no statistically significant differences between the ages of the analyzed patient and control group ($P = 0.068$). They were all non-smokers. Another exclusion criterion was alcohol consumption. All patient and control subjects were recruited from two medical units of the Head and Neck Neoplasm Surgery Department, Department of Otolaryngology and Oncology, at the Medical University of Lodz, Poland. All subjects included in the study were unrelated Caucasians and lived in the Lodz district. The study was approved by the Local Ethic Committee and written consent was obtained from each patient or healthy blood donor before enrolling into the study.

2.2. Cell and cultures

Squamous cancer cells taken from tissue biopsies of primary tumors and metastasis of HNSCC patients, as well as squamous cells isolated from healthy tissue biopsies, were suspended and incubated in a medium containing proteinase K, collagenase and Hanks' Balanced Salt Solution (HBSS) for 1 h at 37 °C. After centrifugation, they were washed twice in phosphate-buffered saline (PBS), suspended in Eagle's Minimum Essential Medium (EMEM) medium at $1–2 \times 10^5$ cells/ml. Peripheral blood lymphocytes from blood of healthy donors and HNSCC patients were collected by centrifugation (15 min, 280*g*) in a density gradient of histopaque-1077 (Sigma, Poland). The viability of the cells measured by the trypan blue exclusion was ~99%. Lymphocytes accounted for ~92% of leukocytes in the obtained cell suspensions, as judged by the characteristic shape of their nuclei. The final concentration of the lymphocytes was adjusted to $1–2 \times 10^5$ cells/ml by adding RPMI-1640 medium (SIGMA, Germany) to the single cell suspension.

The commercially available HTB-43 larynx cancer cell line (ATCC, USA) was used as a control for squamous carcinoma cells isolated from tissue biopsies of patients with head and neck cancer. HTB-43 is a well-characterized cell line of squamous carcinoma; therefore it is an appropriate control for tissue biopsy analysis. HTB-43 larynx cancer cell line was cultivated in EMEM medium with 10% fetal bovine serum at 37 °C in a 5% CO_2 atmosphere for 3–4 days. Prior to examination, cells were trypsinized and suspended in EMEM medium at $1–2 \times 10^5$ cells/ml.

2.3. Irradiation

Cells were γ-irradiated using a ^{60}Co source at 5, 15, 25, 35 Gy at 37 °C in growth medium. Aliquots of cell suspensions were harvested 0, 30, 60 and 120 min thereafter and placed on ice to stop repair reaction. Cells were given for viability test with trypan blue staining immediately after irradiation. The mean percentage of trypan blue positive (live) and negative (dead) cells was counted for each 100 cells from 3 independent experiments.

2.4. Comet assay

The comet assay was performed under alkaline conditions according to the procedure of Singh et al. (1988) with modifications by Klaude et al. (1996). A suspension of cells in 0.75% Low Melting Point (LMP) agarose dissolved in PBS was spread on microscope slides (Superior Marienfeld, Lauda-Königshofen, Germany) precoated with 0.5% normal-melting agarose. The cells were lysed for 1 h at 4 °C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After lysis, the slides were placed in an electrophoresis unit and the DNA was allowed to unwind for 40 min in the electrophoretic solution consisting of 300 mM NaOH, 1 mM EDTA, pH > 13. Electrophoresis was conducted at 4 °C (the temperature of the running buffer did not exceed 12 °C) for 30 min, with an electric field strength of 0.73 V/cm (30 mA). The slides were neutralized with 0.4 M Tris, pH 7.5, stained with 2 μg/ml DAPI and covered with coverslips. To