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Does loss of heterozygosity in critical genome regions predict a local relapse in patients after laryngectomy?

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Abstract

Background: Patients, who had an upper aerodigestive tract malignancy, have a high incidence of succeeding tumor development. This has been attributed to the role of "field cancerization" in carcinogenesis. The aim of this study was analysis of loss of heterozygosity (LOH) in the regions frequently lost during the course of head and neck squamous cell carcinomas (HNSCC), especially at early stages, which could answer the clinicians' question, if LOH analysis has any "predictive" value in relation to tumor occurrence.

Material and methods: Sixty-five larynx cancer patients were examined for loss of heterozygosity on 3p, 7q, 8p, 9p and 18q chromosomal arms with the use of 12 microsatellite markers. The material from a single patient consisted of blood, tumor, safe margin and one or two clinically unchanged mucosal samples. During follow up, the material from brush specimens (14 patients) as well as laryngeal swabs (4 patients) was also examined.

Results: The highest frequency of LOH was detected for marker D3S1234 in tumor tissues (29%). Analysis of margin samples (b) revealed low LOH frequencies (2–5%) and complete retention of heterozygosity for markers: D3S1234, D7S486, D8S261, D8S264, D9S171 and D18S46. Similarly, for normal appearing mucosa from upper part of larynx (c) frequencies of LOH were low (2–6%), with the complete retention of heterozygosity for markers: D3S1284, D3S1304, D3S1234, D8S264 and D9S1870. We did not detect any LOH in the material of normal appearing mucosa from tracheostoma region (d). During follow up, LOH was detected for eight markers, with the highest incidence for markers D18S46 (six cases), D7S486 (four cases) and D3S1300 (three cases).

Conclusions: The data, obtained during this investigation, did not reveal the predictive value of LOH with respect to local relapse occurrence in laryngeal cancer patients. However, time of follow up did not reach 5 years, so that further clinical monitoring should be conducted.

Keywords: Larynx cancer; Loss of heterozygosity; Microsatellite markers; Follow up; Local relapse

1. Introduction

Head and neck squamous cell carcinomas (HNSCC) constitute a significant medical and social problem, with the occurrence of around 5% of all worldwide-diagnosed malignancies. The difficulties in studying the progression of larynx cancer are connected with its complex

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biology, genetics and with a relatively late clinical detection of primary tumors. As the result, local recurrences, lymph node relapses, distant metastases and second primary tumors appear very often [1,2]. Still, in spite of advanced genetic studies and improvements in diagnostics and treatment of HNSCC the survival rates are rather poor, ranging from 40 to 50% [3]. Treatment failures may partially be explained by anatomical predisposition of head and neck region organs—the epithelium of upper respiratory tract, exposed for a prolonged carcinogens activity (in tobacco, alcohol, air, preservatives) is being continuously damaged (the "field cancerization" theory) [4]. Local recurrences can develop within primary tumor resection site, even when surgical margins were histologically tumor-free [5]. These features extorted the investigators to study not only the material derived from tumor, but also from clinically unchanged mucosa resected within the safe margin from the operating field. It is possible that normal-appearing mucosa left after tumor and margin excision may be genetically altered [1,6]. Unfortunately, it means that "field cancerization" process is much more extensive than clinical and histological examinations show [7].

It was estimated, that a cell undertakes an irreversible malignant transformation process after acquiring 6–10 genetic alterations [8]. These alterations concern activation of oncogenes as well as inactivation of tumor suppressor genes, as it was revealed by cytogenetic and molecular studies [9,10]. Loss of heterozygosity (LOH) has been recognized to be an important event in HNSCC, suggesting involvement of the suppressor pathway in the genesis of this disease—LOH is often one of two inactivation steps of tumor suppressor genes (TSGs) [11]. Multiple analyses with the application of microsatellite markers provided the knowledge about chromosomal regions containing critical tumor suppressor genes that are frequently lost or inactivated in HNSCC, like *p16* (9p21), *p53* (17p13.1), *FHIT* (3p14.2), *VHL* (3p25.3), *Rb1* (13q14) and *ING1* (13q34) [9,10]. Moreover, there are numerous regions lost during HNSCC pathogenesis, where putative TSGs were not identified so far (i.e. 8p arm). Microsatellite analysis appears to have a potential application in predicting cancer risk, as shown by studies conducted previously on oral leukoplakias exhibited LOH at chromosomal regions 3p14 and/or 9p21 to carry higher risk for HNSCC development [12].

In the present study, we investigated LOH in the regions frequently lost during the course of HNSCC, harboring known or putative tumor suppressor loci in the material derived from patients treated surgically for larynx cancer. The material consisted of tumor, safe margin and samples of normal appearing mucosa taken from distant, opposite points of surgical specimens. The diversity among clinical material should enable a comparison of genetic alterations in the same patient in various localizations (glottis, trachea). Additionally, during follow up, laryngeal swabs from hypopharynx and brush specimens from tracheostoma were collected to study LOH more than 4 years after surgery. The markers were chosen with the emphasis on localization in regions lost "early" during neoplastic transformation [13,14]. This experiment may answer the clinicians' question, if LOH analysis has any "predictive" value in relation to tumor occurrence.

2. Patients and methods

2.1. Samples collection

Altogether 65 larynx cancer patients (62 men and 3 women) were examined. Material from surgical specimens was obtained from the Department of Otolaryngology and Laryngeal Oncology, K. Marcinkowski Medical University, Poznań, Poland, and was kept frozen at −80 °C right after the surgery till laboratory studies. The detailed data were described previously [15]. In brief, the material from one patient consisted of four or five samples.

In cases of total laryngectomy (46 patients), five samples were taken:

- blood as a reference material;
- tumor (marked as "a");
- safe margin from the operating field ("b");
- two samples from distant, opposite points of the surgical specimen—clinically unchanged mucosa from the epiglottis or hypopharynx (proximal point of specimen called "c") and normal mucosa of the tracheal ring (distal point of specimen called "d").

In cases of partial laryngectomy (19 patients), four samples were taken:

- blood as a reference material;
- tumor (marked as "a");
- safe margin from the operating field ("b");
- normal appearing mucosa from the upper part of the larynx (proximal point of specimen called "c").

Tumor and safe margin tissues were verified histopathologically by microscopy. Normal mucosa was not verified by histology because of unchanged clinical character and a distance from the tumor exceeding 2 cm.

Additionally, during the last follow up visit (June 2005), brush specimens (marked as "z") were taken from 14 patients after total laryngectomy from tracheostomy and laryngeal swabs (marked as "s") were taken from hypopharynx from 4 patients after partial laryngectomy.