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Molecular-Genetic Analysis of *myc* and *c*-Ha-*ras* Proto-oncogene Alterations in Human Carcinoma

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A. Introduction

The results of recent studies on the molecular biology of cancer suggest the abnormal expression of cellular proto-oncogenes in the process of carcinogenesis [1]. The activation of proto-oncogenes, i.e., their conversion into cellular oncogenes, is associated with gene structural alterations and mutations resulting in the unregulated production of oncoproteins, matched by deranged biological function of the latter. Recently, "mixed" variants of *ras*-family gene activation were established. They involve both amplification and point mutations in one of the "hot" codons [2, 3].

The present study deals with the analysis of alterations of *v-myc*-related sequences and *c*-Ha-*ras* proto-oncogenes occurring in primary tumors, metastases, homologous with cancer-intact tissues and peripheral blood leukocytes of cancer patients.

B. Materials and Methods

Genomic DNAs from tumors of the breast, ovary, lung, thyroid, colon, and stomach and homologous normal tissues were prepared as described [4], digested with restriction endonucleases, electrophoresed in 0.8% agarose gels, denatured, and transferred to nitrocellulose filters [5]. Filters were hybridized with ^{32}P-labeled (nick-translated) *v-myc* [6] or *hu-c*-Ha-*ras* [7] probes under stringent conditions, washed, dried, and exposed to ORWO film.

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C. Results and Discussion

Amplification of *myc*-specific sequences was observed in seven of 21 DNA samples obtained from human breast carcinomas (Table 1). Three DNAs (BrC2, BrC1, BrC23) contained additional *myc*-bands, besides a 12.0-kbp *Hind*III-*c-myc* germline, and were characterized by a high degree of amplification (20- and 100- to 150-fold) that may be regarded as *c-myc* gene rearrangement (Fig. 1) [8].

Analysis of DNAs from 11 thyroid carcinomas revealed 5- to 10- and 60- to 80-fold *c-myc* amplification in two samples (ThC3, ThC6). In the DNA with the higher degree of amplification we saw additional amplified *myc*-fragments of the same size as those in breast carcinomas (Fig. 1, Table 1).

Extra copies of the *c-Ha-ras* gene were registered in the samples of breast and thyroid with superamplification of *c-myc* (Fig. 2).

DNAs of 12 patients with ovarian cancer were analyzed, and there was *c-myc* amplification in primary bilateral and metastatic tumors of three of them (OvC7, OvC11, OvC16; Fig. 3, Table 1). *C-myc* alterations can be observed in poorly differentiated tumors characterized by an aggressive clinical course. An increase in the copy number of *myc*-related sequences has been also identified in the DNAs of peripheral blood leukocytes from two ovarian cancer patients (Fig. 3) [9].

The analysis of ten lung tumors showed *c-myc* amplification in two squamous carcinomas. In the only undifferentiated large-cell carcinoma tested amplification of maybe

Table 1. Amplification of *myc*-related sequences in human tumors of different localizations and histotypes

Tumor localization	Tumor histotype	Number of tested tumors/ Number of tumors with amplification	Copy number	Presence of rearrangements[a]
Breast	Ductal invasive carcinoma	17/6	5– 10	
			100–150	+
	Mucinous carcinoma	1/0		
	Medullar carcinoma	1/0		
	Tubular carcinoma	1/1	100–150	+
	Papillary carcinoma	1/0		
	Lobular fibroadenomatosis	1/0		
Total		22/7		
Ovary	Serous cystadenocarcinoma	13/4	5– 10	
	Serous cystadenocarcinoma metastasis	5/1	5– 10	
	Undifferentiated cystadenocarcinoma	6/2	10– 15	
	Undifferentiated cystadenocarcinoma metastasis	4/1	5– 10	
	Mucinous borderline cystadenoma	1/0		
	Endometrioid adenomyoma	1/0		
	Dermoid tumor	1/0		
	Thecoma	1/0		
Total		32/8		
Lung	Keratinizing squamous cell carcinoma	6/1	10– 15	
	Non-keratinizing squamous cell carcinoma	3/1	10– 15	
	Undifferentiated large cell carcinoma	1/1	15– 20	+
Total		10/3		
Thyroid	Papillary carcinoma	9/2	5– 10	
			60– 80	+
	Follicular carcinoma	1/0		
	Undifferentiated spindle-cell carcinoma metastasis	1/0		
	Papillary carcinoma metastasis	1/0		
	Follicular adenoma	1/0		
Total		13/2		
Colon	Adenocarcinoma	11/1	40– 50	+
	Signet-cell carcinoma	1/0		
	Adenoma	6/0		
Total		18/1		
Stomach	Adenocarcinoma	9/0		
	Signet-cell carcinoma	1/0		
	Undifferentiated carcinoma	4/1	15– 25	+
Total		14/1		
Total		110/22		

[a] Presence of *myc*-related sequences containing restriction fragments different from the correspond[illegible] ones for *c-myc* locus

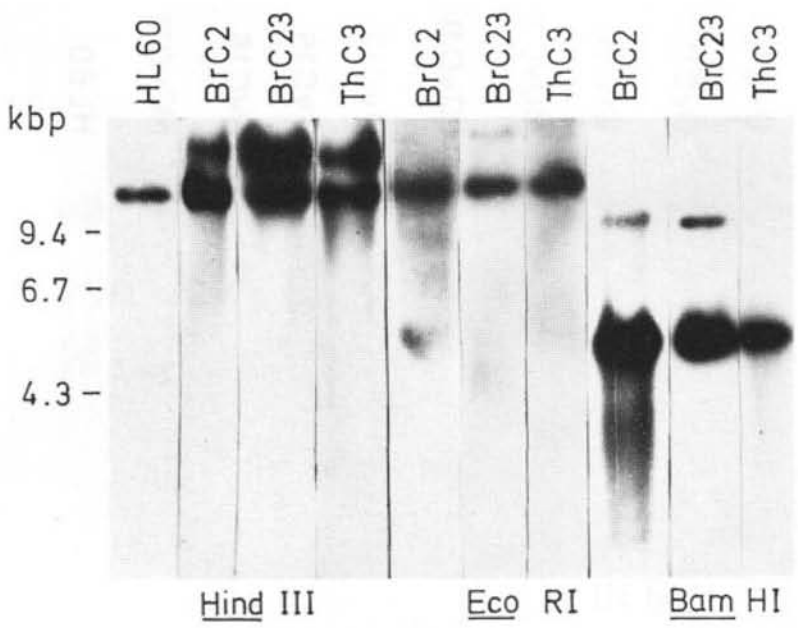

Fig. 1. Restriction analysis of *myc*-related sequences in breast (*BrC*) and thyroid (*ThC*) tumors

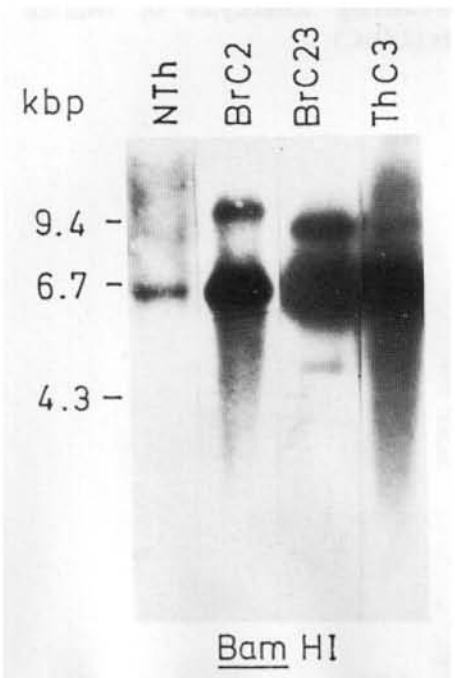

Fig. 2. Restriction analysis of *c-Ha-ras* proto-oncogenes in breast (*BrC*) and thyroid (*ThC*) tumors as compared with normal thyroid tissue (*NTh*)

L-myc (6.6-kbp *Eco*RI-fragment) could be seen. All three tumors were highly metastatic and aggressive.

Myc-amplification is rather frequent in breast, ovarian, and lung tumors (seven of 21, eight of 28, three of ten respectively) and less so in thyroid (two of 12), stomach (one of 14), and colorectal (one of 12) malignancies (Fig. 4, Table 1).

We failed to detect amplification of *myc*-specific sequences in 13 DNA samples from benign tumors of the colon, ovary, thyroid, and breast (Table 1).

Forty-five samples of DNA obtained from normal lung tissue and gastric and colonic mucosa were examined for any alterations of *myc* proto-oncogenes developing in uninvolved tissues homologous with cancer tissues. Extra copies of *myc*-related sequences were identified in three of them. In two cases (lung and gastric tissues) the degree of amplification and length of amplified *myc*-fragments corresponded with those observed in tumors of the same patients. In one instance we were able to identify the amplification of *myc*-specific sequences in the DNA of colonic mucosa only, and there was none in the colon carcinoma of this patient (Fig. 5) [10].

The latter findings, as well as evidence of *myc* gene amplification in blood-circulating leukocytes, corroborate the concept of cancer as a general disease.

Summarizing the results obtained, we can conclude that the phenomenon of *myc*-gene family amplification appears to be common in human primary and metastatic tumors (22 of 98). Our observations are in line with those of J. Yokota and colleagues [11], who detected the above phenomenon in 11% of epithelial tumors tested. Perhaps the somewhat higher frequency of *myc*-gene amplification in our study can be explained by the application of a *v-myc*-specific probe for detecting a *c-myc* germline containing restriction fragments as well as bands characteristic for other genes of the *myc* family or rearranged *c-myc*.

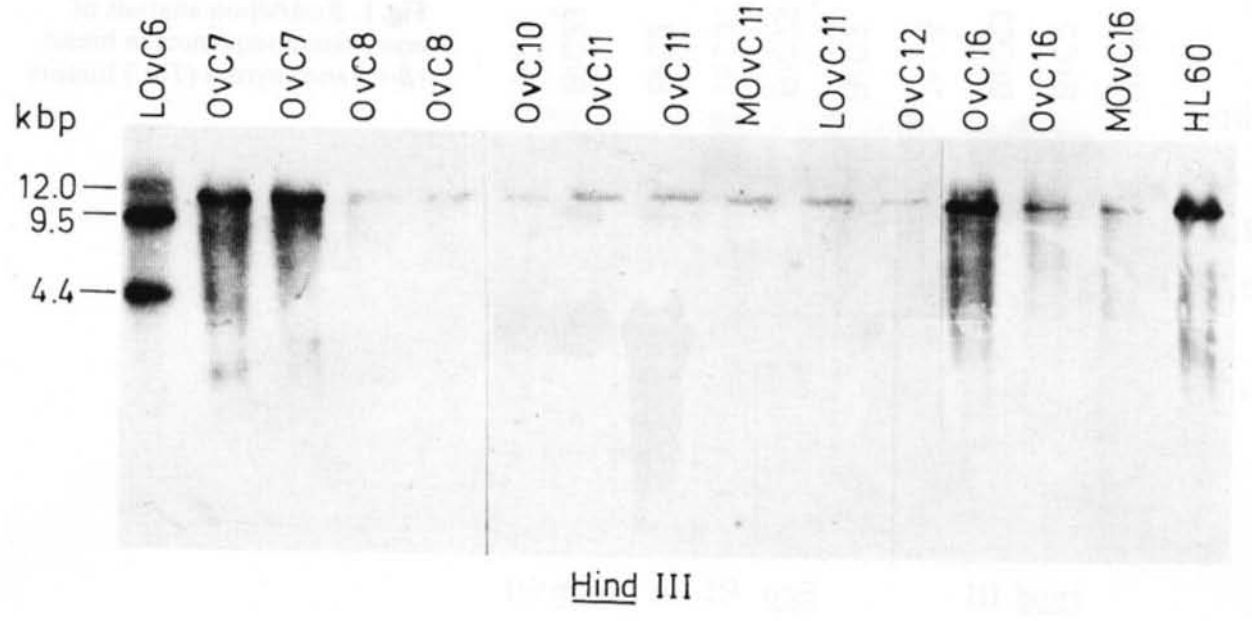

Fig. 3. Restriction analysis of *myc*-related sequences in ovarian tumors (*OvC*), metastatic tumors of patients with ovarian cancer (*MOvC*), and blood-circulating leukocytes of ovarian cancer patients (*LOvC*)

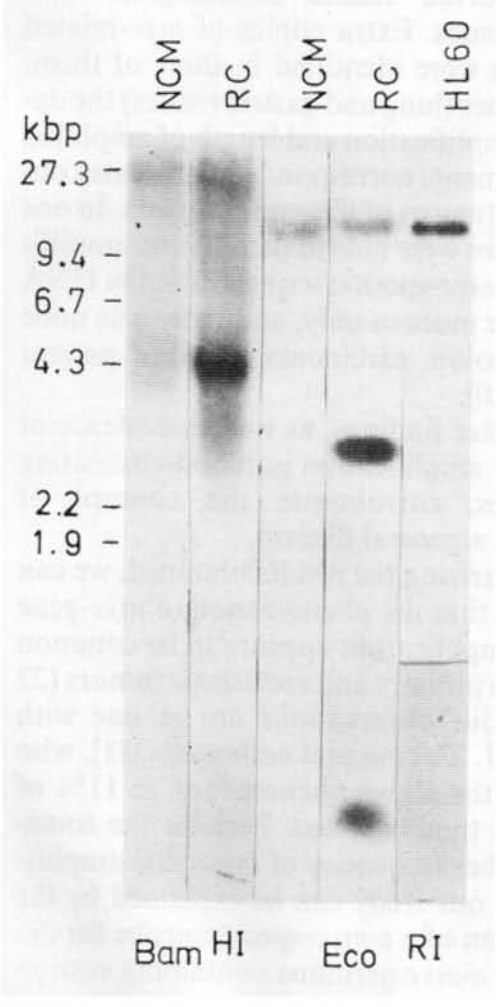

Fig. 4. Restriction analysis of *myc*-related sequences in rectal tumor (RC) and normal colonic mucosa (NCM) of same patient

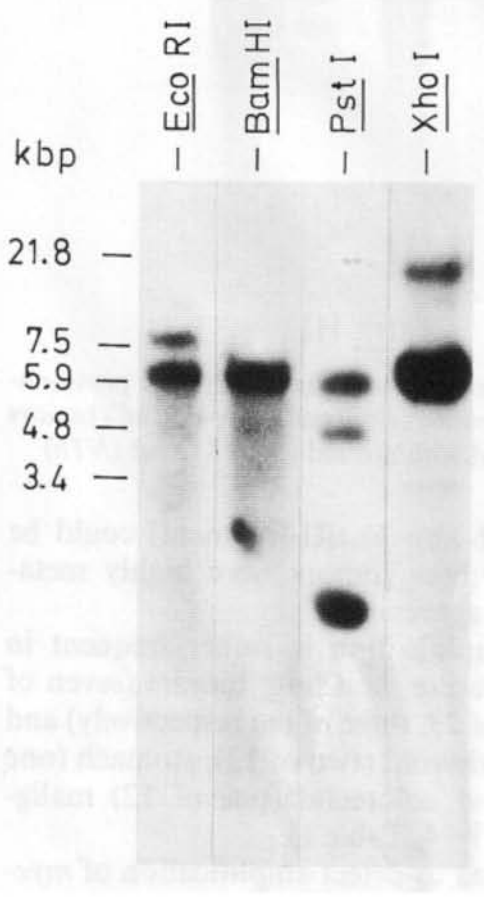

Fig. 5. Restriction analysis of *myc*-related sequences in colonic mucosa of a patient with colonic carcinoma

The finding of abnormal (amplified) *c-myc* and *c*-Ha-*ras* proto-oncogenes in a few breast and thyroid tumors confirms the hypothesis that these two genes somehow cooperate in the process of carcinogenesis.

In most cases *myc*-gene amplification was matched by poor cell differentiation and an aggressive clinical course of the tumor.

The study revealed that *myc* and *c*-Ha-*ras* proto-oncogene activation contributes to tumorigenesis through the mechanism of gene amplification and oncogene cooperation.

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