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The NF1 gene in tumor syndromes and melanoma

Maija Kiuru and **Klaus J. Busam**

Departments of Dermatology and Pathology, University of California Davis, Sacramento, CA; Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

Activation of the RAS/MAPK pathway is critical in melanoma. Melanoma can be grouped into four molecular subtypes based on their main genetic driver: *BRAF*-mutant, *NRAS*-mutant, *NF1*-mutant, and triple wild-type tumors. The NF1 protein, neurofibromin 1, negatively regulates RAS proteins through GTPase activity. Germline mutations in *NF1* cause neurofibromatosis type I, a common genetic tumor syndrome caused by dysregulation of the RAS/MAPK pathway, i.e. RASopathy. Melanomas with NF1 mutations typically occur on chronically sun-exposed skin or in older individuals, show a high mutation burden, and are wild-type for *BRAF* and *NRAS*. Additionally, NF1 mutations characterize certain clinicopathologic melanoma subtypes, specifically desmoplastic melanoma. This review discusses the current knowledge of the *NF1* gene and neurofibromin 1 in neurofibromatosis type I and in melanoma.

Introduction

In the era of personalized medicine and targeted therapies, molecular subtyping of melanoma is replacing the traditional classification based on clinicopathologic features. Based on exome and genome sequencing studies, cutaneous melanoma can be divided into four distinct molecular subtypes: i) B-Raf proto-oncogene, serine/threonine kinase (*BRAF)* -mutant, ii) neuroblastoma RAS viral oncogene homolog *(NRAS)* -mutant, iii) neurofibromin 1 *(NF1)* -mutant, and iv) *BRAF/NRAS/NF1* wild-type (WT) tumors (triple wild-type tumors) [1]. This review discusses the current knowledge of the *NF1* gene and neurofibromin signaling pathways in neurofibromatosis type I and in melanoma, including prospects for therapeutic targeting.

Neurofibromatosis type I

Neurofibromatosis type I (NF1; Online Mendelian Inheritance in Man (OMIM) database 162200) has been recognized for over a century, with the first report by von Recklinghausen [2] and a thorough characterization by Crowe and colleagues in 1956 [3]. It is a common genetic syndrome with an incidence of approximately 1 in 3000 live births caused by mutations in the *NF1* gene [4–6] predisposing to multiple tumors, most commonly derived

Corresponding author: Maija Kiuru, M.D., Ph.D., Departments of Dermatology and Pathology, University of California Davis, 3301 C Street, Suite 1450, Sacramento, CA 95816, Phone: 916-734-0591, Fax: 916-734-6793, mkiuru@ucdavis.edu.

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from the neural crest. NF1 is an autosomal dominant disorder with a high rate, approximately 50%, of new mutations.

Mutations in the *NF1* gene were identified in 1990 by Wallace and coworkers[4], who demonstrated translocations and an insertion of this gene in three NF1 patients. Since then, hundreds of mutations have been reported, with over 80% of patients having a nonsense mutation, an insertion, or a deletion predicted to lead to a truncated protein product [7].

As is characteristic for genetic syndrome caused by a mutation in a tumor suppressor gene, tumors in NF1 patients show loss of heterozygosity (LOH), i.e. somatic mutation inactivating the second, remaining allele [8,9].

Pigmentary disorders and neuroectodermal-derived tumors in NF1

The clinical features of NF1 are numerous and affect multiple organ systems. The most common clinical findings involve the skin, as exemplified by the diagnostic criteria of NF1 (Table 1a). These include café-au-lait-macules, axillary and inguinal freckling, neurofibromas, and plexiform neurofibromas, present in 90%, 80%, 60–90%, and 25% of patients, respectively.

Café-au-lait macule (CALM)

Café-au-lait macules (CALMs) are tan to brown sharply demarcated uniformly pigmented macules or patches typically on the trunk and extremities. In NF1, CALMs are the earliest manifestation, typically appearing at 0–3 years of age. One to a couple of CALMs are common in the general population, therefore, six or more CALMs are required to fulfill one diagnostic criteria of NF1 (Table 1a). Histologically, CALMs show hyperpigmentation of the basal layer keratinocytes of the epidermis with normal to slightly increased number of melanocytes. In CALMs of NF1 patients, giant melanin granules (macromelanosomes) are present in melanocytes [10].

Axillary or inguinal freckling

Axillary or inguinal freckling is characterized by multiple tan to brown sharply demarcated uniformly pigmented macules in the axillae or the groin. They typically develop in early childhood. They are similar to CALMs clinically and histologically except smaller in size.

Lisch nodule

Lisch nodules are melanocytic hamartomas of the eye [11]. They appear as well-defined, dome-shaped papules on the iris and are clear to yellow or brown. They are the most common manifestation of NF1, present in nearly 100% of patients. Histologically, they show an aggregation of melanocytes, admixed with spindle cells and mast cells. Lisch nodules are not known to result in any ophthalmologic complications.

Neurofibroma

Neurofibromas are benign tumors of nerve sheath origin. Clinically, they present as soft skin colored to pink papules or nodules on the trunk, extremities, or head/neck. Though common in the general population as solitary lesions, their number in NF1 patients can vary from a

few to thousands. They typically develop around puberty. Histologically, they are composed of loosely arranged wavy spindled Schwann-like cells, nerve fibers, perineurial cells, and fibroblasts, and infiltrated by mast cells in the dermis or subcutis. Neurofibromas are benign but have a significant impact on quality-of-life mainly due to their appearance [12]. A population of stem/progenitor cells that resides in the dermis termed skin-derived precursors are suggested as the cells of origin of neurofibromas [13]. In addition, non-neoplastic cells in the tumor microenvironment are proposed to play a role in neurofibroma tumorigenesis [13].

Plexiform neurofibroma

Plexiform neurofibromas are much less frequent than neurofibromas, but are considered pathognomonic of NF1. Additionally, they may serve as precursors for malignant peripheral nerve sheath tumor (MPNST), an aggressive sarcoma typically arising in a pre-existing plexiform neurofibroma [14]. Plexiform neurofibromas usually become clinically apparent by 4–5 years of age. Clinically they present as tender, firm subcutaneous nodules often with overlying hyperpigmentation and hypertrichosis. They can infiltrate underlying tissues, cause soft tissue and bony hypertrophy, distortion or compression of adjacent structures, and neurologic deficits. Histologically, they consist of large thick nerves or nerve fibers within a background of neurofibroma.

Optic glioma

Another common tumor in NF1 is optic glioma. Optic gliomas in NF1 are mainly grade I pilocytic astrocytomas, defined as benign tumors that have a favorable prognosis unlike pilocytic astrocytomas in patients without NF1 [6].

Malignant peripheral nerve sheath tumor (MPNST)

MPNSTs occur in 3–15% of NF1 patients, with a peak incidence in young adults. Rapid growth, increased pain or a new neurologic deficit may be a sign of malignant transformation of a pre-existing plexiform neurofibroma in NF1 patients. Histologically, the tumor shows tight wavy or interlacing bundles of spindle cells with cellularity and mitoses determining tumor grade. As MPNSTs may resemble desmoplastic melanoma, exclusion of an associated melanocytic precursor lesion (intra-epidermal or intrafollicular melanoma in situ or melanocytic nevus) is required for the diagnosis of MPNST.

Other tumors in NF1

The overall risk of cancer is increased in NF1. Based on epidemiologic studies, cancer incidence in NF1 is approximately 4-fold higher than in general population [15]. Other reported neoplasms include juvenile myelomonocytic leukemia, pheochromocytoma, central nervous system tumors other than optic glioma, rhabdomyosarcoma, duodenal carcinoid, somatostatinoma, parathyroid adenoma, and gastrointestinal stromal tumor (Table 1b). Additionally, the risk of breast cancer is 5-fold. Epidemiologic studies have also shown an increased risk of cancer of the esophagus, stomach, colon, liver, lung, bone, thyroid, and ovary, non-Hodgkin's lymphoma, and chronic myeloid leukemia [16].

Although somatic mutations in *NF1* are present in melanoma (Tables 2, 3), the risk of melanoma in individuals with NF1 is only minimally increased, to approximately 3.6-

fold [16]. Based on a study of 11 NF1 patients with melanoma, a female preponderance, a higher thickness, and a frequent association with a second neoplasia were identified [17]. Additionally, a desmoplastic melanoma, a subtype of melanoma sharing some morphologic features with MPNST, has been reported in one individual with NF1 [18].

The *NF1* gene

The NF1 gene was mapped to chromosome 17 through linkage analyses in NF1 kindreds [19–23]. Genetic studies were complemented by more refined physical mapping [24]. The complete cDNA sequence of the *NF1* gene [25] and the structure of the large gene consisting of over 50 exons spanning 300 kb of chromosome 17 [26] were later reported.

The *NF1* gene at 17q11.2 is now known to consist of 60 exons and to generate several alternatively spliced isoforms. Although identification of mutations in *NF1* has been challenging due to the very large size and complexity of the gene, lack of mutational hot spots, and the presence of pseudogenes, hundreds of mutations have been reported [7,27]. Through advances in next-generation sequencing technologies, faster and more robust techniques for sequencing of *NF1* have been established [28,29].

Most of the mutations in *NF1* are loss-of-function mutations [4,5,7,30]. These include missense, nonsense, frameshift, and splice site mutations, insertions, deletions, large deletions including *NF1* gene and possibly other flanking genes, and translocations [27]. Therefore, the gene has been classified as a tumor suppressor. Consistent with a role of a tumor suppressor gene, loss of heterozygosity (LOH) or “second-hit” somatic mutations in the inherited wild-type allele are present in many tumor types in NF1 patients, including neurofibromas, malignant peripheral nerve sheath tumors, pheochromocytomas, and myeloid tumors [31–37].

Wide phenotypic variability exists even among patients within the same family, against a definite genotype-phenotype correlation, although with some exceptions [27,38]. A 3-base pair in-frame deletion is associated with the lack of cutaneous neurofibromas, shown in a study of 21 unrelated probands [38]. A microdeletion of *NF1*, the single most common mutation in individuals with NF1, is associated with the development of a large number of neurofibromas at an earlier age and a probable increased risk of MPNSTs [39]. Additionally, an association with splice site mutations and the presence of tumors, especially central nervous system gliomas and MPNSTs, has been reported [40].

The phenotypic variability in NF1 could be explained by modifier genes, including protein-coding sequences, microRNA and long non-coding RNA genes, that may affect the NF1 phenotype [41]. In support of this, cohort studies have demonstrated that the clinical features tend to be similar in close relatives compared with distant relatives. Proof of modifier genes has been sought from mouse models of NF1, candidate gene studies, or whole genome approaches (reviewed in [41]). An array comparative genomic hybridization (aCGH) study of plexiform neurofibromas from NF1 patients showed a recurrent somatic 9p21.3 deletion involving the antisense noncoding RNA in the INK4 locus *(ANRIL)* and an association between a germline SNP rs2151280 resulting in reduced *ANRIL* transcript levels and the

number of plexiform neurofibromas, suggesting that *ANRIL* expression mediated plexiform neurofibromas susceptibility [42]. Polymorphisms in the adenylate cyclase 8 (*ADCY8*) gene correlated with glioma risk in NF1 patients in a sex-specific manner, elevating the risk in females and reducing it in males, also strengthening the evidence of a role of cAMP in gliomagenesis in NF1 [43]. Further studies utilizing genome-wide approaches including genome-wide sequencing appear promising for identification of modifier genes in NF1.

The NF1 protein

NF1 encodes neurofibromin 1, a large protein consisting of over 2800 amino acids [7,44]. Neurofibromin 1 contains multiple functional domains. Early studies on the amino acid sequence of neurofibromin 1 demonstrated a 360-residue region with significant similarity to the catalytic domain of GTPase-activating protein (GAP) and a role in the control of cell growth by interaction with RAS was suggested [45].

The GTPase-activating protein -related domain is the best studied domain of neurofibromin 1 and is know to negatively regulate RAS by converting the active RAS-guanosine triphosphate (RAS-GTP) to the inactive RAS-guanosine diphosphate (RAS-GDP) thereby inhibiting downstream RAS signaling (Figure 1). In support of the critical role of RAS-GTPase function of neurofibromin 1 in NF1 pathogenesis, some of the missense mutations in NF1 patients selectively abolish RAS-GTPase activity without affecting the protein structure and levels [46]. A pleckstrin homology domain interacts with the cytoskeleton and membrane structures. The C-terminal domain can be phosphorylated by protein kinase A (PKA) inhibiting neurofibromin 1 [44].

The upstream regulation of neurofibromin 1 includes the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor, the tyrosine kinase cKIT receptor, the endothelin receptor B (EDNRB), and the tyrosine kinase anaplastic lymphoma kinase (ALK) receptor ([6] and references therein). The most studied downstream pathways regulated by neurofibromin 1 are the RAS/mitogen-activated protein kinase (MAPK) (Figure 1) and phosphoinositide 3-kinase (PI3K)/mechanistic target of rapamycin (mTOR) signaling pathways. In addition, neurofibromin 1 is involved in cyclic adenosine monophosphate (cAMP) signaling. Targeting these pathways offer important avenues for therapies (see “Prospects for targeted therapies”).

The RAS/MAPK pathway

The RAS/MAPK pathway plays a critical role in normal development through regulation of cell growth, differentiation, and senescence. RAS genes include *NRAS*, *HRAS*, and *KRAS*, a multigene family encoding guanosine nucleotide bound GTPases. The signaling pathway starts with the interaction between a ligand and a cell surface receptor, either a G-protein coupled receptor or a receptor tyrosine kinase (Figure 1). This leads to activation of RAS by conversion of RAS-GDP into RAS-GTP. Notably, neurofibromin 1 negatively regulates this step through GTPase activating protein activity converting the active RAS-GTP to the inactive RAS-GDP. Active RAS interacts with many downstream mediators, most importantly by binding to the RAS-binding domain of BRAF or Raf-1 proto-oncogene, serine/threonine kinase (RAF1). This results in homo and heterodimerization and activation

of RAF that then activates the MAP kinases mitogen-activated protein kinase kinase 1 (MAP2K1) and mitogen-activated protein kinase kinase 2 (MAP2K2) via phosphorylation. These in turn phosphorylate and activate mitogen-activated protein kinase 3 (MAPK3 or ERK1) and/or mitogen-activated protein kinase 1 (MAPK1 or ERK2), the effectors of the pathway that control cell cycle progression, differentiation, and growth.

Dysregulation of the RAS/MAPK pathway is one of the key events in oncogenesis, including melanoma. Most melanomas show activation of the RAS/MAPK pathway and the key genes mutated in melanoma, *BRAF*, *NRAS*, *NF1*, *C-KIT*, G protein subunit alpha q (*GNAQ*), and G protein subunit alpha 11 (*GNA11*), all participate in the RAS/MAPK pathway [47].

RASopathies

RASopathies are genetic syndromes caused by germline mutations in genes encoding components or regulators of the RAS/MAPK pathway [48,49]. Given the key role of the RAS/MAPK pathway in normal development, mutations in RAS/MAPK pathway genes in these patients result in developmental abnormalities in multiple organ systems as well as predisposition to cancers. Due to the shared mechanism of RAS/MAPK pathway dysregulation, the syndromes share many clinical features including cutaneous, musculoskeletal, and ocular abnormalities, craniofacial dysmorphology, cardiac malformations, neurocognitive impairment, and increased cancer risk. In addition to NF1, RASopathies include Noonan syndrome caused by activating mutations in protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*), SOS Ras/Rac guanine nucleotide exchange factor 1 (*SOS1*), *RAF1*, *KRAS*, *NRAS*, and SHOC2, leucine rich repeat scaffold protein (*SHOC2*), and inactivating mutations in Cbl proto-oncogene (*CBL*), Noonan syndrome with multiple lentigines (formerly LEOPARD syndrome) caused by activating mutations in *PTPN11*, Costello syndrome caused by activating mutations in *HRAS*, Cardiofacio-cutaneous syndrome caused by activating mutations in *BRAF*, *MAP2K1*, and *MAP2K2*, and Legius syndrome caused by inactivating mutations in sprouty related EVH1 domain containing 1 (*SPRED1*) (Figure 1). Most of these genes are somatically mutated in melanoma and specifically co-occur with *NF1* mutations (see “NF1 mutations in melanoma”).

Cancer risk in these syndromes is increased [50]. The most commonly reported cancers include neuroblastoma, acute lymphoblastic leukemia, low grade glioma, and rhabdomyosarcoma in Noonan syndrome and rhabdomyosarcoma, bladder cancer, and neuroblastoma in Costello syndrome. Cancers reported in Cardio-facio-cutaneous syndrome include acute lymphoblastic leukemia, non-Hodgkin lymphoma, hepatoblastoma and rhabdomyosarcoma. In Noonan syndrome with multiple lentigines (formerly LEOPARD syndrome) acute myeloid leukemia, acute lymphoblastic leukemia, neuroblastoma and one melanoma has been reported. In Legius syndrome one occurrence each of non-small cell lung cancer, Wilms tumor, tubular colon adenoma, acute myeloblastic anemia, tenosynovial giant cell tumor, breast cancer and dermoid tumor of the ovary has been reported [51–54].

The PI3K/mTOR, cAMP, and other pathways

The important role of the PI3K/mTOR pathway is supported by the activation of the pathway in *NF1*-mutant neurofibroma and MPNST cell lines and primary tumors [44,55–58]. The cAMP pathway is deregulated in *NF1*-deficient tumors. In yeast, the NF1 homologues regulate Ras and cAMP signaling. Animal models expressing mutant *Nf1*, including mouse, Drosophila, and zebrafish, show deregulated cAMP levels in various cell types [59–61]. cAMP levels are also altered in human *NF1*-associated tumors, including gliomas [43]. There are many other lesser known neurofibromin 1-regulated effectors downstream of RAS, including afadin, adherens junction formation factor (AFDN), ral guanine nucleotide dissociation stimulator (RALGDS), T-cell lymphoma invasion and metastasis 1 (TIAM1), phospholipase C like 1 (PLCL1), and Ras and Rab interactor 1 (RIN1).

NF1 in the melanocyte lineage

Neurofibromin 1 is proposed to have fundamental functional differences in neural and non-neural tissues, and thus, lead to very different pathologies in different cell lineages [62]. In a rat model system, neurofibromin 1 is broadly distributed during embryogenesis including neural crest migration, but postnatal expression is low or absent in non-neural tissues.

Some of the neural crest derivatives include Schwann cells and melanocytes. Schwann cells are glial cells that wrap around axons in the peripheral nervous system and express relatively high amounts of neurofibromin 1. Neurofibromin plays a role in Schwann cell differentiation [63] and Schwann cell-axonal interactions [64]. Schwann cells appear vulnerable to defects in *NF1,* as exemplified by the many Schwann cell –containing tumors in NF1, including neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors.

Similar to neurofibromas and other NF1-associated tumors [36], biallelic inactivation of *NF1* is observed in melanocytes of CALMs from individuals with NF1 [65]. The downstream effects of *NF1* loss appear cell type dependent. The detailed molecular mechanisms linking loss of *NF1* to hyperpigmentation and melanocyte function have been poorly characterized until the recent years (reviewed in [44]).

Mice deficient of *Nf1* lack pigmentary abnormalities. However, studies using $Nf1^{+/-}$ murine models show that *Nf1* deficiency partially reverts a skin pigmentation defect present in *Kit* and *Mitf* mutant mice, demonstrating that neurofibromin 1 regulates signaling between Kit and melanogenesis associated transcription factor (Mitf) [66]. The $Nf1^{+/-}$ melanocytes also display increased melanogenic gene expression and enhanced Kit-dependent and Kit-independent ERK activity. Additionally, mice with homozygous knock-out of *Nf1* in melanocytes show hyperpigmented skin [67].

Primary human melanocytes are derived from the neural crest and grow extremely slowly in vitro. Therefore, recent protocols for human embryonic stem cell (hESC)- and induced pluripotent stem cell (iPSC)-based derivation of melanocytes have been established [68–70]. Larribere and co-workers [69] reprogammed patient-derived $NF1^{+/-}$ fibroblasts into $NF1^{+/-}$iPSCs. The $NF1^{+/-}$ iPSCs showed an active RAS signaling and differential

expression of genes associated with the RAS/MAPK signaling pathway compared with WT iPSCs. Furthermore, *NF1*$^{+/-}$ iPSC-derived melanocytes displayed a senescence phenotype with a lower melanocyte number, altered cell morphology, and reduced proliferative index compared with WT iPSC-derived melanocytes. The authors concluded that NF1 haploinsufficiency through RAS activation promotes a senescence phenotype in the melanocyte lineage.

Allouche and co-workers [70] used *NF1*$^{+/-}$ hESC-derived melanocytes to study the molecular mechanisms of hyperpigmentation in NF1. *NF1*$^{+/-}$ hESC-derived melanocytes showed an increase in intracellular melanin content, a greater expression of melanogenic enzymes, and an increase in stage III/IV melanosomes compared with WT hESC-derived melanocytes, recapitulating the *in vivo* hyperpigmentation phenotype of NF1 *in vitro*. *NF1*$^{+/-}$ hESC-derived melanocytes displayed an increase in cAMP activity and ERK signaling, the two downstream pathways dysregulated in NF1. Both cAMP and ERK pathways were blocked successfully *in vitro* using a mitogen-activated protein kinase kinase (MEK) inhibitor or a PKA/cAMP inhibitor, holding promise for future therapeutic trials.

NF1 mutations in melanoma

NF1 mutations in cancer

Somatic mutations in *NF1* are present in sporadic cancers. They are the most frequent in MPNSTs, the most common cancer type in individuals with NF1, being present in 40% of tumors [71]. With the expansion of cancer genomics data, online portals offer valuable platforms to explore the available mutation data [72]. According to these data, genetic alterations in NF1 were reported in total of 147 studies (http://www.cbioportal.org). Over twenty published studies included more than 15 specimens and showed *NF1* mutations in more than 10% of samples (Table 2). Of cancer types other than melanoma, these included 40% of MPNST [71], 23% of acute lymphoblastic leukemia [73], 11–18% of glioblastoma [74,75], 12% of non-small cell lung cancer [76], 12% of lung squamous cell carcinoma [77], 13% of lung adenocarcinoma [78], 10–14% of bladder urothelial carcinoma [79–81], 14% of uterine carcinosarcoma [82], 11–12% of uterine endometrial carcinoma [83], 12% of ovarian serous cystadenocarcinoma [84], 11% of pancreatic carcinoma [85], 10% of metastatic cutaneous squamous cell carcinoma [86], and 10% of gastric adenocarcinoma [87,88].

NF1 mutations in melanoma

Before sequencing data were available, the *NF1* locus showed frequent LOH in desmoplastic melanomas, a rare subtype of melanoma [89]. Recent large-scale targeted sequencing, whole exome, and whole genome sequencing efforts have established *NF1* as one of the key drivers of melanoma (Table 3) [1,90,91]. Characteristically for a driver gene, *NF1* shows a high frequency of non-silent exonic mutations and a low frequency of synonymous or intronic mutations, as well as being present in a considerable portion of studied melanoma specimens, approximately 12–18% of all melanomas (Table 3) [91–94](67). Mutations in *NF1* are more common in melanomas occurring on chronically sun-exposed skin or in older patients [90,94], melanomas with higher mutation burden [90] or wild-type for *BRAF* and *NRAS* [92,94,95], and certain cli

NF1 mutations in desmoplastic melanoma

NF1 mutations are found in 45–93% of desmoplastic melanomas [96–98]. This is not surprising, given the morphologic overlap between desmoplastic melanomas and MPNSTs, which commonly show *NF1* mutations [99,100]. Desmoplastic melanoma is a rare subtype of melanoma, with distinct clinical and histopathologic features. It typically occurs on chronically sun-exposed skin of elderly individuals. Histologically it shows fibroblast-like spindled melanocytes surrounded by abundant collagen fibers [101]. Molecularly, a high mutation burden, UV mutation signature, and a diverse activation of the RAS/MAPK pathway characterize desmoplastic melanoma [96,97].

Interestingly, *NF1* loss in melanoma is often associated with concurrent mutations in RASopathy genes [90,102]. These include RAS p21 protein activator 2 (*RASA2*), *PTPN11*, *SOS1*, *RAF1*, and *SPRED1*, mutated in the germline of individuals with Noonan syndrome (*RASA2*, *PTPN11*, *SOS1*, *RAF1*), Noonan syndrome with multiple lentigines (*PTPN11*, *RAF1*), and Legius syndrome (*SPRED1*) (Figure 1). It is suggested that the co-occurring mutations may act synergistically in melanomas [102].

Prospects for targeted therapies

As the major consequence of *NF1* mutation is the increased RAS/MAPK pathway signaling, therapeutic targeting of this pathway both for melanoma and for syndromic NF1-associated tumors is reasonable and supported by preclinical evidence. Additionally, inhibitors of the PI3K/mTOR and cAMP pathways show promise for targeting NF1-deficient tumors. The downstream impact of *NF1* deficiency may be dependent on the cell type, and should be kept in mind in the development of targeted therapies [44].

Preclinical and clinical trials for treatment of NF1-associated tumors, MPNSTs, plexiform neurofibromas, and neurofibromas, include tyrosine kinase inhibitors (imatinib, dasatinib, sunitinib, nilotinib), MEK inhibitors (trametinib, selumetinib), and mTOR inhibitors (rapamycin, sirolimus, everolimus) [103–106]. For treatment of melanoma, the U.S. Food and Drug Administration -approved agents affecting the RAS/MAPK pathway include MEK inhibitors trametinib and cobimetinib and the BRAF inhibitors vemurafenib and dabrafenib. Additionally, non-FDA approved inhibitors include RAF-inhibitors sorafenib, encorafenib, XL281, and R05126766, MEK inhibitors selumetinib and bimetinib, and ERK inhibitor ulixertinib, that have been tested mainly in phase I and II studies in melanoma patients [47,107–111]. In preclinical models, pan-RAF inhibitors, MEK inhibitors, and ERK inhibitors have shown activity in *NRAS*-mutant tumors [112–114], but there are no reports to date on these agents specifically in patients with *NF1*-mutant melanomas.

NF1 may also play a role in driving resistance to RAF/MEK targeted therapies. NF1 loss is thought to mediate resistance to RAF and MEK inhibitors through sustained MAPK pathway activation. Based on murine models, *Nf1* mutation suppresses Braf-induced senescence in melanocytes, promoting melanocyte proliferation and enhancing melanoma development. Nf1/Braf-mutant murine tumors are resistant to BRAF inhibitors but sensitive to combined inhibition of MAPK/ERK and mTOR pathways. In human melanoma cell lines, *NF1* ablation decreases the sensitivity of melanoma to BRAF inhibitors. Based on data from

clinical trials, mutations in NF1 are present in BRAF-mutant tumors intrinsically resistant to BRAF inhibitors as well as from patients showing resistance to BRAF inhibitors [115].

In conclusion, the rapidly increasing understanding of the role of *NF1* mutations in neoplasia and neurofibromin 1 in various cellular signaling pathways will likely produce novel treatment approaches relevant for individuals with NF1 and for sporadic tumors with *NF1* mutations.

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Summary

- **The *NF1* gene is a tumor suppressor gene mutated in the germline of individuals with neurofibromatosis type 1 (NF1). NF1 is one of the genetic syndromes with mutations in the RAS/MAPK pathway, i.e. RASopathies.**
- **NF1 is characterized by pigmented lesions of the skin and the eye, including café-au-lait macules, axillary freckling, and Lisch nodules. The most common tumors in NF1 include neurofibromas, plexiform neurofibromas, malignant peripheral nerve sheath tumors, and optic gliomas.**
- ***NF1* encodes neurofibromin 1 protein, which negatively regulates the RAS/MAPK pathway by converting active RAS-GTP to inactive RAS-GDP. Neurofibromin 1 also functions in the PI3K/mTOR and cAMP signaling.**
- **Somatic mutations in *NF1* are common in cancer including melanoma.**
- **Melanomas characterized by *NF1* mutations typically occur on chronically sun-exposed skin and in older individuals. They typically show a high mutation rate and UV signature mutations. Desmoplastic melanoma, a rare clinicopathologic subtype of melanoma, displays frequent mutations in *NF1*.**
- **Targeting neurofibromin 1-regulated pathways offers potential therapeutic options for the treatment of NF1 and melanoma.**

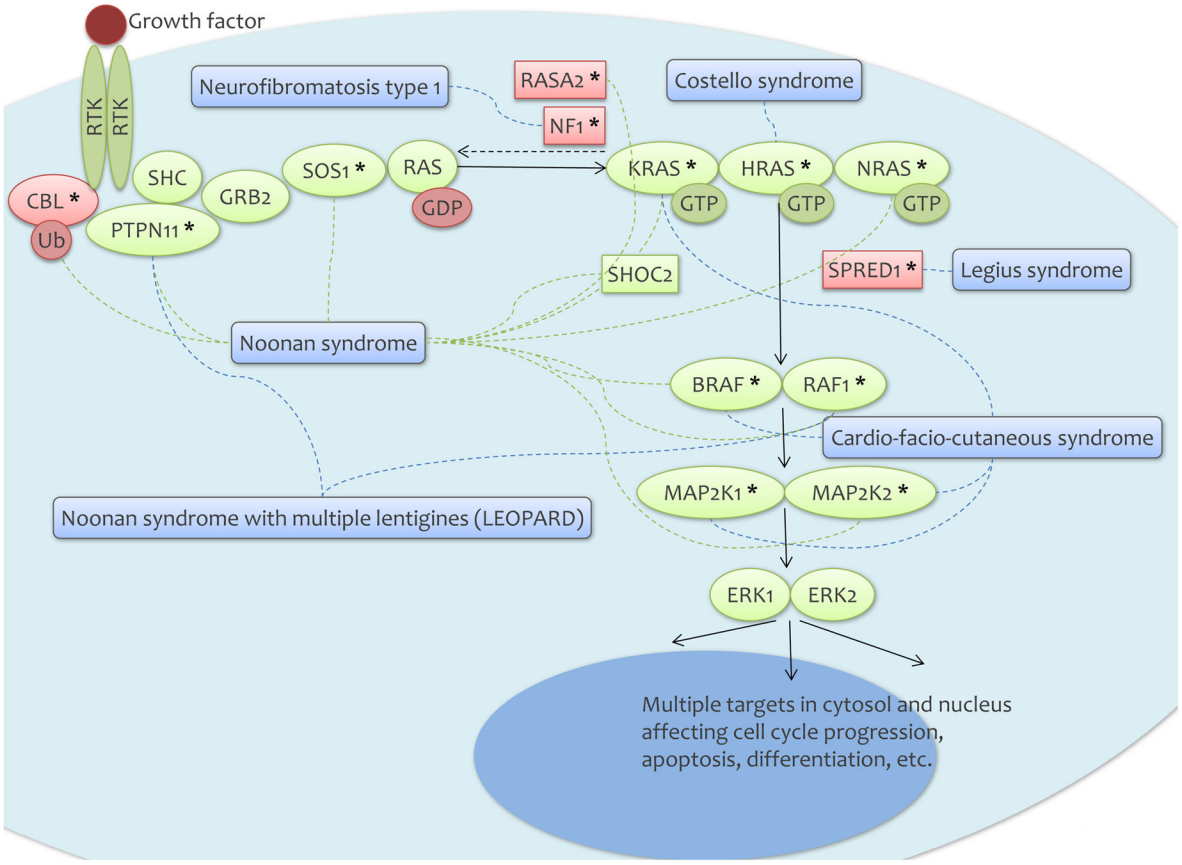

Figure 1.
The RAS/MAPK pathway and RASopathies.

Table 1a

Diagnostic criteria of NF1.

Clinical diagnosis based on presence of two of the following:
1. Six or more café-au-lait macules over 5 mm in diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals.
2. Two or more neurofibromas of any type or one plexiform neurofibroma.
3. Freckling in the axillary or inguinal regions.
4. Two or more Lisch nodules (iris hamartomas).
5. Optic glioma.
6. A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex, with or without pseudarthrosis.
7. First-degree relative (parent, sibling, or offspring) with NF-1 by the above criteria.

Table 1b

Most common tumor types in NF1.

Tumor type
Optic glioma (in 10–15%)
Malignant peripheral nerve sheath tumor (in 3–15%)
Pheochromocytoma
Juvenile myelomonocytic leukemia
CNS tumors other than optic gliomas
Rhabdomyosarcoma (especially of the genitourinary tract)
Duodenal carcinoid
Somatostatinoma
Parathyroid adenoma
Gastrointestinal stromal tumors (GIST)
Breast cancer (~5-fold increased risk in women <50 years of age)

Table 2

NF1 mutations in cancer.

Cancer type	Number of tumors with NF1 mutations	% of tumors with NF1 mutations	Reference
Melanoma	28 of 213	13	90
	16 of 121	13	92
	3 of 25	12	93
	9 of 20	45	97
	46 of 333	14	1
	6 of 34	18	94
	14 of 15	93	96
	13 of 91	14	91
MPNST	6 of 15	40	71
Acute lymphoblastic leukemia	9 of 40	23	73
Glioblastoma	31 of 281	11	75
Non-small cell lung cancer	137 of 1144	12	76
Lung squamous cell carcinoma	21 of 178	12	77
Lung adenocarcinoma	29 of 230	13	78
Bladder urothelial carcinoma	7 of 50	14	79
	12 of 109	11	80
	13 of 127	10	81
Uterine carcinosarcoma	3 of 22	14	82
Uterine endometrial carcinoma	27 of 240	11	83
Ovarian adenocarcinoma	37 of 316	12	84
Pancreatic carcinoma	12 of 109	11	85
Metastatic cutaneous squamous cell carcinoma	3 of 29	10	86
Gastric adenocarcinoma	29 of 287	10	87
	3 of 30	10	88

Table 3

NF1 mutations in melanoma.

Reference	Material	Sequencing method	Number of tumors with NF1 mutation	% of tumors with NF1 mutation	Characteristics of NF1-mutant melanomas	Conclusions
93	Not stated	WGS[1]	3 of 25	12		Revealed genomic evidence of ultraviolet pathogenesis and discovered a new recurrently mutated gene in melanoma
92	Fresh-frozen or short-term cultures (n=121; 15 primary tumors, 30 metastatic tumors, 76 short-term cultures)	WES[2]	16 of 121; 5 of 21 (BRAF/BRAS WT)	13; 25 (BRAF/ NRAS WT)		Development of framework to assess driver and passenger mutations and identification of six novel melanoma genes (PPP6C, RAC1, SNX31, TACC1, STK19, ARID2); provided evidence for direct mutagenic role of UV radiation
91	Fresh-frozen or short-term cultures (n=91)	WES	13 of 91	14		Classification of melanoma into those with high, medium and low mutation count, likely corresponding to chronically exposed, intermittently sun-exposed, and sun-protected lesions, respectively, and identification of new cancer genes such as PPP6C and RAC1
94	Fresh-frozen (n=34)	WES	6 of 34; 5 of 10 (BRAF/NRAS WT)	18; 50 (BRAF/ NRAS WT)		BRAF/NRAS WT melanomas show extensive UV damage and high mutation load and may require different treatment strategies including combination therapies
95	Cell lines		6 of 61 (BRAF/RAS WT); 1 of 10 (BRAF V600E)	10 (BRAF/RAS WT); 10 (BRAF V600E)		Loss of NF1 is common in melanoma and associated with RAS activation, MEK-dependence, and resistance to RAF inhibition
98	FFPE[3] (desmoplastic melanoma with sarcomatoid differentiation; n=1)	Targeted 230 gene panel	1 of 1	100		Report of an unusual desmoplastic melanoma with an undifferentiated sarcomatoid nodule, both components sharing a mutational heritage including mutations in NF1 gene
1	Fresh-frozen (n=333; 67 primary tumors and 266 metastatic tumors)	WES	46 of 333	14	Occur in older individuals, show a higher mutation burden	Classification of melanoma into four genomic subtypes: mutant BRAF, mutant NRAS, mutant NF1, and Triple-wild-type tumors; no significant outcome correlation based on genomic classification; however, immune gene expression associated with histologic lymphocytic infiltration associated with improved survival
90	Fresh-frozen or short-term cultures (n=213)	WES	28 of 213	13	Significantly more somatic mutations; occurred in older patients; associated with similar overall survival; harbor co-mutations in RASopathy genes RASA2, PTPN11, SOS1, RAF1, SPRED1; 60% of NF1-mutant melanoma cell lines sensitive and	NF1 is a key tumor suppressor lost in melanomas, and that concurrent RASopathy gene mutations may enhance its role in melanomagenesis

Reference	Material	Sequencing method	Number of tumors with NF1 mutation	% of tumors with NF1 mutation	Characteristics of NF1-mutant melanomas	Conclusions
					40% resistant to MEK inhibitor selumetinib in vitro	
[97]	Fresh-frozen (desmoplastic melanomas; discovery set; n=20); FFPE (desmoplastic melanomas; validation set; n=42) (total n=62)	WES, WGS (discovery set); targeted 293 gene panel (validation set)	9 of 20 (discovery set); 34 of 62 (total)	45 (discovery set); 54 (total)		Desmoplastic melanomas show high mutation burden, UV mutation signature, diverse activation of the MAPK pathway affecting NF1, CBL, ERBB2, MAP2K1, MAP3K1, BRAF, EGFR, PTPN11, MET, RAC1, SOS2, NRAS, PIK3CA
[96]	FFPE (desmoplastic melanomas; n=15); FFPE (non-desmoplastic melanomas; n=20; 18 metastatic tumors, 2 primary tumors)	Targeted 341 gene panel desmoplastic melanomas)	14 of 15 (desmoplastic melanomas); 4 of 20 (non-desmoplastic melanomas)	93 (desmoplastic melanomas); 20 (non-associated MIS in 12 of 14; depth 1.5–18 mm; pure (n=6), mixed (n=8)	Median age 71.5 (range 24–82); sites scalp (n=5), face (n=4), arm/shoulder (n=3), neck (n=1), chest (n=1); biology of this melanoma type	High frequency of NF1 mutations in desmoplastic melanomas suggests an important role for NF1 in the

[1]WGS= whole genome sequencing;

[2]WES= whole exome sequencing;

[3]FFPE= formalin-fixed, paraffin-embedded