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Mutations of *ras* genes in human tumours (Review)

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Abstract. *Ras* family genes (H-, K- and N-*ras*) are implicated in a wide range of human tumours. Mutations are a major activating mechanism for the *ras* family genes, mainly in codons 12, 13 and 61, resulting in their conversion from proto-oncogenes to activated oncogenes. The detection of mutant *ras* alleles in human tumours has been performed by several investigators in a wide range of tissues. The aim of our review was to summarize the data obtained from these studies and to investigate whether the presence of mutant *ras* alleles was associated with particular clinical parameters.

Contents

1. Introduction

Advances in molecular oncology have revealed various roles the oncogenes and tumour suppressor genes (TSGs) play in the development of cancer (1). These genes usually encode for proteins involved in the control of normal cell growth and differentiation. Alterations in oncogenes and TSGs affecting their expression and function have been recognised as aetiological factors of the disease and are frequently attributed the role of molecular markers in tumour progression.

Among oncogenes, the members of the *ras* family (H-*ras*, K-*ras* and N-*ras*) are the most frequently implicated genes in the development of cancer. *Ras* family genes encode for similar proteins with molecular weight of 21,000 Daltons (p21). p21 is localised in the inner surface of the plasma membrane due to a farnesyl molecule attached to the carboxy terminus of the protein (2). The role of p21 is to transduce molecular signals to the cell nucleus, resulting in the activation of other cellular genes. The first clue for the role of p21 came from the observation that it possesses GTPase activity revealing similarities with the G proteins and thus, activating the adenyl cyclase pathway (3). Although little is known about the expressional patterns and the exact role of the *ras* family genes in human tissues, it is established that p21 is produced constitutively in all human tissues, revealing an important role of *ras* genes in normal cell growth (4-6).

Activation of *ras* genes in human tumours occurs by mutations and aberrant expression. Hot-spots for mutations are the codons 12, 13 and 61 (Fig. 1) which participate in the GTP binding domain of the protein. The mutant p21 loses its ability to become inactivated and thus stimulates cell growth or differentiation constitutively. It is suggested that mutations at codons 12, 13 and 61 confer a proliferative advantage in the cell bearing these mutations and thus they are selected within the cell population as compared to other mutations in different sites of the *ras* genes (7).

The aim of the present report was to review the information as regards the incidence of mutations in the *ras* family genes in human tumours.

2. Methods of detection

Initially, the most common assay for the detection of mutant *ras* alleles was based on the ability of these alleles to transform the mouse NIH/3T3 cell line (8-10). However, although this procedure provided an accurate measure of the transforming potential of the altered *ras* genes, it was not suitable for examining a large set of tumours because it was time-consuming and extremely laborious. Recent advances in the molecular techniques especially the polymerase chain reaction (PCR) and later the characterisation of the hot-spots of the mutations, made it possible to examine directly the tumour DNA for mutations in specific sites of the *ras* genes. This can be performed by hybridisation of the tumour DNA with specific probes for each mutation or alternatively by RNase A mismatch cleavage (11,12). The demand for even more rapid techniques for the detection of mutant *ras* genes led to the development of PCR based assays which distinguish the mutant *ras* alleles due to a restriction fragment length poymorphism (RFLP). These assays are based on the ability of specific restriction endonucleases to recognise sequences in the *ras* genes that overlap with the codons that behave as hot-spots for the mutations (13). In

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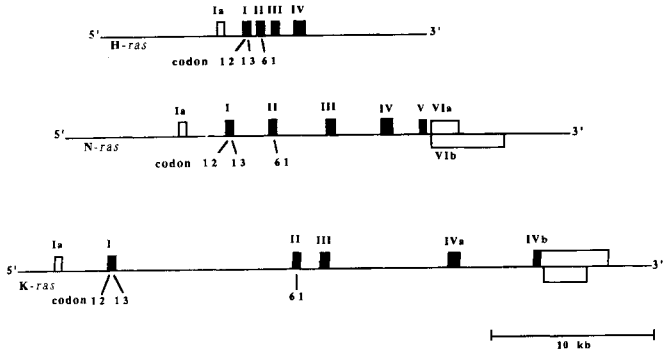

Figure 1. Structure of the H-*ras*, K-*ras* and N-*ras* genes and localisation of the hot spots for mutations. Solid boxes and open boxes indicate coding and non-coding exons respectively.

case that these recognition sites do not naturally occur within the *ras* sequence, they can be entered in the PCR product after the use of a mismatch primer (14). All the forementioned techniques can be followed by sequencing for the precise characterisation of the mutation.

3. Mutations of *ras* genes by site

The frequency of *ras* mutations varies in the different sites of human tumours. A summary indicating the frequencies of *ras* mutations found in tumours located in various sites is shown in Table I.

Pancreas. Tumours of the pancreas are highly malignant and characterised by poor prognosis. These tumours, although they are not very frequent, harbour mutations in the *ras* family genes at the highest frequency as compared with all other human tumours. It has been reported that 90% of the pancreatic adenocarcinomas harbour a K-*ras* mutation (15,16). It is of specific interest that all mutations have been detected in K-*ras* and the majority affecting codon 12. This finding indicates a specificity in K-*ras* proto-oncogene in the development of pancreatic cancer. Despite the high incidence of mutations in carcinomas, the rate of K-*ras* mutations in ductal papillary hyperplasia or intraductal papillary neoplasm (17) was very low.

The strong association of K-*ras* codon 12 point mutations with the development of pancreatic cancer, led several investigators to explore a possible clinical significance of this finding for diagnosis of the disease. Tada *et al* (18) detected K-*ras* codon 12 point mutations in the pancreatic juice of all cases tested and the peripheral blood in 2 of 6 cases with pancreatic adenocarcinoma from circulating metastasising cells. In addition, Kondo *et al* (19) detected mutations at codon 12 of K-*ras* in the pancreatic juice of patients with pancreatic cancer, all negative by cytodiagnosis, and proposed that the detection of K-*ras* codon 12 point mutations may be a valuable diagnostic modality for pancreatic carcinoma.

Colon and rectum. Colorectal cancer represents one of the best studied and characterised human malignancies at the molecular level, mostly due to the availability of the tumour material for research purposes. Several genetic events have been described to play a role in colorectal tumorigenesis, including activating mutations in K-*ras* proto-oncogene. Briefly, a mutation in FAP gene, which is located in 5q, leads to the generation of a hyperproliferative epithelium. DNA hypomethylation and activating mutation in K-*ras* resulted in an adenoma, and the accumulation of deletions to DCC (18q) and p53 (17p) in a carcinoma and metastasis (20).

The role of K-*ras* gene in colorectal tumorigenesis, became apparent after the detection of K-*ras* mutations in approx. 40% of colorectal tumours (21-26). Several studies suggest association between mutations in K-*ras* codon 12 and the clinical parameters of the patients. Halter *et al* (23) found higher incidence of mutations in patients in stage D, patients

Table I. Mutations of *ras* genes in human tumours.

Tumour site	*ras* gene	Frequency range (%)	Reference No.
Pancreas	K-*ras*	80-90	15-16
Colon and rectum	K-*ras*	30-60	21-27
Small intestine	H-*ras*	31	34
Lung	K-*ras*	27-60	35-37
Prostate	H-*ras* K-*ras*	0-25	42-46
Liver	K-*ras* N-*ras*	12-26	47-51
Skin	H-*ras* K-*ras* N-*ras*	0-46	53-61
Ovary	K-*ras*	0-48	69-71
Cervix	K-*ras*	20	72
Endometrium	K-*ras*	10-40	62-66
Stomach	H-*ras*	0-41	74-77
Bladder	H-*ras*	7-66	77-79
Breast	K-*ras*	0-12	80-82
Kidney	K-*ras*	0-50	83-84
Brain	N-*ras* K-*ras*	0-13	85-88
Thyroid	H-*ras* K-*ras* N-*ras*	0-60	89-92
Testis	K-*ras* N-*ras*	12-43	93-95
Leukaemia	N-*ras* K-*ras*	6-40	96-106
Head and neck	H-*ras* K-*ras*	0-30	107-110

with a family history of colon cancer, male patients and long-term survival in stage D. Yamagata *et al* (24) found lower incidence of K-*ras* mutations in flat adenomas than in polypoid adenomas, suggesting that the adenoma-carcinoma sequence through flat adenomas may be different from that through polypoid adenomas. Boughdady *et al* (25) reported that higher incidence of mutations in adenomas associates with the size of the tumour and the severity of the dysplastic changes. Breivik *et al* (26) performed an exhaustive analysis in 251 primary tumours in order to assess the incidence of K-*ras* mutations in colon cancer. They found that 39% of the specimens harboured a mutation at K-*ras* gene. Association was found with sex, age and tumour location. For colonic tumours, young males have fewer mutations than young females while rectal tumors show an inverse but less pronounced relationship. Spandidos *et al* (27) investigated the incidence of K-*ras* and N-*ras* mutations in patients with colorectal cancer. They found that 38% of the patients harboured a K-*ras* mutation while the incidence of mutations in N-*ras* gene was limited to 1.5%. Furthermore, point mutations appear to be more frequent in carcinomas with elements indicating a development from adenoma, in ages below 50 years, in females who had the tumour located at the rest of the large bowel in comparison with rectosigmoid and in higher grade of differentiation.

The incidence of K-*ras* mutations in flat adenomas and adenocarcinomas was investigated by Minamoto *et al* (28) who found relatively low incidence of K-*ras* mutations (16% and 17% respectively), providing further evidence to the hypothesis that this type of tumour is a distinct neoplastic entity.

The data provided on the role of K-*ras* mutations in the development of colorectal cancer, initiated an effort to detect K-*ras* mutations in syndromes predisposing to colorectal cancer. Ulcerative colitis (UC) and Crohn's disease are benign neoplasms that expose patients to an increased risk for the development of colorectal cancer. Although K-*ras* mutations have been detected in approximately 25% of the cases (29), the lower rate in addition to the different site distribution (mutations are more frequent in rectal carcinomas in comparison to colonic carcinomas while the opposite was observed in UC patients) as compared to sporadic colorectal tumours (30,31), suggests that specific genetic differences may underlie the causation of carcinomas arising in these situations.

Pretlow *et al* (32) investigated the mutational activation of the K-*ras* gene in the aberrant crypt foci of human colon. Since 73% (11/15) of these samples harboured a K-*ras* mutation but none was detected in the 27 morphologically normal crypt areas from the same patients they suggested that aberrant crypt foci are the earliest precursors of colon cancer and mutations at the K-*ras* gene are the earliest gene mutational event in colon tumorigenesis.

A low incidence of K-*ras* mutations has also been reported in colonic adenomas from familial polyposis coli patients, a disease which predisposes patients to the development of colorectal cancer, providing evidence that there are common molecular events involved in sporadic and hereditary colorectal tumorigenesis (33).

Small intestine. Although the majority of the studies involves colorectal tumours little is known as regards the implication of the *ras* family genes in small intestinal tumours. Spandidos *et al* (34) investigated the incidence of point mutations in the H-*ras* and K-*ras* genes and found that 4 out of 13 (31%) specimens had a H-*ras* codon 12 point mutation, while no specimens were found positive for a K-*ras* point mutation. These results indicate an association of H-*ras* point mutations with the development of at least a subset of small intestinal tumours.

Lung. Lung cancer is the leading cause of cancer death in the industrialised world, with a high correlation to the smoking habits of the patients. As regards the implication of the *ras* family genes several investigators have described activating mutations, affecting mainly the K-*ras* proto-oncogene. Most of the mutations have been detected in adenocarcinomas and it has been proposed that activating mutations at the K-*ras* proto-oncogene may serve as molecular markers of the disease.

Rodenhuis and Slebos (35) reported that approximately 30% of the adenocarcinomas of the lung harbour an activating K-*ras* codon 12 point mutation with almost all the mutations in the group of the smokers. In addition they found that patients with a K-*ras* point mutation had significantly worse survival than those without an activating mutation at the codon 12. Similar results have been reported by a Japanese group (36) who found that adenocarcinomas of the lung harbour activating mutations at the K-*ras* gene in approx. 20% of the specimens. The incidence of H-*ras* and N-*ras* point mutations, according to the forementioned study, is limited to 1.5% and 4.5% respectively. The same group (36) investigated the incidence of *ras* mutations in squamous cell carcinomas, large cell carcinomas, small cell carcinomas and adenosquamous cell carcinomas of the lung but mutations were found only in squamous cell carcinomas (5.5%) and in large cell carcinomas (14%). The highest incidence of K-*ras* mutations in adenocarcinomas of the lung has been reported by Husgafvel *et al* (37) who detected K-*ras* mutations in 60% of the samples tested. Furthermore, they found a strong association between the presence of mutation and a heavy life-time exposure to tobacco smoke. Apart from smoking, exposure to asbestos have also been described to play a role in the development of K-*ras* mutations (38,39), providing further evidence to the suggestion that *ras* genes may serve as targets of mutagens. Although the majority of the K-*ras* mutations were observed in adenocarcinomas, Rossel *et al* (40) detected higher incidence of K-*ras* mutations in squamous cell carcinomas (21%) than in adenocarcinomas (14%). As regards the clinicopathological parameters of the patients, a strong association has been found between the presence of mutations and the poor survival of patients. Although the aetiology of the different K-*ras* mutation rates, between adenocarcinomas and squamous cell carcinomas, is unknown, it was postulated that geographical variation may play an important role in the K-*ras* mutational activation.

In order to examine if K-*ras* mutations were detectable in cytological material from patients with lung cancer, Kiaris *et al* (41) assessed the incidence of K-*ras* mutations in specimens from fine needle aspiration and bronchoscopy. They found that approximately 23% of the specimens contained a mutant K-*ras* allele indicating that the detection of K-*ras* mutations may serve as a molecular marker for the detection of the disease.

Summarizing, K-*ras* mutational activation represent a frequent event in lung carcinogenesis. The majority of the studies described that K-*ras* mutations occur more frequently in adenocarcinomas, however detection of relatively high rates of K-*ras* mutations in other histological entities of lung tumours (such as squamous cell carcinomas) remains as a possibility which has to be clarified with future investigations. Furthermore, the detection of K-*ras* mutations was associated with poor prognostic indicators, most strikingly with poor survival. Apart from the detection of *ras* mutations in the tumour tissue, the detection of activated members of the *ras* family is possible in cytological material of the patients, indicating that the detection of mutant *ras* alleles may serve as a molecular marker for the development of the disease.

Prostate. Prostate cancer is a major cause of death from cancer in males in the Western world. However, the implication of the *ras* family genes in the development of prostatic cancer has not been studied in depth. Generally, a minor role for the *ras* family genes has been proposed in prostatic cancer. Most of the studies demonstrated a low incidence of *ras* mutations (4-10%) and almost exclusively restricted to the H-*ras* proto-oncogene, while mutations at the K-*ras* and N-*ras* gene have rarely been detected (42-44). However, reports from Greece (45) and from Japan (46) demonstrated a relatively high incidence (approximately 25%) of *ras* mutations in prostatic cancer, affecting mainly the K-*ras* proto-oncogene. These reports indicate the presence of particular environmental factors that may result in the activation of the K-*ras* gene.

Although the implication of the *ras* family genes in the development of prostatic cancer is not clearly understood as yet, a role of the *ras* genes in the development of the disease should be considered, particularly in association with certain environmental factors.

Liver. Hepatic cancer is characterised by poor prognosis and is associated with specific carcinogens such as aflatoxins. Mutations in the *ras* family genes are not very frequent in hepatic cancer but when present they are associated with specific histopathology of the tumour.

Tada *et al* (47) investigated the incidence of *ras* mutations in primary hepatic malignant tumours and found that 26% of the tumours tested exhibited evidence of a mutant *ras* allele. All mutations were found in the K-*ras* gene. Furthermore, 66% of the cholangiocarcinomas harboured K-*ras* mutations while no mutations were found in hepatocellular carcinomas and hepatoblastomas. These results suggest that *ras* gene mutations (K-*ras* in particular) play an important role in the pathogenesis of cholangiocarcinoma. The same group also examined a larger set of cholangiocarcinomas and confirmed their previous results (48). In this report they found that K-*ras* mutations appear more frequently in the hilar type of intrahepatic cholangiocarcinomas and suggested the presence of similar etiologic factors in hepatic and colon carcinomas since the incidence and spectrum of *ras* mutations were the same in both types of the disease. In addition, K-*ras* point mutations in angiosarcomas of the liver were considered as a consequence of vinyl-chloride DNA adduct formation (49). In contrast to these results, Challen *et al* (50) reported a low incidence of *ras* mutations in a subset of hepatocellular carcinomas tested. However, 3 among 4 mutations that were detected in the 19 patients, were found to affect the N-*ras* gene. This is noteworthly because the incidence of N-*ras* mutations in human solid tumours is rare in the Western world. A relatively high incidence of K-*ras* point mutations was reported by Nikolaidou *et al* (5/41, 12%) in patients from Greece (51).

In conclusion, activation of the *ras* family genes is associated with a particular subtype of hepatic cancers, cholangiocarcinomas.

Skin. H-*ras* proto-oncogene is the most frequently activated member of the *ras* family in non-melanoma human skin cancer, which is consistent with a model proposed for the mouse skin tumorigenesis (52). In most cases, the mutations occur at the pyrimidine-rich sequences of the *ras* genes, indicating that these sites are the targets of the DNA induced damage (53). However, the high incidence of *ras* mutations in non-melanoma skin cancer has been questioned by Campbell *et al* (54) who failed to detect any mutations in 40 basal cell carcinomas, 12 squamous cell carcinomas and 12 cases of Bowen's disease.

Melanomas represent a subset of the skin tumours which are characterised by high metastatic potential. Initial studies suggested that approximately 20% of the cases presented *ras* mutations the majority of which was found in the N-*ras* gene (55,56). However, these results were not confirmed by other investigators who found very low incidence of N-*ras* mutations both in uveal and cutaneus melanomas (57-59). Mutations have also been described frequently to activate K-*ras* gene in melanomas (60) but other studies failed to confirm these results (58,61).

Female reproductive tract. Mutations in the *ras* family genes have been detected by several investigators in endometrial carcinoma in variable frequence. These mutations affected mainly the K-*ras* proto-oncogene at a rate of approximately 10-40% (62,63) of the specimens. Furthermore, Enomoto *et al* (63) in order to further define the role of the *ras* family genes in the development of endometrial carcinoma investigated a set of premalignant cases of the uterine endometrium. Although they failed to find a clear association between the presence of a mutation and the development of the disease, their results suggested that frequently the presence of a mutation is associated with a more aggressive histological type. The incidence of K-*ras* point mutations in premalignant cases of endometrium was studied also by Duggan *et al* (64) who suggested that it is an early event in the development of the disease. Furthermore, Mizuuchi *et al* (65) suggested that K-*ras* activation represents an independent risk factor which is important in determining the aggressiveness of the disease. Association between the presence of the K-*ras* point mutations and the country of origin of the samples has been proposed by Sasaki *et al* (66) who reported that this particular genetic aberration occurs more frequently in patients from Japan. In addition, the same group suggested that the presence of K-*ras* mutations is associated with a good prognosis.

Although K-*ras* point mutations are a relatively uncommon event in ovarian carcinomas (67), in different subtypes of ovarian neoplasm *ras* mutations appear to be a more frequent feature. In borderline tumours K-*ras* point mutations were

detected in 48% of the specimens (68). Enomoto *et al* (69) detected K-*ras* mutations in 27% of the specimens and found that they occur more frequently in mucinous adenocarcinomas than in other epithelial tumours. These results were confirmed by a different group (70) who also suggested an association between the codon 12 point mutation in mucinous adenomas with the occurrence of intestinal type adenomas. Teneriello *et al* (71) detected K-*ras* mutations in 30% of low malignant potential tumours. It could be argued that K-*ras* mutations are associated with particular subtypes of ovarian neoplasms such as borderline tumours, mucinous adenomas and adenocarcinomas and low malignant potential tumours.

In cervical cancer the incidence of *ras* mutations is relatively low as compared to this of the endometrium. Koffa *et al* (72) found that approx. 20% of the cervical tumours harbour an activating K-*ras* mutation of which 28% is found in malignant tumours and 5.4% in benign.

Stomach. The molecular alterations that follow the development of gastric cancer are not clearly understood as yet. The implication of the *ras* family genes has been investigated by several groups and the majority of the studies suggested that *ras* genes play a minor role in gastric cancer. Victor *et al* (73) found no evidence of H-, K- and N-*ras* point mutations in an analysis involving patients from South Africa, where high incidence of gastric cancer was described. These results were confirmed by other studies involving patients from the Western world (74) and Japan (75) who recognised low incidence of activated *ras* family genes in patients with gastric cancer. Although the majority of the studies suggest that the members of *ras* family of genes are rarely activated in gastric tumours, Deng *et al* (76) reported that 11 out of 27 (41%) of the cases tested exhibited evidence of H-*ras* mutations. Furthermore, in the forementioned study the presence of H-*ras* mutations was associated with distal metastases and the survival time of gastric cancer patients after surgical operations.

As regards gastric cancer in general, it is of specific interest that the majority of the mutations have been detected in H-*ras* proto-oncogene while colorectal tumours exhibit mutations in the K-*ras* gene (22-27). We may postulate that this is due to the different carcinogens present in each tissue.

Bladder. Bladder tumours harbour activating mutations in the *ras* family genes in approximately 7-17% of the samples (77,78). Contrary to the majority of the tumours that harbour an activated K-*ras* allele, the H-*ras* proto-oncogene is activated in bladder tumours, providing evidence for the tissue specificity of the *ras* family genes. High incidence of *ras* mutations in bladder tumours has been reported by Haliassos *et al* (79) who detected H-*ras* codon 12 point mutations in 66% of the specimens with a combined PCR-RFLP assay. The same group detected the mutant H-*ras* allele in the urine of 47% of the patients with bladder tumour suggesting that the detection of this aberration may have a prognostic value for the detection of the neoplasia (79).

Breast. Breast cancer represents a major cause of death in adult females. Several alterations at the molecular level have been associated with the development of the disease, such as overexpression of the c-*erb*B-2 and mutations and aberrant expression of the p53 tumour suppressor gene. In addition, in hereditary breast cancer recent studies revealed three altered loci with deletions and/or mutations, that led to the development of cancer.

The implication of *ras* genes in breast cancer have been studied mainly at the level of overexpression. Mutations in the *ras* family is generally considered as a rare event in the development of the disease (80,81). Koffa *et al* (82) found that 8 among 65 (12%) harbour an activating K-*ras* mutation. All the mutations were restricted to high grade tumours (II and III) indicating that this particular aberration is a late event in the development of the disease.

Kidney. The implication of the *ras* genes in the development of cancer of the kidney has not been studied in depth as yet. However the available data indicate that *ras* mutations are rare events in the development of this cancer (83) However, a special category of cancer of the kidney, occurring in patients after kidney transplantations, may exhibit higher incidence of *ras* mutations. This has been proposed by Skalkeas *et al* (84) who suggested that K-*ras* codon 12 point mutations is a common event in kidney transplanted patients who develop neoplasia, even in the least aggressive forms of the disease, contrary to the sporadic cases.

Brain. The absence of *ras* mutations in glioblastomas and neuroblastomas has been reported by two groups (85,86) while a role for the *ras* genes in the development of brain tumours has been proposed by Brustle *et al* (87) who detected an activated K-*ras* gene in one among 9 neuroectodermal tumours tested. In addition, Ireland (88) reported 2 specimens positive for N-*ras* mutations among 15 (13%) in neuroblastomas. However, future studies involving larger set of specimens should be performed in order to define the role of the *ras* genes in the development of brain tumours.

Thyroid. Mutations in all three *ras* family genes have been found in thyroid tumours. The highest incidence of mutations was found in follicular and undifferentiated carcinomas (89,90) while in papillary carcinomas the incidence of *ras* mutations was limited (89,91,92). Macrofollicular hyperplasias are characterised by the absence of mutations in the members of the *ras* family (90).

Testis. A significant incidence of *ras* mutations has been reported in testicular tumours, mainly in seminomas. The majority of the mutations were detected in K-*ras* and N-*ras* proto-oncogenes (93). However the high incidence of *ras* mutations in testicular cancer was not confirmed by other investigators (94,95), probably due to the small number of specimens included in these studies. Investigation of a larger set of tumours should be performed in order to establish the precise role of mutant *ras* alleles in the development of testicular cancer.

Leukaemia. An activated member of the *ras* family has been detected in approximately 30% of the patients with acute myeloid leukaemia (96-99). The point mutations occur in the N-*ras* (mainly) and K-*ras* while in the H-*ras* gene point mutations have rarely been detected. As regards the clinical

aspects of the patients harbouring mutant *ras* alleles it appears that the cell clones with *ras* mutations exhibit more resistance to chemotherapy as compared to the cell clones with normal *ras* genes (100). However, no particular association has been found between the *ras* mutation and the pathological features of the patients. Mutations in the *ras* family genes have also been detected in patients with myelodysplastic syndromes (101), at significant frequency. However, the presence of the mutation was not associated with the development of acute leukemia (AL), indicating that this particular aberration in the *ras* genes could not serve as a prognostic factor (101,102). Vashiukin *et al* (103) detected activated N-*ras* alleles in the blood plasma of patients with AL or myelodysplasia syndrome (MDS) and proposed that plasma could be a useful material for monitoring myeloid disorders.

The incidence of *ras* mutations in lymphoid malignancies is not as high as that reported in myeloid disorders (104,105). It might be postulated that the tumorigenic potential conferred by the mutant *ras* alleles in lymphocytes is lower than this in myeloid cells. However, although Lubbert *et al* (106) detected N-*ras* mutations in only 6% of the patients they found a strong association between the presence of N-*ras* mutations and poor prognosis.

Head and neck. Activated members of the *ras* family are rarely detected in head and neck tumours and the average incidence of *ras* mutations is approx. 5% only (107-109). However, Saranath *et al* (110) detected *ras* mutations in 35% of the specimens and found that mutations were associated with the chewing of tobacco. This finding provides further evidence to the suggestion that *ras* genes frequently behave in a carcinogen specific manner.

4. Alternative methods of activation for the *ras* family genes

Apart from point mutations, a polymorphism of the *ras* alleles (at least H-*ras*) may be associated with the development of the malignancy (111) corresponding to the number of the repetition of a core 28 bp repeat, at the 3' end of the gene (VTR). Four main VTR alleles have been recognised and several rare alleles with intermediate length. The presence of rare VTR alleles has been proposed to associate with increased probability for the development of cancer. In addition, Kiaris *et al* (109) suggested that instability of this region may be associated with the deregulation of the H-*ras* gene. Furthermore, loss of heterozygosity of the H-*ras* locus (112), amplification of K-*ras* and N-*ras* genes (113) and abnormal methylation (114) of the *ras* family genes have also been described in human tumours and may be associated with the development of the disease.

Structural alterations represent the major, but not the only activating mechanism for the *ras* family genes in human tumours. Overexpression of $p21^{ras}$ is frequently recognised in several human cancers. The majority of the studies have involved the immunohistochemical detection of p21 (115-117) but a subset of the studies also recognised elevated levels of *ras* mRNA in human tumours (109,113,118,119). The overexpression of *ras* family genes does not necessarily requires the existence of point mutated *ras* alleles because the normal *ras* alleles have been proved to confer a tumorigenic potential when overexpressed (120).

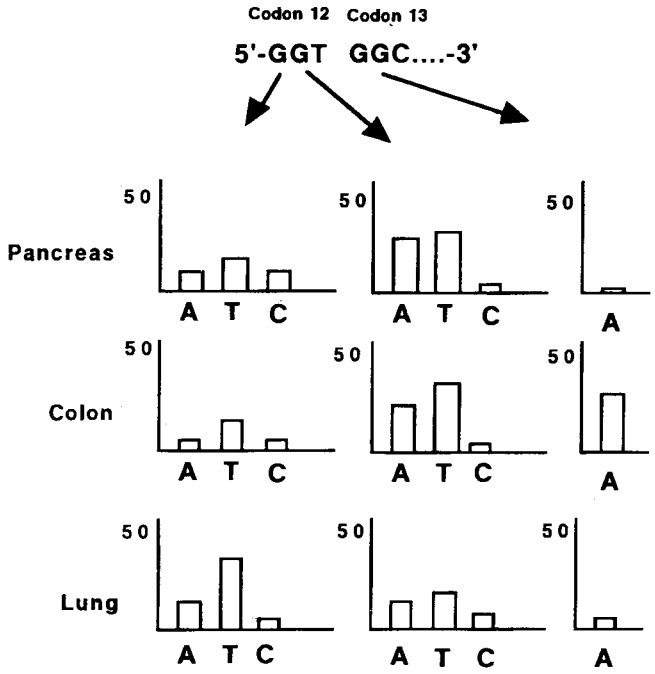

Figure 2. Typing of the commonest K-*ras* codon 12 point mutations detected in pancreatic, colorectal and lung tumours. Data were obtained from literature cited in the text.

5. Conclusions and perspectives

The forementioned data summarise the present state of information as regards the implication of the *ras* family genes in the development of human tumours *in vivo*. The analysis concentrated on the detection of activating point mutations and excluded alternative methods of activation. It is obvious that *ras* family genes are involved in a wide range of human tumours and in particular cases (such as pancreatic, lung and colon cancer) at a significant rate (Table I, Fig. 2). Furthermore, the detection of activating point mutations is frequently associated with an aggressive type of the disease and with specific clinical characteristics (Table II).

The clarification of the role of activated *ras* alleles in human tumours, may have significant implication in the clinical practice. The presence of mutant *ras* alleles may serve

Table II. Association of *ras* mutations with prognosis.

Tumour tissue	mutant *ras*	Prognosis
Colon (stage D)	K-*ras*	favourable
Lung	K-*ras*	poor
Leukaemia	N-*ras*	poor
Endometrium	K-*ras*	poor
Stomach	H-*ras*	poor

as molecular markers for the development of the disease. Biopsy specimens from surgically resected tumours may be assayed for the presence of *ras* mutations and this may help to predict the course of the disease or to establish treatment strategies (i.e. in leukemias). Furthermore, the detection of *ras* mutations may provide useful information as regards the early detection of the disease. In this case cytological material might be used (i.e. in lung cancer) in order to screen the population for the presence of mutant K-*ras* alleles (121).

A more challenging possibility is the use of the forementioned information for the therapy of cancer. Such an approach has been successfully carried out *in vitro* by specific compounds (antisense oligonucleotides and inhibitors of farnesylation) that block *ras* genes at the level of transcription or post-transcriptional modifications respectively (122,123).

Studies involving large number of specimens, in association with detailed clinical parameters should be performed, in order to reveal the precise role of the *ras* family genes in human cancer and to apply this information in clinical practice.

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