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Deoxyribonucleic acid content as an indicator of progression of squamous cell carcinogenesis in the esophagus: Comparative analysis on imprint-cytospin and tissue section preparation

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Abstract

Background: The purpose of this study was to explore the potential use of image analysis on tissue sections preparation as a predictive marker of early malignant changes during squamous cell (SC) carcinogenesis in the esophagus. Results of DNA ploidy quantification on formalin-fixed, paraffin-embedded tissue using two different techniques were compared: imprint-cytospin and 6 μm thick tissue sections preparation. *Methods*: This retrospective study included 26 surgical specimens of squamous cell carcinoma (SCC) from patients who underwent surgery alone at the Department of Surgery in CHUV Hospital in Lausanne between January 1993 and December 2000. We analyzed 53 samples of healthy tissue, 43 tumors and 7 lymph node metastases. *Results*: Diploid DNA histogram patterns were observed in all histologically healthy tissues, either distant or proximal to the lesion. Aneuploidy was observed in 34 (79%) of 43 carcinomas, namely 24 (75%) of 32 early squamous cell carcinomas and 10 (91%) of 11 advanced carcinomas. DNA content was similar in the different tumor stages, whether patients presented with single or multiple synchronous tumors. All lymph node metastases had similar DNA content as their primary tumor. *Conclusions*: Early malignant changes in the esophagus are associated with alteration in DNA content, and aneuploidy tends to correlate with progression of invasive SCC. A very good correlation between imprint-cytospin and tissue section analysis was observed. Although each method used here showed advantages and disadvantages; tissue sections preparation provided useful information on aberrant cell-cycle regulation and helped select the optimal treatment for the individual patient along with consideration of other clinical parameters.

Keywords: Image analysis; Predictive makers; Squamous cell carcinoma; Esophageal carcinogenesis; Statistical analysis; Survival data; DNA histogram; Genetic instability; Histopathologic diagnosis; Tumor differentiation; Cell cycle regulation; Clinical parameters; Molecular analysis; Imprint cytospin; DNA ploidy; Cytometry; Early cancer

1. Introduction

Squamous cell carcinoma (SCC) of the esophagus has an exceedingly poor prognosis because of its late symptomatology, unrelenting progression, difficult access for treatment and uncertainty about its prognostic factors. In spite of technical advances in surgery and radiation therapy, there have been few improvements over the past 20 years. In most western countries, the 5-year survival rates have stayed consistently under 10%. The significance of this malignancy lies in the major therapeutic challenge it poses, from the magnitude of surgery required to excise the tumor, to the other techniques now available to relieve patient's dysphagia. More predictive and prognostic factors would be advantageous to stratify patients into those believed to be at "high risk", and thus, likely to benefit from chemo- and or radiotherapy, and those believed to be at "low risk" who might be better treated with immediate resection alone. This strategy could select the patient for the appropriate treatment; thereby sparing certain

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subgroups from the morbidity associated with more aggressive treatment modalities.

Nuclear non-diploid deoxyribonucleic acid (DNA) content has been shown to be related to poor prognosis in many malignant tumors [1–7]. We thus conducted a retrospective study on formalin-fixed, paraffin-embedded tissues to further explore the potential use of the nuclear DNA content as a diagnostic tool at different developmental stages of esophageal SCC. The correlation between imprint-cytospin and tissue sections preparation was assessed to determine the most sensitive way for detecting aneuploid clones in small foci of early malignant lesions.

2. Materials and methods

2.1. Patients, tissue collection and processing

Between January 1993 and December 2000, 26 esophagectomy specimens were obtained by radical surgery at the Department of Surgery, CHUV Hospital, Lausanne, Switzerland, and were subsequently included in this study. Fifteen patients underwent surgery for early esophageal SCCs ranging from dysplasia to T1 SCC while 11 other patients had an esophagectomy for advanced T2 or T3 SCC. No patient received neo-adjuvant radiation therapy or chemotherapy. The series consisted of 19 male and 7 female patients whose ages ranged from 47 to 81 years (mean age, 62 years). In all esophagectomy specimens, the tumor and surrounding healthy tissues were precisely mapped and paraffin-embedded in totality. This allowed a detailed histological examination by routine hematoxylin and eosin (HE) stained preparations. The tumors were classified according to the criteria proposed by the International Union Against Cancer, 5th ed., 2001 [8]. For each case, the samples of distant and adjacent healthy mucosa as well as one or more neoplastic lesions were examined. This allowed us to analyze 53 healthy tissues (27 distant and 26 adjacent to the main lesion), 43 tumors (32 early SCCs including 4 dysplasias, 9 in situ carcinoma, 9 micro-invasive carcinoma, 10 T1 SCC and 11 advanced T2-T3 SCCs) as well as 7 lymph node metastases. Thirteen patients had a single tumor localization, among whom eight had several tumor stages (such as in situ or micro-invasive carcinoma adjacent to a more advanced lesion) and five patients had multicentric synchronous tumors. To be considered as multicentric tumors, two or more lesions had to be separated by at least 2 cm of non-neoplastic mucosa [9]. Tumor differentiation was classified as well, moderate or poor according to standard histopathologic criteria [10]. Well-differentiated SCC was characterized by horny pearls and visible inter-cellular junctions. The cells were usually fairly uniform in size and shape, and their nuclei were evenly stained with scanty mitoses. The morphology of moderately or poorly differentiated SCC was characterized by more or less diffuse clusters of neoplastic cells and scanty or no obvious keratin pearls. The cells had a large variation in size and shape with irregular nuclear morphology and increased number of mitoses.

2.2. Measurements of DNA content

The number of samples for cytophotometric DNA analysis in each case was calculated as a function of the maximal diameter of the tumor (one sample per centimeter/maximal length). In addition, the samples of normal esophageal mucosa adjacent and distant to the tumor were examined for each case.

2.3. Imprint-cytospin preparation

The first part of the study was dedicated to the evaluation of DNA ploidy by means of imprint-cytospin, a technique readily available in our laboratory. The adjacent and distant healthy tissues, early lesions (such as dysplasia, in situ and micro-invasive carcinomas) and infiltrative tumors were separately sampled on the corresponding paraffin blocks by means of a dissecting microscope [11]. A histological control of the blocs following micro-dissection was carried out. One hundred and twenty blocs were processed for DNA assessment, with an average number of 4.6 blocs per case, including samples from healthy esophageal mucosa and lymph node metastases for some cases.

All dissected blocs were processed following the technique described by Hedley et al. [12]. DNA image analysis was performed on Feulgen-stained single-cell preparations using an image analysis system (Zeiss, AxioScop2, AutoCyte Quic DNA 1.1, original magnification $\times 40$). Minimums of 250 nuclei were measured per smear (300 nuclei in most cases). Only well preserved nuclei were selected by the investigator using the gallery program of the AutoCyte Quic DNA system. As an internal control, we used six lymphocytes or at least six tissue polymorphonuclear leukocytes when available, with a coefficient of variation less than 2%.

Histograms were interpreted according to the European Society of Analytical Cellular Pathology (ESACP) recommendations which allow DNA stemlines to be identified as abnormal (or aneuploid) if they deviate more than 10% from the diploid (2c) or tetraploid regions (4c) [13]. Considering that in normal tissues and in most low-grade or slowly proliferating neoplasms, approximately 85% of the cell population forms the G0/1 peak and 15% of the cells in the S-phase and G2/M phases [14,15], we only considered as aneuploid the samples with more than 15% of cells with a DNA-index in excess of 1.10.

Rare events in DNA histograms were also considered abnormal cells often-called 5c or 9c exceeding events because representing non-proliferating abnormal cells with different chromosomal anomalies.

2.4. Tissue section preparation

Conjointly the second part of study was dedicated to evaluating the feasibility and usefulness of tissue sections