Mutations of \textit{ras} genes in human tumours (Review)

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Abstract. \textit{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 \textit{ras} family genes, mainly in codons 12, 13 and 61, resulting in their conversion from proto-oncogenes to activated oncogenes. The detection of mutant \textit{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 \textit{ras} alleles was associated with particular clinical parameters.

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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 \textit{ras} family (H-ras, K-ras and N-ras) are the most frequently implicated genes in the development of cancer. \textit{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 \textit{ras} family genes in human tissues, it is established that p21 is produced constitutively in all human tissues, revealing an important role of \textit{ras} genes in normal cell growth (4-6).

Activation of \textit{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 \textit{ras} genes (7).

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

2. Methods of detection

Initially, the most common assay for the detection of mutant \textit{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 \textit{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 \textit{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 \textit{ras} genes led to the development of PCR based assays which distinguish the mutant \textit{ras} alleles due to a restriction fragment length polymorphism (RFLP). These assays are based on the ability of specific restriction endonucleases to recognise sequences in the \textit{ras} genes that overlap with the codons that behave as hot-spots for the mutations (13). In
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.**

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

**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.
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) 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 aforementioned 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.
Liver. Hepatic cancer is characterised by poor prognosis and reports indicate the presence of particular environmental factors that may result in the activation of the K-ras gene. Furthermore, Mizuuchi et al (65) suggested that K-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.

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.

Female reproductive tract. Mutations in the ras family genes have been detected by several investigators in endometrial carcinoma in variable frequency. 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 not detected in the 19 patients, were found to affect the N-ras gene. This is noteworthy 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).

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 cutaneous 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).

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 noteworthy 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.
been associated with the development of the disease, such as breast cancer, which represents a major cause of death in adult females. Several alterations at the molecular level have been detected in malignant adenocarcinomas and low malignant potential tumours. In cervical cancer, the incidence of ras mutations is relatively low as compared to this of the endometrium. As regards gastric cancer in general, it is of specific interest that the majority of the mutations have been detected in H-ras, K-ras and N-ras. 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. 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). Mutations in all three ras family genes have been detected 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). 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 different carcinogens present in each tissue.

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. The majority of the mutations were detected in K-ras and N-ras while in colorectal tumours the incidence of ras mutations was limited (89,91,92). 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). Mutations in all three ras family genes have been detected 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).

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<sup>ras</sup> 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).

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).

Table II. Association of ras mutations with prognosis.

<table>
<thead>
<tr>
<th>Tumour tissue</th>
<th>mutant ras</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon (stage D)</td>
<td>K-ras</td>
<td>favourable</td>
</tr>
<tr>
<td>Lung</td>
<td>K-ras</td>
<td>poor</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>N-ras</td>
<td>poor</td>
</tr>
<tr>
<td>Endometrium</td>
<td>K-ras</td>
<td>poor</td>
</tr>
<tr>
<td>Stomach</td>
<td>H-ras</td>
<td>poor</td>
</tr>
</tbody>
</table>
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 aforementioned information for the therapy of cancer. Such an approach has been successfully carried out in vitro by specific compounds (antisense oligonucleotides and inhibitors of farnesylatation) 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 precise information in clinical practice.

References