

Ras in Cancer and Developmental Diseases

Alberto Fernández-Medarde and Eugenio Santos

Abstract

Somatic, gain-of-function mutations in *ras* genes were the first specific genetic alterations identified in human cancer about 3 decades ago. Studies during the last quarter century have characterized the Ras proteins as essential components of signaling networks controlling cellular proliferation, differentiation, or survival. The oncogenic mutations of the H-*ras*, N-*ras*, or K-*ras* genes frequently found in human tumors are known to throw off balance the normal outcome of those signaling pathways, thus leading to tumor development. Oncogenic mutations in a number of other upstream or downstream components of Ras signaling pathways (including membrane RTKs or cytosolic kinases) have been detected more recently in association with a variety of cancers. Interestingly, the oncogenic Ras mutations and the mutations in other components of Ras/MAPK signaling pathways appear to be mutually exclusive events in most tumors, indicating that deregulation of Ras-dependent signaling is the essential requirement for tumorigenesis. In contrast to sporadic tumors, separate studies have identified germline mutations in Ras and various other components of Ras signaling pathways that occur in specific association with a number of different familial, developmental syndromes frequently sharing common phenotypic cardiofaciocutaneous features. Finally, even without being a causative force, defective Ras signaling has been cited as a contributing factor to many other human illnesses, including diabetes and immunological and inflammatory disorders. We aim this review at summarizing and updating current knowledge on the contribution of Ras mutations and altered Ras signaling to development of various tumoral and nontumoral pathologies.

Keywords: Ras, oncogenes, mutation, cancer, Ras-MAPK pathway, developmental syndromes

The Ras oncogene family has been very extensively studied over the last 3 decades, with more than 40,000 scientific articles published on the subject during this period. The fundamental implication of Ras proteins in pathological processes such as cancer and in physiological processes controlling cellular proliferation, differentiation, and survival justifies the interest seen in the scientific literature, currently showing a rate of 200–300 articles published per month.

The H-*ras*, N-*ras*, and K-*ras* oncogenes were the first human oncogenes discovered in human tumors more than 30 years ago and are the founding members of the wide Ras gene superfamily, composed by more than 150 distinct cellular members. As reviewed in other articles of this journal issue, the members of the Ras GTPase family are crucial players in many signaling networks connecting a great variety of upstream signals to an even wider set of downstream effector pathways linked to the functional control of a great assortment of cellular outcomes including cell cycle progression, growth, migration, cytoskeletal

changes, apoptosis, and senescence. The crosstalk between this plethora of signaling pathways and others controlled by different sets of signaling molecules creates molecular networks whose balance is crucial to determine the final outcome of cellular responses in the cell.^{1,2} The complexity of all these events controlling cell life reflects the difficult puzzle that has to be solved when these networks are altered in pathological situations and stresses the importance of their examination to find proper therapeutic approaches able to drive the cells back to a healthy signaling balance.

Within cellular signaling networks, participation of H-Ras, N-Ras, or K-Ras in the Ras-Raf-MAPK pathway has been proven essential for control of proliferation, differentiation, and survival of eukaryotic cells. Indeed, the evolutionary relevance and importance of this pathway are underlined by the growing number of pathological conditions that have been linked to alterations in some of its components. Thus, in addition to the frequent mutation of *ras* genes occurring in various types of cancer that was initially discovered about 30 years

ago,^{3–5} molecular alterations of many other components of the signaling pathway, such as B-Raf, EGFR, and NF-1, have been described in association with the development of a number of different types of malignancies.^{6–8} In most cases, the experimental data indicate that the mutations of different components of the signaling pathway are mutually exclusive events, as documented for BRAF and RAS oncogenes in the case of malignant melanomas.⁹ However, in some cases, simultaneous molecular alterations of more than one component of this pathway may co-exist. This is significant, for example, in the case of solid tumors where simultaneous amplification of EGFR related genes and presence or absence of K-Ras mutations are predictive of the response to novel drugs targeting the EGFR.¹⁰

Centro de Investigación del Cáncer, IBMCC (CSIC-USAL), University of Salamanca, Salamanca, Spain

Corresponding Authors:
 Alberto Fernández-Medarde
 Email: afm@usal.es

Eugenio Santos
 Email: esantos@usal.es

The experimental observations accumulated for the last 30 years document that somatic mutations are the typical genetic lesions affecting Ras and other oncogenes linked to the development of sporadic human tumors. In contrast, more recent observations have uncovered the occurrence of germline mutations in Ras and other members of the Ras-MAPK pathway that result also in constitutive activation of this pathway, although to a lesser extent than that found in tumors, and are specifically linked to the development of a number of distinct but related developmental syndromes. The first report of such type of mutations concerned the neurofibromatosis 1 (*NFI*) locus, a Ras GTPase activating protein (RasGAP) that is the causative agent for the neurofibromatosis type 1.¹¹ Later on, germline mutations in many other members of the Ras pathway (including the 3 Ras genes, signaling molecules as *PTPN11*, *MEK1*, and *MEK2* and *SPRED1*; positive and negative Ras regulators as *SOS1* or *Rasa1*; or downstream effectors such as *BRAF*) have been detected in relation to various other inherited developmental syndromes including Noonan, Costello, cardiofaciocutaneous, Legius, or Leopard syndromes.¹²⁻¹⁴

Altered Ras signaling may also contribute to the development of other types of pathologies besides cancer and developmental syndromes. For example, H-Ras activation has been associated with nonobese diabetes and diabetic retinopathy,^{15,16} where it is associated with abnormal vascular development.^{17,18} Increased amounts of cellular farnesylated Ras proteins may also account for some detrimental phenotypes observed in hyperinsulinemia.^{15,16,19} Changes in the expression patterns of H-Ras and K-Ras have been implicated in glomerulonephritis.²⁰ In AD neurons vulnerable to neurodegeneration, N-Ras accumulation and co-localization with nNOS have been described.²¹ Mutations in *ZHHD9*, a H-Ras and N-Ras palmitoyltransferase, have been reported to cause a particular type of X-Linked mental retardation,²² which may thus be

considered for inclusion as a potential new member of an increasing family of rasopathies. Finally, aberrant *Sos1* levels and Ras signaling have been described in patients with chronic idiopathic urticaria.²³

We will focus the following sections on updating and analyzing the experimental evidence and mechanisms linking the contribution of altered Ras signaling caused by somatic or germline mutations of *ras* genes or other genes coding for different components of Ras signaling pathways to the development of human diseases including cancer and developmental syndromes.

Incidence of Somatic Ras Mutations in Cancer

Mutations in any 1 of the 3 canonical H-*ras*, N-*ras*, or K-*ras* genes are among the most common events in human tumorigenesis. Multiple studies on different human tumors accumulated over the last 3 decades have identified 2 hot spots for *ras* oncogenic mutation, located respectively around codons 12 and 61 of their highly conserved coding sequences. A number of different databases have been generated during this period to include all the information generated regarding the presence of specific mutations of *ras* genes in different forms of human tumors.^{24,25} Currently, the Sanger Center keeps and periodically updates a comprehensive database involving the nature and frequency of *ras* mutations in different human tumors (catalogue of somatic mutations in cancer: <http://sanger.ac.uk/cosmic>) (Table 1). Overall, up to about 30% of all human tumors screened are found to carry some mutation in any of the canonical *ras* genes. Remarkably, these oncogenic mutations predominantly affect the K-*ras* locus, with oncogenic K-*ras* mutations being detected in 25-30% of all tumor samples screened.²⁴ The high frequency of K-*ras* mutations and the observation that they mostly appear during early stages of tumor progression provide strong argument supporting a causative role of K-Ras in human tumorigenesis.

By comparison, the rates of oncogenic mutation occurring in the N-*ras* and H-*ras* family members are much lower (8% and 3% of samples screened, respectively). The predominant involvement of K-Ras in pathological tumor development is also consistent with the superior physiological relevance suggested by the study of the phenotypes of knockout mice strains showing that N-Ras and H-Ras are dispensable, but K-Ras is essential for normal mouse development.^{26,27}

Analysis of the very extensive sets of tumor samples studied during the last 3 decades has revealed that there is a prevalent (although not bi-univocal) association of specific mutated Ras isoforms with particular types of tumors (Table 1).^{24,25} Thus, K-Ras mutations are present in a majority of pancreatic ductal adenocarcinoma and significantly high percentages of lung and colon tumors but are very uncommon in bladder tumors, where H-Ras is the most frequently mutated Ras isoform detected. In contrast, the studies have revealed a high incidence of N-Ras mutations in hematopoietic tumors and in malignant melanomas, whereas the rate of K-Ras or H-Ras mutations in the latter tumors is marginal.²⁴

In summary, although the specificity between tumor type and mutated Ras oncogene is not absolute (even in pancreatic adenocarcinomas where K-Ras mutations are prevalent, a low percentage of mutations can be found in N-Ras), in general, K-*ras* mutations are more frequently found in adenocarcinomas and solid tumors, whereas N-*ras* is the prevalent Ras gene mutated in leukemias, thyroid carcinomas, or malignant melanoma (where is mutually exclusive with B-Raf mutations) and H-*ras* mutations are sparingly found, with a prevalence in bladder carcinoma and low incidence cancers such as seminomas or Hurthle cell carcinomas (Table 1).²⁴

Oncogenic mutations are concentrated within 2 hotspots (around codons 12 and 61) of the primary nucleotide sequence of all ras family members. However, the incidence of mutation at

Table 1. Distribution and Frequency of *ras* Mutations in Human Tumors

Organ/Tissue	Tumor Type	H- <i>ras</i>	N- <i>ras</i>	K- <i>ras</i>
Biliary tract	Adenocarcinoma	0 (151)	2 (194)	35 (934)
Bladder	Transitional cell carcinoma	12 (1166)	2 (322)	4 (427)
Breast	Carcinoma	1 (542)	2 (330)	4 (544)
Cervix	Adenocarcinoma	9 (249)	3 (64)	8 (611)
Colon	Adenocarcinoma	0 (76)	2 (55)	36 (4310)
	Adenoma	0 (3)	0 (11)	22 (3545)
Ganglia (autonomic)	Neuroblastoma	0 (64)	8 (103)	3 (63)
	Other	N/A	N/A	27 (298)
Leukemias	AML	0 (1216)	12 (3404)	4 (1778)
	CML	0 (265)	3 (532)	2 (313)
	CMML	1 (118)	15 (157)	11 (84)
	JMML	0 (41)	19 (165)	7 (143)
Lymphomas	ALL	0 (284)	10 (703)	7 (549)
	Burkitt's lymphoma	0 (30)	10 (30)	3 (30)
	Hodgkin's lymphoma	2 (44)	16 (45)	0 (44)
	Plasma cell myeloma	2 (185)	20 (484)	6 (403)
Liver	Hepatocellular carcinoma	0 (163)	4 (202)	4 (307)
Lung	Large cell carcinoma	4 (50)	4 (49)	21 (189)
	Non small cell carcinoma	0 (683)	1 (695)	16 (3575)
	Squamous cell carcinoma	1 (261)	0 (360)	6 (1407)
	Other (neoplasia)	N/A	N/A	22 (563)
Pancreas	Ductal adenocarcinoma	0 (110)	1 (138)	69 (3483)
	Endocrine tumor	0 (2)	75 (4)	1 (68)
Prostate	Adenocarcinoma	6 (489)	2 (509)	8 (1002)
Skin	Basal cell carcinoma	7 (180)	1 (147)	4 (147)
	Squamous cell carcinoma	9 (236)	7 (107)	5 (107)
	Malignant melanoma	1 (904)	20 (3466)	2 (924)
Soft tissue	Angiosarcoma	0 (6)	0 (6)	49 (53)
	Leiomyosarcoma	3 (30)	0 (13)	8 (173)
	Liposarcoma	6 (72)	0 (21)	4 (45)
	Rhabdomyosarcoma	4 (158)	11 (151)	4 (162)
	Myxoma	0 (19)	0 (19)	11 (19)
	Malignant fibrous histiocytoma-pleomorphic sarcoma	15 (117)	2 (57)	16 (131)
Stomach	Adenocarcinoma	4 (218)	2 (205)	6 (2054)
	Other	11 (9)	0 (1)	6 (241)
Testis	Germinoma	0 (56)	7 (115)	7 (190)
	Seminoma	17 (30)	0 (30)	0 (23)
Thyroid	Anaplastic carcinoma	4 (440)	17 (436)	9 (433)
	Follicular carcinoma	5 (381)	17 (392)	4 (372)
	Papillary carcinoma	2 (1525)	4 (1941)	2 (1654)
	Hurthle cell carcinoma	16 (44)	4 (26)	0 (41)

Note: Data obtained from the Sanger Catalogue of Somatic Mutations in Cancer, at <http://sanger.ac.uk/genetics/CGP/cosmic/>.²⁴ Values presented as the total percentage of clinical samples analyzed (*n* shown within parentheses) for that particular tumor type. **Boldface** corresponds to tumors presenting significantly high rates (>10) of mutation in *ras* genes. ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CML = chronic myeloid leukemia; CMML = chronic myelomonocytic leukemia; JMML = juvenile myelomonocytic myeloid leukemia; N/A = not available.

both sites varies among the different 3 main *ras* family members. Thus, in K-Ras, the tandem Glycine 12-Glycine 13 (G12-G13) accounts for about 99% of the mutations detected (86% and

13%, respectively), whereas mutations affecting Glutamic acid 61 (Q61), the other main hotspot in Ras proteins, account for the remaining 1%²⁴ (<http://sanger.ac.uk/genetics/CGP/cosmic/>).

The biological significance of some other mutations found along the K-*ras* locus is largely unknown. A recent report has shown that exon 4 mutations may predict a more favorable prognosis.²⁸ Another report has described a novel transforming mutation combination affecting codons 19 and 20 in colorectal carcinoma (L19F and T20A).²⁹

Oncogenic mutations of N-*ras* genes in human tumors follow a different distribution pattern, with highest rates of mutation found at Q61 (about 60% of total N-*ras* mutations) and lower percentages detected at G12 (24.4%) and G13 (12.7%).²⁴ Finally, H-*ras* mutations show their own specific pattern, with highest percentage of mutations detected in codon 12 (about a 54%), followed by codon 61 (34.5%) and codon 13 (9%).

Although other mechanisms may also lead to *ras* activation in vitro or in cell lines,³⁰ oncogenic mutation appears to be the almost exclusive mechanism linking *ras* genes to in vivo human tumor development. Thus, despite some early reports describing amplification of K-Ras^{31,32} or N-Ras³³ in some tumors and cell lines, the bulk of experimental data accumulated show that Ras amplification is not a common phenomenon in cancer. Furthermore, a recent report describing Ras overexpression in a colon carcinoma failed to find a relationship with prognosis, suggesting that Ras overexpression cannot be used as a predictive factor.³⁴ The very infrequent detection of Ras amplification in tumors might be related to recent observations showing that the relative percentages of expressed H-Ras, N-Ras, and K-Ras proteins are almost constant in various tissues and cells analyzed, regardless of whether they are tumoral or normal.³⁵

Genetics and Biology of Tumors Harboring *ras* Mutations

As shown in Table 1, *ras* mutations are frequent in some of the cancers with the worst prognoses. The following sections will describe clinical and molecular

aspects of various tumor types in which *ras* genes are frequently mutated and will analyze the contribution of altered Ras signaling to the progression and the causal features of those tumors.

Pancreatic Ductal Adenocarcinoma

About 95% of tumors arising in the pancreas affect the duct epithelial cells. Although pancreatic adenocarcinomas are not among the most frequently detected tumors worldwide, they are among the most aggressive and with worst prognosis/outcome in humans. These tumors harbor the highest reported incidence of *ras* mutations among all human cancers. These mutations seldom affect H-Ras or N-Ras and concentrate almost exclusively on the K-Ras locus, with reports of mutation rates ranging from 95%³⁶ to a 69%³⁷ in the scientific literature (Table 1). These discrepancies may arise from different analytic methods of mutation analysis or may reflect the fact that K-*ras* mutations increase during pancreatic cancer evolution, with rates of 30% reported in early neoplasms and almost 100% in advanced cancer.³⁸ The bulk of reported mutations affect K-*ras* codon 12 (changing glycine to either aspartic acid, arginine, or valine) and result in constitutive activation of the outcoming Ras proteins.²⁴ The available data indicate that K-*ras* mutations are early events in pancreatic cancer evolution as even samples from chronic pancreatitis present a high percentage of K-*ras* mutations.³⁹

There are contradictory reports concerning the prognostic value of K-*ras* mutations in pancreatic cancer. Whereas early reports did not find correlation between presence of K-*ras* mutations and survival rates,^{40,41} more recent studies have described a worse prognosis of non-resectable pancreatic cancers harboring K-*ras* mutations⁴² and shorter survival rates associated with the detection of K-*ras* mutations in tissue surrounding the surgical margins of resected pancreatic tumors.⁴³ Separate studies have also reported different aggressiveness of pancreatic adenocarcinomas depending on

the particular K-*ras* mutation occurring in them. For example, tumors bearing K-*ras* G12R and G12A mutations were reported to have worse survival rates than tumors harboring G12V or G12S mutations.^{44,45} Surprisingly, (interestingly) for mutations resulting in the same G12D amino acid substitution, tumors harboring GaT mutations were described as more aggressive than those harboring GaC mutations at codon 12.⁴⁵

The high prevalence of K-*ras* mutations and their likely contribution promoting early events in pancreatic tumorigenesis have prompted the developmental use of therapeutic trials interventions using K-Ras as a target. However, despite promising preclinical results with cell lines and mouse xenografts,⁴⁶⁻⁴⁸ the results obtained in clinical trials with farnesyltransferase inhibitors aimed at blocking posttranslational modifications of the K-Ras proteins have been deeply disappointing.⁴⁹⁻⁵¹ These negative results may be explained, at least in part, because K-Ras posttranslational processing may also involve geranylation, in addition to farnesylation.⁵² In addition, because of the pre-supposed role of K-Ras in initiation of pancreatic tumorigenesis, rather than in establishment of advanced pancreatic cancer, it is conceivable that the accumulation of other genetic modifications could overcome or bypass the inhibition of K-Ras in the pancreatic cancer cells. However, despite the data questioning the use of Ras as a therapeutic target, new anti-Ras approaches are still being tested. For example, a vaccination approach against K-Ras oncogenic peptide has been recently reported to increase survival rates in surgically resected pancreatic cancer patients.⁵³

Colorectal Carcinoma

K-*ras* mutations are common events detected in 40-45% of all colorectal carcinoma (CRC) samples analyzed (Table 1), suggesting that K-Ras proteins are important players in tumor development.⁵⁴ Most K-*ras* mutations affect codons 12 and 13 (80% and 20%,

respectively), and G12D is the most common amino acid change resulting from such mutations. In contrast, much lower mutation rates have been found in N-*ras* (1-3% of CRC samples analyzed).^{54,55} No activating mutations have been reported so far for H-*ras* in CRC.

The detection of mutated K-*ras* in both early and late CRC stages suggests that, as in pancreatic cancer, K-*ras* mutations may be early events in tumor development.⁵⁶⁻⁵⁸ Although still controversial, it has been proposed in this regard that in some CRCs, K-*ras* mutations may occur as early events in formation of aberrant crypt foci that could later progress to hyperplastic polyps and eventually to CRC.⁵⁹ Nevertheless, unlike pancreatic carcinomas where K-*ras* mutations are prevalent, many other genetic alterations besides K-*ras* mutations may occur in CRC that could be responsible for tumor initiation and progression in this case.

In contrast to early reports,⁶⁰ many recent studies have documented a correlation between K-*ras* mutations and poor prognosis of aggressive colorectal carcinomas.⁶¹⁻⁶³ Separate studies have also reported that the rate of K-*ras* mutation is enhanced in CRC patients with lung metastasis⁶⁴ and that the presence of K-*ras* mutations in CRC patients with liver metastasis is predictive of bad prognosis.⁶⁵

Analysis of the mutational state of *ras* genes has proven to be very significant for selection of therapeutic approaches in CRC. Thus, for tumors with high EGFR expression levels and WT K-*ras*, significant clinical benefit derives (35% overall response rate) from treatment with specific monoclonal antibodies against EGFR (cetuximab, panitumumab),^{10,66} whereas negligible benefit (response rate 3%) is observed in patients carrying mutant K-*ras*.^{67,68} Furthermore, tumor free progression or overall survival shows better results in patients carrying WT K-*ras* than in those harboring oncogenic mutations.⁶⁹ Although these data are promising for patients with WT K-*ras*, results are still poor. Therefore, various ongoing clinical trials are testing new

additional combinations of anti-EGFR, Mab-based therapies as well as alternative therapeutic approaches such as vaccines against mutant K-Ras, inhibitors of downstream kinases, and so on (see trials in NCT00019006, NCT00019084, NCT00019331, NCT00326495, or NCT01085331 at <http://clinicaltrials.gov/show/>).

Non-Small Cell Lung Carcinoma

As with other carcinomas, non-small cell lung carcinomas (NSCLCs) display a high frequency of *K-ras* mutations and low rates of oncogenic changes in either *N-ras* or *H-ras* (Table 1). The total reported rate of *K-ras* mutations in NSCLC varies from 16% to 40% of samples analyzed.⁷⁰⁻⁷² Approximately 94% of all *K-ras* mutations result in changes of the Gly residue coded for by codon 12 of WT *K-ras*. G12C accounts for about a 40% of total mutations, followed by G12V (22%) and G12D (16%).^{24,73} This is likely attributable to the origin of NSCLC, which is usually associated with tobacco smoking.⁷⁴ Indeed, G-C or G-T transversal mutations of guanine nucleotide residues located in normal *K-ras* codon 12 are known to be produced by tobacco smoke and are rare events in NSCLC found in nonsmokers.⁷⁵

The study of animal models⁷⁶ (reviewed in O'Hagan & Heyer, this issue) suggests that *K-ras* mutations may have a causative role in NSCLC development. For example, in a mouse model mimicking the apparition of somatic, human *K-ras* mutations by means of intrachromosomal *in vivo* recombination leading to activation of the mutant allele, the animals developed lung carcinomas resembling human NSCLC and evolving through a series of morphological alterations similar to those described in staging of human NSCLC.⁷⁷ The notion of *ras* mutations as early events triggering human NSCLC is further supported by their detection in precancerous lesions⁷⁸ and the observation of such mutations arising upon long-term exposure to ambient chemicals such as tobacco, asbestos, and smoky coal.⁷⁹⁻⁸¹

Most recent studies suggest that the presence of *K-ras* mutations in NSCLC is indicative of more aggressive tumors,⁸²⁻⁸⁴ although some previous reports may suggest otherwise.⁸⁵⁻⁸⁷ Separate studies have also suggested that a relationship might exist between the final prognosis and the type of *K-ras* mutation occurring in the NSCL tumor. For example, it has been reported that G12D mutations are associated with tumors with better prognosis than those bearing G12V or G12R substitutions.⁸⁸ In addition, a recent report using a NSCLC cancer cell line has shown that different amino acid substitutions may account for different drug sensitivities in those tumors.⁷³

As already described for CRC, the mutation status of K-Ras is very important when selecting a therapeutic approach in NSCLC. The response and survival rates of NSCLC patients treated with EGFR inhibitors are much higher when their tumors harbor WT K-Ras.^{67,89} Thus, specific monoclonal antibodies or other inhibitors blocking EGFR action are a front-line therapeutic approach for lung cancers without K-Ras mutations, although current clinical trials are also trying to test and find novel combinatory therapeutic approaches aimed at achieving better long-term survival rates. With regard to the tumors harboring mutant K-Ras, similar approaches to those previously mentioned for pancreatic and colorectal cancer are being tried at present in clinical trials that explore anti-active Ras vaccines or a variety of downstream kinase inhibitors (see NCT00655161, NCT00019006, NCT00005630, NCT00098254 at <http://clinicaltrials.gov/show/>).

Malignant Melanoma

Together with bladder carcinomas, melanomas are the only high-incidence/high-mortality solid tumors in humans in which *K-ras* mutations are not prevalent over *N-ras* or *H-ras* mutations. Specifically, *N-ras* mutations are found in 20-30% of malignant melanoma samples analyzed (Table 1).^{90,91} Substitutions of Q61 account for most (about

86%) *N-ras* mutations detected, whereas changes of G12 or G13 are significantly less frequent (7% and 4.5%, respectively). Indeed, the most common mutations found are, in this order, Q61K, Q61R, Q61L, and G12D.^{24,90} This is likely attributable to the preference for dicyclobutane formation at the Q61 site upon UV irradiation, which is a major cause of skin mutations leading to malignant melanoma.⁹² It is also relevant to mention here the frequent detection of activated BRAF oncogenes in human melanomas. The fact that BRAF is a Ras downstream effector and the observation that *BRAF* and *N-ras* mutations appear to be mutually exclusive in melanomas indicate that altered Ras signaling is a crucial initiating factor triggering melanomagenesis.^{9,93,94}

Screening of melanoma samples at different stages of tumor progression has shown that *N-ras* mutations are early events in melanomagenesis. Analysis of primary tumors and metastasis from the same patients does not show increased rates of *N-ras* mutation in the metastatic samples and also documents the presence of *N-ras* mutations already at the early stages such as even at the nevi stage.⁹¹

Some studies have suggested that the presence of *N-ras* mutations is linked to better prognosis in malignant melanomas,⁹⁵ possibly because melanoma cells carrying *N-ras* mutations may become targets for cytotoxic T-Lymphocytes.⁹⁶ This is even more dramatic in melanoma patients carrying an A18T mutation that showed a markedly better prognosis than those carrying mutations in Q61.⁹⁷ However, other studies have not detected significant correlation between mutations in *N-ras* codon 61 and overall survival.⁹¹

Regarding therapeutic approaches, the use of the farnesyltransferase inhibitor R115777 (tipifarnib) for treatment of melanoma patients⁹⁸ has not yielded any positive clinical response. In addition, an ongoing clinical trial (<http://clinicaltrials.gov/show/NCT00281957>) is analyzing the effect of combining this drug with the BRAF inhibitor sorafenib

in nonresectable melanoma patients. As mutations of other upstream activators or components of downstream pathways are also frequently found in melanoma tumors, most ongoing or future strategies for melanoma treatment are focused on targeting these other signaling molecules (see NCT01320085, NCT00866177, NCT00304525 at <http://clinicaltrials.gov/show/>).

Urinary Bladder Carcinoma

Bladder cancer is the sixth most frequent malignancy in Europe and the United States⁹⁹ (<http://apps.nccd.cdc.gov/uscs/toptencancers.aspx>). However, mortality from bladder carcinomas is significantly lower than in other carcinomas, probably because most tumors (75-85%) are detected as early-stage, still noninvasive carcinomas.¹⁰⁰

Although T24 bladder carcinoma cells were the source of the first human oncogene detected,³⁻⁵ the rates of *H-ras* mutations detected in human bladder carcinomas are not high, with reports ranging from as low as 0% up to 12% or even 30% of all bladder carcinomas analyzed (Table 1).^{101,102} Among these mutations, G12V substitutions predominate (about 60% of total mutations), followed by G12D and Q61R (8% and 7%, respectively).²⁴

Despite the medium-low *ras* mutation levels detected, a recent report has highlighted the crucial role of Ras proteins in bladder cancer by showing that overexpression of at least 1 of the 3 Ras canonical proteins is a common event in this illness. In this report, 77% of the analyzed tumors expressed higher Ras levels than the surrounding normal tissue.¹⁰¹ Remarkably, overexpression of K-Ras and N-Ras was found mainly in bladder carcinomas, whereas H-Ras was more frequently overexpressed in transitional cell carcinomas.

H-ras mutations have been described as early events in tumor development^{100,103} and have been linked mainly to low-grade tumors that rarely evolve to more aggressive stages. Despite this, a study has also found that a single

nucleotide polymorphism (81T>C) in the *H-ras* locus is associated with a higher risk of developing bladder carcinomas and more specifically with advanced, more aggressive types of cancer.¹⁰²

H-Ras is nowadays not being used as a target for bladder cancer treatment (<http://clinicaltrials.gov/ct2/results?term=Bladder+carcinoma>). Nevertheless, several attempts have been made in the search of H-Ras targeted therapies. Thus, an anti-H-Ras ribozyme designed and used against cell lines and a mouse bladder cancer model showed the ability to reduce tumor growth and even lead to complete regression after a set of multiple adenoviral injections.¹⁰⁴ Similarly, other studies have succeeded using dominant negative H-Ras constructs and adenoviral vectors for treatment of orthotopically induced bladder tumors in mice.¹⁰⁵ Unfortunately, these promising data have not resulted in clinically available treatments, mainly because the applicability of these therapies must overcome toxicity of the adenoviral vectors and small infection efficiency. Interestingly, some patients may benefit from carrying *H-ras* mutation in their bladder tumors. As the oncogenic H-Ras proapoptotic ability is stimulated upon treatment with histone deacetylase (HDAC) inhibitors,¹⁰⁶ an ongoing clinical trial (<http://clinicaltrials.gov/show/NCT00087295>) is using romidepsin (HDAC Inhibitor FR901228) for treatment of bladder carcinomas.

Thyroid Carcinomas

Ras mutations are found in a discrete percentage of thyroid cancers. The Sanger Catalogue of Somatic Mutations in Cancer cites significant rates of *N-ras* mutations in anaplastic and follicular carcinomas (17%) and *H-ras* mutations in Hurthle cell carcinomas (16%) (Table 1).²⁴ In contrast, other studies have reported predominant rates of mutation in *K-ras* genes (24.3%) and much lower frequencies of mutation for *N-ras* or *H-ras* (8.4% and 4.7% respectively).¹⁰⁷ These discrepancies may be due to

different methods of mutation analysis, regional or racial differences between patients, or different criteria when selecting the patients for analysis.

As in melanoma, the *N-ras* mutations concentrate on codon 61. Thus, Q61R (68%) and Q61K (15%) are the most frequent amino acid substitutions detected, and mutations in G12 or G13 are rare events (Sanger Catalogue of Somatic Mutations in Cancer).²⁴ In contrast, mutations in *K-ras* and *H-ras* affect mainly codons G12 and 13.¹⁰⁷

BRAF mutations are also detected in thyroid carcinomas, even at higher frequencies than Ras mutations, and are also mutually exclusive with these.¹⁰⁸ Nevertheless, mutations in the upstream tyrosine kinase receptor *RET* are probably the most frequently detected alterations in thyroid cancer (about 50% of these tumors) and their main target for therapeutic approaches. Whereas *RET* mutations appear mainly in medullary and papillary thyroid carcinomas, *N-ras* alterations are found mostly in follicular and anaplastic tumors, and *BRAF* mutations are more common in papillary and anaplastic carcinomas.^{24,109}

As in other malignancies, the fact that *ras* mutations have been detected in thyroid adenomas suggests that these mutations are early events in thyroid cancer development. Nevertheless, *ras* mutations in these tumors are associated with undifferentiated phenotype, high vascularization, and bigger tumoral mass, which is indicative of poor prognosis in thyroid carcinomas, where they correlate with more aggressive tumors and higher chance of distant metastasis.^{107,110}

Radioiodine remains the first-line treatment for thyroid carcinomas, but its side effects¹¹¹ have triggered the search for less aggressive therapeutic approaches. Most current research on treatment of these illnesses is focused on targeting mutant *BRAF*. Thus, a majority of the ongoing clinical trials are using Sorafenib (BAY 43-9006), a specific B-RAF inhibitor as the therapeutic approach (NCT00095693, NCT01263951, NCT00098592 at <http://clinicaltrials.gov/>

show/). Indeed, phase 2 studies have shown very favorable results¹¹² and have raised expectations for the effectiveness of this drug for thyroid cancer treatment. Other approaches are still targeting Ras proteins, usually in combination with B-RAF inhibitors. Thus, a combination of Sorafenib with the FT inhibitor Tipifarnib was reported to yield significant increases in progression-free survival in papillary or medullary thyroid carcinoma patients.¹¹³

Hematopoietic Malignancies

Ras mutation rates vary widely in hematopoietic cancers, with values ranging in leukemias from as low as 5% in chronic myeloid leukemia (CML) to 27% in chronic myelomonocytic leukemia (CMML) (Table 1). Some studies have also reported exceedingly higher percentages (70%) in CMML and plasma cell myeloma (reviewed in Reuter *et al.*¹¹⁴). A prevalence of activating mutations of both K-*ras* and N-*ras* has been described in multiple myeloma.¹¹⁵ In general, mutations are almost inexistent in H-*ras*, are rare events for K-*ras* (with the exception of CMML), and are much more frequent for N-*ras*, reaching rates of up to 20% in juvenile myelomonocytic myeloid leukemia (JMML) or plasma cell myeloma (Table 1).

Despite sharing this genetic modification with melanomas or thyroid carcinomas, the pattern of N-*ras* mutations in hematopoietic tumors is very different²⁴ (Sanger Catalogue of Somatic Mutations in Cancer). Thus, in sharp contrast to solid tumors, lymphomas concentrate N-*ras* mutations on codon 61, with rather similar frequencies for the 3 most commonly detected amino acid substitutions: 61Q (38%), G12 (36%), and G13 (25%). These differences are even more markedly found in leukemias, where the N-*ras* mutation pattern resembles that observed for K-*ras* in solid tumors, with G12 mutations clearly prevailing over G13 and Q61 mutations (G12, 53%; G13, 29%; Q61, 17%). The most common amino acid changes do not differ from those observed in other malignancies, with G12D or G13D and Q61R or

Q61K being the more frequent alterations. The significance and importance of *ras* mutations in the origin of hematological cancers are underscored by studies of animal mouse models whose bone marrow was repopulated with cells infected by a retroviral construct expressing a N-Ras oncogenes.¹¹⁶ These mice developed myeloproliferative disorders resembling CML, indicating that N-*ras* mutations are sufficient for development of this type of hematological syndrome.

Mutations affecting various other components of Ras signaling pathways (such as upstream receptor tyrosine kinase receptors, as c-Kit, c-FMS, or FLT3, or other signaling molecules modulating Ras activation) have been reported in hematological malignancies (reviewed in Reilly¹¹⁷). For example, inactivating mutations of NF1 (a GAP for Ras), and the subsequent hyperactivation of Ras,¹¹⁸ are probably involved in development of JMML. Interestingly, patients with neurofibromatosis have a higher risk of developing JMML,¹¹⁹ and about 15% of children with JMML, but without clinical neurofibromatosis, present inactivating mutations in the NF1 gene and hyperactivation of Ras.¹¹⁸ Likewise, increased levels of Ras activation have been linked to CML resulting from the Bcr/Abl translocation creating the Philadelphia chromosome.¹²⁰ A separate report has also shown that mutations in FLT3, NRAS, KRAS, or PTPN11 are mutually exclusive in childhood acute lymphoblastic leukemia (ALL).¹²¹

The correlation between the presence of *ras* mutations and the prognosis of hematopoietic malignancies is rather unclear and depends largely on the type of hematopoietic cancer under consideration. For example, there are contradictory reports concerning acute myelogenous leukemia (AML), as some publications reported a link of N-*ras* mutations to worse prognosis,¹²² whereas others described them as unrelated to the final outcome of the disease^{123,124}, a particular study even links specific mutations at codon 13 to a better outcome of

the disease.¹²⁵ Likewise, in acute lymphoblastic leukemia, *ras* mutations may have a role in tumor development, but there are contradictory reports regarding their relation to final survival rate, with some early studies reporting that tumors with N-*ras* mutations have worse survival rate than those carrying WT copies¹²⁶ and more recent reports failing to show a correlation between N-*ras* mutations and final outcome.¹²⁷ N-*ras* mutations have also been associated with a worse prognostic in myelodysplastic syndrome, mainly attributable to a higher risk of developing AML.¹²⁸ Finally, the occurrence of N-*ras* mutations in multiple myeloma (MM) appears to be independent of clinical stage, but oncogenic Ras is associated with disease progression, aggressive phenotype, and shorter survival.^{115,129}

As already mentioned, the use of farnesyl transferase inhibitors (FTI) in treatment of solid tumors is a history of disappointment, especially given that preclinical studies created such high expectations (reviewed in Appels *et al.*¹³⁰ and Mazieres *et al.*¹³¹). Unfortunately, the history of FTIs in the treatment of leukemias and lymphomas is no different, and poor results have followed great preclinical observations.¹³² Despite this, some clinical trials for hematopoietic malignancies are still ongoing that focus on the use of FTIs, either as single anti-Ras agents (see NCT00093990, NCT00354146, NCT00082888, etc., <http://clinicaltrials.gov/show/>) or in combination with different drugs targeting other components of relevant signaling pathways (NCT00101153, NCT00096122, etc., at <http://clinicaltrials.gov/show/>). Ongoing clinical trials are testing the usefulness of anti-Ras monoclonal antibodies trial (<http://clinicaltrials.gov/show/NCT00003959>) as well as various inhibitors of downstream kinases such as the Raf inhibitor sorafenib¹³³ (<http://clinicaltrials.gov/show/NCT00131989> and NCT00303966, etc.) or the MEK inhibitors AS703026 (<http://clinicaltrials.gov/show/NCT-00957580>) and GSK1120212 (<http://clinicaltrials.gov/show/00920140>).

Targeting upstream receptor and nonreceptor tyrosine kinases has proven clinically useful in the case of imatinib (Gleevec), a direct inhibitor of the Bcr/Abl oncogene that is being worldwide used for CML treatment,¹³⁴ although analysis of data accumulated during the last 5 years shows that 30% of the patients had to abandon treatment and therefore new strategies have to be designed for them.¹³⁵

Ras Mutation in Other Tumor Types

Ras mutations are rather uncommon in other high-incidence cancers such as prostate, breast, or liver carcinomas.

Regarding breast cancer, it was reported that WT H-Ras expression correlates with better survival of node-free breast cancer patients, probably by inducing apoptosis of the cancer cells at an early stage,¹³⁶ and that elevated H-Ras levels in more advanced breast cancer patients could be indicative of a worse prognosis.¹³⁷

In hepatocellular carcinomas, where *ras* mutations are found in less than 10% of tumors, it has been recently shown that WT Ras proteins become hyperactivated through a mechanism involving the inactivation of Ras-GAPs that occurs in most samples analyzed.¹³⁸ As with other tumors involving alteration of Ras signaling pathways, this study also showed that *ras* mutations and GAP promoter hypermethylation and silencing are mutually exclusive events.¹³⁸

Neuroblastomas, cervix adenocarcinomas, or stomach cancers also harbor low rates of *ras* mutation.²⁴ Nevertheless, overexpression of WT H-Ras in neuroblastoma has been reported as a good prognostic predictor.¹³⁹ Furthermore, even if *ras* mutations are not common, cervix adenocarcinomas are reported to show overexpression of H-Ras and N-Ras and normal levels of K-Ras compared with surrounding normal tissue,^{140,141} although no association between Ras overexpression and prognosis has been found.¹⁴⁰ In gastric cancer, where Ras mutations are also uncommon, a different mechanism has

been proposed for abnormal Ras activation. MicroRNA mir-204 has been reported to be downregulated in these tumors, thus leading to higher Ezrin expression, higher Ras activation, and poorer prognosis.¹⁴²

Significant frequencies of *K-ras* mutations locus are detected in some lower incidence cancers such as biliary tract adenocarcinomas (35%), angiosarcomas (49%), or malignant fibrous histiocytoma (16%), where H-*ras* mutations have also been found (15%) (Table 1) (source: <http://sanger.ac.uk/genetics/CGP/cosmic/>). Finally, H-*ras* and N-*ras* mutations have been found in neck and head cancer, where H-Ras overexpression has been also described¹⁴³ (Sanger Catalogue of Somatic Mutations in Cancer). In this case, the Ras alterations may be associated with better prognosis, as some reports described better survival rates for patients carrying H-*ras* mutations in oral cancer¹⁴⁴ and overexpression of WT H-Ras in squamous cell carcinomas of the head and neck.¹⁴⁵

Rasopathies Mediated by Germline Mutations in *ras* Genes or in Other Components of Ras Signaling Pathways

The wealth of experimental data accumulated during the last 3 decades have clearly established and documented the frequency and importance of somatic *ras* mutations in development of a variety of sporadic, human tumors appearing during adult life. Conversely, more recent observations accumulated within the last decade have brought to light the occurrence of various germline *ras* mutations occurring in association with various hereditary familial developmental syndromes (Table 2). Indeed, the genetic and molecular characterization of multiple clinical samples of this collection of inherited developmental diseases has shown that their transmission may be linked not only to the presence of germline *ras* mutations but also to the occurrence of germline mutations in various other upstream or downstream

components of Ras signaling pathways.^{13,14,146-150} It is therefore evident that disruption of correct Ras signaling is the main mechanism and driving force leading to development of this collection of distinct developmental syndromes, which otherwise exhibit a number of shared, overlapping phenotypic features.

We summarize in the next sections some of the most relevant features of different syndromes associated with germline mutations that affect canonical Ras proteins or other members of the Ras dependent signaling pathways.

Neurofibromatosis Type 1 (NF1)

NF1 was the first congenital rasopathy described,¹⁵¹ with an approximate incidence of 1/3,000 in the general population. It is an autosomal dominant disease caused by inactivating genetic modifications in the *NF1* gene, coding for a GTPase activating protein (GAP) acting on Ras. In contrast to other rasopathies, where mutations have been observed in more than 1 member of the Ras signaling pathway, modifications of the *NF1* gene are the only genetic alterations detected that are responsible for NF1, suggesting that at least some of its clinical features may be attributable to functions of the NF1 protein that are not related to Ras signaling. The genetic alterations observed for the *NF1* locus include deletions, insertions, or mutations that are often (about 50% of cases) de novo events happening in the parent's germline and cannot be related to a familiar NF1 background.

The accepted clinical features for NF1 diagnosis include 2 or more of the following: café-au-lait spots (6 or more, bigger than 5 mm in infants or 15 mm in postpubertal patients), neurofibromas (2 or more), axillar or inguinal freckling, osseous lesions, optic pathway tumors, and 2 or more iris hamartomas.¹⁵² The NF1 patients normally have reduced life spans mainly because of the development of tumors, as they present higher rates of CNS tumors (gliomas and astrocytomas), neurofibrosarcomas, and leukemias than

Table 2. Congenital Syndromes Associated with Mutational Alterations of Components of Ras Signaling Pathways

Syndrome	Mutated Gene	Protein Function	Mutations/Other Changes Observed	Associated Neoplasias
Neurofibromatosis type1 ^{156,185}	<i>NF1</i> (Neurofibromin)	RasGAP	Small and large deletions; insertions; mutations throughout the protein; intron mutations have also been described. Nonsense R1947X mutation is the most frequent event (~ 2%)	Increased cancer risk: neurofibrosarcomas, central nervous system tumors, myeloid leukemias
Leopard syndrome ^{147,157,173}	<i>PTPN11</i> (SHP2)	RTK phosphatase	Y279C/S, A461T, G464A, T468M/P, R498W/L, Q506P, Q510P/E/G	Myelodysplasia, acute myelogenous leukemia, neuroblastoma
Noonan syndrome ^{149,161,166}	<i>RAF1</i> (c-Raf)	Kinase	S257L, L613V	Cancer an uncommon outcome of the illness; higher risk of myeloproliferative disease and leukemia, especially juvenile myelomonocytic leukemia
	<i>B-RAF</i> (B-Raf)	Kinase	T241P, L245F	
Legius syndrome ^{170,186}	<i>PTPN11</i> (SHP2)	RTK phosphatase	Over 58 different mutations. The most frequent: D61N/G, Y63C/G, A72S/G, T73I, E76D, Q79R, E139D, Y279C, N308D/S, T468M, M504V	Possible increased risk of cancer
	<i>SOS1</i> (Sos1)	RasGEF	T266K, M269R/T, D309Y, Y337C, G434R, C441Y, P478 R/L, S548R, L550P, R552G/K/S, F623I, P655L, Y702H, W729L, I733F, E846K	
	<i>K-RAS</i> (K-Ras)	GTPase	V14I, Q22R, P34L/Q, I36M, T58I, V152G, D153V, F156I	
	<i>N-RAS</i> (N-Ras)	GTPase	T50I, G60E	
	<i>RAF1</i> (c-Raf)	Kinase	R256S, S257L, S259F, T260R/I, P261S/L/A, V263A, D486N/G, T491I/R, S612T, L613V	
Costello syndrome ^{14,173}	<i>B-RAF</i> (B-Raf)	Kinase	E501K, K499E, L597V	Rhabdomyosarcoma, transitional cell carcinoma, neuroblastoma
	<i>SHOC2</i> (SHOC2)	Scaffold	S2G	
	<i>MEK1</i> (MEK1)	Kinase	D67N	
Cardio-facio-cutaneous syndrome ^{150,173}	<i>SPRED1</i> (SPRED1)	Interactor	Deletions; amino acid switching mutations: V44D, S149N, M1T; nonsense mutations: R16X, R64X, E73X, R117X, Q213X, Q215X, K322X, R325X	Cancer predisposition uncertain; possible acute lymphoblastic leukemia
	<i>H-RAS</i> (H-Ras)	GTPase	G12S/A/V/C/E, G13C, K117R, A146T	
Hereditary gingival fibromatosis type 1 ¹⁸⁰	<i>K-RAS</i> (K-Ras)	GTPase	K5N, V152G, F156L	No increased risk of cancer
	<i>B-RAF</i> (B-Raf)	Kinase	G534R, D638E	
	<i>K-RAS</i> (K-Ras)	GTPase	Q22E, P34R, G60R, D153V, F156I	
Autoimmune lymphoproliferative syndrome ¹⁸²	<i>B-RAF</i> (B-Raf)	Kinase	A246P, Q257K/R, S467A, F468S, G469E, L485F, V487G, K499E, E501K/G, G534R, N580D, N581D, F595L, G596V, D638E	Increased risk of hematological malignancies
	<i>MEK1</i> (MEK1)	Kinase	F53S, P124L, Y130C	
Capillary malformation–arteriovenous malformation ^{183,187}	<i>MEK2</i> (MEK2)	Kinase	F57C, K61E, P128R, G132V	Vascular tumors
	<i>SOS1</i> (Sos1)	RasGEF	Single nucleotide insertion (C) between nt 3248 and nt 3249	
	<i>N-RAS</i> (N-Ras)	GTPase	G13D	
	<i>Rasa1</i> (p120RasGAP)	RasGAP	Deletions; duplications; and mutations: G829A, C853T, C1336T, Q446X, C540Y, G1619A	

See accompanying text and references for more detailed information about symptoms and mechanisms involved in the development of each syndrome.

the normal population.^{153,154} A likely cause underlying the development of these tumors is the constitutive hyperactivation of Ras signaling that occurs in cells of these patients as a consequence of the absence of the downregulatory GAP activity of NF1.^{152,155,156}

Leopard Syndrome (LS)

This rasopathy is caused by mutations in the *PTPN11* locus (85%), whose gene product is a receptor tyrosine kinase phosphatase, and it can also be developed upon mutations in the *B* and *C-Raf* genes. The name of the syndrome, Leopard, in addition to reflect the characteristic spotted skin of the patients, is an acronym for a list of the main symptoms used for diagnosis of the illness: lentiginos, ECG abnormalities, ocular hypertelorism (distance between the eyes), pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness.¹⁵⁷

The *PTPN11* gene product, SHP2, is a phosphatase acting as an important mediator of signaling initiated through many growth factor receptors, cytokines, and hormones, and several studies have shown that the Ras-MAPK pathway is one of its main downstream targets. Nevertheless, the exact mechanism used by this phosphatase to promote Ras activation is still unclear.¹⁵⁸ Most mutations found in LS are missense mutations, and it has been proposed that these phosphatase defective mutations in the *PTPN11* gene have gain-of-function effects,¹⁵⁹ although other reports suggest dominant negative effects for these mutations.¹⁶⁰

Noonan Syndrome (NS)

Closely related to the previous illness, NS is a more common condition affecting 1 in 2,000 individuals. In addition, it is more genetically heterogeneous. Thus, although it is also produced by modifications in the *PTPN11* locus (~50%),¹² lower incidence mutations in other genes, including *Sos1* (~13%), *K-Ras* (<2%), *N-Ras*, *B-* and *C-Raf*, *MEK1*, and *SHOC2*, have been found.^{148,161-166} In comparison to LS, the *PTPN11* mutations detected in NS produce clearly gain-of-function effects. They

affect the interaction regions of the N-SH2 and the phosphatase domains, implicated in switching from the inactive to the active conformation, thus unbalancing the stoichiometry toward an active SHP-2 protein.¹² Similarly, the mutations of the Ras guanine exchange factor *Sos1* occurring in NS are known to promote *Sos1* open conformation and activity, thus leading to higher cellular Ras-GTP levels and general pathway activation.¹⁶⁶ This is also true for the NS mutations directly affecting Ras family members. The K-Ras and N-Ras mutants found in NS are reported to activate the Ras-ERK pathway at a greater extent than wild-type Ras proteins although to a lesser extent than the Ras mutants commonly found in cancer.^{161,167}

Although LS and NS share many phenotypical characteristics, Noonan patients lack the café au lait spots in the skin. Noonan diagnosis is based on several facial abnormalities, including alterations of the ears (posteriorly rotated, low set, or with a thick helix), eyes (drooping of the eyelid or ptosis, hypertelorism, strabismus) or neck (webbed). Additional symptoms used to diagnose NS include short stature, cryptorchidism, thorax abnormalities, congenital heart disease, or mental retardation.¹⁶⁸ The constitutive activation of the Ras-MAPK pathway found in this syndrome predisposes individuals with NS to an increased risk of developing cancer, including acute lymphoblastic leukemia, rhabdomyosarcoma, or neuroblastoma. In an attempt to clarify the molecular mechanisms underlying the cardiovascular symptoms of some NS cases, a mouse model carrying the NS *A-Raf* mutation L613V has been recently generated¹⁶⁹ that supports the notion that enhanced MEK-ERK activity is crucial for at least some of the symptoms observed in NS patients.

Legius Syndrome (NF1-like)

This is an illness related to NF1 and Noonan that is produced by mutations in the *SPRED1* gene.¹⁷⁰ Its protein product is a negative regulator of Raf activation by Ras.¹⁴ Frequent phenotypical characteristics of the Legius syndrome include

café-au-lait spots, macrocephaly, and developmental delays. A variety of tumors have also been observed in patients with this syndrome, including NSCLC, Wilms' tumor, or breast cancer, among others, although it is still unclear whether *SPRED1* mutations underlie the development of these tumors.¹⁷¹

Costello Syndrome (CS)

CS is an autosomal dominant illness for which mutations in the *H-Ras* gene are the predominant cause.¹⁷² Substitutions of glycine 12 of this protein account for almost 80% of total CS mutations, although the mutations found in CS are usually less activating than those observed in tumors. Thus, whereas G12V mutants prevail in tumors, the most frequently mutations found in CS include G12S, G12A, or G13D.¹⁷³ The CS mutations affecting lysine 117 (K117R) and alanine 146 (A146T) are known to induce higher guanine exchange dissociation rates as well as higher pathway activation and increased proliferation.¹⁴⁶

The main symptoms used to diagnose CS include delayed development, mental retardation, cardiomyopathy, coarse face, or loose skin (especially in hands and feet). In addition, Costello patients are at higher risk of developing tumors, mainly rhabdomyosarcomas, neuroblastomas, or bladder cancer.¹⁴⁶ Mouse models involving oncogenic G12V *H-ras* mutations introduced in the wild-type *H-ras* locus by means of homologous recombination provide helpful biological systems to analyze the molecular mechanisms responsible for generation of the various phenotypic defects of CS patients.^{174,175}

Cardiofaciocutaneous Syndrome (CFC)

CFC is a rare syndrome linked to mutations occurring in *K-Ras* (scarce), *B-Raf* (~75%),¹⁷⁶ *MEK1*, and *MEK2* (~25%).¹⁷⁷ The symptoms of this syndrome are very similar to those of CS and NS, as it shows characteristic facial abnormalities together with postnatal growth deficit, almost the same cardiac defects as CS or NS, and cognitive defects.¹⁵⁰ Despite

these similarities, the cause of CFC is clearly different. As mentioned above, the prevalent mutations in CS affect the *H-ras* locus, whereas no changes in this gene have been observed in CFC. Mutations in *B-Raf* are the most common cause of CFC and affect its cysteine-rich and kinase domains. Similar to the mutations found in cancer, the *B-Raf* CFC mutations can result in either gain or loss of kinase activity and downstream MEK, ERK, or Elk activation.^{176,177} A functional explanation of these apparent contradictions is still missing, but it might be related to the implication of alternative signaling components, such as C-Raf, whose crosstalk with B-Raf is known to have an important role in resistance to B-Raf inhibitors in melanoma^{178,179} and whose potential role in CFC has not yet been analyzed.

Hereditary Gingival Fibromatosis (HGF) Type 1

HGF type 1 is caused by insertional mutations of the *Sos1* locus¹⁸⁰ resulting in a frame-shift that causes loss of the C-terminal polyproline SH3 binding region, constitutive plasma membrane localization, increased GEF activity, and overexpression of many cell cycle regulators such as cyclins C, D1/D2, E1/E2, E2F transcription factors 1/2, and PCNA.¹⁸¹ Although mutations in NS and HGF may affect the same *Sos1* locus, no developmental defects are observed in HGF, where only a much more benign phenotype is observed that involves a slowly progressive fibrous growth of the gingival.¹⁸⁰

Autoimmune Lymphoproliferative Syndrome (ALPS)

This is an illness characterized by non-malignant accumulation of mature lymphocytes in the body and autoimmunity. Usually it is caused by defects in the apoptotic pathway of the lymphocytes, with defects in the Fas receptor (Type Ia), Fas ligand (Type Ib) (accounting for 80% of the cases) or caspases 10 (Type IIa) and 8 (Type IIb) (3%). In a small percentage of ALPS patients without

genetic alterations in those loci, a causal mutation (G13D) in the *N-ras* locus has also been described,¹⁸² opening a new class of ALPS, termed Type IV. This mutation results in gain-of-function, activation of the Ras/MAPK pathway, a reduction of apoptosis inhibitor BIN expression, and increased apoptosis.

Capillary Malformation–Arteriovenous Malformation (CM-AVM)

The causal genetic alterations in CM-AVM are mutations in *RASA1*, the gene encoding for p120-RasGAP, that result in an increased Ras-ERK pathway activation. This happens in a subset of patients with capillary malformation who, in addition, show arteriovenous malformations, arteriovenous fistulas, or Parkes Weber syndrome (characterized by small arteriovenous malformations associated with soft tissue and bone hypertrophy). Some changes in the *RASA1* locus are hereditary, but almost 50% are de novo alterations and include frameshift mutations or changes of the amino acid at position 540 from cysteine to tyrosine. As in many other rasopathies, CM-AVM patients are at a higher risk of developing cancer, especially central nervous system tumors similar to those found in neurofibromatosis.^{183,184}

Most mutations in these disorders usually affect a wider spectrum of amino acids in the Ras proteins than those observed in cancer and produce a discrete but stable increase in signaling through the Ras-Raf-MEK-ERK pathway. This is in clear contrast to the mutations responsible for oncogenic transformation, which affect mainly codons 12, 13, and 61 of the *ras* genes and produce a much stronger and constitutive increase in signaling through this pathway. Nevertheless, most patients suffering those illnesses are more prone to develop tumors specific for each disease attributable to the Ras-ERK pathway hyperactivation (Table 2).¹⁷³

Acknowledgments

The authors apologize to many authors of original reports not included in the References section because of space limitations.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by grants from FIS (Fondo de Investigación Sanitaria) [PS09/01979]; RTICC (Red Temática de Investigación Cooperativa en Cáncer) [RD06/0020/000], and JCYL (Junta de Castilla y León) [SA044A08 and GR93].

References

- Rajalingam K, Schreck R, Rapp UR, Albert S. Ras oncogenes and their downstream targets. *Biochim Biophys Acta*. 2007;1773(8):1177-95.
- Stites EC, Ravichandran KS. A systems perspective of ras signaling in cancer. *Clin Cancer Res*. 2009;15(5):1510-3.
- Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci U S A*. 1982;79(11):3637-40.
- Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. *Nature*. 1982;297(5866):474-8.
- Santos E, Tronick SR, Aaronson SA, Pulciani S, Barbacid M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. *Nature*. 1982;298(5872):343-7.
- Cacev T, Radosevic S, Spaventi R, Pavelic K, Kapitanovic S. NF1 gene loss of heterozygosity and expression analysis in sporadic colon cancer. *Gut*. 2005;54(8):1129-35.
- Dhomen N, Marais R. New insight into BRAF mutations in cancer. *Curr Opin Genet Dev*. 2007;17(1):31-9.
- Khoukaz T. Administration of anti-EGFR therapy: a practical review. *Semin Oncol Nurs*. 2006;22(1 Suppl 1):20-7.
- Davies H, Bignell GR, Cox C, *et al*. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-54.
- Amado RG, Wolf M, Peeters M, *et al*. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26(10):1626-34.
- Marchuk DA, Saulino AM, Tavakkol R, *et al*. Cdna cloning of the type 1 neurofibromatosis gene: complete sequence of the NF1 gene product. *Genomics*. 1991;11(4):931-40.
- Tartaglia M, Mehler EL, Goldberg R, *et al*. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet*. 2001;29(4):465-8.
- Denayer E, de Ravel T, Legius E. Clinical and molecular aspects of RAS related disorders. *J Med Genet*. 2008;45(11):695-703.
- Tidyman WE, Rauen KA. The rasopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev*. 2009;19(3):230-6.

15. Kanwar M, Kowluru RA. Diabetes regulates small molecular weight G-protein, H-Ras, in the microvasculature of the retina: implication in the development of retinopathy. *Microvasc Res.* 2008;76(3):189-93.
16. Rapoport MJ, Weiss L, Mor A, *et al.* Prevention of autoimmune diabetes by linomide in non-obese diabetic (NOD) mice is associated with up-regulation of the TCR-mediated activation of p21(ras). *J Immunol.* 1996;157(10):4721-5.
17. Benter IF, Yousif MH, Canatan H, Akhtar S. Inhibition of Ca²⁺/calmodulin-dependent protein kinase II, RAS-gtpase and 20-hydroxyeicosatetraenoic acid attenuates the development of diabetes-induced vascular dysfunction in the rat carotid artery. *Pharmacol Res.* 2005;52(3):252-7.
18. Yousif MH. Signal transduction through Ras-GTPase and Ca²⁺/calmodulin-dependent protein kinase II contributes to development of diabetes-induced renal vascular dysfunction. *Cell Biochem Funct.* 2006;24(4):299-305.
19. Draznin B, Miles P, Kruszynska Y, *et al.* Effects of insulin on prenylation as a mechanism of potentially detrimental influence of hyperinsulinemia. *Endocrinology.* 2000;141(4):1310-6.
20. Kocher HM, Moorhead J, Sharpe CC, *et al.* Expression of Ras GTPases in normal kidney and in glomerulonephritis. *Nephrol Dial Transplant.* 2003;18(11):2284-92.
21. Luth HJ, Holzer M, Gertz HJ, Arendt T. Aberrant expression of nNOS in pyramidal neurons in Alzheimer's disease is highly co-localized with p21ras and p16ink4a. *Brain Res.* 2000;852(1):45-55.
22. Raymond FL, Tarpey PS, Edkins S, *et al.* Mutations in ZDHHC9, which encodes a palmitoyltransferase of NRAS and HRAS, cause X-linked mental retardation associated with a Marfanoid habitus. *Am J Hum Genet.* 2007;80(5):982-7.
23. Confino-Cohen R, Aharoni D, Goldberg A, *et al.* Evidence for aberrant regulation of the p21Ras pathway in PBMCs of patients with chronic idiopathic urticaria. *J Allergy Clin Immunol.* 2002;109(2):349-56.
24. Forbes SA, Bindal N, Bamford S, *et al.* COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 2011;39(Database issue):D945-50.
25. Shimizu N, Ohtsubo M, Minoshima S. Mutation-View/KMccancerDB: a database for cancer gene mutations. *Cancer Sci.* 2007;98(3):259-67.
26. Esteban LM, Vicario-Abejon C, Fernandez-Salguero P, *et al.* Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development. *Mol Cell Biol.* 2001;21(5):1444-52.
27. Johnson L, Greenbaum D, Cichowski K, *et al.* K-ras is an essential gene in the mouse with partial functional overlap with N-ras. *Genes Dev.* 1997;11(19):2468-81.
28. Janakiraman M, Vakiani E, Zeng Z, *et al.* Genomic and biological characterization of exon 4 KRAS mutations in human cancer. *Cancer Res.* 2010;70(14):5901-11.
29. Naguib A, Wilson CH, Adams DJ, Arends MJ. Activation of K-RAS by co-mutation of codons 19 and 20 is transforming. *J Mol Signal.* 2011;6:2.
30. Pulciari S, Santos E, Long LK, Sorrentino V, Barbacid M. Ras Gene amplification and malignant transformation. *Mol Cell Biol.* 1985;5(10):2836-41.
31. Heighway J, Hasleton PS. C-Ki-ras amplification in human lung cancer. *Br J Cancer.* 1986;53(2):285-7.
32. Taya Y, Hosogai K, Hirohashi S, *et al.* A novel combination of K-ras and myc amplification accompanied by point mutational activation of K-ras in a human lung cancer. *EMBO J.* 1984;3(12):2943-6.
33. Graham KA, Richardson CL, Minden MD, Trent JM, Buick RN. Varying degrees of amplification of the N-ras oncogene in the human breast cancer cell line MCF-7. *Cancer Res.* 1985;45(5):2201-5.
34. Akkiprik M, Celikel CA, Dusunceli F, *et al.* Relationship between overexpression of ras p21 oncoprotein and K-ras codon 12 and 13 mutations in Turkish colorectal cancer patients. *Turk J Gastroenterol.* 2008;19(1):22-7.
35. Wang Q, Chaerkady R, Wu J, *et al.* Mutant proteins as cancer-specific biomarkers. *Proc Natl Acad Sci U S A.* 2011.
36. Almoguera C, Shibata D, Forrester K, *et al.* Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell.* 1988;53(4):549-54.
37. Kipp BR, Fritcher EG, Clayton AC, *et al.* Comparison of KRAS mutation analysis and FISH for detecting pancreaticobiliary tract cancer in cytology specimens collected during endoscopic retrograde cholangiopancreatography. *J Mol Diagn.* 2010;12(6):780-6.
38. Hezel AF, Kimmelman AC, Stanger BZ, Bardesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 2006;20(10):1218-49.
39. Caldas C, Hahn SA, Hruban RH, *et al.* Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. *Cancer Res.* 1994;54(13):3568-73.
40. Dergham ST, Dugan MC, Kucway R, *et al.* Prevalence and clinical significance of combined K-ras mutation and p53 aberration in pancreatic adenocarcinoma. *Int J Pancreatol.* 1997;21(2):127-43.
41. Hruban RH, van Mansfeld AD, Offerhaus GJ, *et al.* K-ras oncogene activation in adenocarcinoma of the human pancreas: a study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol.* 1993;143(2):545-54.
42. Chen H, Tu H, Meng ZQ, *et al.* K-ras mutational status predicts poor prognosis in unresectable pancreatic cancer. *Eur J Surg Oncol.* 2010;36(7):657-62.
43. Kim J, Reber HA, Dry SM, *et al.* Unfavourable prognosis associated with K-ras gene mutation in pancreatic cancer surgical margins. *Gut.* 2006;55(11):1598-605.
44. Immervoll H, Hoem D, Kugarajh K, Steine SJ, Molven A. Molecular analysis of the EGFR-RAS-RAF pathway in pancreatic ductal adenocarcinomas: lack of mutations in the BRAF and EGFR genes. *Virchows Arch.* 2006;448(6):788-96.
45. Kawesha A, Ghaneh P, Andren-Sandberg A, *et al.* K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbb-2 and erbb-3 in resected pancreatic ductal adenocarcinoma. *Int J Cancer.* 2000;89(6):469-74.
46. Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. *J Natl Cancer Inst.* 2001;93(14):1062-74.
47. End DW, Smets G, Todd AV, *et al.* Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer Res.* 2001;61(1):131-7.
48. Kohl NE, Wilson FR, Mosser SD, *et al.* Protein farnesyltransferase inhibitors block the growth of ras-dependent tumors in nude mice. *Proc Natl Acad Sci U S A.* 1994;91(19):9141-5.
49. Cohen SJ, Ho L, Ranganathan S, *et al.* Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. *J Clin Oncol.* 2003;21(7):1301-6.
50. Martin NE, Brunner TB, Kiel KD, *et al.* A phase I trial of the dual farnesyltransferase and geranylgeranyltransferase inhibitor L-778,123 and radiotherapy for locally advanced pancreatic cancer. *Clin Cancer Res.* 2004;10(16):5447-54.
51. Van Cutsem E, van de Velde H, Karasek P, *et al.* Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol.* 2004;22(8):1430-8.
52. Whyte DB, Kirschmeier P, Hockenberry TN, *et al.* K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J Biol Chem.* 1997;272(22):14459-64.
53. Weden S, Klemp M, Gladhaug IP, *et al.* Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. *Int J Cancer.* 2011;128(5):1120-8.
54. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer.* 2011;50(5):307-12.
55. Irahara N, Baba Y, Noshio K, *et al.* NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol.* 2010;19(3):157-63.
56. McLellan EA, Owen RA, Stepniwska KA, Sheffield JP, Lemoine NR. High frequency of K-ras mutations in sporadic colorectal adenomas. *Gut.* 1993;34(3):392-6.
57. Nash GM, Gimbel M, Cohen AM, *et al.* KRAS mutation and microsatellite instability: two genetic markers of early tumor development that influence the prognosis of colorectal cancer. *Ann Surg Oncol.* 2010;17(2):416-24.
58. Yuen ST, Davies H, Chan TL, *et al.* Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. *Cancer Res.* 2002;62(22):6451-5.
59. Pretlow TP, Pretlow TG. Mutant KRAS in aberrant crypt foci (ACF): initiation of colorectal cancer? *Biochim Biophys Acta.* 2005;1756(2):83-96.
60. Andersen SN, Lovig T, Breivik J, *et al.* K-ras mutations and prognosis in large-bowel carcinomas. *Scand J Gastroenterol.* 1997;32(1):62-9.

61. Chang YS, Yeh KT, Chang TJ, *et al.* Fast simultaneous detection of K-RAS mutations in colorectal cancer. *BMC Cancer*. 2009;9:179.
62. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJ. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut*. 2005;54(9):1283-6.
63. Zavodna K, Konecny M, Krivulcik T, *et al.* Genetic analysis of KRAS mutation status in metastatic colorectal cancer patients. *Neoplasia*. 2009;56(3):275-8.
64. Cejas P, Lopez-Gomez M, Aguayo C, *et al.* KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. *Plos One*. 2009;4(12):e8199.
65. Nash GM, Gimbel M, Shia J, *et al.* KRAS mutation correlates with accelerated metastatic progression in patients with colorectal liver metastases. *Ann Surg Oncol*. 2010;17(2):572-8.
66. Lievre A, Bacht JB, Boige V, *et al.* KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol*. 2008;26(3):374-9.
67. Raponi M, Winkler H, Dracopoli NC. KRAS mutations predict response to EGFR inhibitors. *Curr Opin Pharmacol*. 2008;8(4):413-8.
68. Saif MW, Oettle H, Vervenne WL, *et al.* Randomized double-blind phase II trial comparing gemcitabine plus LY293111 versus gemcitabine plus placebo in advanced adenocarcinoma of the pancreas. *Cancer J*. 2009;15(4):339-43.
69. Deschoolmeester V, Boeckx C, Baay M, *et al.* KRAS mutation detection and prognostic potential in sporadic colorectal cancer using high-resolution melting analysis. *Br J Cancer*. 2010;103(10):1627-36.
70. Graziano SL, Gamble GP, Newman NB, *et al.* Prognostic significance of K-ras codon 12 mutations in patients with resected stage I and II non-small-cell lung cancer. *J Clin Oncol*. 1999;17(2):668-75.
71. Nelson MA, Wymer J, Clements N Jr. Detection of K-ras gene mutations in non-neoplastic lung tissue and lung cancers. *Cancer Lett*. 1996;103(1):115-21.
72. Suzuki Y, Orita M, Shiraishi M, Hayashi K, Sekiya T. Detection of ras gene mutations in human lung cancers by single-strand conformation polymorphism analysis of polymerase chain reaction products. *Oncogene*. 1990;5(7):1037-43.
73. Garassino MC, Marabese M, Rusconi P, *et al.* Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. *Ann Oncol*. 2011;22(1):235-7.
74. Ahrendt SA, Decker PA, Alawi EA, *et al.* Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. *Cancer*. 2001;92(6):1525-30.
75. Riely GJ, Kris MG, Rosenbaum D, *et al.* Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res*. 2008;14(18):5731-4.
76. Singh M, Lima A, Molina R, *et al.* Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. *Nat Biotechnol*. 2010;28(6):585-93.
77. Johnson L, Mercer K, Greenbaum D, *et al.* Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature*. 2001;410(6832):1111-6.
78. Yokota J, Kohno T. Molecular footprints of human lung cancer progression. *Cancer Sci*. 2004;95(3):197-204.
79. DeMarini DM, Landi S, Tian D, *et al.* Lung tumor KRAS and TP53 mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. *Cancer Res*. 2001;61(18):6679-81.
80. Husgafvel-Pursiainen K, Hackman P, Ridanpaa M, *et al.* K-ras mutations in human adenocarcinoma of the lung: association with smoking and occupational exposure to asbestos. *Int J Cancer*. 1993;53(2):250-6.
81. Rodenhuis S, Slebos RJ, Boot AJ, *et al.* Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. *Cancer Res*. 1988;48(20):5738-41.
82. Gautschi O, Huegli B, Ziegler A, *et al.* Origin and prognostic value of circulating KRAS mutations in lung cancer patients. *Cancer Lett*. 2007;254(2):265-73.
83. Mascaux C, Iannino N, Martin B, *et al.* The role of KRAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer*. 2005;92(1):131-9.
84. Sasaki H, Okuda K, Kawano O, *et al.* Nras and Kras mutation in Japanese lung cancer patients: Genotyping analysis using lightcycler. *Oncol Rep*. 2007;18(3):623-8.
85. Camps C, Sirera R, Bremnes R, *et al.* Is there a prognostic role of K-ras point mutations in the serum of patients with advanced non-small cell lung cancer? *Lung Cancer*. 2005;50(3):339-46.
86. Lu C, Soria JC, Tang X, *et al.* Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. *J Clin Oncol*. 2004;22(22):4575-83.
87. Ramirez JL, Sarries C, de Castro PL, *et al.* Methylation patterns and K-ras mutations in tumor and paired serum of resected non-small-cell lung cancer patients. *Cancer Lett*. 2003;193(2):207-16.
88. Keohavong P, demichele MA, Melacrinis AC, *et al.* Detection of K-ras mutations in lung carcinomas: relationship to prognosis. *Clin Cancer Res*. 1996;2(2):411-8.
89. Eberhard DA, Johnson BE, Amler LC, *et al.* Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol*. 2005;23(25):5900-9.
90. Hocker T, Tsao H. Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. *Hum Mutat*. 2007;28(6):578-88.
91. Omholt K, Karsberg S, Platz A, *et al.* Screening of N-ras codon 61 mutations in paired primary and metastatic cutaneous melanomas: mutations occur early and persist throughout tumor progression. *Clin Cancer Res*. 2002;8(11):3468-74.
92. Van der Lubbe JL, Rosdorff HJ, Bos JL, Van der Eb AJ. Activation of N-ras induced by ultraviolet irradiation in vitro. *Oncogene Res*. 1988;3(1):9-20.
93. Brose MS, Volpe P, Feldman M, *et al.* BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res*. 2002;62(23):6997-7000.
94. Singer G, Oldt R 3rd, Cohen Y, *et al.* Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. *J Natl Cancer Inst*. 2003;95(6):484-6.
95. Ugurel S, Thirumaran RK, Bloethner S, *et al.* B-RAF and N-RAS mutations are preserved during short time in vitro propagation and differentially impact prognosis. *Plos One*. 2007;2(2):e236.
96. van Elsas A, Scheibenbogen C, van der Minne C, *et al.* UV-induced N-ras mutations are T-cell targets in human melanoma. *Melanoma Res*. 1997;7(Suppl 2):S107-13.
97. Demunter A, Ahmadian MR, Libbrecht L, *et al.* A novel N-ras mutation in malignant melanoma is associated with excellent prognosis. *Cancer Res*. 2001;61(12):4916-22.
98. Gajewski TF, Niedzwiecki D, Johnson J, *et al.* Phase II study of the farnesyltransferase inhibitor R115777 in advanced melanoma: CALGB 500104. In: *Journal of Clinical Oncology*, 2006 ASCO Annual Meeting Proceedings (Post-Meeting Edition), Vol 24, No 18S (June 20 Supplement). 2006; p. 8014.
99. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer*. 2010;46(4):765-81.
100. Castillo-Martin M, Domingo-Domenech J, Karni-Schmidt O, Matos T, Cordon-Cardo C. Molecular pathways of urothelial development and bladder tumorigenesis. *Urol Oncol*. 2010;28(4):401-8.
101. Boulalal I, Zaravinos A, Karyotis I, Delakas D, Spandidos DA. Activation of RAS family genes in urothelial carcinoma. *J Urol*. 2009;181(5):2312-9.
102. Johne A, Roots I, Brockmoller J. A single nucleotide polymorphism in the human H-ras proto-oncogene determines the risk of urinary bladder cancer. *Cancer Epidemiol Biomarkers Prev*. 2003;12(1):68-70.
103. Goebell PJ, Knowles MA. Bladder cancer or bladder cancers? Genetically distinct malignant conditions of the urothelium. *Urol Oncol*. 2010;28(4):409-28.
104. Irie A, Kashani-Sabet M, Scanlon KJ, Uchida T, Baba S. Hammerhead ribozymes as therapeutic agents for bladder cancer. *Mol Urol*. 2000;4(2):61-6.
105. Watanabe T, Shinohara N, Sazawa A, *et al.* Adenovirus-mediated gene therapy for bladder cancer in an orthotopic model using a dominant negative H-ras mutant. *Int J Cancer*. 2001;92(5):712-7.
106. Choudhary S, Wang HC. Proapoptotic ability of oncogenic H-Ras to facilitate apoptosis induced by histone deacetylase inhibitors in human cancer cells. *Mol Cancer Ther*. 2007;6(3):1099-111.
107. Garcia-Rostan G, Zhao H, Camp RL, *et al.* Ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. *J Clin Oncol*. 2003;21(17):3226-35.
108. Greco A, Borrello MG, Miranda C, Degl'Innocenti D, Pierotti MA. Molecular

- pathology of differentiated thyroid cancer. *Q J Nucl Med Mol Imaging*. 2009;53(5):440-53.
109. Wells SA Jr, Santoro M. Targeting the RET pathway in thyroid cancer. *Clin Cancer Res*. 2009;15(23):7119-23.
 110. Hara H, Fulton N, Yashiro T, *et al*. N-ras mutation: an independent prognostic factor for aggressiveness of papillary thyroid carcinoma. *Surgery*. 1994;116(6):1010-6.
 111. Lee SL. Complications of radioactive iodine treatment of thyroid carcinoma. *J Natl Compr Canc Netw*. 2010;8(11):1277-86; quiz 1287.
 112. Duntas LH, Bernardini R. Sorafenib: rays of hope in thyroid cancer. *Thyroid*. 2010;20(12):1351-8.
 113. Hong DS, Sebt SM, Newman RA, *et al*. Phase I trial of a combination of the multikinase inhibitor sorafenib and the farnesyltransferase inhibitor tipifarnib in advanced malignancies. *Clin Cancer Res*. 2009;15(22):7061-8.
 114. Reuter CW, Morgan MA, Bergmann L. Targeting the Ras signaling pathway: a rational, mechanism-based treatment for hematologic malignancies? *Blood*. 2000;96(5):1655-69.
 115. Steinbrunn T, Stuhmer T, Gattenlohner S, *et al*. Mutated RAS and constitutively activated Akt delineate distinct oncogenic pathways, which independently contribute to multiple myeloma cell survival. *Blood*. 2011;117(6):1998-2004.
 116. MacKenzie KL, Dolnikov A, Millington M, Shouman Y, Symonds G. Mutant N-ras induces myeloproliferative disorders and apoptosis in bone marrow repopulated mice. *Blood*. 1999;93(6):2043-56.
 117. Reilly JT. Receptor tyrosine kinases in normal and malignant haematopoiesis. *Blood Rev*. 2003;17(4):241-8.
 118. Shannon KM, O'Connell P, Martin GA, *et al*. Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. *N Engl J Med*. 1994;330(9):597-601.
 119. Stiller CA, Chessells JM, Fitchett M. Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. *Br J Cancer*. 1994;70(5):969-72.
 120. Zou X, Calame K. Signaling pathways activated by oncogenic forms of Abl tyrosine kinase. *J Biol Chem*. 1999;274(26):18141-4.
 121. Paulsson K, Horvat A, Strombeck B, *et al*. Mutations of FLT3, NRAS, KRAS, and PTPN11 are frequent and possibly mutually exclusive in high hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 2008;47(1):26-33.
 122. De Melo MB, Lorand-Metze I, Lima CS, Saad ST, Costa FF. N-ras gene point mutations in Brazilian acute myelogenous leukemia patients correlate with a poor prognosis. *Leuk Lymphoma*. 1997;24(3-4):309-17.
 123. Kiyoi H, Naoe T, Nakano Y, *et al*. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood*. 1999;93(9):3074-80.
 124. Shih LY, Huang CF, Wang PN, *et al*. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. *Leukemia*. 2004;18(3):466-75.
 125. Coghlan DW, Morley AA, Matthews JP, Bishop JF. The incidence and prognostic significance of mutations in codon 13 of the N-ras gene in acute myeloid leukemia. *Leukemia*. 1994;8(10):1682-7.
 126. Lubbert M, Mirro J Jr, Miller CW, *et al*. N-ras gene point mutations in childhood acute lymphocytic leukemia correlate with a poor prognosis. *Blood*. 1990;75(5):1163-9.
 127. Perentesis JP, Bhatia S, Boyle E, *et al*. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. *Leukemia*. 2004;18(4):685-92.
 128. Paquette RL, Landaw EM, Pierre RV, *et al*. N-ras mutations are associated with poor prognosis and increased risk of leukemia in myelodysplastic syndrome. *Blood*. 1993;82(2):590-9.
 129. Chng WJ, Gonzalez-Paz N, Price-Troska T, *et al*. Clinical and biological significance of RAS mutations in multiple myeloma. *Leukemia*. 2008;22(12):2280-2284.
 130. Appels NM, Beijnen JH, Schellens JH. Development of farnesyl transferase inhibitors: a review. *Oncologist*. 2005;10(8):565-78.
 131. Mazieres J, Pradines A, Favre G. Perspectives on farnesyl transferase inhibitors in cancer therapy. *Cancer Lett*. 2004;206(2):159-67.
 132. Braun T, Fenaux P. Farnesyltransferase inhibitors and their potential role in therapy for myelodysplastic syndromes and acute myeloid leukaemia. *Br J Haematol*. 2008;141(5):576-86.
 133. Mori S, Cortes J, Kantarjian H, *et al*. Potential role of sorafenib in the treatment of acute myeloid leukemia. *Leuk Lymphoma*. 2008;49(12):2246-55.
 134. Goldman JM. Chronic myeloid leukemia: a historical perspective. *Semin Hematol*. 2010;47(4):302-11.
 135. Marin D. Current status of imatinib as frontline therapy for chronic myeloid leukemia. *Semin Hematol*. 2010;47(4):312-8.
 136. Schondorf T, Rutzel S, Andrack A, *et al*. Immunohistochemical analysis reveals a protective effect of H-ras expression mediated via apoptosis in node-negative breast cancer patients. *Int J Oncol*. 2002;20(2):273-7.
 137. Watson DM, Elton RA, Jack WJ, *et al*. The H-ras oncogene product p21 and prognosis in human breast cancer. *Breast Cancer Res Treat*. 1991;17(3):161-9.
 138. Calvisi DF, Ladu S, Conner EA, *et al*. Inactivation of Ras GTPase-activating proteins promotes unrestrained activity of wild-type Ras in human liver cancer. *J Hepatol*. 2011;54(2):311-9.
 139. Kitanaka C, Kato K, Ijiri R, *et al*. Increased Ras expression and caspase-independent neuroblastoma cell death: possible mechanism of spontaneous neuroblastoma regression. *J Natl Cancer Inst*. 2002;94(5):358-68.
 140. Leung TW, Cheung AN, Cheng DK, Wong LC, Ngan HY. Expressions of c-erbB-2, epidermal growth factor receptor and pan-ras proto-oncogenes in adenocarcinoma of the cervix: correlation with clinical prognosis. *Oncol Rep*. 2001;8(5):1159-64.
 141. Mamas IN, Zafiroopoulos A, Koumantakis E, Sifakis S, Spandidos DA. Transcriptional activation of H- and N-ras oncogenes in human cervical cancer. *Gynecol Oncol*. 2004;92(3):941-8.
 142. Lam EK, Wang X, Shin VY, *et al*. A microRNA contribution to aberrant Ras activation in gastric cancer. *Am J Transl Res*. 2011;3(2):209-18.
 143. Coutinho CM, Bassini AS, Gutierrez LG, *et al*. Genetic alterations in Ki-ras and Ha-ras genes in juvenile nasopharyngeal angiofibromas and head and neck cancer. *Sao Paulo Med J*. 1999;117(3):113-20.
 144. Sathyan KM, Nalinakumari KR, Kannan S. H-Ras mutation modulates the expression of major cell cycle regulatory proteins and disease prognosis in oral carcinoma. *Mod Pathol*. 2007;20(11):1141-8.
 145. Kiaris H, Spandidos DA, Jones AS, Vaughan ED, Field JK. Mutations, expression and genomic instability of the H-ras proto-oncogene in squamous cell carcinomas of the head and neck. *Br J Cancer*. 1995;72(1):123-8.
 146. Schubert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer*. 2007;7(4):295-308.
 147. Schubert S, Bollag G, Shannon K. Deregulated Ras signaling in developmental disorders: new tricks for an old dog. *Curr Opin Genet Dev*. 2007;17(1):15-22.
 148. Schubert S, Zenker M, Rowe SL, *et al*. Germline KRAS mutations cause Noonan syndrome. *Nat Genet*. 2006;38(3):331-6.
 149. Tartaglia M, Zampino G, Gelb BD. Noonan syndrome: clinical aspects and molecular pathogenesis. *Mol Syndromol*. 2010;1(1):2-26.
 150. Tidyman WE, Rauen KA. Noonan, Costello and cardio-facio-cutaneous syndromes: dysregulation of the Ras-MAPK pathway. *Expert Rev Mol Med*. 2008;10:e37.
 151. Cawthon RM, O'Connell P, Buchberg AM, *et al*. Identification and characterization of transcripts from the neurofibromatosis 1 region: the sequence and genomic structure of EVI2 and mapping of other transcripts. *Genomics*. 1990;7(4):555-65.
 152. Jett K, Friedman JM. Clinical and genetic aspects of neurofibromatosis 1. *Genet Med*. 2010;12(1):1-11.
 153. Ferner RE. Neurofibromatosis 1 and neurofibromatosis 2: a twenty first century perspective. *Lancet Neurol*. 2007;6(4):340-51.
 154. Korf BR. Malignancy in neurofibromatosis type 1. *Oncologist*. 2000;5(6):477-85.
 155. Bollag G, Clapp DW, Shih S, *et al*. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat Genet*. 1996;12(2):144-8.
 156. Rasmussen SA, Friedman JM. NF1 gene and neurofibromatosis 1. *Am J Epidemiol*. 2000;151(1):33-40.
 157. Sarkozy A, Digilio MC, Dallapiccola B. Leopard syndrome. *Orphanet J Rare Dis*. 2008;3:13.
 158. Matozaki T, Murata Y, Saito Y, Okazawa H, Ohnishi H. Protein tyrosine phosphatase SHP-2: a proto-oncogene product that promotes Ras activation. *Cancer Sci*. 2009;100(10):1786-93.

159. Oishi K, Zhang H, Gault WJ, *et al.* Phosphatase-defective LEOPARD syndrome mutations in PTPN11 gene have gain-of-function effects during *Drosophila* development. *Hum Mol Genet.* 2009;18(1):193-201.
160. Kontaridis MI, Swanson KD, David FS, Barford D, Neel BG. PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. *J Biol Chem.* 2006;281(10):6785-92.
161. Cirstea IC, Kutsche K, Dvorsky R, *et al.* A restricted spectrum of NRAS mutations causes Noonan syndrome. *Nat Genet.* 2010;42(1):27-9.
162. Cordeddu V, Di Schiavi E, Pennacchio LA, *et al.* Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. *Nat Genet.* 2009;41(9):1022-6.
163. Nava C, Hanna N, Michot C, *et al.* Cardiofacio-cutaneous and Noonan syndromes due to mutations in the RAS/MAPK signalling pathway: genotype-phenotype relationships and overlap with Costello syndrome. *J Med Genet.* 2007;44(12):763-71.
164. Pandit B, Sarkozy A, Pennacchio LA, *et al.* Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat Genet.* 2007;39(8):1007-12.
165. Razzaque MA, Nishizawa T, Komoike Y, *et al.* Germline gain-of-function mutations in RAF1 cause Noonan syndrome. *Nat Genet.* 2007;39(8):1013-7.
166. Roberts AE, Araki T, Swanson KD, *et al.* Germline gain-of-function mutations in SOS1 cause Noonan syndrome. *Nat Genet.* 2007;39(1):70-4.
167. Gremer L, Merbitz-Zahradnik T, Dvorsky R, *et al.* Germline KRAS mutations cause aberrant biochemical and physical properties leading to developmental disorders. *Hum Mutat.* 2010;32(1):33-43.
168. Jorge AA, Malaquias AC, Arnhold JJ, Mendonca BB. Noonan syndrome and related disorders: a review of clinical features and mutations in genes of the RAS/MAPK pathway. *Horm Res.* 2009;71(4):185-93.
169. Wu X, Simpson J, Hong JH, *et al.* MEK-ERK pathway modulation ameliorates disease phenotypes in a mouse model of Noonan syndrome associated with the Raf1(L613V) mutation. *J Clin Invest.* 2011;121(3):1009-25.
170. Brems H, Chmara M, Sahbatou M, *et al.* Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nat Genet.* 2007;39(9):1120-6.
171. Denayer E, Chmara M, Brems H, *et al.* Legius syndrome in fourteen families. *Hum Mutat.* 2011;32(1):E1985-98.
172. Aoki Y, Niihori T, Kawame H, *et al.* Germline mutations in HRAS proto-oncogene cause Costello syndrome. *Nat Genet.* 2005;37(10):1038-40.
173. Aoki Y, Niihori T, Narumi Y, Kure S, Matsubara Y. The RAS/MAPK syndromes: novel roles of the RAS pathway in human genetic disorders. *Hum Mutat.* 2008;29(8):992-1006.
174. Chen X, Mitsutake N, LaPerle K, *et al.* Endogenous expression of Hras(G12V) induces developmental defects and neoplasms with copy number imbalances of the oncogene. *Proc Natl Acad Sci U S A.* 2009;106(19):7979-84.
175. Schuhmacher AJ, Guerra C, Sauzeau V, *et al.* A mouse model for Costello syndrome reveals an Ang II-mediated hypertensive condition. *J Clin Invest.* 2008;118(6):2169-79.
176. Niihori T, Aoki Y, Narumi Y, *et al.* Germline KRAS and BRAF mutations in cardiofacio-cutaneous syndrome. *Nat Genet.* 2006;38(3):294-6.
177. Rodriguez-Viciana P, Tetsu O, Tidyman WE, *et al.* Germline mutations in genes within the MAPK pathway cause cardiofacio-cutaneous syndrome. *Science.* 2006;311(5765):1287-90.
178. Heidorn SJ, Milagre C, Whittaker S, *et al.* Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell.* 2010;140(2):209-21.
179. Montagut C, Sharma SV, Shioda T, *et al.* Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res.* 2008;68(12):4853-61.
180. Hart TC, Zhang Y, Gorry MC, *et al.* A mutation in the SOS1 gene causes hereditary gingival fibromatosis type 1. *Am J Hum Genet.* 2002;70(4):943-54.
181. Jang SI, Lee EJ, Hart PS, *et al.* Germ line gain of function with SOS1 mutation in hereditary gingival fibromatosis. *J Biol Chem.* 2007;282(28):20245-55.
182. Oliveira JB, Bidere N, Niemela JE, *et al.* NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc Natl Acad Sci U S A.* 2007;104(21):8953-8.
183. Eerola I, Boon LM, Mulliken JB, *et al.* Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet.* 2003;73(6):1240-9.
184. Revencu N, Boon LM, Mulliken JB, *et al.* Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat.* 2008;29(7):959-65.
185. Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). *J Med Genet.* 1996;33(1):2-17.
186. Jim HP, Upadhyaya M. Novel human pathological mutations. Gene symbol: SPRED1. Disease: Legius syndrome. *Hum Genet.* 2010;127(1):111-2.
187. Hershkovitz D, Bercovich D, Sprecher E, Lapidot M. RASA1 mutations may cause hereditary capillary malformations without arteriovenous malformations. *Br J Dermatol.* 2008;158(5):1035-40.