Oncology 43: 36–39 (1986)

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0030–2414/86/0431–0036 $ 2.75/0

Comparative Study of Harvey-*RAS* Oncogene Expression with Conventional Clinicopathologic Parameters of Breast Cancer

Niki J. Agnantis[a], *Panayota Parissi*[a], *Demetrios Anagnostakis*[a], *Demetrios A. Spandidos*[b, 1]

[a] Hellenic Anticancer Institute, Athens, Greece; [b] Beatson Institute for Cancer Research, Bearsden, Glasgow, UK

Key Words. Oncogene expression · Breast cancer

Abstract. We have previously examined the expression of the Harvey-*ras* (Ha-*ras*) oncogene related transcripts in human malignant breast tumors and in their respective normal tissue [*Spandidos and Agnantis*. Anticancer Res. *4:* 269–272, 1984]. Our results revealed a significant elevation of Ha-*ras* transcripts in malignant compared to normal tissue. In the present follow-up study we have examined the relationship of Ha-*ras* oncogene expression to the various clinicopathological parameters of these tumors. Although elevated expression was observed in all breast tumors as compared to their respective normal breast tissue there was no correlation with the tumor stage as defined by the TNM system. However, several correlations between Ha-*ras* oncogene expression and histologic parameters were found and comparisons of the mean value of Ha-*ras* oncogene expression with the parameters examined showed the following: the stellate tumor margin and the larger tumor size had the lowest mean value; the infiltrating duct histologic type had the highest mean value; the mean value was lower in the presence of lymphocytic infiltration in the tumor; a higher mean value was obtained in cases with lymph node metastases.

Introduction

Several transforming cellular oncogenes have been isolated from a variety of human tumors and their characterization has contributed significantly to our understanding of cancer at the molecular level [for a review see ref. 1]. It has also been found that some of these oncogenes are expressed at higher levels in tumor as compared to normal tissue [2–5]. Although study of both qualitative (structural mutation) and quantitative (altered levels) aspects of oncogene expression in tumor cells may well shed some light on mechanisms of cell transformation [6] we still know very little concerning the abnormal expression of these genes in relation to variations in the clinical behavior of the tumors.

Current staging methods include evaluation of tumor histology and size, stages of axillary lymph nodes and estrogen receptor status of the primary tumor [7]. However, these parameters comprise only an approximate prognostic index of clinical behavior and in particular of the metastatic potential of the tumor.

In the present study we have examined the relationship of Harvey-*ras* (Ha-*ras*) oncogene expression in malignant and normal breast tissue to the various clinicopathological parameters of these tumors.

Patients and Methods

All cases were female patients who underwent treatment for breast cancer at the Breast Clinic of the Hellenic Anticancer Institute in Athens, Greece. The tumor size in all cases was bigger than

[1] We thank Dr. *I. B. Kerr* for critical appraisal of the manuscript.

Table I. Expression of human Ha-*ras* oncogenes in malignant tumors and normal breast tissue studied by RNA spot hybridization analysis compared with the pTNM

Patient No.	pTNM[1]	Tissue histology (malignant/normal)		
		Ha-*ras*[2]		rRNA[2]
1	T_{1a} N_{1a} M_0	11	10	0.9
2	T_{1a} N_{1b} M_0	14		1.1
3	T_{1a} N_{1b} M_0	5.8		1.1
4	T_{1a} N_{1b} M_0	9.3		1.0
5	T_{2a} N_0 M_0	2.5	11.4	1.3
6	T_{2a} N_{1a} M_0	24		1.0
7	T_{2a} N_{1a} M_0	3.0		1.0
8	T_{2a} N_{1a} M_0	4.1		1.1
9	T_{2a} N_{1a} M_0	10		1.1
10	T_{2a} N_{1b} M_0	14		1.0
11	T_{2a} N_{1b} M_0	14		0.9
12	T_{2a} N_{1b} M_0	28		1.0
13	T_{2a} N_{1b} M_0	7.3		1.0
14	T_{2a} N_{1b} M_0	7.8		1.0
15	T_{2a} N_{1b} M_0	13		0.9
16	T_{2a} N_{1b} M_0	7.7		0.9
17	T_{2a} N_{1b} M_0	9.3		1.1
18	T_{2a} N_2 M_0	15		1.0
19	rT_{3a} N_0 M_0	14	7.3	1.1
20	T_3N_0 M_0	2.8		1.2
21	T_{3a} N_0 M_0	5.3		0.8
22	T_{4b} N_0 M_0	2.6	4.7	1.1
23	T_{4b} N_0 M_0	4.3		1.3
24	T_{4b} N_{1b} M_0	7.3		1.1

[1] All tissue specimens were obtained from patients operated on for breast cancer at the Breast Clinic of the Hellenic Anticancer Institute, Athens, Greece. During the frozen section time a slab of tumor tissue and of normal tissue from the breast biopsy material was stored at −70 °C until RNA was isolated. pTNM was defined according to UICC classification.

[2] The autoradiographs were scanned and the concentrations of Ha-*ras* or rRNA specific RNAs were determined at arbitrary units for each probe. The ratios of RNAs in malignant/normal tissue from the same patient are given.

1 cm. Tissue specimens were obtained when frozen section examination was being undertaken from 24 cancer cases including a specimen of normal breast tissue at a distance of at least 2 cm from the primary tumor. All the material was stored at −70 °C until RNA was isolated as previously described [3]. Expression of the Ha-*ras* oncogene was studied using spot hybridization [8] and Northern blot [9] hybridization analyses [5]. Quantitation of the Ha-*ras* related transcripts was obtained by the spot hybridization analysis after autoradiographic scanning as previously described [8].

Histopathological evaluation was based on paraffin-embedded sections stained with hematoxylin and eosin.

Results

The relationship between pTNM (tumor staging) and the relative expression of human HA-*ras* related transcripts in the 24 cases examined is shown in table I. Whereas between stage T1 and T2 of the disease there is a small increase of the mean value, in the next two stages the mean value is decreased.

The relationship of the expression of human Ha-*ras* oncogenes to patient age, the different histologic parameters of the mastectomy specimen and its gross characteristics are shown in table II. Comparisons of the mean value of Ha-*ras* oncogene expression with the parameters examined shows that: (1) the range between 50 and 60 years of age showed the higher mean value; (2) the stellate tumor margin and the bigger tumor size showed the lowest mean value; (3) the infiltrating duct histologic type had the highest mean value; (4) tumor grade III had a lower mean value in comparison with grade II. There were no grade I cases in our study; (5) whenever lymphocytic infiltration was present in the tumor, the mean value was lower, and (6) cases with lymph node metastases showed a higher mean value.

Discussion

Our results indicate that although elevated expression was observed in all breast tumors as compared to their respective normal breast tissue there was no correlation with the tumor stage as defined by the TNM system. This may not be surprising if there is indeed a heterogeneity of cells with metastatic potential in the primary tumor as has been suggested [10]. However, as shown in table II and pointed out in the 'Results' section several correlations between Ha-*ras* oncogene expression and histologic parameters can be found. Despite the trends documented in the 'Results' section there appears to be no very clear correlation between the elevation in Ha-*ras* expression which we have observed and the conventional staging criteria, nor does it seem likely that variation in Ha-*ras* expression correlates with probable clinical outcome. Nonetheless, further study of both qualitative and quantitative aspects of oncogene expression [1, 6] may still be important in fully defining the behavior of these tumors.

Table II. Relationship of the expression of human Ha-*ras* oncogenes with patient age, the different histologic parameters of the mastectomy specimen and its gross characteristics.

Patient No.	Patient age	Topography	Tumor margin			Tumor size cm	Cancer type	Tumor grade	Lym. Inf.
			Ci.	ID	St.				
1	58	CUH		+		1.5	IFD + tubular	II	+
2	58	COH			+	1.5	In SD + L + IFL	II	+
3	39	LI			+	1.7	In SD + IFD n.o.s.	III	+
4	56	CUH			+	1.5	IFD + L + In SD	III	+
5	45	UO			+	4.2	IFD n.o.s.	III	–
6	52	CIH	+			2	In SD IFD n.o.s.	II	–
7	79	LI			+	4	IFD mucinous	II	+
8	42	UI			+	3	In SD + IFD n.o.s.	II	+
9	64	UO		+		4.2	IFD n.o.s. + tubular	II	–
10	65	CIH		+		2.5	IFD n.o.s. + apocrine	III	–
11	33	COH		+		3	IFD + L + In SD	II	+
12	60	Central		+		2.7	In SD + IFD mucinous	II	–
13	66	UO			+	3.5	In SD + L + IFD n.o.s. + L	III	+
14	50	UO			+	2.5	In SL + IFL	II	–
15	55	UO			+	2.5	IFD n.o.s.	II	–
16	70	CUH			+	2.5	IFD n.o.s.	II	–
17	52	CLH			+	2	In SD + IFD n.o.s.	II	+
18	75	UO		+		3.2	IFD n.o.s.	III	+
19	62	UO	+			6	IFL	II	–
20	52	LO		+		6.5	IFD n.o.s. + medullary	III	+
21	23	UH			+	8.5	In SD + IFD n.o.s.	III	+
22	64	CUH		+		14	IFD n.o.s. + medullary	III	+
23	72	LI			+	4	IFD n.o.s.	II	+
24	72	UO	+			4.5	IFD n.o.s. + In SD + Paget	III	+

Ci. = circumscribed
ID = ill-defined
St. = stellate
Inv. = invasion
Ves. = vessel
Lym. = lymphocytic
Inf. = infiltration
In S. = in situ
Sec. Micr. = secondary microscopic
CD = cystic disease
LNM = lymph node metastases
UO = upper outer
UI = upper inner
C = center
LO = lower outer
LI = lower inner
In SL = in situ lobular
In SD = in situ duct
IFD n.o.s. = infiltrating duct, not otherwise specified
IFL = infiltrating lobular
Q = quadrant
H = half

[1] Total number of lymph nodes/lymph nodes and metastases.

Ves. Inv.	Skin Inv.	Sec. foci	Micr.	Associated CD		LMM per level[1]		
		In S.	Inv.	simple	com-plex	I	II	III
+	−	+ LOQ	−	+	−	4/0		
−	−	−	−	+	−	4/2	7/1	3/1
tumorectomy						1/1		
+	−	+ UOQ	+	−	−	6/6	3/3	3/2
tumorectomy								
−	−	−	−	−	−	6/0	4/0	
−	−	−	−	−	+	4/0		
−	−	−	−	+	−	6/0	5/0	5/0
−	−	−	−	−	−	11/0	5/0	2/0
−	−	−	−	+	−	9/2	6/0	
−	−	−	−	−	+	11/4	8/2	4/1
−	−	−	−	−	−	5/1	4/0	2/0
−	−	−	−	+		5/2	3/0	5/0
−	−	−	−	+		7/4	2/0	1/0
−	−	−	+ LOQ	−	−	18/14	11/10	8/5
−	−	−	−	−	−	9/1	3/0	1/0
−	−	−	−	−	+	7/2		
−	−	−	−	+	−	6/6	3/3	
−	local recurrence							
tumorectomy								
tumorectomy								
−	+	−	−	+	−	simple mastectomy		
	+ tumorectomy							
−	+	+ U	+ IQ	−	−	3/3		

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Dr. N.J. Agnantis,
Hellenic Anticancer Institute,
171 Alexandras Avenue,
Athens (Greece)