REVIEW ARTICLE

Human cancer and cellular oncogenes

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Introduction

Studies on the involvement of cellular oncogenes (c-onc, proto-oncogene) in human cancer have progressed rapidly since 1979 when Weinberg and his group [1] and subsequently other workers [2–4] succeeded in the transformation *in vitro* of NIH3T3 cells (a mouse fibroblast cell line) by transfection with DNA isolated from various tumour cell lines. Establishment of an *in vitro* assay system to screen for active transforming genes was a breakthrough in molecular biological studies on human cancer. The active transforming genes detected in this way were subsequently cloned using recombinant DNA techniques, and their nucleotide sequences were determined. With the aid of these specific gene probes, it soon became apparent that altered expression of certain cellular oncogenes appeared to be closely associated with many human cancers. In this article we have attempted to provide an up-to-date review of the various mechanisms (point mutation, gene amplification, gene rearrangement, etc.) which may lead to oncogene activation, and suggest some of the ways in which the altered expression of these gene products might lead to tumorigenesis.

Activated *ras* oncogenes

Surprisingly, the transforming gene first isolated from human tumour cells was homologous to a retroviral oncogene. It was the point-mutated cellular Harvey *ras* gene (c-H-*ras*) that coded for a protein with an M_r of 21000 (p21) with a single amino acid substitution at either position 12 or 61 [5]. Activated c-H-*ras* genes with point mutations were isolated not only from established human tumour cell lines, but also from surgical specimens of human tumour tissues [6]. Active c-H-*ras* was not however present in normal cells of cancer patients whose tumours contain the active gene. Therefore, generation of point-mutated c-H-*ras* genes was concluded to be a result of somatic mutation. The discovery of point-mutated cellular oncogenes was of great importance in understanding the mechanism of chemical carcinogenesis. Epidemiological studies have indicated that most human cancers are caused by environmental mutagens or carcinogens [7]. Carcinogens must damage cellular DNA, but the crucial damage to DNA that induces cancer is unknown. Presumably point mutation of cellular oncogenes, generated by the action of chemical carcinogens, is an important causative event in the induction of human cancer. Point mutations in other *ras* family genes (c-K-*ras*, N-*ras*) were also found in human cancers as well as in tumours induced by chemical carcinogens in experimental animals. Tables 1 and 2 summarize the activated *ras* family genes found so

far in various human cancers and tumours in experimental animals. Activation of *ras* family genes may be brought about by mutation causing amino acid substitution of glycine at position 12 or glutamine at position 61 in the p21 protein. Recently, activation of the gene by substitution of the amino acid at position 13 was also found [59]. Transfection experiments using c-H-*ras* mutant genes prepared by site-directed mutagenesis, in fact, revealed that the transforming activity of c-H-*ras* is obtained by substitution of glycine at position 12 in p21 by any other amino acid except proline [73]. A similar conclusion was also reached for p21 with substitution of glutamine at position 61 [74]. Random bisulphite-induced mutagenesis of the cloned human c-H-*ras* proto-oncogene also revealed that, besides position 12 or 61, single amino acid substitutions at positions 13, 59 or 63 of p21 activate the transforming potential of the c-H-*ras* gene [75].

A puzzling problem with respect to point-mutational activation of *ras* family genes is that the frequency of detection of activated genes is not high in the human cancer tissues examined (generally 5–10%), although activated *ras* family genes are found in almost all types of tumours. The question of whether point-mutational activation is really involved in initiation of cellular transformation or is related to a later stage of progression of cancer is still not clarified. However, involvement of point-mutational activation of the c-H-*ras*-gene in initiation of the carcinogenic process has been suggested from experiments in animal systems. When female rats were given a single dose of highly labile *N*-nitroso-*N*-methylurea (NMU), efficient induction of mammary carcinomas was observed and more than 80% of the carcinomas carried a specific G→A transition in codon 12 of the c-H-*ras* gene [63]. Furthermore, Quintanilla *et al.* [61] analysed the molecular changes at different stages of carcinogenesis in mouse skin tumours induced by initiating and promoting agents. Almost all the tumours and even premalignant papillomas initiated with dimethylbenzanthracene had a specific A→T transversion in codon 61 of the c-H-*ras* gene. The frequency of this mutation depended on the initiating agent used, but not the promoter, suggesting that the mutation occurred at the time of initiation [61]. Contrary to these observations, among five melanoma cell lines originating from separate metastatic deposits in a single patient, only one contained an activated N-*ras* gene, suggesting that activation of the *ras* gene is involved in the progression state [47]. In this connection, it is noteworthy that the content of c-H-*ras* p21 (although not known whether it is normal or activated) in human gastric carcinomas analysed by an immunohistochemical technique was higher in metastatic tumours than in

Table 1. Activated *ras* family genes isolated from human tumour cells

Surgical specimens are indicated by *. Point mutations determined by hybridization of oligonucleotides are indicated by ☆

Origin	Tumour	Oncogenes	Mutation	References
Bladder carcinoma	ET	H- <i>ras</i>	12 Gly(GGC) → Val(GTC)	[8–13]
	T24	H- <i>ras</i>	12 Gly(GGC) → Val(GTC)	[13–22]
	J82	H- <i>ras</i>		[9,23]
	A1698	K- <i>ras</i>	12 Gly(GGT) → Arg(CGT)	[17,24]
	JBT44*	H- <i>ras</i>	61 Gln(CAG) → Leu(CTG)	[6,25]
	JBT55*	H- <i>ras</i>	11 or 12 Gly	[6,25]
	A1663	K- <i>ras</i>	12 Gly(GGT → Asp(GAT)	[26]
Lung carcinoma	*	N- <i>ras</i>	61 Gln(CAA) → Arg(CGA)	[27]
	Calu-1	K- <i>ras</i>	12 Gly(GGT) → Cys(TGT)	[16,28–31]
	Hs242	H- <i>ras</i>	61 Gln(CAG) → Leu(CTG)	[32]
	A2182	K- <i>ras</i>	12 Gly(GGT) → Arg(CGT)☆	[17,18,24]
	A427	K- <i>ras</i>	12 Gly(GGT) → Asp(GAT)	[17,26]
	PR371	K- <i>ras</i>	12 Gly(GGT) → Cys(TGT)	[33]
	PR310	K- <i>ras</i>	61 Gln(CAA) → His(CAT)	[33,34]
	SW1271	N- <i>ras</i>	61 Gln(CAA) → Arg(CGA)	[35]
	1615*	K- <i>ras</i>		[17]
	SK-Lu-1	K- <i>ras</i>		[16,36]
	(adenocarcinoma) A549	K- <i>ras</i>	12 Gly(GGT) → Ser(AGT)☆	[26,37]
	(squamous carcinoma) LC-10*	K- <i>ras</i>		[24]
	(small cell carcinoma) LX-1	K- <i>ras</i>		[23,37,38]
	(giant cell carcinoma) LU-65	K- <i>ras</i>	12 Gly(GGT) → Cys(TGT)	[39]
Colon carcinoma	SK-CO-1	K- <i>ras</i>		[16,30]
	SW-620	K- <i>ras</i>	12 Gly(GGT) → Val(GTT)☆	[27]
	SW-116	K- <i>ras</i>	12 Gly(GGT) → Ala(GCT)☆	[27]
	SW-1398	K- <i>ras</i>	12 Gly(GGT) → Cys(TGT)☆	[27]
	(adenocarcinoma) SW-480	K- <i>ras</i>	12 Gly(GGT) → Val(GTT)	[28,37,40]
	A2233	K- <i>ras</i>		[17]
Pancreas carcinoma	*	N- <i>ras</i>	13 Gly(GGT) → Arg(GGT)	N. Nitta, M. Ochiai, M. Nagao & T. Sugimura, personal communication
	A1165	K- <i>ras</i>	12 Gly(GGT) → Asp(GAT)☆	[26]
	1189*	K- <i>ras</i>		[17]
	(adenocarcinoma) *	K- <i>ras</i>	12 Gly(GGT) → Arg(CGT)	[41,42]
Gastric cancer	*	K- <i>ras</i>	12 Gly(GGT) → Ser(AGT)☆	[27]
Gall bladder carcinoma	A1604	K- <i>ras</i>		[17]
Renal pelvic carcinoma	JPT26*	H- <i>ras</i>	61 Gln(CAG) → Arg(CGG)	[6,25]
Ovary carcinoma	OVCA-1*	K- <i>ras</i>		[43]
Mammary carcinoma	HS578T	H- <i>ras</i>	12 Gly(GGC) → Asp(GAC)	[44]
Malignant melanoma	SK-2	H- <i>ras</i>	61 Gln(CAG) → Leu(CTG)	[45,46]
	SK-MEL-119	N- <i>ras</i>		[47]
	SK-MEL-146	H- <i>ras</i>		[47]
	K-MEL-147	N- <i>ras</i>		[47]
	SK-MEL-93	N- <i>ras</i>		[47]
Neuroblastoma	SK-N-SH	N- <i>ras</i>	61 Gln(CAA) → Lys(AAA)	[16,30,48–50]
Fibrosarcoma	HT1080	N- <i>ras</i>	61 Gln(CAA) → Lys(AAA)	[17,18,51–53]
Rhabdomyosarcoma	RD	N- <i>ras</i>	61 Gln(CAA) → His(CAY)☆	[53,54]
	1085*	K- <i>ras</i>		[17]
Promyelocytic leukaemia	HL-60	N- <i>ras</i>	61 Gln(CAA) → Leu(CTA)	[40]
Chronic myelogenous leukaemia	IM9	N- <i>ras</i>		[55]
T-cell acute lymphocytic leukaemia	CCRF-CEM	K- <i>ras</i>		[55]
	PRM18402	N- <i>ras</i>		[56]
	T-ALL-1	N- <i>ras</i>		[56]
	p-12	N- <i>ras</i>		[56]
Acute myelogenous leukaemia	31-26-146	N- <i>ras</i>		[55]
	*	N- <i>ras</i>	12 Gly(GGT) → Asp(GAT)	[57,58]
	AML1	N- <i>ras</i>	13 Gly(GGT → Asp(GAT)	[59]
	33*	N- <i>ras</i>	13 Gly(GGT → Asp(GAT)	[59]
	49*	N- <i>ras</i>	13 Gly(GGT → Val(GTT)	[59]
	77*	N- <i>ras</i>	13 Gly(GGT → Val(GTT)	[59]

Table 2. Activated *ras* family genes isolated from animal tumour cells

Abbreviations: DMBA, 7,12-dimethylbenzanthracene; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; NMU, *N*-methyl-*N*-nitrosourea; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; 1,8-DNP, 1,8-dinitropyrene; MCA, methylcholanthrene; MAV, myeloblastosis-associated virus.

Tumour	Inducing reagents	Oncogenes	Mutation	References
Mouse skin squamous cell carcinoma	DMBA/TPA	H- <i>ras</i>	61 Gln(CAA)→Leu(CTA)	[60,61]
Rat mammary carcinoma	NMU	H- <i>ras</i>	12 Gly(GGA)→Glu(GAA)	[62,63]
Mouse benign skin papilloma	DMBA/TPA	H- <i>ras</i>	61 Gln(CAA)→Leu(CTA)	[61,64]
Mouse thymoma	γ-Radiation	K- <i>ras</i>	12 Gly(GGT)→Asp(GAT)	[65,66]
	NMU	N- <i>ras</i>		[65]
Rat hepatocellular carcinoma	IQ	H- <i>ras</i>		[67]
Rat fibrosarcoma	1,8-DNP	K- <i>ras</i>	12 Gly(GGT)→Cys(TGT)	[68,69]
Mouse spontaneous hepatocellular adenocarcinoma	–	H- <i>ras</i>		[70]
Avian nephroblastoma	MAV	H- <i>ras</i>		[71]
Mouse fibrosarcoma	MCA	K- <i>ras</i>		[72]

primary tumours, and patients with c-H-*ras* p21-positive carcinomas were found to have a significantly worse prognosis than those with p21-negative carcinomas [76].

Activated oncogenes other than those of the *ras* family

Most activated cellular oncogenes so far isolated from human cancer cells by DNA transfection of NIH3T3 cells are members of the *ras* family of genes, although more than 25 different cellular oncogenes have been identified in studies on retroviruses. However, improvements in the DNA transfection assay have recently led to the isolation of several new types of activated oncogenes from human tumour cells and experimental animal tumours, and these are summarized in Table 3. The exact natures of some of these oncogenes are still unknown.

As discussed later, products of proto-oncogenes in normal cells may function in the process of cell proliferation, regulation of cellular metabolism through signal transfer, or cell differentiation. Modulation of the structures of proto-oncogenes, which cause (1) overproduction of gene products, (2) increased specific activity of gene products or (3) deregulated synthesis of gene products, might be the trigger for induction of cancer. Activated *ras* family genes can be classified in the second category.

The following mechanisms are involved in activation of proto-oncogenes.

(1) Point-mutational activation. In addition to the *ras* family genes, carcinogen (ethylnitrosourea)-induced activation of the *neu* gene in rat neuro/glioblastoma is also due to a point mutation. The T→A transversion causes replacement of valine at residue 664 by glutamic acid in the transmembrane domain of the encoded p185 protein [94]. The rat *neu* gene corresponds to the human *c-erbB-2* gene. However, point-mutational activation of the *c-erbB-2* gene in human cancer has not been reported so far, although amplification of the gene was found in several human primary tumours [100].

(2) Gene amplification. Amplification of oncogenes has been observed in many different tumour tissues [101]. The amplified oncogenes found, including those of the *ras* family, are summarized in Table 4. The extent of

amplification ranged from several-fold to several-hundred-fold. Some of the amplified genes are located on chromosomes as homogeneously staining regions or independently as double minute chromosomes. Tumour cells with amplified oncogenes generally synthesize much larger amounts of the corresponding mRNA, resulting in overproduction of the protein products. It should be mentioned that the frequency of amplification of oncogenes is not high: it is generally found in less than 10% of the tumours examined. No correlation has been found between a particular cancer and the amplified oncogene. Thus it is still premature to use oncogene amplification as a tool for cancer diagnosis, except in the cases of certain genes. The N-*myc* gene is known to be commonly expressed in neuronal cells, and amplification of the N-*myc* gene (10~300-fold) is frequently observed in surgical specimens of neuroblastoma, retinoblastoma and cells cultured from them [112,120,121,123–125,127]. Amplifications of the N-*myc* and L-*myc* genes are also found specifically in small cell lung carcinoma [126,129,144]. The extent of N-*myc* amplification is suggested to be correlated with the progressive stage of small cell lung carcinoma [101; F. Alt, personal communication].

(3) Gene rearrangement. The clearest and most extensively studied case of this category is translocation of the *c-myc* gene from chromosome 8 to chromosome 14 in Burkitt's lymphoma [145]. Expression of the translocated *c-myc* gene is controlled by the immunoglobulin heavy chain locus, which is located on chromosome 14. The breakpoints identified for the translocated *c-myc* genes are summarized in Fig. 1. The position of the chromosomal breakpoint relative to the *c-myc* gene may be reflected by different genetic mechanisms and also by the stage of B-cell differentiation. In the case of sporadic Burkitt's lymphoma which is not related to infection by Epstein-Barr virus (EBV), the recombination site of the *c-myc* gene with the immunoglobulin locus is clustered in the region of exon 1, as for example in DK179, MC116, IM35, ST486 and IM31 (Fig. 1). On the other hand, in endemic Burkitt's lymphoma, which is closely related to EBV infection, the recombination site is far upstream from the 5'-end of the *c-myc* gene [152]. Another type of

Table 3. Activated transforming genes other than *ras* family genes

Oncogene	Origin	Mechanism of activation	Function	References
<i>met</i>	<i>N</i> -Methyl- <i>N'</i> -nitronitrosoguanidine-transformed human osteosarcoma cell line (MNNG-HOS)	Rearrangement	Tyrosine-specific	[77-79]
<i>mcf</i>	Human mammary carcinoma cell line	Rearrangement (occurring during transfection)	Protein kinase (Ser/Thr)	[80]
<i>ret</i>	Primary human T-cell lymphoma			[81]
<i>dbl</i>	Primary human diffuse B-cell lymphoma	Rearrangement (occurring during transfection)	Protein kinase (Ser/Thr)	[82]
<i>raf</i>	Primary human stomach cancer			[83]
	Normal human colon mucosa of colon cancer patient	Rearrangement (occurring during transfection)	Tyrosine-specific protein kinase	T. Tahira & M. Nagao, unpublished work
<i>hst</i>	Human glioblastoma cell line			[84]
	Primary human stomach cancer	Rearrangement	Tyrosine-specific protein kinase	[85]
	Noncancerous portion of the same patient			[85]
	Lymphnode metastasis of stomach cancer from another patient	Rearrangement	Tyrosine-specific protein kinase	[85]
<i>oncD(trk)</i>	Human colon carcinoma			[86]
<i>Blym</i>	Burkitt's lymphoma	Rearrangement (occurring during transfection)	Tyrosine-specific protein kinase	[23,87-89]
<i>Tlym</i>	T-cell lymphoma			[88]
<i>mas</i>	Human epidermoid carcinoma			[90]
<i>lca</i>	Human hepatocellular carcinoma	Point mutation Rearrangement (occurring during transfection)	Tyrosine-specific	[91]
Unknown	Ewing sarcoma cell line			[84]
	Leiomyosarcoma cell line			[84]
	Pancreatic adenocarcinoma cell line			[92]
	Human melanoma cell line			[93]
<i>neu</i>	Rat neuro/glioblastoma	Point mutation Rearrangement (occurring during transfection)	Tyrosine-specific	[94-97]
<i>raf</i>	2-Amino-3-methylimidazo[4,5- <i>f</i>]-quinoline-induced rat hepatocellular carcinoma			[98,99]

activation of the *c-myc* gene found in Burkitt's lymphoma is translocation of the immunoglobulin light chain locus, which is originally located on either chromosome 2 or 22, to chromosome 8 [145]. In these cases, the breakpoints were observed in the 3' proximal regions of the *c-myc* genes (Fig. 1). It should be noted that translocated *c-myc* genes sometimes code for proteins with altered *N*-terminal amino acid sequences [150,156,164]. However, the mutated gene was found to retain reduced transforming ability in a rat embryo fibroblast focus formation assay [156]. The frequency of translocation of the *c-myc* gene in Burkitt's lymphoma is very high and the translocated *c-myc* gene is always expressed, while the gene at another allele of the normal chromosome 8 is not expressed [165]. Thus translocation of the *c-myc* gene is probably relevant to tumorigenesis of Burkitt's lymphoma. Proof that deregulated expression of the *c-myc* gene is really involved in tumour induction was obtained in recent studies with transgenic mice in which an immunoglobulin enhancer-linked or retroviral long terminal repeat-linked *c-myc* gene was integrated [166,167]. Tumours, including lymphomas, were induced with high frequency in these transgenic mice.

In the case of acute or chronic lymphocytic leukaemia originating from T-cell lymphocytes, translocation of the *c-myc* gene and deregulation of *c-myc* gene expression

are also observed. In this case, the *c-myc* gene remains on chromosome 8, but the region containing the α -chain gene of the T cell receptor on chromosome 14 is translocated onto chromosome 8, proximal to the *c-myc* gene [158,163,168]. Some of the breakpoints of these rearranged *c-myc* genes are illustrated in Fig. 1.

A translocation involving the *c-myc* gene has also been found in cells other than haematopoietic cells. In a hereditary renal cell carcinoma, a translocation between chromosome 3 and 8 has been reported [169]. Recently, rearranged *c-myc* genes were found in a human cell line and also primary tissue of a giant cell carcinoma of the lung. The rearrangements in these carcinomas were in regions about 7 kb upstream from the transcription initiation site [36].

In chronic myelogenous leukaemia, a reciprocal translocation between chromosome 9 and chromosome 22 was observed with 95% frequency. In this case, it was shown that the *c-abl* gene located on chromosome 9 was joined to the *bcr* (breakpoint cluster region) gene of chromosome 22, resulting in the formation of a fused gene that encodes a larger *c-abl* protein (210 kDa) with an extra polypeptide in the *N*-terminal region [170,171]. This modified protein may play a crucial role in induction of this particular type of leukaemia [122,172].

Specific translocation of chromosomes is observed with high frequency in other leukaemic cancers such as

Table 4. Amplification of human oncogenes

Abbreviations: DMs, double minute chromosomes; HSRs, homogeneously staining regions.

Oncogene	Cell and tumour type	Amplification (fold)	Presence of DMs or HSRs	References
<i>c-myc</i>				
Cell lines				
HL60	Promyelocytic leukaemia	8–32		[102,103]
COLO320	Neuroendocrine tumour from colon carcinoma	32	DMs, HSR	[104]
NCI-H60	Variant class of small cell lung carcinoma	38	DMs	[105]
NIC-N177		76	–	[106]
NCI-H82		25	HSR	[105,107]
NCI-N417		47	HSR	[105]
NCI-H446		20	–	[105]
NCI-N390	Pure small cell lung carcinoma	4	–	[105]
NCI-N231		6		[105]
SCLC-MV	Small cell lung carcinoma	5–76		[108]
Lu65	Giant cell carcinoma of the lung	8		[39]
St-4	Gastric carcinoma	8–10		[109]
NSC-3		13–15		[68]
Hut 23	Lung carcinoma	5		[110]
NC-147	Small cell carcinoma			[110]
SF-188	Glioblastoma	25	DMs	[111]
BT	Ductal adenocarcinoma	2		[112]
SKBR-3	Breast metastasis pleural effusion cells	8–16		[112]
Cama-1		1–2		[112]
MCF-7		1–3		[112]
Primary tumours				
Acute promyelocytic leukaemia		16–32	DMs	[103]
Gastric carcinoma		4–8		[113,114]
Pancreatic carcinoma		50		[42]
Primary metastatic tumour in lymph node LN-1		50		[42]
Primary metastatic tumour in lymph node LN-2		50		[115]
Breast cancer (38 of 121)		2–15		[115]
Squamous carcinoma of head and neck (2 of 7)				[116]
Bronchioloalveola carcinoma of lung (1 of 2)				[116]
Carcinomas of colon and rectum (2 of 32)				[116]
Carcinomas of breast (2 of 10)		3–8		[116]
Carcinomas of ovary (1 of 5)				[116]
Osteogenic sarcoma (1 of 2)				[116]
Rhabdomyosarcoma (1 of 1)				[116]
Small cell carcinoma of the lung (5 of 45)		3		[117]
Experimental animals				
SEWA				
Osteosarcoma 4031Tc3		15–20	DMs	[118]
Osteosarcoma Stam A		15–20	HSR	[118]
Osteosarcoma RecATc2		20–30	CM	[118]
Morris hepatoma 7794A		5–10		[119]
<i>N-myc</i>				
Cell lines				
Kelly	Neuroblastoma	100–120	HSR	[120,121]
NGP		120–140	HSR	[120]
NLF		20–25	HSR	[120]
CHP-134		20–25/700	HSR	[115,120–122]
CHP-126		100–120	HSR/DMs	[120,121,123]
IMR-32		15–20	HSR	[120,121,123]
NMB		100–120	HSR	[120]
MCN-1		5–8	DMs	[120]
NB9		300	DMs	[123]
NB16		300	DMs	[123]
NB19		600	HSR	[123]
LA-N-L		100	HSR	[123]
LA-N-5		50	DMs	[123]
CHP-382/JK		40	HSR	[124]
Y79	Retinoblastoma	25/100–200		[121,125]
LA-RB63-1A		10–20	DMs or HSR	[125]

Table 4 (cont.) Amplification of human oncogenes

Oncogene	Cell and tumour type	Amplification (fold)	Presence of DMs or HSRs	References
<i>L-myc</i>	NCI-H69	85		[126]
	NCI-H249	150		[126]
	NCI-H372	35		[126]
	NCI-H526	135		[126]
	NCI-H689	170		[126]
	Primary tumours			
	Neuroblastoma (24 of 63)	3–300		[127]
	Neuroblastoma (12 of 67)	10–270		[112]
	LA-RB70 retinoblastoma	100–200		[128]
	Recurrent rhabdomyosarcoma	10		[128]
	Cell lines			
	NCI-H298	10		[129]
	NCI-H378	20		[129]
	NCI-H510	15		[129]
	NCI-N691	10		[129]
<i>c-myb</i>	Cell lines			
	ML 1	5–10		[130]
	ML 2	5–10		[130]
	ML 3	5–10		[130]
	COLO201	10		[131]
<i>Ki-ras</i>	COLO205	10		[131]
	Cell lines			
	SW480	5		[28,37]
	Lx-1	3		[28,37]
	Lu65	10	DMs	[39,131]
	KT883	10		[132]
	Primary tumours			
	ABT165	40		[6]
	Pancreatic tumour			
	Metastatic lymph node tumour LN-1	3–6		[42]
	Metastatic lymph node tumour LN-2	3–6		[42]
	Experimental animals			
	Y1	30	HSR	[133]
		60	DMs	[133]
<i>Ha-ras</i>	Cell lines			
	SK-2	10–20		[134]
	Primary tumours			
<i>abl</i>	Bladder carcinoma	5–10		[135]
	Cell lines			
<i>ets-1</i>	K-562	4–8		[136,137]
	Primary tumour			
<i>c-erbB-1</i>	Patient 1	40	HSR	[138]
	Patient 2	10	–	[138]
	Cell lines			
<i>c-erbB-2</i>	A431	30/3–110	–	[139, 140]
	TE 1	2–3		[141]
	HSC-1	50		[141]
	NA	30	–	[141]
	Ca9-22	4		[141]
<i>c-yes-1</i>	Primary tumours			
	Stomach adenocarcinoma (2 of 9)	5–50		[100]
	Kidney adenocarcinoma	5–50		[100]
	Breast adenocarcinoma (2 of 10)	5–50		[100,142]
<i>c-yes-1</i>	Primary tumours	4–5		[143]
	Gastric cancer

Fig. 1. The breakpoints identified for the translocated *c-myc* genes in Burkitt's lymphomas and T-cell leukaemias

Approximate positions determined for the following Burkitt's lymphomas are indicated, Burkitt's lymphomas with t(8:14) translocation, Daudi [146,147], Seraphina [148], BL31 [148,149], Raji [146,148,150], BL22 [145,148,151], BK179 [152], As283A [152], DK288 [152], MC166 [152], CW678 [152], EB3 [152], AW-Ramos [146,153], BL42 [148], JOY [146], BM [146], W1 [146], IM35 [152], DK34 [152], Namalwa [152], Manca [153-155], LY65 [145,148,156], CA46 [152], ST486 [152,157], JD38 [152], FM100 [152], FM101 [152], IM31 [152], PP984 [152] and BL29 [146]; Burkitt's lymphomas with t(2:8) translocation, JBL2 [158-160], JI [159], LY91 [159]; Burkitt's lymphomas with t(8:22) translocation, BL37 [161], BL2 [159], Maku [147], PA682 [159], LY47 [159] and LY67 [159]. Breakpoints for t(8:14) translocations in acute lymphocytic leukaemia (ALL [162]) and in T-Cell leukaemias (Hut78 [163], Def [158] and SKW3 [158]) are indicated by asterisks. The DNA sizes indicated in parentheses are the lengths between the expected breakpoints and the *c-myc* exons.

chronic lymphocytic leukaemia of B-cells [173], acute B-cell lymphoblastic leukaemia [174] and T-cell chronic lymphocytic leukaemia [175,176], and putative oncogenes *bcl-1*, *bcl-2* and *tcl-1* have been isolated from these respective leukaemias. The genes *bcl-1* on chromosome 11 and *bcl-2* on chromosome 18 are translocated to the immunoglobulin heavy chain locus of chromosome 14. On the other hand, by an inversion of the region within the long arm of chromosome 14, the *tcl-1* gene is translocated to a proximal region of the genes for the α -chain of the T-cell receptor.

Activations of the *ret* [81], *mas* [90] and *raf* (normal human colon mucosa of colon cancer patient and 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced rat

hepatocellular carcinoma) [99] genes were also caused by gene rearrangement (Table 3). However, these rearrangements were presumed to be artifacts occurring during DNA transfection experiments *in vitro*. It should be worth noting that even point mutations can occur during transfection of NIH3T3 cells [177,178].

Restriction fragment length polymorphism (RFLP) analysis, used for diagnosis of hereditary diseases [179,180], is a good method for the detection of gene rearrangements involved in tumorigenesis, if a suitable DNA fragment is available as a probe. In this way, the translocation of chromosomes has also been characterized in other tumours [38,181-185]. For example, RFLP could be detected with 100% frequency in T-cell acute

lymphoblastic leukaemia, when a gene for the α -chain of the T-cell receptor was used as a probe in Southern blot analysis [186]. Since RFLP is not found in the DNA of normal lymphocytes of patients, the gene rearrangement observed in the leukaemic cells is clearly due to somatic mutation. However, in many other cases, a clear correlation between a specific chromosomal translocation and the corresponding tumours has not yet been obtained. The possibility that some of the chromosomal translocations take place as secondary events during progression of cancers cannot be excluded.

Activation of multiple oncogenes in human cancers

Tumorigenic transformation of rodent primary embryo fibroblasts requires the co-expression of an activated c-H-*ras* gene and c-*myc* oncogene [187] or adenovirus early region 1A [188] or activated c-H-*ras* gene expression in cells immortalized by carcinogens [189]. Thus at least two oncogenes are necessary for tumour formation [190]. NIH3T3 cells used as recipient cells in the transfection assay have already undergone some degree of transformation, and are not considered as totally normal cells: their transformation can be achieved by introduction of a single activated oncogene. In fact, in many human cancer tissues and tumour cell lines, expression or activation of multiple oncogenes was observed [191]. For example, in a human promyelocytic leukaemia cell line (HL-60), the activated N-*ras* gene coexists with an amplified version of the c-*myc* gene [192]. In the case of a human giant cell carcinoma of the lung propagated in nude mice, and a surgical specimen of pancreatic cancer, activations of the c-K-*ras* gene by a point-mutation (replacement of glycine at position 12 of p21 by cysteine or arginine, respectively) were accompanied by amplification of c-*myc* genes [39,42]. In a Burkitt's lymphoma, in addition to the altered c-*myc* locus, activation of the *Blym* oncogene was proposed [23,89,193]. However, the mechanism of activation of *Blym* is unknown [87]. Oncogenes can be roughly classified into two groups, i.e. those functioning in nuclei, and those functioning in the cytoplasm, including the cell membrane [194]. Activation of one oncogene of each group may be necessary for carcinogenesis. In chemical carcinogenesis, two or more steps are required for induction of cancer; namely initiation, promotion and progression [195]. Studies on chemical carcinogenesis and epidemiological studies indicate that a long latent period is needed for induction of cancer after the initiation reaction (damage of DNA) has taken place. It is therefore possible that activation of an additional oncogene(s) may occur during these later processes of carcinogenesis. However, it is still not clear what type of alteration in the genomic structure of oncogenes takes place during the promotion and progression stages. In the induction of mammary carcinomas in female rats by administration of NMU, no tumours are induced if the ovaries are removed after application of NMU [196]. These results indicate that activation of c-H-*ras* by NMU is not the only factor responsible for induction of cancer, and that additional hormonal modulation is also necessary. Perhaps these other factors are also involved in the process of promotion and progression of human cancer.

Transcription of the c-*myc* and c-H-*ras* genes in chick embryo fibroblast cells and rat cells was greatly increased by Rous sarcoma virus infection [197; Y. Kuchino &

S. Nishimura, unpublished result]. Recently, synthesis of mRNA of the *fgr* proto-oncogene was also reported to be induced by EBV infection in lymphocytes [198]. The increased expression of these oncogenes did not seem to be due to gene amplification or gene rearrangement, so some other mechanism for the co-operative expression of multiple oncogenes must be considered.

Recessive mutation related to human cancers

Genetic studies indicated that recessive mutation of a particular gene is involved in hereditary cancers such as retinoblastoma and Wilms tumour [199–201]. In the case of retinoblastoma, if both alleles of the Rb-1 gene located on chromosome 13q14 are inactivated, the disease will be pronounced. In the case of Wilms tumour, the recessive mutation is located on chromosome 11p13. Attempts to identify these mutated alleles are being carried out in many laboratories, and recently Friend *et al.* isolated a cDNA segment corresponding to the Rb-1 locus [202]. However, their exact natures are still not fully understood. The targets of recessive mutations may be the genes coding for repressor proteins, which negatively regulate expression of particular oncogenes. In other hereditary cancers such as familial polyposis syndrome [203] and hereditary thyroid carcinoma [204], even the chromosome locus responsible for the disease is still unknown. Abnormalities of known oncogenes (point mutation, gene amplification and gene rearrangement) are generally found only at frequency of up to 20%. An obvious possibility is that other important oncogenes with a dominant phenotype still remain to be identified. Another possibility is that recessive mutation of a gene is involved in general cancers in addition to hereditary cancers. It should be noted that the transfection assay used to screen for oncogenes is useful only in detecting activated oncogenes that are dominant at the cellular level.

Functional aspects of oncogene products

As shown in Table 5, proteins coded by oncogenes can be classified into several categories with respect to their biochemical properties [4,205–208]. From these properties, oncogene products are suggested to be involved in biochemical processes connected with either the proliferation or differentiation of cells. An important question is how the biochemical functions of these oncogene products are connected with their transforming potentials.

Most biochemical investigations have so far been carried out on the p21 protein encoded by the c-H-*ras* gene, or the pp60^{src} protein encoded by the v-*src* gene. In this section, we discuss the biochemical and biological properties of p21 protein as an example of an oncogene product. For this analysis, recombinant *E. coli* cells were used to express large amounts of p21 proteins with amino acid replacements in specific positions or truncated p21 proteins [106,251–257]. Although these bacterially expressed proteins do not have the usual post-translational addition of palmitic acid on a cysteine residue in the C-terminal region, they were still found to be useful for biochemical studies. Normal p21 was found to have both GTP-binding activity and GTPase activity, while the activated p21 protein showed greatly reduced GTPase activity [86,255,258]. Addition of palmitic acid to the cysteine residue at position 186 of p21 is necessary

Table 5. Classification of oncogene products with respect to their functions

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1. DNA-binding proteins or proteins mostly located in nuclei
fos [209], *c-myc* [126,210,211], *L-myc* [126], *N-myc* [212], *myb* [213], *p53* [214], *ski* [215]
erbA [216,217], *ceal* (*erbA*-related) [T. Yamamoto, personal communication]
 2. Proteins in locations other than nuclei
 - (a) Protein kinase
 - (i) Tyrosine-specific protein kinase
abl [113,122,218,219], *erbB1* [220–223], *erbB2* [224–226], *fgr* [227], *fms* [228], *fps(fes)* [229]
eph (*fps*-related) [H. Hirai, personal communication], *kit* [230], *met* [78], *neu* [96,231,232], *oncD(trk)* [233]
ros [234], *src* [4], *yes* [235], *syn* (*yes*-related) [236], *lck* (*yes*-related) [237,238],
lyn (*yes*-related) [239]
 - (ii) Serine- and threonine-specific protein kinase
mos [240], *raf* [241,242], *pks* (*raf*-related) [243]
 - (b) Guanine nucleotide binding protein (see the text)
H-ras, *K-ras*, *N-ras*
 - (c) Growth factor-related protein
sis [244–246]
 - (d) Growth factor receptor-related protein
erbB1, *erbB2(neu)*, *fms* [247,248], *ros* [249], *met*
 - (e) Integral membrane protein
mas [90]
 3. Unknown
dbl [82], *hst* [85], *lca* [91], *mcf-2* [80], *ret* [81], *rel* [250]
-

for its transforming activity, probably for its attachment to the cell membrane, although the amino acid sequence in the C-terminal region of p21 is not essential for its guanine nucleotide binding activity [259,260].

Comparison of the amino acid sequences of GTP binding proteins (EF-Tu, G proteins, transducin, p21 protein, etc.) revealed a conserved region in these proteins which may be important for their GTP binding activity [261–263]. The three-dimensional structure of EF-Tu determined by X-ray crystallography gave some insight into the conformational characteristics of p21 [264]. Clearly, X-ray and n.m.r. data on the conformation of p21 itself are needed for a further understanding of this subject.

Since normal p21 possesses both GTP-binding and GTPase activities, it can be considered to be a member of the G proteins that regulate adenylate cyclase activity [138,262,265–267]. In fact, in *Saccharomyces cerevisiae*, the proteins encoded by the yeast *ras* genes (*RAS*) turned out to be regulatory components of adenylate cyclase [268]. However, in animal systems, no evidence that p21 is involved in the cellular regulation of adenylate cyclase has yet been obtained. Microinjection of p21 protein into *Xenopus* oocytes did not result in an increase in their level of cyclic AMP [269]. Addition of p21 in large excess to the membrane fraction of S49 lymphoma cells, which lack *N_s* protein (a G protein), did not restore adenylate cyclase activity [270]. Like other GTP binding proteins, p21 probably modulates signal transfer through its conformational change caused by the binding of guanine nucleotides. Hydrolysis of bound GTP to GDP by its own GTPase activity regulates the function of normal p21, but activated p21 with reduced GTPase activity always remains in the active form. However, the transforming activities of c-H-*ras* genes with various point mutations in codon positions 12 or 61 were recently found not to be strictly correlated with the GTPase activity of p21 encoded by these artificially mutated c-H-*ras* genes [74]. Moreover, p21 with threonine in place of alanine at position 59 was found to

have high transforming activity, although its GTPase activity was almost the same as that of normal p21 [190].

Amino acid substitutions at positions 12 or 61 are known to reduce the GTPase activity of p21, as described above. However, they have no effect on GTP binding [48,74]. Since microinjection of an antibody inhibiting p21 GTP-binding activity *in vitro* caused transient reversion of cells transformed by the *ras* oncogene to those with the normal phenotype [271], GTP-binding activity may play a crucial role in the function of p21 functions, including transforming activity. Mutant genes have been constructed to elucidate the involvement of the GTP-binding activity of p21 in the transformation of cells. A single amino acid substitution at codons 83, 119 or 144 decreased the affinity of p21 for GTP by a factor of 25–100. However, these mutant genes induced transformation of NIH3T3 cells with efficiencies comparable with that of wild-type v-H-*ras* [257,272]. On the other hand, replacement of asparagine at position 116 by lysine or tyrosine completely abolished the GTP-binding activity of p21. In this case, the mutant DNAs did not transform NIH3T3 cells [273]. These results suggest that residual GTP-binding activity is sufficient for the transforming ability of p21, but that complete absence of GTP-binding activity abolishes transformation.

Summary of virus-associated human cancers

This review has focused on cellular oncogenes. However, RNA or DNA of viral origin is apparently involved in some human cancers [274]. HTLV I (human T-cell lymphotropic virus type I) was found to be the causative agent of adult T-cell leukaemia, cases of which are clustered in the southern part of Japan, the Caribbean Islands and Africa [117]. HTLV I does not contain any oncogene derived from a cellular gene. A protein(s) coded by the *tat* region of HTLV I enhances transcription of the genes controlled by its own long terminal repeat sequence in a *trans*-acting manner, and is thought to be involved in leukaemogenesis [117].

A close correlation was found epidemiologically between the integration of a specific type of papillomavirus and the incidence of cervical carcinoma [275–277]. Recently, a genomic DNA from cervical cancer tissue, containing human papillomavirus type 16, was found to induce malignant transformation of NIH3T3 cells [278].

EBV is also linked to a specific type of Burkitt's lymphoma [279]. Early infection with hepatitis B virus is closely correlated with later induction of hepatoma [280]. The molecular mechanisms of carcinogenesis by these DNA viruses are not understood at present. Elucidation of the mechanisms involved may be difficult, because of the long latent periods between viral infection and induction of cancer. However, these problems are very important, since a large proportion of cancers are associated with viruses, especially in some countries.

Concluding remarks

Since 1979, rapid progress has been made in the field of cellular oncogene research. Now it seems likely that, by extending oncogene research in the present direction, the molecular mechanisms of carcinogenesis in humans will be understood in the near future. But more biochemical studies on oncogene products are necessary for a clear understanding of cancer. Cloning of oncogenes and determination of their nucleotide sequences are rather straightforward approaches, although such investigations have provided much information. As a next step, more painstaking studies, namely isolation of oncogene products and analyses of their biochemical properties and biological functions, are needed. Such investigations are important not only for understanding the mechanisms of tumorigenesis, cell proliferation and differentiation, but also for finding ways for preventing cancer and for developing more effective anti-cancer reagents.

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