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Ras in Cancer and Developmental Diseases

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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]

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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 (*NF1*) 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 hiperinsulinemia.[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-*ras*	N-*ras*	K-*ras*
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-Ras[33] 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 Table1, *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* mutations[42] 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 presupposed 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 hyperplasic 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 metastasis[64] 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 models[76] (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 NCSLC.[77] The notion of *ras* mutations as early events triggering human NSCLC is further supported by their detection in precancerous lesions[78] 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 approached 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, NCT-00005630, 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 mayor 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 patients[98] 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 States[99] (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 pro-apoptotic 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 effects[111] 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, NCT-00098592 at http://clinicaltrials.gov/

show/). Indeed, phase 2 studies have shown very favorable results[112] 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 different[24] (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 Reilly[117]). 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 Brc/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 copies[126] 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 sorafenib[133] (http://clinicaltrials.gov/show/NCT00131989 and NCT00303966, etc.) or the MEK inhibitors AS703026 (http://clinicaltrials.gov/show/NCT-00957580) and GSK1120212 (http://clinicaltrials.gov/how/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 described[143] (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 cancer[144] 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]	*NF1* (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]	*PTPN11* (SHP2)	RTK phosphatase	Y279C/S, A461T, G464A, T468M/P, R498W/L, Q506P, Q510P/E/G	Myelodysplasia, acute myelogenous leukemia, neuroblastoma
	RAF1 (c-Raf)	Kinase	S257L, L613V	
	B-RAF (B-Raf)	Kinase	T241P, L245F	
Noonan syndrome[149,161,166]	*PTPN11* (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	Cancer an uncommon outcome of the illness; higher risk of myeloproliferative disease and leukemia, especially juvenile myelomonocytic leukemia
	SOS1 (Sos1)	RasGEF	T266K, M269R/T, D309Y, Y337C, G434R, C441Y, P478 R/L, S548R, L550P, R552G/K/S, F623I, P655L, Y702H, W729L, I733F, E846K	
	K-RAS (K-Ras)	GTPase	V14I, Q22R, P34L/Q, I36M, T58I, V152G, D153V, F156I	
	N-RAS (N-Ras)	GTPase	T50I, G60E	
	RAF1 (c-Raf)	Kinase	R256S, S257L, S259F, T260R/I, P261S/L/A, V263A, D486N/G, T491I/R, S612T, L613V	
	B-RAF (B-Raf)	Kinase	E501K, K499E, L597V	
	SHOC2 (SHOC2)	Scaffold	S2G	
	MEK1 (MEK1)	Kinase	D67N	
Legius syndrome[170,186]	*SPRED1* (SPRED1)	Interactor	Deletions; amino acid switching mutations: V44D, S149N, M1T; nonsense mutations: R16X, R64X, E73X, R117X, Q213X, Q215X, K322X, R325X	Possible increased risk of cancer
Costello syndrome[14,173]	*H-RAS* (H-Ras)	GTPase	G12S/A/V/C/E, G13C, K117R, A146T	Rhabdomyosarcoma, transitional cell carcinoma, neuroblastoma
	K-RAS (K-Ras)	GTPase	K5N, V152G, F156L	
	B-RAF (B-Raf)	Kinase	G534R, D638E	
Cardio-facio-cutaneous syndrome[150,173]	*K-RAS* (K-Ras)	GTPase	Q22E, P34R, G60R, D153V, F156I	Cancer predisposition uncertain; possible acute lymphoblastic leukemia
	B-RAF (B-Raf)	Kinase	A246P, Q257K/R, S467A, F468S, G469E, L485F, V487G, K499E, E501K/G, G534R, N580D, N581D, F595L, G596V, D638E	
	MEK1 (MEK1)	Kinase	F53S, P124L, Y130C	
	MEK2 (MEK2)	Kinase	F57C, K61E, P128R, G132V	
Hereditary gingival fibromatosis type 1[180]	*SOS1* (Sos1)	RasGEF	Single nucleotide insertion (C) between nt 3248 and nt 3249	No increased risk of cancer
Autoimmune lymphoproliferative syndrome[182]	*N-RAS* (N-Ras)	GTPase	G13D	Increased risk of hematological malignancies
Capillary malformation–arteriovenous malformation[183,187]	*Rasa1* (p120RasGAP)	RasGAP	Deletions; duplications; and mutations: G829A, C853T, C1336T, Q446X, C540Y, G1619A	Vascular tumors

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: *l*entigines, *E*CG abnormalities, *o*cular hypertelorism (distance between the eyes), *p*ulmonic stenosis, *a*bnormal genitalia, *r*etardation of growth, and sensorineural *d*eafness.[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 generated[169] 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 is still unclear whether *SPRED1* mutations underlie the develop 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* locus[180] 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 nonmalignant 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]

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