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Cell Cycle Distribution and Ornithine Decarboxylase Activity in Head and Neck Cancer in Response to Enteral Nutrition

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Abstract—*Flow cytometric DNA distribution and the activity of the enzyme ornithine decarboxylase (ODC) have been measured in tumor biopsies from patients with malignant head and neck tumors before and after 6–8 days on nasogastric tube feeding. Thirteen patients were randomized to the study group (defined enteral nutrition), and 13 patients to the control group (spontaneous oral intake).*

The relative size of the aneuploidic compartment was significantly increased in tumor biopsies from patients on enteral nutrition compared to controls on ad libitum *oral intake. The aneuploidic compartment and DNA index were unrelated to histologic differentiation and to 1 year patient survival. Poorly differentiated tumors had higher ODC activity than moderately to highly differentiated tumors. ODC activity was positively correlated* ($r = 0.63$, $P < 0.01$) *to the aneuploidic compartment size in tumors. Patients with less than 1 year survival and T4 tumors had a trend to higher* ($P < 0.05$) *ODC activity compared to those with more than 1 year survival.*

In conclusion, this study demonstrates that nutritional support can change DNA distribution in human cancer. Such changes may either be related to activation of cell proliferation or tumor dedifferentiation.

INTRODUCTION

EXPERIMENTAL STUDIES have provided evidence that nutritional modulation of the host can affect the growth rate of malignant tumors [1–4]. In general, it has been difficult to confirm such effects in human cancer [5]. However, in a recent study evidence was presented to support the idea that cancer patients who received parenteral nutrition had increased polyamine levels in blood erythrocytes [6]. Urinary levels of such polyamines have been shown to be associated with disease activity and tumor burden [7]. In addition, it has been reported that total parenteral nutrition (TPN) may alter cell cycle kinetics in tumors from patients with head and neck cancer [8]. The objective of the present study was therefore to investigate whether enteral refeeding of patients with head and neck cancer would demonstrate evidence to support the well-recognized fear that nutrition may stimulate tumor cell proliferation. For this purpose we have measured nuclear DNA distribution by flow cytometry in tumor specimens before and after enteral nutrition as nasogastric tube feeding. We have also measured ornithine decarboxylase activity (ODC) in tumor samples since ornithine decarboxylase constitutes a rate-limiting enzyme for the synthesis of polyamines which are recognized as some kind of growth factors in dividing cells [9].

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MATERIALS AND METHODS

Patients

Thirty-one patients with various head and neck tumors were examined. The diagnoses were: oral carcinoma 21, hypopharyngeal carcinoma six, laryngal carcinoma two, maxillar carcinoma one, salivary gland carcinoma one. Most of the patients had advanced tumors (T4, $n = 19$; T3, $n = 5$; T2, $n = 7$). All but four tumors were histologically classified as squamous cell carcinomas. All patients had a history of recent weight loss exceeding >5%. None of the patients had received any kind of

cytostatic treatment, radiotherapy, antiinflammatory drug or hormonal substitution before our investigation.

Study design

Initial tumor biopsies were taken for histologic examination, flow cytometric analyses and in most patients determination of ornithine decarboxylase activity (ODC). One tissue sample was also taken from the apparently normal mucosal membrane in the oral cavity to be used for blank measurements in the various assays.

The patients were then randomized by chance to a study and control group. In the study group, 13 patients received enteral nutrition (Clinifeed, Protein rich® Roussel Nutrition, Stockholm, Sweden, see Table 1) by use of a nasogastric tube. The gastric infusion continued for 20–22 h/day for 6–8 days depending on when it was practically possible to take a second biopsy for investigation. All study patients received non-protein calories corresponding to 120–150% of their estimated resting energy expenditure and proteins between 0.8 and 1.0 g/kg/day in addition to their spontaneous oral intake. This additional intake was estimated to be 400–600 kcal, with a ratio of non-protein calories 145 kcal per protein. The purpose was thus to assure that the basal energy requirement was provided as a minimum support according to our standard hospital routine use of artificial enteral nutrition. The other group served as control patients (13 patients). Accordingly, they continued to eat corresponding to their spontaneous appetite. The caloric intake was estimated to be less than 1000 kcal/day and 0.5 g protein/kg/day in these patients.

Following the 6–8 days on nutritional support a second sample of tumor tissue was taken from all patients as far as possible from the location of the original biopsy and possible tissue injury. All patients had fasted overnight before any tissue biopsies were taken.

Another five patients were also subjected to an initial tumor biopsy investigation without any second biopsy as follow up.

Table 1. *Composition of the enteral diet per* 375 ml (500 kcal)

Protein 30 g	
Fat 11 g	
Carbohydrates 70 g of which is:	
Glucose	0.83 g
Lactose	7.13 g
Sucrose	19.9 g
Maltose	2.6 g
Polysaccharides	39.48 g
Minerals	
Calcium	210 mg
Phosphate	319 mg
Sodium	296.5 mg
Potassium	938 mg
Magnesium	45 mg
Iron	3.75 mg
Copper	0.3 mg
Zinc	3.75 mg
Manganese	1.5 mg
Vitamins	
Vitamin A	0.6 mg
Vitamin D	2.5 mcg
Vitamin E	7.5 mg
Thiamine	2.5 mg
Riboflavin	2.5 mg
Vitamin B_6	2.0 mg
Niacin	10 mg
Folic acid	100 mcg
Vitamin B_{12}	0.45 mcg
Pantothenic acid	7.5 mg
Vitamin C	75 mg
Biotin	5.2 mcg

Flow cytometry

Tumor materials consisted of two to three pieces and each of them represented between 100–200 mg wet tissue weight. These pieces were immediately fixed and kept in 70% ethanol. The storage was never longer than 1 week. The subsequent cytometric measurements were performed in detail as described by Barlogie *et al.* [10]. We have defined the aneuploidic compartment size as the area of aneuploidy in relation to the total DNA histographic area.

Ornithine decarboxylase activity

Tumor tissue was rapidly excised and washed in phosphate buffered saline, pH 7.4. After homogenization in 0.25 mol/l sucrose containing 1 mmol/l dithiothreitol (20 ml/g tumor wet weight), centrifugation was performed at 18,000 ***g*** for 2 h. The supernatant was used for determination of the enzyme activity as described by Noguchi *et al.* [11]. All samples were run in duplicates. Protein was measured as described by Lowry *et al.* [12] with bovine albumin as the standard. Blank reactions in the enzyme assay were always run and consisted of all reagents and solutions without the enzyme. The enzyme activity was expressed as nmol of $^{14}CO_2$ per hour per mg protein.

Altogether 19 patients were subjected to investigation of ODC activity in tumor biopsies. Nine of these patients were in the study group receiving enteral nutrition while seven patients were in the control group. Another three patients were subjected to an initial ODC measurement.

This study was approved by The Committee for Ethics at The Faculty of Medicine, University of Gothenburg, Sweden. All patients gave informed consent before investigation.

FIGURE 1

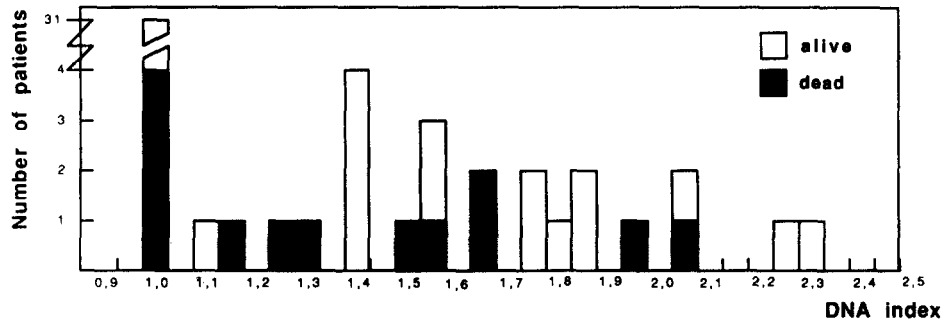

Fig. 1. DNA index in tumor tissue before nutrition and its relationship to 1 year survival of the patients. Twenty-two patients had aneuploid and polyploid tumors in addition to diploid (D1 = 1.0) cell clone. Two of the patients had two aneuploidic clones.

RESULTS

DNA pattern in tumor tissue before nutrition and prognostic evaluation

Aneuploid and polyploid cell populations in addition to a diploid $G_{0:1}$ population were found in 22 (71%) out of 31 patients with tumor. No significant correlation was demonstrated between the histopathologic differentiation and the DNA index or the aneuploidic compartment in squamous cell carcinoma. Thus, poorly differentiated carcinomas ranged from single diploid populations to diploid stem lines with an additional aneuploidic clone (DI: 1.1–2.3). Such a heterogeneity was also found in the group of moderate to well-differentiated carcinoma, demonstrating either single diploid populations occasionally in combination with one or two additional cell clones (DI: 1.4–2.25). Patients with aneuploidic tumors had no significant increase in mortality within 1 year (9/22 = 41%) compared to patients with a monoclonal diploid tumor (4/9 = 44%). The distribution of DNA index and its relationship to the one year patient survival is shown in Fig. 1. The non-surviving patients were evenly distributed along the axis of DNA index. Around 50% of these patients had DNA index <1.4.

The percentage of the aneuploid or total SG_2 compartment did not correlate to the degree of cell differentiation or to the patient mortality. In some cases, however, it was not possible to measure the total SG_2 compartment size due to overlapping of a variety of stem lines in the histogram. Therefore, measurement of the relative aneuploidic compartment size seemed to provide a more reliable parameter in the flow cytometric studies.

ODC activity, differentiation, aneuploidy before nutrition and prognosis

ODC activity was determined in 19 tumors. Sixteen of these were squamous cell carcinomas. Undifferentiated squamous cell carcinomas had a significantly increased ODC activity (3.9 ± 1.1 nmol/h/mg protein) when compared to moderately and well-differentiated tumors (1.0 ± 0.4 nmol/h/mg protein) ($P < 0.01$). There was a positive correlation ($r = 0.63$) between ODC activity and the percentage of aneuploidy (Fig. 2). Figure 3 shows that patients who survived less than 1 year ($n = 8$) seemed to have a trend to a higher ODC activity in tumor tissue (3.6 ± 1.1 vs. 1.6 ± 0.6 nmol/kg/h/mg protein, $P < 0.05$) compared to patients who survived 1 year ($n = 11$).

DNA distribution and ODC activity in response to enteral nutrition

New stem lines were detected in three tumors out of 13 patients receiving enteral nutrition. Altogether, eight of these patients demonstrated an aneuploidic compartment (Table 2). Two new cell clones appeared with DNA indices of 1.1 and 2.0 in one of these tumors. However, it cannot be ruled out that these clones in fact originated from the diploid stem line and that the peak with index 2.0

FIG 2

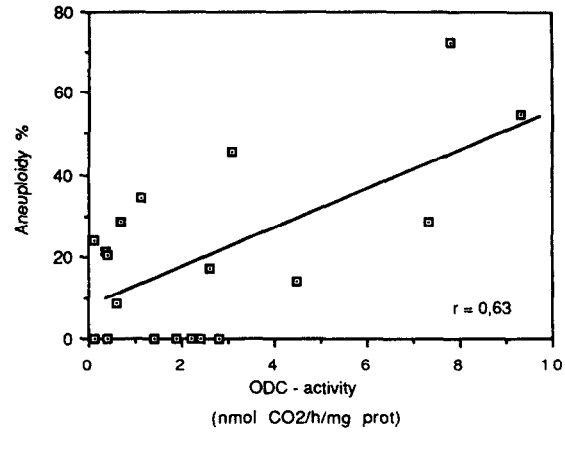

Fig. 2. The relationship between ornithine decarboxylase activity (ODC) and the percentage of aneuploidy in tumor biopsies before nutrition.

Table 2. Flow-cytometric analysis and ODC activity in tumour tissue before and after enteral nutrition

	Aneuploid compartment				Ornithine decarboxylase activity (nmol CO_2/h/mg protein)	
	No. of subjects	% Mean ± S.E.M.	Increase	Decrease	No. of subjects	Mean ± S.E.M.
Nutrition						
Before		26.7 ± 7.0				2.0 ± 0.7
	8		7†	1	9	
After		35.3 ± 8.5*				1.7 ± 0.5
Controls						
Before		29.5 ± 4.6				1.3 ± 0.4
	12		2	10	7	
After		18.1 ± 5.6				1.7 ± 0.4

*Difference from values of control patients is significant after nutrition ($P < 0.01$; Students t-test).
†Difference between prenutritional state and following nutrition is significant compared to the controls ($P < 0.01$; Fisher's exact test).

FIG 3

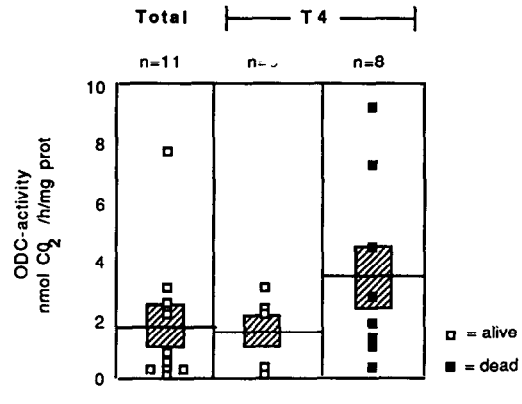

Fig. 3. Mean values of ornithine decarboxylase activity (ODC) in patients who did or did not survive 1 year. Shaded bars represent ± S.E. T4 refers to patients with tumor stage T4; n = *number of patients. ODC activity was significantly increased* ($P < 0.05$) *in patients with less than 1 year survival compared with patients with more than 1 year survival. Tumor ODC activity in all patients* (n = 11; *T2–T4 tumors*) *who survived more than 1 year is also shown for comparison.*

consequently was compatible with an increment of the G_2M compartment. In the control patients, one out of 13 tumors demonstrated an additional stem line in the second biopsy, while aneuploidic clones did not occur in four tumors. Altogether, 12 of these patients demonstrated an aneuploidic compartment. The relative size (%) of the aneuploidic compartment was thus increased in seven patients and decreased in one patient who received enteral nutrition, while it increased in only two patients and decreased in 10 patients in the control group. This difference between the groups could either be confirmed by Fisher's exact test for the 2 × 2 contingency table [13] or by Student's t-test. There were no significant alterations between the first and second biopsies in either the percentage of total SG_2 or ODC activity among the nutritional and control patients. S-phase values specifically were not possible to determine in a systematic way due to the fact that several tumors contained more than one cell clone, which led to overlapping in the S-phase region of the cytogram.

DISCUSSION

This study provides cicumstantial evidence that enteral nutrition may affect DNA distribution in human malignant tumors, since the aneuploidic compartment size increased in a significantly larger proportion in cancer patients who received nutritional support compared with controls eating spontaneously. Such cytocompartment changes may either be compatible with activation of tumor cell proliferation or dedifferentiation of tumor cells. Our results are in agreement with a recent study [8] also suggesting stimulated tumor growth following total parenteral nutrition (TPN) in cancer patients as evaluated by measures of flow cytometry. However, in that study the actual protein and caloric intake was not significantly different from that of the control group not receiving TPN. Therefore, it was suggested that the tumor cytometric changes were rather an effect of the TPN administration as such instead of being induced by the substrates. Only sparse information is available about the effect of nutrition on human cancer, although it has been well recognized that animal tumors are sensitive to nutritional alterations [3]. The results in the present study give circumstantial support to the suggestion that certain aneuploidic stem lines may respond differently to nutrition than diploid cell clones which may harbour a large mass of normal cells, frequently inflammatory and connective tissue sometimes accounting for 90% of the total cell number [14].

Altered responsiveness to nutrition in different stem lines may explain why new stem lines were detected from three patients receiving nutritional support and why clones were missing from the second biopsy specimens among four of the control patients. Although three different samples were taken from different regions of each tumor at every occasion, it must be considered that there may be a considerable heterogeneity of DNA distribution in tumors [15, 16]. In our studies, however, we found remarkable similarities between and within samples with respect to cell clone identification and percentage of aneuploidy. However, we found that there was a broad variation of the total SG_2 compartment. This may in part be due to the uncertainty to distinguish SG_2 cells from aneuploidic G_1 background cells, particularly in the hypertriploid region.

Experimentally, it is easy to demonstrate an upregulation of ODC activity in response to refeeding following a period of undernutrition (our own unpublished results). However, nutritional support showed no evidence of increasing the ODC activity in the present study, although there was a correlation between the percentage of aneuploidy and the ODC activity before nutrition (Fig. 2). Studies on the cell cycle progression have indicated that ornithine decarboxylase may be a marker of G_1 progression [17–19] and also of the near G_2M transition phase [20]. One possible explanation for the lack of ODC activation in the present study may be that all patients were fasted overnight prior to taking tumor biopsies. The DNA profile may not rapidly respond to nutritional deprivation while the ODC activity does so due to a half life of the enzyme less than 1 h as found in various cells [19, 21, 22].

The occurence of DNA aneuploidy was 71% among the patients in our study. However, the number of patients with early cancer was too small to evaluate whether aneuploidy was correlated to the clinical stage as reported in a variety of tumor types [14–16, 23, 24]. In addition we could not find any dominant stem line. The cell clones ranged evenly between 1.0 and 2.35 DNA index units. None of these clones showed significant clusterings in patients with short survival. This is in contrast with a previous study demonstrating shorter survival for hypertriploid abnormalities in different solid tumors [25]. It may be possible that similar DNA abnormalities have different prognostic strength in different tumor types [25]. There was a positive correlation between the degree of aneuploidy and ODC activity although a direct relationship between aneuploidy and clinical outcome could not be found (Fig. 2). Furthermore, high enzyme activities were accompanied by a low histopathologic differentiation and shorter patient survival (Fig. 3). This fact suggests that measurements of ODC activity may be of value for the assessment of tumor aggressiveness, which is supported by experimental investigations [9].

In conclusion, this study provides evidence to support the idea that aneuploidic stem lines in head and neck cancer may be activated in some patients following enteral nutritional support. This is one of few clinical investigations giving support to the fear that clinical nutrition may stimulate tumor growth under certain conditions. The clinical importance of our observations remains to be determined.

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