DNA-flow cytometry of head and neck carcinoma: the importance of uniform tissue sampling and tumor sites

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Summary. Flow cytometric DNA ploidy measurements using deparaffinized tumor specimens were performed on 46 squamous cell carcinomas of the head and neck, including 22 carcinomas of the oropharynx, 18 carcinomas of the larynx and six carcinomas of the oral cavity. Aneuploidy was found in 14 of these tumors with carcinomas of the larynx and oral cavity showing almost equal percentages of DNA aneuploidy (10/18 and 3/6, respectively). In contrast, only 1 of the oropharyngeal carcinomas was aneuploid. Accurate microscopy-controlled sampling of tumor tissue from the histological tissue blocks was found to be mandatory in order to obtain reliable ploidy measurements.

Key words: DNA-flow cytometry – Head and neck cancer – Tissue sampling

Introduction

Traditionally, the prognosis of head and neck cancer patients is largely based on TNM classification. However, cases of the same stage and treated similarly may still show very different clinical outcomes. During the last decade ploidy measurements by means of DNA flow cytometry (FCM) has proven to be of value as an additional prognostic parameter in malignant tumors of a variety of organs [14]. In general, patients with diploid solid tumors were found to have a more favorable prognosis than those with aneuploid cancers [2, 13, 15, 17, 18]. However, exceptions occur and aneuploid neuroblastomas have been reported to carry a better prognosis than diploid ones [12].

With regard to carcinomas of the head and neck, a great diversity of aneuploid percentages has been reported, ranging from 38% to 86% [1]. In the majority of reports aneuploidy has been correlated with a decreased survival, although this finding is in some dispute and has even been reported to have a better prognosis in patients with laryngeal cancer [5]. In certain cases discrepancies in reported ploidy percentages may be caused by poor standardization or subjective evaluation of ploidy [4]. In addition, the exact sites of tumor involvement in the head and neck have often not been specified. Moreover, a non-uniform method of tissue sampling may also have contributed to the apparently conflicting results.

Recent technical advances have made it possible to use paraffin-embedded tissue for FCM, so that ploidy assessments can be performed on archival material [7, 8]. In this study we have analyzed 46 squamous cell carcinomas of the head and neck, with special attention to the ploidy status of the tumors in relation to their site of origin and to the method of tissue sampling used.

Materials and methods

Tumors. FCM DNA analysis was performed on formalin-fixed, paraffin-embedded material from 46 primary head and neck tumors. This group consisted of 22 oropharyngeal carcinomas (19 tonsillar region and 3 base of tongue), 18 laryngeal carcinomas, and 6 oral cavity carcinomas. In 21 cases it was possible to analyze both the primary tumor and an associated lymph node metastasis. All tissue blocks used in this study were histologically verified to contain at least 15% tumor cells.

DNA measurements by FCM. Paraffin sections of tumors were processed according to the method described by Hedley et al. [7, 8]. Following this, FCM analysis was performed with a Becton-Dickinson Facstar flow cytometer (Becton-Dickinson, Erenbodegem, Belgium) equipped with a 5 W argon laser set at an excitation wavelength of 488 nm. A dot plot of red fluorescence width versus red fluorescence area was used to exclude debris and doublets. Individual nuclear fluorescence was measured by photomultipliers through a 585 nm band filter, 30 nm band width, and was processed electronically. All data were plotted on a 1024-channel histogram. Histogram analysis was done with a Hewlett-Packard computer using cytological software (Becton-Dickinson, Erenbodegem, Belgium). Non-neoplastic tissue present in each of the 50 µm sections was taken as an internal control. Fluorescence from 50,000 nuclei was recorded in all cases.
Results

Histograms were classified as aneuploid or diploid. All major peaks had a coefficient of variation under 10%. All of the 46 blocks of the primary tumors were analyzed and yielded acceptable histograms. Tetraploid tumors cells (containing twice the amount of DNA as compared with non-malignant cells), when representing a small fraction of the whole section were difficult to detect, since those tumor cells in the G0 and G1 phases of the cell cycle had the same DNA content as normal cells in G2 and M phase. For the purpose of this study, a 4c peak representing more than 20% of the whole cell population and a detectable peak at the 8c position was considered indicative of aneuploidy, since it was unlikely that normal cells would have such a high G2/M peak.

Representative DNA histograms of head and neck tumors from this study are shown in Fig. 1.

In 21 cases, consisting of 18 oropharyngeal and 3 laryngeal carcinomas, both primary tumor and a lymph node metastasis were analyzed. Histologically, no significant differences were found between primary and metastatic tumors. A concordant ploidy status was found in 15 cases but a discrepancy was uncovered in 6 cases. Three diploid tumors had aneuploid metastases and 3 aneuploid primaries had diploid metastases. These unexpected differences in ploidy contents [3] may have been caused by inaccurate sampling methods. We therefore removed as much normal tissue as possible from the blocks containing the tumor. A repeat analysis of the 6 previously discrepant cases then resulted in 4 identical ploidy values. In the remaining 2 cases, the primary tumors remained diploid while the metastases showed aneuploid peaks.

In 14 specimens (30%), an aneuploid peak was found. The distribution of the aneuploid cases according to the site of the primary tumor is shown in Fig. 2.