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Human Malignant Tumours of the Breast, as Compared to their Respective Normal Tissue, Have Elevated Expression of the Harvey *ras* Oncogene

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Abstract. *Analysis of expression of Harvey-ras related oncogenes in human malignant breast tumours and in their respective normal tissue has revealed a significant elevation of Harvey-ras transcripts in malignant as compared to normal tissue. In contrast, the sis oncogene is not expressed significantly in either type of malignant or normal tissue. These results suggest that Harvey-ras oncogene may be specifically activated in the development of human malignant breast tumours.*

Several recent studies have described expression of cellular oncogenes analogous to retroviral oncogenes in fresh and culture derived human hematopoietic neoplastic cell types and established cell lines from a variety of tumours (1-3). Similar studies have not been reported on human solid tumours. It has been found that the cellular homologous of several retroviral oncogenes exhibit tissue-specific patterns of transcription (1,2,4). Moreover expression of cellular oncogenes during mouse development (5,6) and liver regeneration (7) has lent further support to the hypothesis that cellular oncogenes play a role in normal development. Inappropriate expression of these genes could be important in the development of the malignant phenotype of tumour cells. In this study we have examined the presence of RNA transcripts from the Ha-*ras* and *sis* human oncogene families in malignant and normal breast tissues. We have found an elevated expression of the human Harvey-*ras* oncogene in malignant tumours as compared to their respective normal breast tissue.

Patients and Methods

All cases in this study involved female patients who were treated for breast cancer at the Breast Clinic of the Hellenic Anticancer Institute of Athens, Greece.

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Tissue specimens were stored at −70°C. These were subsequently pulverized under liquid nitrogen and RNA was extracted as previously described (8). Briefly, the tissues were homogenized in guanidine-HCl buffer (8.0M guanidine HCl, 20 mM sodium acetate, 50 mM EDTA, 5% 6-mercaptoethanol, pH 7.0). Cell lysates were made 2% with SDS and heated at 65°C for 2 min. After vortexing for 30 sec, 5 ml of the cell lysate were placed on a 3 ml cushion of CsCl solution (5.7 M CsCl, 50 mM EDTA pH 8.0) and centrifuged for 48 h at 40K rpm at 15°C in a 10 × 10 Ti rotor. The RNA pellet was resuspended in 2.0 M $LiCl_2$, 4.0 M urea and left at 4° C for 24 h. RNA was pelleted at 10K rpm for 15 min in a Sorval centrifuge, resuspended in 0.1 × MOPS buffer (1 × MOPS=20mM Na MOPS, 5 mM sodium acetate, 1 mM EDTA, pH 7.0) and dialyzed in the same buffer for 2 h before lyophilizing. Before each experiment the quality of RNA preparations was examined by formaldehyde agarose gel electrophoresis, followed by ethidium bromide staining, transfer to nitrocellulose and hybridization to DNA probes (see below). Ten µg of total cell RNA was spotted per dot as described (9). Hybridizations were performed in 5 × SSC, 50% formamide for 24 h at 42°C with 20 ng/ml probe as described (10) using 2 × Denhardt's solution (11). ^{32}P-labelled DNA probes with specific activities 2-3 × 10^8 cpm/µg DNA were made by nick-translation. The nitrocellulose sheets were washed in 0.1 × SSC at 60°C and exposed to hypersensitized X-ray films at -70°C (12). The filters were hybridized sequentially with ^{32}P-labelled nick-translated pT24C3 (13), pL335 (14) or pHR28 (A Sproul and G Birnie, unpublished results) recombinant probes carrying the cellular Ha-***ras*1**, cellular ***sis*** and 28S ribosomal human DNA sequences respectively.

Results

RNA Spot Hybridization Analysis. First, we wished to determine the relative levels of human Harvey-***ras*** and ***sis*** transcripts in total cell RNA made from malignant breast tumours and respective normal tissue using an RNA spot hybridization assay (9). The intensities of the autoradiographic spots were quantitated using densitometric scanning as previously described (9). Preliminary analysis with RNA from six patients was carried out to determine conditions for probe excess during hybridization. The results with RNA from patient No. 6 are shown in Fig. 1. Probe excess was confirmed by obtaining a linear autoradiographic response to serial dilutions of RNA. Results of the RNA spot hybridization analysis from twelve patients are shown in Fig. 2 and Table I. The following observations can be made from these results: First, transcripts from the human Ha-***ras*** related oncogenes

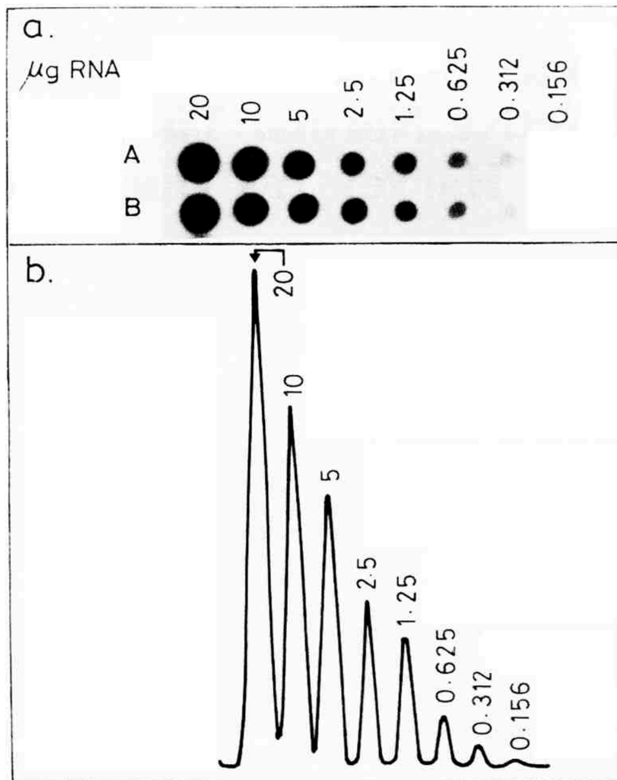

Fig. 1. *Determination by the RNA spot hybridization asay of Ha-***ras** *oncogene RNA transcripts in total cell RNA isolated from breast carcinoma tissue of patient No 6. Extraction of RNA cells and spotting onto nitrocellulose is described in Patients and Methods. Duplicate (A and B) samples containing serial 2-fold dilutions of RNA were spotted. The filter was hybridized with* ^{32}*P-labelled pT24C3 DNA probe. The autoradiograph is shown in (a) and the scanning across A in (b).*

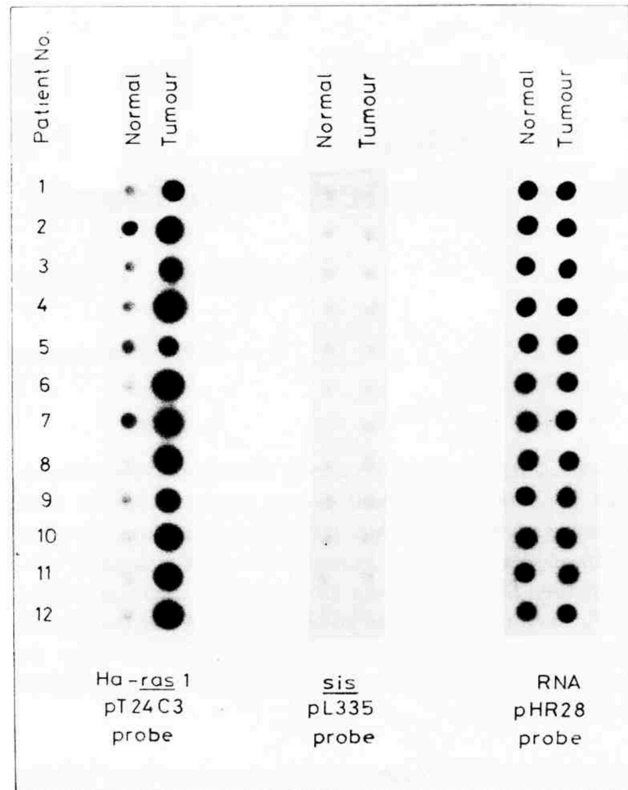

Fig. 2. *RNA spot hybridization analysis of Ha-***ras** *and* **sis** *oncogené expression in human cells. M=malignant breast carcinoma. N=normal breast tissue.*

could easily be detected in most malignant breast tissues but are less detectable in normal tissue. The human ***sis*** oncogene is expressed at very low levels in both types of tissue examined. Second, the amount of human Ha-***ras*** specific RNAs varied in tissues from different patients whereas little variation was observed in ***sis*** RNA levels. The relative levels of Ha-***ras*** transcripts varied between 2.5 (patient No. 5) to 15 (patient No. 6) × higher in malignant tissues as compared to normal tissue from the same patient. We also checked the relative amount of RNA from each sample spotted onto nitrocellulose, as an additional control, by hybridizing the filter with pHR28 a human ribosomal RNA probe. As shown in the autoradiograph (Fig. 2C) and confirmed by scanning the spots (Table I) there is no substantial difference in the amount of ribosomal RNA present in these samples.

Northern Blot Hybridization Analysis. The sizes of Ha-***ras*** related transcripts expressed in malignant and normal human breast tissues was investigated using Northern blot hybridization analysis. A Ha-***ras***l probe, pT24C3 recombinant (13) revealed the presence of one main band at about 1.2 Kb in total cellular RNA from malignant tissues but barely detectable in normal tissue (Fig. 3). These results confirm and extend the spot hybridization analyses. The nature of these transcripts was further investigated by isolating poly A^+ RNA and Northern blot hybridization analysis. As shown in Fig. 4 Ha-***ras*** related transcripts of 1.2 Kb in size were again found to be elevated in malignant as compared to normal breast tissues. Northern blot hybridization analysis of total or poly A^+ RNA using a ***sis*** probe has failed to show any discrete RNA transcripts.

Discussion

Expression of ***ras*** related transcripts in human cells has been studied in some detail over the past few years. It has been found that a 1.2 Kb Ha-***ras*** related transcripts are present in the T24 human bladder carcinoma cell line (13,15) and two approximately 6.0 Kb Ha-***ras*** related transcripts in human haematopoietic cell lines (2). Using an N-***ras*** probe 3 different sized transcripts of 5.8, 2.2 and 1.5 Kb have been found in normal human fibroblasts and established human cell lines (16). It was deduced that the 2.2 Kb transcript is related to

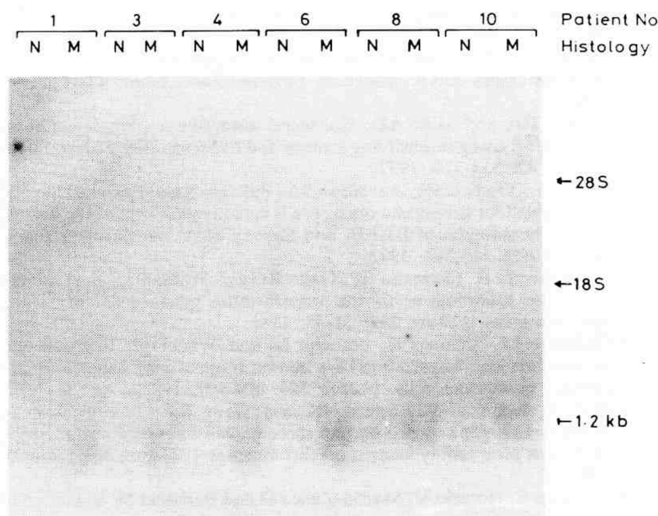

Fig. 3. *Northern blot hybridization analysis of transcripts related to human Ha-***ras** *oncogenes in RNAs from samples of normal (N) and malignant (M) breast tissues. Twenty μg of total cell RNA per lane were analysed in 1.0% agarose-formaldehyde-containing gels, blotted onto nitrocellose and hybridized with the pT24C3 recombinant carrying the human Ha-***ras1** *oncogene probe.*

the N-***ras*** orcogene (16). Our results demonstrate that cellular sequences related to the Ha-***ras***1 oncogene are transcribed in human malignant and normal breast tissue. The size of these transcripts is approximately 1.2 Kb. The demonstration that Ha-***ras*** related transcripts are elevated in malignant as compared to normal breast tissues shows that the expression

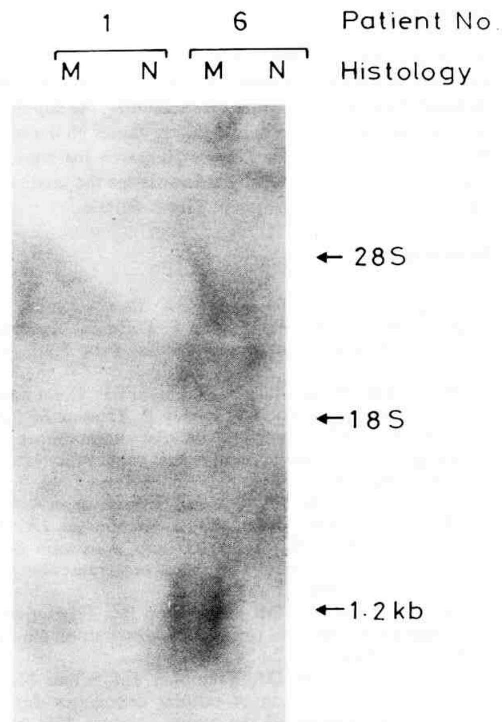

Fig 4. *Northern blot hybridization analysis of poly* A^+ *RNA transcripts related to human Ha-***ras** *oncogene mRNAs of normal (N) and malignant (M) breast tissues. Poly* A^+ *RNA were analysed in 1.0% agarose-formaldehyde-containing gels, blotted onto nitrocellulose and hybridized with the pT24C3 probe.*

Table I. *Expression of human Ha-***ras** *oncogenes in malignant tumours and normal tissue of the breast studied by RNA spot hybridization analysis*[a].

Patient No	Tissue histology[b] (Malignant/Normal)	
	Ha-ras	rRNA
1	5.3	0.8
2	4.3	1.3
3	5.8	1.1
4	9.3	1.0
5	2.5	1.3
6	15	1.0
7	4.1	1.1
8	13	0.9
9	7.7	0.9
10	10	1.1
11	9.3	1.1
12	7.3	1.1

[a] The autoradiographs (Fig. 2) were scanned and the concentrations of Ha-**ras** or rRNA specific RNAs were determined at arbitrary units for each probe. The ratios of RNAs malignant/normal tissue from the same patient are given.
[b] All tissue specimens were obtained from patients in the Hellenic Anticancer Institute, Athens, Greece. Histological examination was carried out in part of the specimen and the remaining tissue was stored at -70°C until RNA was isolated.

of this gene is associated with the transformed state of the cells and may be important in the process of carcinogenesis.

Activation of Ha-***ras*** related genes in chemically induced mammary carcinomas of the rats has recently been demonstrated (17). It has been shown that at least in some cases the Ha-***ras*** gene was activated by a point mutation in amino acid 12 of the p21 Ha-***ras*** gene product. However, in mammary carcinomas induced in C3H mice by the mouse mammary tumour virus (MMTV) elevated transcription of a putative oncogene called int-1, caused by proviral insertion seems to be involved in the induction of these tumours (18). Similar studies of carcinogen induced mouse skin papillomata (19) and human premalignant and malignant tumours of the colorectum (20) have shown elevated Ha-***ras*** oncogene expression.

Oncogene amplification has been shown to be responsible for elevated expression of cellular oncogenes such as ***myc*** and Kl-***ras*** (23) in some human cell lines. Whether gene amplification plays a role in the malignant breast tumours described here remains to be seen. Such studies as well as transfection studies using DNA from these tumours are in progress in our laboratory.

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