Eur J Cancer, Vol. 28A, No. 4/5, pp. 755–757, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Association Between Chromosome 11q13 Amplification and Prognosis of Patients with Oesophageal Carcinomas

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Ninety pairs of normal and tumour tissue DNAs were isolated from paraffin-embedded blocks of advanced oesophageal carcinoma cases and examined for gene amplification at chromosome 11q13 by dot-blot hybridisation using the *int*-2 gene as a probe. 22 of 90 carcinomas (24%) showed more than two times amplification. Although no significant correlation was observed between gene amplification and histological type or metastasis to lymph node, a tendency for deeper invasion to be associated with more frequent amplification was observed. In relation to prognosis, patients with amplification had a lower survival rate than those without amplification. This tendency was evident both in the group with well differentiated type carcinoma and in the group which had no metastasis to lymph node. Thus, gene amplification of the *int*-2 locus may be a useful prognostic factor.
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INTRODUCTION

AMPLIFICATION OF proto-oncogenes in malignant tumours such as N-*myc* in neuroblastoma or *erb*B-2/*HER*-2 in carcinoma of the breast has been reported to be frequent, the indication being that this may be a prognostic factor in these types of carcinomas [1–5].

In oesophageal carcinomas, frequent gene amplification at chromosome 11q13, which contains the *hst* and *int*-2 genes, has been described [6]. This region was also found to be amplified in some breast and gastric carcinomas, although at a frequency much less than that apparent in oesophageal carcinomas [5–7]. Amplification of this region was considered to have a relation with metastasis to lymph nodes [6], and may also have an important role to play during malignant progression of head and neck squamous cell carcinomas [8]. However, whether an association exists between amplification at chromosome 11q13 in oesophageal carcinomas and prognosis of patients remains unclear. To address this question, we have investigated gene amplification at chromosome 11q13 in 90 patients with oesophageal carcinomas who underwent operations more than 5 years ago and were followed-up carefully thereafter. In the present report possible association with prognosis or other pathological parameters is discussed.

MATERIALS AND METHODS

DNA extraction

90 pairs of normal and tumour tissues from patients with advanced oesophageal carcinomas, who underwent operation during the period 1980–1985, were used in this study.

DNAs were extracted from paraffin embedded tissues by the modified method of Goelz *et al.* [9]. Tissues were separated into cancerous and non-cancerous portions, sliced with a razor blade and incubated in TE9 (500 mmol/l Tris, 20 mmol/l EDTA, 10 mmol/l NaCl, pH 9.0), 1% sodium dodecyl sulphate and 0.9 mg/ml proteinase K for 24 h at 50°C. DNAs were purifed by phenol/ chloroform extraction and ethanol precipitation.

Slot blotting

2 μg DNA were denatured in 0.5 N NaOH, 1.5 M NaCl and blotted onto Hybond-N$^+$ nylon membranes (Amersham) using a HYBRI-SLOT apparatus (Bethesda Research Laboratories).

DNA probes

pSS6(*int*-2) and total human lymphoblast DNA were labelled by the random priming method with ^{32}P-dCTP [10]. Hybridisation and washing were performed according to the method reported by Nakamura *et al.* [11]. After hybridisation with pSS6, the filters were stripped and rehybridised with total human DNA for a quantitative control.

Radioactivity for each slot was measured using a BAS 2000 image analyser (Fuji film). To correct for amounts of DNA between cases, the amplification ratio was calculated as (Ti/Ni)/(Tc/Nc); where Ti, radioactivity in tumours for *int*-2; Ni, radioactivity in normal tissue for *int*-2; Tc, radioactivity in tumours for total human DNA; Nc, radioactivity in normal tissue for total human DNA. A ratio > 2 was counted as positive, and < 1.5 as negative. Ratios between 1.5–2.0 were classified as intermediate.

Statistical analysis

Analysis of survival data was conducted by the method of Cox [12] and Kaplan–Meier [13]. In the analysis of contingency tables, Fisher's exact test for small expected frequencies was performed [14].

RESULTS

Figure 1 illustrates representative results of slot hybridisation using pSS6(*int*-2) or total human DNA as probes. 22 (24%) of 90 cases were classifed into the amplified group, 61 were negative

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Revised 23 Oct. 1991; accepted 29 Nov. 1991.

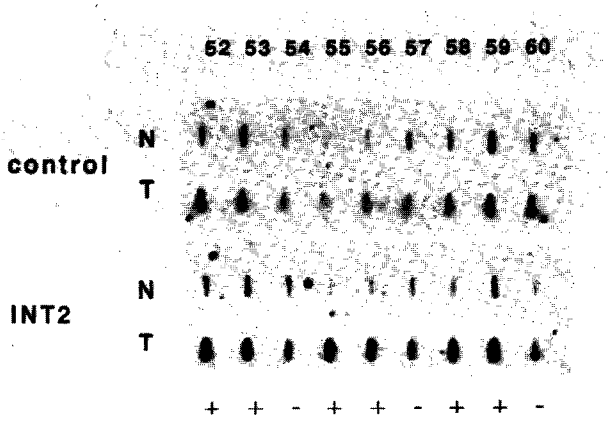

Fig. 1. *Int-2* amplification in oesophageal carcinomas. Dot-blot hybridisation of nine sets of DNA is shown. Numbers above figure are case number. N: normal tissue; T: tumour. Filter was hybridised with pSS6(*int-2*), stripped and re-hybridised with total human DNA as control. +: amplified; −: non-amplified.

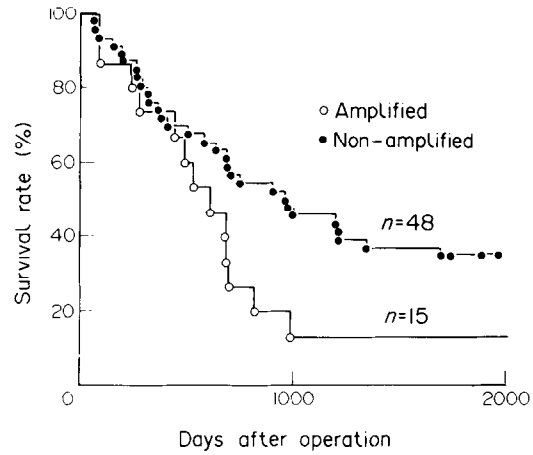

Fig. 2. Association between *int-2* amplification in oesophageal carcinomas and survival rate. Cases with non-curative resection were excluded. Patients who died within 30 days were also excluded as direct operative deaths. The Kaplan–Meier method was used to estimate the distribution of each group. The two survival curves are not significantly different ($P > 0.05$; generalised Wilcoxon test).

Table 1. Int-2 *amplification in oesophageal carcinomas*

	Non-amplified	Intermediate	Amplified	Total
Male	50 (65)	7 (9)	20 (26)	77 (100)
Female	11 (85)	0	2 (15)	13 (100)
Total	61 (68)	7 (8)	22 (24)	90 (100)

(%).

and 7 were considered as intermediate (Table 1). The mean age at operation of these 90 patients was 62.3 years and the frequency of the amplification among different age groups was almost the same (data not shown). Gene amplification was detected more frequently in males (20/77; 26%) than in females (2/13; 15%), although the difference was not statistically significant.

The 7 intermediate cases were excluded from further analysis leaving 83 to be used for comparison with data for histological analysis (Table 2). The incidence of gene amplification was 28%

Table 2. *Histological factors and* int-2 *amplification*

	Non-amplified	Amplified	
Histological type			
Well differentiated	23	9	(28)
Moderately differentiated	26	10	(28)
Poorly differentiated	9	2	(20)
Undifferentiated	3	1	(20)
*Invasion depth			
pm, a1	7	1	(13)
a2, a3	54	21	(28)
Lymph node metastasis			
Negative	18	5	(22)
Positive	43	17	(28)

(%).

* pm, invasion to muscularis propria; a1, invasion reaching the adventitia; a2, definite invasion to adventitia; a3, invasion into the neighbouring structures.

in both well differentiated types and moderately differentiated types, and 20% in poorly or undifferentiated types, this difference being without significance. The depth of local invasion of tumours and the status of lymph node metastasis, which are significant factors for prognosis, were also examined. A higher incidence of amplification was observed in the deeply invasive group (28%) as compared with the less invasive group (13%). A little difference was observed between the positive lymph node (LN) group (28%) and the negative LN group (22%).

For the analysis of prognosis, 17 cases with non-curative operation were excluded. 3 patients who died within 30 days after operation were also excluded as direct operative deaths. The Kaplan–Meier comparison procedure was performed between 15 amplified and 48 non-amplified cases (Fig. 2). Although patients with amplification demonstrated a shorter survival than those without amplification, the two survival curves were not significantly different ($P > 0.05$; generalised Wilcoxon test).

Survival rate was also compared for each group, classified on the basis of pathological phenotype. In the moderate and poorly differentiated groups, no significant differences were observed. In contrast, in well differentiated group, all 3 patients whose carcinomas had gene amplification, died within 2 years, while more than half the patients without gene amplification survived for 5 years (Table 3).

Figure 3 illustrates the survival rates of patients with or without lymph node metastasis. In the positive LN group, the two survival curves were not significantly different between amplified and non-amplified cases. However, in the negative LN group, the two survival curves were significantly different ($P < 0.01$; generalised Wilcoxon test).

DISCUSSION

The present analysis of co-association between gene amplification at chromosome 11q13 and histopathological diagnosis or prognosis of oesophageal carcinoma patients revealed that of 90 cases examined, 22 (24%) to have an amplified *int-2* locus. The reason for the lower frequency than that reported previously [6] is unclear.

No significant correlation between gene amplification and histological type or metastasis to lymph nodes was observed. However, our data do indicate that gene amplification might

Table 3. Survival rate for each histological type

Histological type	Amplification	2-year survival	5-year survival
Well	Non-amplified	12/16 ($P = 0.036$)*	10/16 ($P = 0.087$)*
	Amplified	0/3	0/3
Moderately	Non-amplified	11/23 (NS)	6/23 (NS)
	Amplified	3/9	2/9
Poorly + undifferentiated	Non-amplified	4/9 (NS)	1/9 (NS)
	Amplified	1/3	0/3

Non-curative cases and direct operative deaths were excluded.
* P values were calculated by Fisher's exact test.

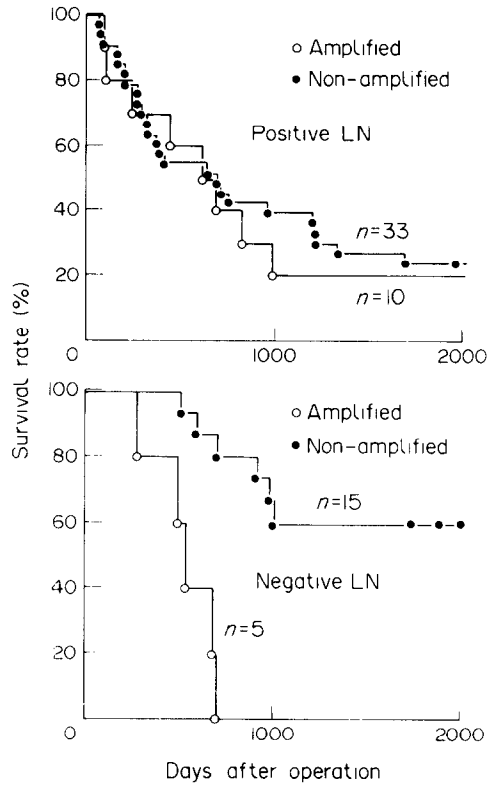

Fig. 3. Association between *int*-2 amplification, lymph node metastasis and survival rate. Cases with non-curative resection and direct operative deaths were excluded. In the positive LN group, the two survival curves are not significantly different. In contrast, in the negative LN group, the two survival curves are significantly different ($P < 0.01$; generalised Wilcoxon test).

be a valuable prognostic factor for patients with oesophageal carcinomas, since the positive gene amplification group tended to have a poorer prognosis.

Although no statistically significant difference was observed in the moderate and poorly differentiated groups, the difference in 2-year survival rate observed between amplified and non-amplified cases in the well differentiated group was significant. Two reasons for this specificity may be speculated. One is that in the moderate and poorly differentiated groups, gene amplification might be masked by contamination by large number of stromal cells due to the invasive character of these tumours. The other reason is that other genetic changes accumulate in moderate or poorly differentiated types which might exert more important influence on patient prognosis.

Negative lymph node group with gene amplification had a poorer prognosis than those without gene amplification and distant metastasis (liver, lung, paraaortic lymph node in the abdomen) were detected in 3 of 5 cases with gene amplification who had no lymph node metastasis at operation. These results suggest that amplification of the *int*-2 locus might be associated with metastasis through the blood vessels, but not via the lymph ducts.

In conclusion our results indicate the possibility that chromosome 11q13 gene amplification may be a useful prognostic factor for patients with oesophageal carcinomas, particularly for those with well differentiated types or without lymph node metastasis.

1. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984, **224**, 1121–1124.
2. Seeger RC, Brodeur GM, Sather H, *et al.* Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985, **313**, 1111–1116.
3. Slamon DJ, Clark CM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: Correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. *Science* 1987, **235**, 177–182.
4. Slamon DJ, Godolphin W, Jones LA, *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
5. Adnane J, Gaudray P, Simon MP, Simony-Lafontaine J, Jeanteur P, Theillet C. Proto-oncogene amplification and human breast tumor phenotype. *Cancer Res* 1989, **4**, 1389–1395.
6. Tsuda T, Tahara E, Kajiyama G, Sakamoto H, Terada M, Sugimura T. High incidence of hst-1 and int-2 genes in human esophageal carcinomas. *Cancer Res* 1989, **49**, 5505–5508.
7. Yoshida M, Wada M, Satoh H, *et al.* Human HST1(HSTF1) gene maps to chromosome band 11q13 and coamplifies with the INT2 gene in human cancer. *Proc Natl Acad Sci USA* 1988, **85**, 4861–4864.
8. Somers KD, Cartwright SL, Schecter GL. Amplification of the *int*-2 gene in human head and neck squamous cell carcinomas. *Oncogene* 1990, 5, 915–920.
9. Goelz SE, Hamilton SR, Vogelstein B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem Biophys Res Comm* 1985, **130**, 118–126.
10. Feinberg A, Vogelstein B. A technique for radiolabeling DNA restrictive endonuclease fragments to high specific radioactivity. *Anal Biochem* 1984, **137**, 266–267.
11. Nakamura Y, Carlson M, Krapcho K, Kanamori M, White R. New approach for isolation of VNTR markers. *Am J Hum Genet* 1988, **43**, 854–859.
12. Cox DR. *Analysis of Survival Data.* London, Chapman and Hall, 1984.
13. Kaplan EL, Meier P. Nonparametric estimation for incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
14. Everitt BS. *The Analysis of Contingency Tables.* London, Chapman and Hall, 1980.