Cancer Risk and Spectrum in Individuals with RASopathies

17

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Contents

17.1 Introduction

The identification of Ras emerged from the study of highly potent transforming retroviruses isolated from animals [1]. Specifically, in the 1960s, it was shown that the Harvey murine sarcoma virus and the Kirsten murine sarcoma virus induced sarcomas when injected into rats [2, 3]. It would later be established that two *ra*t *s*arcoma (ras; Harvey ras or Ha-ras or H-ras; Kirsten ras, or Ki-ras or K-ras) genes encoding for their respective 21 kD protein products were the transformative

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G. Tadini et al. (eds.), *Multidisciplinary Approach to Neurofibromatosis Type 1*,
https://doi.org/10.1007/978-3-319-92450-2_17

elements of the viruses and that many cancers in man harbor mutations in the *HRAS* and *KRAS* human homologs [1]. Subsequently, additional studies elucidated over 150 related genes and their corresponding protein products that function as small guanosine nucleotide GTPases termed the Ras superfamily of proteins [4]. The Ras subfamily is a branch of the superfamily.

In humans, the RAS subfamily of proteins consists of 36 members [4]. HRAS, KRAS, and NRAS are the best studied because defects are associated with disease. These three RAS isoforms are key components of the RAS/mitogen-activated protein kinase (MAPK) signaling pathway which functions to relay extracellular signals into the cell. Notably, RAS and other components of RAS/MAPK pathway interact with other cellular proteins and pathways in an intricate and complex manner. Activation of the pathway is initiated when an extracellular ligand such as a growth factor binds to the transmembrane tyrosine kinase receptor (RTK), which leads to autophosphorylation and recruitment of adaptor proteins (GRB1, CBL, SHP2, and SOS1) to the intracellular domain. Thereafter, SOS1 performing as a guanosine nucleotide exchange factor (GEF) facilitates the exchange of GDP for GTP on RAS. The GTP-bound RAS then binds to and activates the first protein of the MAPK pathway, RAF (ARAF, BRAF, or CRAF). Next, MEK1/2 and then ERK1/2 are successively activated. The stimulated ERK1 and ERK2 interact with membrane, cytosolic, and nuclear substrates to regulate cell growth, differentiation, and proliferation. NF1 and SPRED1 serve as negative regulators of the RAS/MAPK pathway (Fig. 17.1). Dysregulation of the RAS/MAPK pathway has important

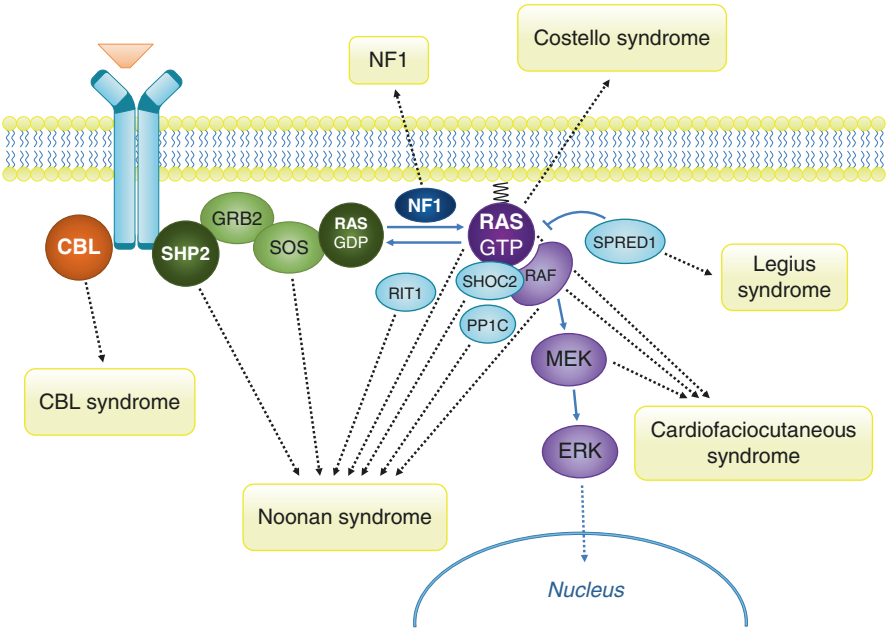

Fig. 17.1 RAS/MAPK pathway and corresponding RASopathies

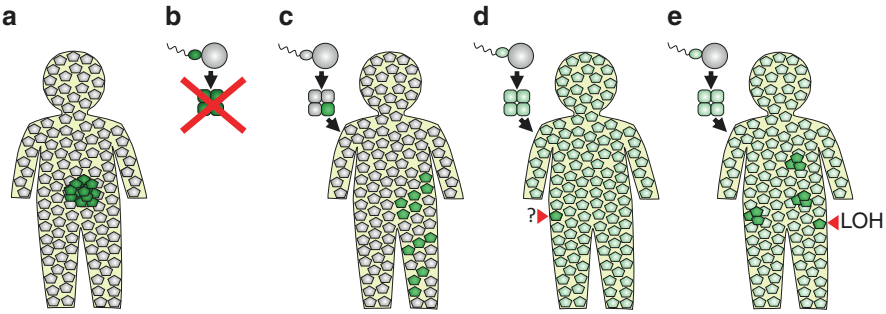

Fig. 17.2 Various consequences of RAS/MAPK pathway dysregulation: (**a**), Oncogenic mutation in a sporadic tumor: a strongly activating (oncogenic) mutation (green color) occurs as a somatic event in a cell and—together with other genetic events—gives rise to proliferation and tumor formation. (**b**), Oncogenic mutation in the germline: strongly activating mutations may arise in germ cells (more likely in sperm), but after fertilization perturbed embryonic development will lead to prenatal lethality in most cases. (**c**), Mosaic RASopathy due to postzygotic ocurrence of an oncogenic mutation: If the same mutation as in (**a**) and (**b**) emerges as a postzygotic mutational event in the embryo, it may be able to survive in the mosaic state and lead to specific lesions in regions affected by the mutation. (**d**), Mildly activating mutation in Noonan syndrome: a mildly activating mutation (light green color) is tolerated in the germline, but the generalized dysregulation of RAS/MAPK signalling leads to characteristic developmental anomalies. The switch from mild to strong activation (arrowhead, green color) by a single genetic event is unlikely and oncogenesis usually requires independent mutational events. (**e**), Inactivating NF1 mutations in neurofibromatosis: The defect of one allele is present in the germline and leads to a mild generalized overactivation of RAS/MAPK signaling that gives rise to mild Noonan syndrome-like features (haploinsufficiency). The switch to severe RAS/MAPK pathway dysregulation may occur with a single secondary genetic event of loss of heterozygosity at the NF1 locus (arrowhead). This is likely to occur at multiple sites during lifetime and, if it is sufficient to lead to tumor formation from certain cells, numerous tumors of that kind will occur

implications for human health. Germline, mosaic, and somatic aberrations in the genes of RAS/MAPK pathway are associated with developmental syndromes, congenital disorders, and cancers, respectively (Fig. 17.2). Herein, we review the cancer risk and spectrum associated with disorders related to germline or mosaic RAS/MAPK pathway disturbances as well as examine practical surveillance guidelines and therapeutic implications.

17.2 RASopathies and Cancer: General Thoughts

Mutations of various genes leading to dysregulation of the RAS signaling pathway occur in the germline and cause syndromes including neurofibromatosis type 1 (NF1), Noonan syndrome (NS), NS with multiple lentigines (NSML), Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFCS), NF1-NS, Legius syndrome, and NS-like disorder with or without juvenile myelomonocytic leukemia (NSLL or CBL syndrome). Although certain clinical characteristics distinguish one syndrome from another, due to a common mechanism of RAS/MAPK pathway dysfunction,

many affected individuals exhibit overlapping NS-like phenotypic features such as dysmorphic craniofacial features, congenital heart defects, short stature, skin abnormalities, and variable intellectual disability [5]. It is considered that the expression of NS-like abnormalities mainly reflects the degree of systemic dysregulation of the RAS/MAPK pathway in the developing embryo and child. In fact, the genetic relatedness of these clinically overlapping conditions was postulated long prior to identifying their related genetic basis. Collectively, these syndromes are called the RASopathies, defined by the combination of a germline defect of a RAS signaling component and NS-like features.

It is important to recognize that, in the case of activating mutations of RAS pathway genes, the mutation spectrum observed in the germline differs substantially from the mutation spectrum observed in cancer. This difference can be explained by the notion that in the case of germline mutations biochemically "strong" cancer-associated defects have a higher chance of being embryonic lethal, while in somatic (cancer) cells, "strong" mutations represent key steps during cancerogenesis. The prenatal selection against germline mutations with strong activating effects on the RAS/MAPK pathway is supported by the observation that prenatal testing of *PTPN11* (and other genes) in fetuses with severe abnormalities (such as hydrops fetalis) may detect mutations that are otherwise typically found as somatic mutations in cancer ([6]; and unpublished findings from our lab). Consistently, rare live-born children with "strong" activating mutations usually harbor de novo mutations and tend to have severe developmental defects and a high risk of developing cancer. The occurrence and viability of "strong" mutations in the germline may also depend on the biologic function of the mutated gene [7]. For example, mutations of residues 12 or 13 of HRAS (leading to CS) is observed more frequently in the germline than identical mutations of KRAS. Another chance for prenatal survival of severely dysregulating "oncogenic" RAS/MAPK pathway mutations is in a postzygotic mosaic state which leads to mosaic RASopathies discussed later in this chapter.

In contrast, biochemically "mild" (hypomorphic) mutations are tolerated more commonly as a germline defect without leading to embryonic death. Due to their milder effect, these mutations are rarely seen as somatic cancer-related lesions. The cancer risk of individuals with "mild" mutations is only moderately elevated. From the cancer risk perspective, these "mild" defects represent examples of rare moderate cancer risk alleles (such rare moderate disease risk alleles, unless they have a systemic phenotype, are largely unknown because they typically escape standard gene discovery approaches) [8]. These mutations may also occur as familial mutations, depending on the degree of physical and intellectual impairment and their effects on fertility.

In the case of predominantly loss-of-function germline mutations in negative regulators of the RAS/MAPK pathway (e.g., NF1), the systemic dysregulation of the pathway caused by the defect of one allele (haploinsufficiency) may have settled disadvantageous effects on the developing embryo that lead to mild NS-like manifestations. These genes act in a recessive manner on the cellular level and loss or mutation of the second allele in somatic cells is the key step for tumor development. This explains the observation that the tumor risk can be high in patients with

syndromes caused by this mechanism despite the fact that the primary lesions are well tolerated during embryogenesis. Of note, mutations can be both, activating for some biological properties and inactivating for others. Hence, the differentiation is not always clear cut.

While in this theoretical model, the degree of systemic RAS/MAPK dysregulation determines the expression of NS-like developmental anomalies, the tumor risk for a certain entity is not solely depending on the oncogenic potential of the underlying germline event but also on the probability of occurrence of additional genetic hit(s) that are sufficient to confer the transformation in a sensitive cell. For example, in individuals with NF1 the risk of developing neurofibroma is substantially higher than the risk of developing malignant peripheral nerve sheet tumors because the latter requires a more complex pattern of somatic defects. The tumor types that develop in individuals with RASopathies are biologically highly plausible. For example, chromosome 11 band p15 rearrangements are a common event in sporadic embryonal rhabdomyosarcoma which is also associated with somatic defects of *HRAS* (also located on chromosome 11p15). In CS, *HRAS* mutations are present in all cells, including mesenchymal cells with the potential to transform into rhabdomyosarcoma cells. Additional acquired rearrangements of 11p15 in this premalignant tissue contributes to the high rhabdomyosarcoma risk in individuals with CS [9]. With these general thoughts in mind we now briefly review various RASopathies focusing on the cancer risk and spectrum associated with each condition.

17.3 Cancer and Noonan Syndrome

The most common neurodevelopmental syndrome associated with an aberrant RAS/MAPK pathway is NS. Approximately 1 in 1000–2000 newborns are affected. Individuals with NS have distinctive craniofacial dysmorphism including broad forehead, hypertelorism, ptosis, down-slanting palpebral fissures, and low-set posteriorly rotated ears. As noted above, many of these craniofacial features are also observed in other RASopathies and give clue to the presence of a RAS/MAPK pathway anomaly. In addition, in NS congenital cardiac defects, reduced growth, bleeding diathesis, and neurocognitive delay may be present. Disease causative mutations have been identified in *PTPN11* (50%), *SOS1* (13%), *RAF1* (5%), *RIT1* (5%), *KRAS*, *NRAS*, *BRAF*, *MAP 2K1*, *RRAS*, *MRAS*, *RASA2*, *SOS2*, and *LZTR1*. Mutations of *SHOC2* und *PPP1CB* cause NS-like disorders with loose anagen hair. Affected individuals show cardiac anomalies (NS spectrum, semilunar valve dysplasias), characteristic hair, diffuse hyperpigmentation, mild mental retardation, short stature, and a recognizable facial phenotype.

As noted above, the RAS pathway mutations observed in patients with NS differ from the variations typically driving tumorigenesis. As far as this has been studied functionally, NS-associated mutations have been shown to confer less severely dysregulating effects on signaling compared to oncogenic mutations in the respective genes [10, 11]. Consequently, the cancer risk among individuals with NS is not strongly increased and cancer is a rare complication encountered in affected

families. As expected, a moderate increase in cancer risk can be confirmed epidemiologically—a recent study observed 8 cases of childhood cancer among 632 patients, while 1 neoplasm was expected when comparing it to the healthy population (Standardized Incidence Ratio: 8.1 [3.5–16.0]) [8]. Tumors that occur in individuals with NS include embryonal rhabdomyosarcoma, neuroblastoma, and astrocytoma, among others. Interestingly, several cases of dysembryoplastic neuroepithelial tumor (DNET) have been reported suggesting that DNET is a rare complication in NS [8]. Moreover, several NS patients with acute lymphoblastic leukemia have been reported. Most patients had a hyperdiploid karyotype [12] providing evidence for a non-random association between ALL and NS. A non-malignant tumor manifestation in NS is multiple giant cell lesions predominantly affecting the jaw bones. These tumors may also occur in other RASopathies [13].

Infants with NS and specific mutations of *PTPN11* (or rarely *KRAS*) are at increased risk of developing a polyclonal myeloproliferative disease that resembles juvenile myelomonocytic leukemia (JMML). Although myeloproliferative disease in children with NS can resolve without treatment, malignant transformation can occur [14–16]. Taken together, the cancer risk is only slightly increased in individuals with NS.

Individuals with NSML have clinical features most akin to NS but these patients display a high penetrance for multiple lentigines and hypertrophic cardiomyopathy. The disorder was previously called LEOPARD syndrome as an acronym for lentigines, electrocardiogram abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and deafness. The disorder is caused by specific mutations in *PTPN11* (particularly p.T468M, p.Y279C) and rarely in other genes. Rare cases of childhood cancer have been reported, suggesting that the risk is slightly elevated [8]. Recent evidence suggested that neurofibroma-like nerve-sheath tumors and hypertrophic neuropathy is associated with NSML in adulthood [17, 18]. Like in NS, the cancer risk is not strongly increased.

17.4 Cancer and Cardio-Facio-Cutaneous Syndrome

CFCS is a more rare disorder caused by germline mutations of the RAS/MAPK pathway. CFCS patients have macrocephaly, broad forehead, bitemporal narrowing, hypoplastic supraorbital ridges, sparse or missing eyebrows, down-slanting palpebral fissures, ocular abnormalities (strabismus, nystagmus, myopia, ptosis, hyperopia, and astigmatism), short nose with low nasal bridge and anteverted nares, high-arched palate, and low-set posteriorly rotated ears. As indicated by the name, CFCS patients may display more severe ectodermal abnormalities including sparse, curly, thin or thick, woolly and occasionally brittle hair, hyperkeratosis, keratosis pilaris, hemangioma, ichthyosis, and nevi. Moreover, approximately 75% of individuals with CFCS have cardiac defects that include pulmonic stenosis, atrial and ventricular septal defect, hypertrophic cardiomyopathy, and heart valve anomalies [19]. Musculoskeletal and neurologic abnormalities are also frequently encountered

in CFCS patients. The degree of intellectual impairment is usually much more severe than in NS. Finally, during infancy, CFCS patients may experience failure to thrive, gastrointestinal reflux, vomiting, oral aversion, and constipation. Mutations in *BRAF*, *MEK1*, *MEK2*, and *KRAS* are responsible for the syndrome. Like in NS, cancer is a rare complication in patients with CFCS. Leukemia, lymphoma, hepatoblastoma, and rhabdomyosarcoma have been reported in rare cases [20]. No risk figure is available, but it seems that cancer risk in CFCS does not exceed the risk that has been reported for NS.

17.5 Cancer and Costello Syndrome

Besides NF1, CS is the RASopathy with the highest cancer risk. First symptoms of the disorder may be observed even during pregnancy. In utero, there may be polyhydramnios secondary to poor swallowing, fetal overgrowth with macrocephaly, and cardiac arrhythmia [5, 19, 21, 22]. Prematurity and generalized edema are not uncommon [21, 23]. During neonatal period, patients usually display feeding difficulties and may show gastro-esophageal reflux secondary to pyloric stenosis and secondary failure to thrive [22, 24]. Likewise, hypotonia, irritability, and hypersensitivity of sound and touch, shyness, and sleep disturbances have been noted in CS infants [25]. The craniofacial features of CS include macrocephaly with a prominent forehead, epicanthal folds, down-slanting palpebral fissures, short nose with depressed nasal bridge and a broad base, low-set posteriorly rotated ears with thick helices and lobes, full cheeks, and large mouth and lips. Dermatologic manifestations consist of soft loose skin, excessive wrinkling, redundancy of the skin of the hands and feet with deep palmar and plantar creases. Hypertrophic cardiomyopathy is also present in CS patients as well as valve anomalies, septal defects, and arrhythmia. CS is caused by de novo germline mutations in *HRAS* [26–28]. The typical CS-associated *HRAS* mutations are located at codons 12 or 13 which are also mutated in solid tumors. Notably, however, the more common CS-associated substitutions (e.g., p.G12S) are less common in cancer.

The fact that CS-associated germline *HRAS* mutations resemble those found as somatic events in tumors is considered to explain the high risk for benign and malignant tumors such as cutaneous papillomas, embryonal rhabdomyosarcoma, transitional cell carcinoma of the bladder, and neuroblastoma in CS. Cancer occurs in 15% of patients by age 20 years [29, 30] justifying cancer surveillance.

17.6 Cancer and CBL Syndrome

The condition NSLL or CBL syndrome (CBLS) is caused by germline mutations of *CBL* and many patients typically display craniofacial and other anomalies reminiscent of NS [31–33]. However, the NS-like phenotype is not always clinically obvious. A particular complication in CBLS is a vasculopathy of medium-sized arteries. As discussed further below, CBLS patients appear to have a high risk of developing

JMML. CBLS-related JMML is a clonal condition and characterized by loss of heterozygosity in hematopoietic cells. Despite the fact that CBLS-related JMML is a clonal disease, some individuals demonstrate spontaneous regression with a benign course.

17.7 Cancer and RASopathies That Lack a NS-Like Appearance

Capillary malformation arteriovenous malformation syndrome (CM-AVM) is another RAS/MAPK pathway disease. Here, multifocal capillary malformations which may be associated with arteriovenous malformations and fistulas. The vascular lesions may be located in many tissues including skin, muscle, bone, heart, and brain. Inactivating mutations in the *RASA1* gene cause CM-AVM. *RASA1* encodes for a RAS-GAP, and the CM-AVM associated mutation results in decreased hydrolysis of RAS-GTP and increased RAS/MAPK signaling [34]. Interestingly, capillary and vascular malformations do also occur as rare cutaneous anomalies in NS and related disorders.

Synaptic RAS GTPase-activating protein 1 (SYNGAP1) is in humans encoded by the *SYNGAP1* gene. SYNGAP1 is a RAS GTPase-activating protein that is critical for the development of cognition and proper synapse function. The protein is expressed exclusively in the brain and mutations in humans can cause intellectual disability or epilepsy. Specifically, Hamdan et al. identified de novo truncating mutations in *SYNGAP1* in approximately 3% of patients with nonsyndromic mental retardation [35]. Patients do not have NS features likely due to expression of *SYNGAP1* limited to the brain. There are no data indicating that individuals with these two conditions have an increased cancer risk.

17.8 Cancer and Mosaic RASopathies

The presence of two genetically distinct populations of cells within an individual derived from a postzygotic mutation is termed mosaicism. In the 1930s it was recognized that some individuals developed NF1 features such as café-au-lait spots, freckling, and neurofibromas limited to a segment of the body. Termed segmental NF1, it would later be established that in most cases, the disorder represented a form of mosaicism of the disease [36]. Subsequently, we have come to learn that there is a broad range of symptoms and disorders involving mosaicism for RASopathy-associated mutations. Indeed, patients with classic features of CS have been recognized who have both normal and mutation-harboring cells within one tissue type demonstrating mosaicism [37, 38]. These are examples for mosaicism of typical mutations associated with a typical—albeit sometimes milder or regionally expressed phenotype.

The term “mosaic RASopathies” has been introduced to describe mosaicism for “oncogenic” RAS/MAPK pathway mutations that are usually embryonically lethal when occurring in the germline, but may survive in the mosaic state. The conditions

belonging to the group of mosaic RASopathies are neurocutaneous disorders that usually lack NS-like features, because the percentage of mutated cells in the body is too small to cause manifestations that are seen with a systemic (generalized) RAS/MAPK pathway overactivation. The majority of mosaic RASopathies belong to the group of systematized or isolated congenital nevi. The great variability of mosaic disorders is related not only to the genotype but also to the localization and distribution of mutated cells in the body.

Keratinocytic epidermal nevi and sebaceous nevus as well as their systematized forms, epidermal nevus syndrome, and Schimmelpenning syndrome, respectively, are caused by mosaic *RAS* mutations with *HRAS* being the most commonly affected *RAS* gene and the p.G13R substitution representing the predominant mutation [39, 40]. The mutations can typically be found in affected but not in unaffected tissue and are usually absent from blood cells [40]. Congenital melanocytic nevi and systematized variant named neurocutaneous melanocytosis typically have oncogenic missense mutations in *NRAS* as mosaic in affected tissue. More recent studies have associated oculoectodermal syndrome with specific *KRAS* mosaic mutations [41], extracranial arteriovenous-malformations with *MAP2K1* (*MEK1*) mutations [42], and *KRAS* mutations in arteriovenous-malformations of the brain [43].

For epidermal nevi there is probably a small risk for malignant transformation. Melanoma occurs in roughly 1–2% of patients with congenital melanocytic nevi and those patients with larger nevi have a higher risk for malignant transformation [44, 45].

The wide-ranging phenotype of the mosaic RASopathies may be explained by the timing of the mutation, the type of the mutation, the particular lineage and the viability of the cells bearing the mutation, as well as by the body region involved [36]. However, there are other yet to be elucidated factors that may be important for a mosaic presentation of RASopathies. A report of a young patient with NSML where the left trunk and arm lacked lentigines that were present on other parts of the body. Fibroblasts from normal appearing skin as well as skin with lentigines showed the *PTPN11* mutation, suggesting an unknown mechanism of "silencing" of the mutation in the normal skin [46].

17.9 Cancer Surveillance for Individuals with RASopathies

Individuals affected by a RASopathy often have multi-system involvement and comprehensive care is often necessary. However, only a subset of patients, specifically those with NF1 (not discussed in detail here), CS, and CBL syndrome, have a clinically relevant (i.e., >5%) increase in cancer risk, and for individuals with these conditions, cancer surveillance is recommended. In patients with CS < 10 years of age, physical exam, and abdominal-pelvic ultrasound are recommended—to screen for rhabdomyosarcoma and neuroblastoma—every 3–4 months. After 10 years of age an annual urinalysis should be completed as a screening tool to detect bladder cancer [29, 30]. CBL syndrome patients have a high but not precisely defined JMML risk during early childhood. Therefore, from 0 to 5 years of age, physical exam (with assessment

of spleen) and complete blood count with white blood cell differential every 3–6 months has been recommended [30].

Some patients with mosaic RASopathies, such as patients with multiple/large congenital melanocytic naevi with involvement of the CNS, may also have a clinically relevant increase in cancer risk [47]. Patients affected by a mosaic RASopathy should be monitored clinically on a regular basis. The melanoma risk in patients with large and/or multiple congenital melanocytic nevi justifies screening with MRI of the CNS starting within the first year of life [47] and close clinical monitoring. In addition to what is recommended in the literature [47], annual brain MRIs may be justified due to the significant risk of cerebral melanoma in patients with CNS involvement.

If cancer occurs in patients with RASopathies, there is no evidence that standard therapies are not effective. Nevertheless, due to the likely involvement of the RAS signaling pathway in these cancer patients, the use of targeted therapies (such as MEK inhibitors) may be discussed and considered, especially in the absence of effective standard protocols.

17.10 Conclusion

Germline RAS pathway mutations lead to an increased cancer risk. For most RASopathies, the elevated cancer risk is mild and does not warrant cancer surveillance. Exceptions include NF1, CS, and CBL syndrome. Patients with specific *PTPN11* or *KRAS* mutations may benefit from surveillance (3-monthly blood counts and clinical monitoring during the first 5 years of life) due to their risk of developing a JMML-like myeloproliferation. Depending on the timing and cell type expression of the mutant allele mosaic mutations putatively lead to variable phenotypes. The risk of melanoma observed in patients with congenital melanocytic nevi justifies MRI of the brain and regular physical exams. The wide range of disorders associated with RAS/MAPK pathway dysfunction highlights the critical role of the pathway in man. Excitingly, novel therapies based on inhibition of the RAS pathway are being developed for these disorders.

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