Cell Cycle 12:1, 43–50; January 1, 2013; © 2013 Landes Bioscience

# Mosaic RASopathies

Christian Hafner* and Leopold Groesser

Department of Dermatology; University of Regensburg; Regensburg, Germany

**Keywords:** RAS, HRAS, KRAS, NRAS, mutation, mosaic RASopathy, epidermal nevus, mosaicism, sebaceous nevus, epidermal nevus syndrome

"RASopathies" are a group of developmental syndromes with partly overlapping clinical symptoms that are caused by germline mutations of genes within the Ras/MAPK signaling pathway. Mutations affecting this pathway can also occur in a mosaic state, resulting in congenital syndromes often distinct from those generated by the corresponding germline mutations. For syndromes caused by mosaic mutations of the Ras/MAPK signaling pathway, the term "mosaic RASopathies" has been proposed. In the following article, genetic and phenotypic aspects of mosaic RASopathies will be discussed.

## The RAS Signaling Pathway

Ras proteins are small GTPases playing a key role in transducing extracellular growth factor stimuli into the intracellular environment. After the binding of growth factors to their corresponding receptors on the cell surface, guanine-nucleotide exchange factors (e.g., SOS proteins) are activated. These factors cause a switch from inactive GDP-Ras to active GTP-Ras. On the other hand, GTPase-activating proteins, such as neurofibromin and p120GAP, catalyze the hydrolysis of GTP-Ras to inactive GDP-Ras, thus terminating Ras signaling.[1] Active GTP-Ras can cross-talk with further signaling proteins and stimulate downstream pathways. The two most important Ras-dependent pathways are the Ras-Raf-MEK-ERK and the PI3K-Akt signaling pathways. Activation of these pathways finally influences cell survival/apoptosis, proliferation and differentiation in various tissues. Three genes encode for the classical Ras proteins: *HRAS*, *KRAS* and *NRAS*. The protein sequences of the different Ras proteins have been highly conserved in phylogenesis, especially the N-terminal G domain, whereas the C-terminal sequence that is important for the targeting of Ras proteins to the cell membrane show a higher degree of variability.[2]

Considering the central position of Ras proteins in cellular signaling processes, it is not surprising that the disturbance of Ras protein function is fundamentally involved in the pathogenesis of many human disorders; e.g., activating mutations in *RAS* genes are found in about 30% of human cancers.[3] These oncogenic mutations are somatic alterations, thus restricted to the tumor tissue and absent in non-neoplastic tissues of the patients.

*Correspondence to: Christian Hafner; Email: christian.hafner@ukr.de
Submitted: 10/14/12; Accepted: 12/03/12
http://dx.doi.org/10.4161/cc.23108

According to current concepts, they are most likely acquired during life, e.g., by exposure to mutagenic factors. Interestingly, different cancer types often show the specific involvement of one of the three *RAS* genes, and the activating mutations occur preferentially at hotspot loci of codons 12, 13 and 61.

## Germline RASopathies

In addition to the somatic mutations in cancer, mutations of *RAS* genes or other components of the Ras-Raf-MEK-ERK signaling pathway can also occur in germline. These germline mutations result in developmental syndromes, with craniofacial dysmorphology and abnormalities of the heart, skin, eyes, brain and musculoskeletal system. Furthermore, some of these patients harbor an increased risk for the development of cancer during life. The term "RASopathies" has been suggested for congenital syndromes caused by germline mutations in genes of the Ras-Raf-MEK-ERK signaling pathway.[4] This class of genetic syndromes shows a considerable phenotypic overlap, which is explained by the common pathologic activation of the same pathway. Some RASopathies even show mutations of the same gene. The RASopathies currently comprise Noonan syndrome (*PTPN11*, *SOS1*, *KRAS*, *NRAS*, *RAF1*), neurofibromatosis type 1 (*NF1*), LEOPARD syndrome (*PTPN11*, *RAF1*), hereditary gingival fibromatosis (*SOS1*), capillary malformation-arteriovenous malformation syndrome (*RASA1*), Costello syndrome (*HRAS*), autoimmune lymphoproliferative syndrome (*NRAS*), cardio-facio-cutaneous syndrome (*BRAF*, *KRAS*, *MAP2K1*, *MAP2K2*) and Legius syndrome (*SPRED1*). In most cases, the mutations in RASopathies are heterozygous, result in an activation of the Ras/MAPK signaling pathway and follow an autosomal dominant inheritance. Though there may be some overlap (e.g., *HRAS* p.G12S is found both in Costello syndrome and in sporadic cancer), in most cases the mutational spectrum of the affected genes is different between germline RASopathies and sporadic cancer. While some RASopathies are rather frequent, such as the Noonan syndrome (1/1,000 to 1/2,500 newborns) or neurofibromatosis type 1 (1/3,000 newborns), others are rare disorders such as Costello and LEOPARD syndrome. However, some RASopathies are caused by inactivating mutations. *RASA1* acts like a tumor-suppressor gene,[5,6] promoting the conversion of active GTP-bound Ras to inactive GDP-bound Ras. It has been hypothesized that in the background of a germline *RASA1* mutation, a second hit in the wild-type allele randomly occurring at different body sites causes the vascular anomalies, according to the two-hit hypothesis of Knudson.

**Table 1.** Mosaic RASopathies

| Mosaic RASopathy | Gene | Reported mosaic mutations | References |
|---|---|---|---|
| Keratinocytic epidermal nevus | *HRAS* | G12C, G12V, G13R, Q61L | 8 |
| | *KRAS* | G12D | 8 |
| | *NRAS* | G12D, P34L, Q61R | 8 |
| | *(FGFR3)* | R248C, S249C, G372C | 8,14,45 |
| Keratinocytic epidermal nevus syndrome | *HRAS* | G12S | 19 |
| | *KRAS* | G12D | 18 |
| | *(FGFR3)* | R248C, S249C | 20-23 |
| Sebaceous nevus | *HRAS* | A11S, G12C, G12D, G12S, G13R | 25 |
| | *KRAS* | G12D, G12V | 25 |
| Schimmelpenning syndrome | *HRAS* | G13R | 25 |
| | *KRAS* | G12D | 25 |
| Mosaic Costello syndrome | *HRAS* | G12S | 32,33 |
| Segmental neurofibromatosis type I | *NF1* | Various point mutations, deletions, insertions | 38,39 |

## Mosaic RASopathies

Genetic mosaicism is defined by the presence of at least two genetically distinct cell populations in the same organism. It results from postzygotic mutations. The clinical phenotype of mosaic disorders is determined by the timing of mutation, the level of pathway activation as well as the affected cell type. Happle has hypothesized that the phenotypical consequences of some activating mutations in the germline may be so fundamental that they are not compatible with development and life. Therefore, these mutations may only survive in a mosaic state with a limited number of affected tissues and cells.[7] For some of the established RASopathies, like Costello syndrome or neurofibromatosis type I, patients with mosaic mutations have been described. In these cases, the phenotype was not significantly different from the germline variants. Apart from that, mosaic mutations affecting the Ras-Raf-MEK-ERK signaling pathway have been identified in congenital cutaneous disorders like epidermal nevi. The fact that they show a distinct phenotype compared with the germline RASopathies prompted us to propose the designation "mosaic RASopathies" for this group of congenital disorders (**Table 1**).[8]

**Keratinocytic epidermal nevi.** Epidermal nevi represent a paradigm for cutaneous mosaic disorders.[9,10] The lesions are congenital and are arranged in a linear fashion following Blaschko's lines. They may be either visible at birth or become manifest during the first years of life. According to the affected skin components, epidermal nevi have been divided into organoid (with abnormal adnexal components, such as the hair follicles, sebaceous and sweat glands) and non-organoid (keratinocytic) types (with only epidermal changes).[11] The common keratinocytic epidermal nevus is the most frequent non-organoid epidermal nevus type.[12] It is benign, although in some cases, development of malignant tumors such as basal cell carcinomas and squamous cell carcinomas on the basis of a pre-existing keratinocytic epidermal nevus has been reported. A part of these nevi is caused by mosaic *FGFR3* and *PIK3CA* mutations.[13,14] More recently, it has been shown that common keratinocytic epidermal nevi also result from mosaic *RAS* mutations.[8] In a series of 72 nevi, *RAS* mutations were found in 39% of the lesions. The most frequent mutations occurred in the *HRAS* gene, with the G13R mutation representing a hotspot mutation. In addition, *KRAS* and *NRAS* mutations were identified. In some samples, the *HRAS* mutation co-occurred with a *PIK3CA* mutation, while *RAS* and *FGFR3* mutations were mutually exclusive. The *RAS* mutations were present in a mosaic state, as skin tissue adjacent to the epidermal nevus and blood leukocytes of the patients showed a wild-type sequence. These findings prove a close genotype-phenotype association and incorporate common keratinocytic epidermal nevi to the mosaic RASopathies. Because the identified *FGFR3* point mutations in keratinocytic epidermal nevi have been shown to activate, at least in part, the Ras-Raf-MEK-ERK signaling pathway in keratinocytes and urothelial cells,[15,16] keratinocytic epidermal nevi caused by *FGFR3* mosaic mutations may also be added to the group of mosaic RASopathies. This classification is further supported by the fact that *FGFR3* mutant and *RAS* mutant keratinocytic epidermal nevi show an undistinguishable clinical and histologic phenotype.

**Keratinocytic epidermal nevus syndrome.** Epidermal nevus syndromes are defined by the association of an epidermal nevus with extracutaneous abnormalities, such as cerebral, musculoskeletal, cardiac, renal and ocular defects.[17] Of note, this definition includes cases in which extracutaneous tissues are affected by the same mosaic mutation as the epidermal nevus, however, do not show an aberrant phenotype. The keratinocytic epidermal nevus syndrome in particular is characterized by a keratinocytic epidermal nevus, whose underlying mosaic mutation has spread to other organs. Although the exact incidence of this syndrome is unknown, it is less frequent than keratinocytic epidermal nevi without involvement of other organs. The corresponding extracutaneous abnormalities comprise neuronal defects, such as seizures, mental retardation, hemimegalencephaly, ventricular abnormalities, cortical atrophy and hemiparesis. Skeletal manifestations include incomplete bone formation, hypertrophy or hypoplasia of bones, bone cysts, kyphoscoliosis and vitamin D-resistant rickets.

Recently, *RAS* mutations have been identified in patients diagnosed with keratinocytic epidermal nevus syndrome. For

instance, the co-occurrence of a keratinocytic epidermal nevus, a rhabdomyosarcoma, polycystic kidneys and growth retardation in an infant could be attributed to an oncogenic mosaic *KRAS* G12D mutation.[18] In a second patient with a systematized keratinocytic epidermal nevus, who had developed multiple urothelial carcinomas, a mosaic *HRAS* G12S mutation was identified in the keratinocytic epidermal nevus, the urothelium, the urothelial cell carcinomas and in the blood leukocytes.[19] Both patient cases are examples of mosaic RASopathies. Further studies will have to determine the prevalence of mosaic *RAS* mutations in keratinocytic epidermal nevus syndrome. Besides *RAS* mutations, the keratinocytic epidermal nevus syndrome can also be caused by *FGFR3* mosaic mutations.[20-23] The reported patients demonstrate mild facial dysmorphism, cerebral abnormalities with seizures and mental retardation, scoliosis and involvement of blood leukocytes. In analogy to the *FGFR3*-mutant keratinocytic epidermal nevi, the corresponding *FGFR3*-mutant keratinocytic epidermal nevus syndromes can be designated as mosaic RASopathies. It will be interesting to analyze in a larger cohort of patients whether the different *RAS* and *FGFR3* mutations in keratinocytic epidermal nevus syndrome show a distinct clinical spectrum of symptoms. Both patients with a *RAS*-mutant keratinocytic epidermal nevus syndrome developed cancers (rhabdomyosarcoma and urothelial carcinoma), whereas the four reported patients with a *FGFR3*-mutant keratinocytic epidermal nevus syndrome had no history of cancer. It will be particularly important for the prognosis of the patients and clinical surveillance programs whether this association is significant or occurred by chance.

**Sebaceous nevus.** Sebaceous nevi are organoid epidermal nevi that are preferentially localized in the head and neck region. Histologically they are characterized by abundant sebaceous glands, epidermal hyperplasia and apocrine elements.[24] A peculiar feature of sebaceous nevi is the secondary development of mostly benign tumors in about 25% of lesions during life. These nevi present clinically as hairless, yellow-orange plaques of varying size and shape. Recently it has been shown that sebaceous nevi are caused by mosaic mutations of *HRAS* and *KRAS* genes.[25] *HRAS* mutations were found in 95%, and *KRAS* mutations in 5%, of the lesions. Some nevi showed double mutations of *RAS* genes. In total, 97% of sebaceous nevi harbored a *RAS* mutation. Analysis of mutiple non-lesional tissues revealed a wild-type sequence, indicating mosaicism. The *HRAS* G13R mutation was the predominant mutation present in 91% of the nevi. Cell culture of lesional keratinocytes demonstrated that these cells are the carrier of the *HRAS* mutation. Since sebaceous nevi are rather frequent with an incidence of approximately 1 in 1,000 live births, and *RAS* mutations have been found in a high percentage of the lesions, sebaceous nevi probably represent the mosaic RASopathy with the highest prevalence.

**Schimmelpenning syndrome.** In analogy to the keratinocytic epidermal nevi, sebaceous nevi can be associated with extracutaneous abnormalities, such as cerebral, ocular and skeletal defects. This syndrome is called Schimmelpenning syndrome or linear sebaceous nevus syndrome.[26] In a large series of 196 patients with sebaceous nevus, 7% revealed neurological abnormalities like mental retardation, seizures and hemimegalencephaly.[27] Ocular abnormalities comprise coloboma and lipodermoids, and skeletal defects include hypoplastic bones, short stature, incomplete formation of bony structures and vitamin D-resistant hypophosphatemic rickets, similar to the keratinocytic epidermal nevus syndrome. Two patients with Schimmelpenning syndrome[28,29] were recently analyzed, and both exhibited *RAS* mosaic mutations.[25] One patient was a 52-y-old woman revealing a sebaceous nevus on her scalp, neck, trunk and right arm, which was associated with growth retardation, ocular abnormalities, disproportionate hyposomia, multiple bone fractures and bone deformation due to hypophosphatemic rickets. In this patient, analysis of multiple lesional tissues showed the *HRAS* G13R mutation, which was absent in blood leukocytes, suggesting a mosaicism of the *HRAS* mutation. The second subject with Schimmelpenning syndrome was a monozygotic twin. While his brother showed no abnormalities, the patient displayed a severe Schimmelpenning syndrome, with a sebaceous nevus on the face, ocular abnormalities, an isolated cleft palate, a patent ductus arteriosus and cerebral defects. Analysis of a biopsy from the sebaceous nevus of this subject showed the *KRAS* G12D mutation, which was absent in normal skin and blood leukocytes, thus confirming mosaicism. Therefore, Schimmelpenning syndrome can be considered as a mosaic RASopathy, although the frequency of mosaic *RAS* mutations in this syndrome has to be further evaluated in a larger cohort of patients.

**Costello syndrome.** Costello syndrome is a very rare RASopathy caused by *HRAS* germline mutations.[30] It is characterized by growth retardation, skin changes, including deep palmar and plantar creases, papillomata, loose skin, abnormal fingernails, spatulate finger pads and increased pigmentation, cardiomyopathy, coarse face and cancer predisposition.[31] Two patients with a mosaic Costello syndrome have been reported in the literature, both of them being caused by a mosaic *HRAS* mutation. One patient displayed a phenotype suggestive for Costello syndrome, however, lacked an *HRAS* mutation in her blood leukocytes.[32] In contrast, analysis of buccal swabs showed that approximately 30% of the cells carried an *HRAS* G12S mutation, thus confirming mosaicism of this mutation. Another report described a father who had a mosaic *HRAS* G12S mutation.[33] This mutational mosaicism obviously involved the gonads of the subject, because he had an offspring with an *HRAS* G12S germline mutation and Costello syndrome, suggesting a father-to-son transmission of the mutation. Both reports provide evidence that in rare cases, Costello syndrome may manifest as a mosaic RASopathy.

**Segmental neurofibromatosis type I.** Neurofibromatosis type I is an autosomal dominant inherited RASopathy with an incidence of 1 in 2,500 to 1 in 3,000 individuals.[34] The clinical findings comprise cafe-au-lait spots, neurofibromas of the skin, Lisch nodules in the eye and plexiform neurofibromas. Furthermore, this syndrome is associated with abnormalities of the vascular, skeletal and central nervous system. Of note, patients with neurofibromatosis type I have an increased risk to develop benign and malignant tumors, such as optic pathway gliomas or malignant peripheral nerve sheath tumors. Neurofibromatosis type I is caused by germline mutations of the *NF1* gene, which encodes

for the tumor-suppressor protein neurofibromin. In contrast to many other RASopathies that are characterized by activating heterozygous mutations, the mutations in the *NF1* gene are inactivating. According to the two-hit hypothesis of Knudson, the second wild-type allele of *NF1* is inactivated by somatic mutations, small deletions or insertions (loss of heterozygosity).[35,36] Some Schwann cells in neurofibromas of neurofibromatosis type I patients show loss of heterozygosity of *NF1*, while the other cells still retain one functional wild-type allele.[37] According to these findings, benign and malignant tumors, cafe-au-lait spots and other lesions in neurofibromatosis type I are presumably the result of inactivation of both *NF1* alleles, one by an inherited mutation and the other by a second genetic alteration. The variability of the phenotype of the lesions and their localization in neurofibromatosis patients might be explained by the element of chance in determining what cell types are involved by the second hit and at which localization this happens. Interestingly, some abnormalities observed in neurofibromatosis type I, such as general hyperpigmentation of the skin, are obviously caused by the loss of one *NF1* allele (haploinsufficiency).

Somatic mosaicism of *NF1* deletions has been reported in two patients with generalized neurofibromatosis type I that were phenotypically indistinguishable from patients with germline mutations.[38,39] Moreover, some patients display a segmental form of neurofibromatosis type I. These patients may have pigmentary changes only, neurofibromas only, both pigmentary changes and neurofibromas or isolated plexiform neurofibromas that are restricted to a segmental area of the body.[40] Patients with segmental neurofibromas interestingly show a neural distribution in dermatomes, because the genetic mutation appears to be restricted to Schwann cells. In contrast, patients with solely pigmentary segmental changes show a distribution that follows the lines of Blaschko, and melanocytes have been identified as an affected cell type. Segmental neurofibromatosis type I is thought to be about 30 times less frequent than the germline variant.[41] The segmental neurofibromatosis type I results from postzygotic genetic alterations in the *NF1* gene such as microdeletions.[42] A second hit in a cell within the affected segment will cause a lesion (e.g., neurofibroma), whereas inactivation of an *NF1* allele outside this segment will not result in a lesion because of the remaining wild-type allele. All cases of postzygotic *NF1* alterations ("first hit") that lead to mosaicism can be categorized as mosaic RASopathies. The mosaicism might result in a segmental neurofibromatosis type I, but also in a generalized disease indistinguishable from germline neurofibromatosis, depending on the time point at which the mutation occurs during embryogenesis.

## Other Mosaic RASopathies

Further established RASopathies may occur in mosaicism in rare cases. For example, a patient with LEOPARD syndrome has been published whose left trunk and arm were devoid of the classical lentigines observed on other body sites.[43] This phenotype suggests a mosaicism, but the underlying mechanism was unknown, as both fibroblasts from lesional and non-lesional skin areas harbored the *PTPN11* mutation. Silencing of the mutated gene by an unidentified second mutation or by epigenetic factors as well as revertant mosaicism may account for the observed phenotype in this case.

## Functional Aspects

Most of the mutations underlying mosaic RASopathies are point mutations resulting in an activation of the Ras-Raf-MEK-ERK signaling pathway. These mutations can be designated "oncogenic," as they are also found in a variety of benign and malignant tumors and have been shown to be tumorigenic in vitro and in vivo.[44] Interestingly, the mutation spectrum of mosaic RASopathies shows a considerable overlap with that of tumors but is often different from germline RASopathies. Another interesting fact is that in several mosaic RASopathies, the mutational spectrum is characterized by one dominant hotspot mutation. For example, common keratinocytic epidermal nevi show a predominance of the *HRAS* G13R and the *FGFR3* R248C mosaic mutations.[8,14,45,46] Furthermore, the *HRAS* G13R mosaic mutation is found in approximately 90% of sebaceous nevi.[25] The reason for this predominance of one single hotspot mutation remains unknown. Carcinogens with a site-specific mutagenic effect in embryogenesis may explain this phenomenon. Another possibility is that in comparison to other mutations occurring during embryogenesis, hotspot mutations provide a growth advantage for the affected cell clones, thus resulting in a positive selection of the respective clones. In contrast to the mosaic RASopathies, the spectrum of somatic mutations of the same genes in tumors is more heterogeneous. For example, in seborrheic keratosis, a benign epidermal skin tumor that is histologically almost identical to keratinocytic epidermal nevus, the spectrum of *FGFR3* and *RAS* mutations is considerably more diverse than in the epidermal nevi.[47-49]

For some genes such as *HRAS*, the observed phenotypical pleiotropy of the same mutation in mosaic RASopthies vs. germline RASopathies is intriguing. The *HRAS* G12S mutation, for example, is the most frequent germline mutation in Costello syndrome.[31] The same mutation has been identified in sebaceous nevi[25] and in a case of keratinocytic epidermal nevus syndrome.[19] Though patients with Costello syndrome show a skin phenotype,[50] it is markedly different from that observed in mosaic RASopathies. We have hypothesized that the cell type affected by the mutation (e.g., both epithelial and mesenchymal cells in Costello syndrome vs. epithelial cells in epidermal nevi), as well as the time point at which the mutation occurs in embryogenesis, may be critical determinants for the observed phenotypic difference between germline and mosaic RASopathies.

Moreover, even in mosaic RASopathies, the observed pleiotropy is remarkable. The mosaic *HRAS* G13R mutation, for example, can result both in a sebaceous nevus and a keratinocytic epidermal nevus, the latter lacking abundant sebaceous glands and the dilated apocrine glands that are observed in sebaceous nevi.[8,25] We hypothesize that this variability of phenotypes caused by an identical mosaic mutation is best explained by the varying differentiation potential of a mutated progenitor cell. The differentiation of cells is a complex process that is strictly organized

and controlled depending on the developmental stage of the organism and the body site. Keratinocytic progenitor cells in the head and neck region, for example, will differentiate into epidermal keratinocytes and sebaceous glands, whereas other body sites show less sebaceous glands. Thus, if the mosaic *HRAS* mutation affects a progenitor cell in the head and neck region with a sebaceous differentiation potential, a sebaceous nevus might result. If the mosaic mutation occurs in a progenitor cell of the trunk, the sebaceous differentiation program will not be activated, because skin at this region contains less sebaceous glands, and the resulting phenotype will be a keratinocytic epidermal nevus without abundant adnexal structures. According to this concept, the cellular context of the mosaic mutation (i.e., regulatory mechanisms that depend on the cell type, the stage of embryonic development and the specific anatomical region) will determine the resulting phenotype of mosaic RASopathies.

## Mosaic RASopathies and Cancer

The Ras-Raf-MEK-ERK signaling pathway has a central role in tumorigenesis, with approximately 30% of human tumors harboring oncogenic *RAS* mutations.[3,51] Other genes of this pathway are also reported to show genetic alterations in benign and malignant tumors such as *BRAF* in malignant melanoma[52] and in melanocytic nevi.[53] Therefore, it is not surprising that several RASopathies are associated with an increased risk of developing (malignant) tumors during life.[54] This association between RASopathies and cancers is biologically plausible, as both germline and sporadic *RAS* mutations activate the same pathway. In a retrospective analysis, 4% of patients with Noonan syndrome were found to develop various cancers, such as myeloproliferative disease, neuroblastoma, low-grade glioma, rhabdomyosarcoma and acute lymphoblastic leukemia. In Costello syndrome, 10% of patients had a history of cancer, including rhabdomyosarcoma, bladder cancer and neuroblastoma. A cancer incidence peak in childhood was observed in both syndromes.[54] In the same study, 4% of patients with cardio-facio-cutaneous syndrome had been identified with acute lymphoblastic leukemia, non-Hodgkin lymphoma, rhabdomyosarcoma and hepatoblastoma. In neurofibromatosis type I, the frequent development of neurofibromas is a criterion for the diagnosis of the syndrome. The risk of malignant myeloid disorders in children with neurofibromatosis type I is 200–500 times the normal risk.[55] Moreover, these patients have a higher susceptibility for malignant peripheral nerve sheath tumors,[56] optic pathway gliomas,[57] pheochromocytomas[58] and other malignancies.

Mosaic RASopathies also harbor an increased risk for tumor development. Of the two reported patients with an epidermal nevus syndrome caused by a *RAS* mutation, one patient developed a rhabdomyosarcoma in childhood.[18] A postzygotic *KRAS* G12D mutation was identified as the underlying mutation, suggesting that mosaicism of this genetic alteration caused the keratinocytic epidermal nevus and contributed to the rhabdomyosarcoma. This assumption is compatible with the reported dysregulation of the RAS signaling pathway in human rhabdomyosarcoma.[59] The other patient developed bladder cancer at the unusually young age of 19 y.[19] After a period of 29 y, the patient was again found to have urothelial cancer of the bladder and the renal pelvis. Furthermore, a metastasis of the urothelial carcinoma was found in the lung. The *HRAS* G12S mutation was detected in the epidermal nevus tissue, the urothelial carcinomas, the lung metastasis as well as in the normal urothelium, whereas it was absent in the muscle layer of the bladder, thus confirming mosaicism. These examples demonstrate that congenital mosaic mutations of oncogenes may predispose to cancer in children and adults. Malignant tumors that occur in young patients without a history of familial cancer or tumors that develop in a multicentric manner may thus indicate congenital mosaicism of an oncogenic mutation.

An association between epidermal nevus syndromes and cancers, such as urothelial carcinoma and rhabdomyosarcoma, had already been known from the literature.[60-64] However, it remains unknown whether these cases were caused by mutations of the RAS signaling pathway, as these reported syndromes have not been genetically analyzed. Further studies are necessary to determine the incidence of malignancies in epidermal nevus syndromes and a possible correlation between the underlying gene mutations and specific cancer types. The risk for the development of a malignant tumor might depend on the activation potential of the respective mutation, the size of the mutated mosaic patch and the tissue and cell types affected by the mutation. We hypothesize that even though the mosaicism can be rather widespread and involve many different organs, only specific tissue types may be prone to develop cancer in the presence of a predisposing mosaic mutation.

Approximately 25% of sebaceous nevi develop secondary tumors during life.[24] The majority of these tumors is benign, comprising mainly trichoblastoma, syringocystadenoma papilliferum and further benign adnexal tumors, but malignant tumors have also been reported.[65-67] These tumors derive directly from sebaceous nevus cells, as they were shown to carry the same *HRAS* mutation as the underlying nevus.[25] Therefore, *RAS* mutations in sebaceous nevi may predispose to the development of benign and malignant tumors. Although the exact mechanisms remain elusive, either a second genetic hit or other pathogenetic factors could foster the tumor growth in the context of constitutive RAS pathway activation by the mosaic mutation. This view is supported by the fact that *RAS* mutations are associated with keratoacanthomas and squamous cell carcinomas that develop in patients with metastasized malignant melanomas receiving a therapy with a BRAF inhibitor. While the prevalence of *RAS* mutations in sporadic human squamous cell carcinomas is rather low,[68] *RAS* mutations are significantly more frequent in these tumors developing under BRAF inhibitor therapy.[69] These findings suggest a model of tumor growth by paradoxical pathway activation by BRAF inhibitors in RAS-primed mutant keratinocytes. This hypothesis is supported by a mouse model in which tumor growth of *HRAS* mutant keratinocytes was not initiated but accelerated by a BRAF inhibitor.[70] There are also a few reports of neoplasms growing on a pre-existent keratinocytic epidermal nevus.[71] However, it is unknown why the prevalence of secondary tumors in sebaceous nevi is considerably higher than

in keratinocytic epidermal nevi. Once more, the mutant cell type (epidermal keratinocytic stem cell vs. sebaceous stem cell), paracrine factors as well as varying exogenous factors, resulting in additional genetic hits, may play a role for the observed difference.

## Concluding Remarks

Mosaicism is an important contributor to human disease, and mosaic RASopathies have emerged as a new class of congenital disorders. Though mosaic RASopathies share a common pathogenesis and show some clinical overlap with germline RASopathies, the occurrence of the mutations in a mosaic state often leads to a phenotype that is not merely an incomplete manifestation of the corresponding germline RASopathy, but results in a distinct clinical entity such as epidermal nevi. For some genes, like *HRAS*, the associated mosaic RASopathies (keratinocytic epidermal nevi and sebaceous nevi) are considerably more frequent than the corresponding germline RASopathy (Costello syndrome). The knowledge of the clinical and pathogenetic characteristics of mosaic RASopathies is important for physicians. In contrast to classical germline genetic disorders, the diagnosis often cannot be made by analysis of blood DNA, because in many patients, the mosaicism does not involve the bone marrow. It is crucial to analyze lesional tissue from the patients for the detection of the underlying mosaic mutation, which sometimes may be challenging or even impossible. While mosaic disorders of the skin can be analyzed rather easily by a biopsy due to the good visibility and accessibility of the lesions, this may be not the case for abnormalities of other organs, such as the brain or the skeletal system. The identification of the mutations underlying mosaic RASopathies help to classify the disorders and to make the exact diagnosis in patients with equivocal clinical symptoms. It might also be helpful for genetic counseling of the patients and their relatives. However, estimation of the risk for transmission of the mosaic mutation to the next generation is very difficult, because an involvement of the gonads by the mosaicism usually cannot be excluded. If the mosaic mutation is transmitted to the next generation, the offspring will harbor the mutation in all cells, which can be linked to a severe clinical phenotype that sometimes may be not compatible with life. For example, the mosaic *FGFR3* R248C mutation that is frequently found in keratinocytic epidermal nevi is associated with thanatophoric dysplasia in germline,[72] a severe skeletal dysplasia syndrome that is lethal at the time of birth or in the first years of life. The most frequent *HRAS* mutation in mosaic RASopathies, the G13R substitution, has not been described in germline up to now. It is unknown whether this gene mutation is lethal in germline. In contrast, other *HRAS* mutations, such as G12S, have been found in a mosaic state, for example, in sebaceous and keratinocytic epidermal nevi, but are also common in Costello syndrome patients. Thus, if an *HRAS* G12S mosaic mutation in a patient with epidermal nevus (syndrome) would affect the gonads and be transmitted to the offspring, the mutation would result in a classical Costello syndrome. The fact that most patients with epidermal nevi have healthy offspring suggests that in these patients, the risk of transmission is very low. However, the exact mechanisms of the distribution of the mutant cell clone during embryogenesis are not understood, and even a small visible mosaic skin lesion may not entirely rule out a gonadal involvement. In these cases, the identification of the underlying mutation in the mosaic (skin) lesion of the parents will allow a targeted prenatal genetic analysis.

Besides that, the knowledge of mosaic RASopathies is relevant due to their increased risk for the development of malignancies in children and adults. Depending on the affected tissues, as well as the respective gene mutations, these cancers may occur at an unusual age or multifocally. While the mosaic lesions in the skin are right before our eyes and can easily be monitored for the growth of tumors, this is not the case for internal organs such as the urinary tract. In patients with a widespread mosaic RASopathy, monitoring of organs that harbor a potential risk for cancer development might be recommended.

It is not unlikely that in the future, further mosaic RASopathies will be discovered. The predominance of mosaic RASopathies with a skin phenotype may be due to the better visibility of the lesions rather than to a true higher incidence. Mosaic RASopathies of internal organs may contribute to cancer and non-neoplastic disorders as well but remain unnoticed. The continuing improvement of genetic techniques (e.g., whole exome and deep sequencing approaches) will provide new insights in the prevalence and pathogenetic relevance of mosaic RASopathies in man in the near future.

### References


1. Donovan S, Shannon KM, Bollag G. GTPase activating proteins: critical regulators of intracellular signaling. Biochim Biophys Acta 2002; 1602:23-45; PMID:11960693
2. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: conserved structure and molecular mechanism. Nature 1991; 349:117-27; PMID:1898771; http://dx.doi.org/10.1038/349117a0
3. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 2007; 7:295-308; PMID:17384584; http://dx.doi.org/10.1038/nrc2109
4. Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev 2009; 19:230-6; PMID:19467855; http://dx.doi.org/10.1016/j.gde.2009.04.001
5. Boon LM, Mulliken JB, Vikkula M. RASA1: variable phenotype with capillary and arteriovenous malformations. Curr Opin Genet Dev 2005; 15:265-9; PMID:15917201; http://dx.doi.org/10.1016/j.gde.2005.03.004
6. Eerola I, Boon LM, Mulliken JB, Burrows PE, Dompmartin A, Watanabe S, et al. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. Am J Hum Genet 2003; 73:1240-9; PMID:14639529; http://dx.doi.org/10.1086/379793
7. Happle R. Lethal genes surviving by mosaicism: a possible explanation for sporadic birth defects involving the skin. J Am Acad Dermatol 1987; 16:899-906; PMID:3033033; http://dx.doi.org/10.1016/S0190-9622(87)80249-9
8. Hafner C, Toll A, Gantner S, Mauerer A, Lurkin I, Acquadro F, et al. Keratinocytic epidermal nevi are associated with mosaic RAS mutations. J Med Genet 2012; 49:249-53; PMID:22499344; http://dx.doi.org/10.1136/jmedgenet-2011-100637
9. Happle R. Mosaicism in human skin. Understanding the patterns and mechanisms. Arch Dermatol 1993; 129:1460-70; PMID:8239703; http://dx.doi.org/10.1001/archderm.1993.01680320094012
10. Happle R, Rogers M. Epidermal nevi. Adv Dermatol 2002; 18:175-201; PMID:12528406
11. Happle R. The group of epidermal nevus syndromes Part I. Well defined phenotypes. J Am Acad Dermatol 2010; 63:1-22, quiz 23-4; PMID:20542174; http://dx.doi.org/10.1016/j.jaad.2010.01.017
12. Sugarman JL. Epidermal nevus syndromes. Semin Cutan Med Surg 2004; 23:145-57; PMID:15295924; http://dx.doi.org/10.1016/j.sder.2004.01.008
13. Hafner C, López-Knowles E, Luis NM, Toll A, Baselga E, Fernández-Casado A, et al. Oncogenic PIK3CA mutations occur in epidermal nevi and seborrheic keratoses with a characteristic mutation pattern. Proc Natl Acad Sci USA 2007; 104:13450-4; PMID:17673550; http://dx.doi.org/10.1073/pnas.0705218104

14. Hafner C, van Oers JM, Vogt T, Landthaler M, Stoehr R, Blaszyk H, et al. Mosaicism of activating FGFR3 mutations in human skin causes epidermal nevi. J Clin Invest 2006; 116:2201-7; PMID:16841094; http://dx.doi.org/10.1172/JCI28163
15. Hafner C, Di Martino E, Pitt E, Stempfl T, Tomlinson D, Hartmann A, et al. FGFR3 mutation affects cell growth, apoptosis and attachment in keratinocytes. Exp Cell Res 2010; 316:2008-16; PMID:20420824; http://dx.doi.org/10.1016/j.yexcr.2010.04.021
16. di Martino E, L'Hôte CG, Kennedy W, Tomlinson DC, Knowles MA. Mutant fibroblast growth factor receptor 3 induces intracellular signaling and cellular transformation in a cell type- and mutation-specific manner. Oncogene 2009; 28:4306-16; PMID:19749790; http://dx.doi.org/10.1038/onc.2009.280
17. Vujevich JJ, Mancini AJ. The epidermal nevus syndromes: multisystem disorders. J Am Acad Dermatol 2004; 50:957-61; PMID:15153903; http://dx.doi.org/10.1016/S0190-9622(02)61547-6
18. Bourdeaut F, Hérault A, Gentien D, Pierron G, Ballet S, Reynaud S, et al. Mosaicism for oncogenic G12D KRAS mutation associated with epidermal nevus, polycystic kidneys and rhabdomyosarcoma. J Med Genet 2010; 47:859-62; PMID:20805368; http://dx.doi.org/10.1136/jmg.2009.075374
19. Hafner C, Toll A, Real FX. HRAS mutation mosaicism causing urothelial cancer and epidermal nevus. N Engl J Med 2011; 365:1940-2; PMID:22087699; http://dx.doi.org/10.1056/NEJMc1109381
20. Bygum A, Fagerberg CR, Clemmensen OJ, Fiebig B, Hafner C. Systemic epidermal nevus with involvement of the oral mucosa due to FGFR3 mutation. BMC Med Genet 2011; 12:79; PMID:21639936; http://dx.doi.org/10.1186/1471-2350-12-79
21. Collin B, Taylor IB, Wilkie AO, Moss C. Fibroblast growth factor receptor 3 (FGFR3) mutation in a verrucous epidermal naevus associated with mild facial dysmorphism. Br J Dermatol 2007; 156:1353-6; PMID:17441958; http://dx.doi.org/10.1111/j.1365-2133.2007.07869.x
22. García-Vargas A, Hafner C, Pérez-Rodríguez AG, Rodríguez-Rojas LX, González-Esqueda P, Stoehr R, et al. An epidermal nevus syndrome with cerebral involvement caused by a mosaic FGFR3 mutation. Am J Med Genet A 2008; 146A:2275-9; PMID:18642369; http://dx.doi.org/10.1002/ajmg.a.32429
23. Ousager LB, Bygum A, Hafner C. Identification of a novel S249C FGFR3 mutation in a keratinocytic epidermal naevus syndrome. Br J Dermatol 2012; 167:202-4; PMID:22229528; http://dx.doi.org/10.1111/j.1365-2133.2012.10812.x
24. Moody MN, Landau JM, Goldberg LH. Nevus sebaceous revisited. Pediatr Dermatol 2012; 29:15-23; PMID:21995782; http://dx.doi.org/10.1111/j.1525-1470.2011.01562.x
25. Groesser L, Herschberger E, Ruetten A, Ruivenkamp C, Lopriore E, Zutt M, et al. Postzygotic HRAS and KRAS mutations cause nevus sebaceous and Schimmelpenning syndrome. Nat Genet 2012; 44:783-7; PMID:22683711; http://dx.doi.org/10.1038/ng.2316
26. Schimmelpenning GW. [Clinical contribution to symptomatology of phacomatosis]. Fortschr Geb Rontgenstr Nuklearmed 1957; 87:716-20; PMID:13512450; http://dx.doi.org/10.1055/s-0029-1213358
27. Davies D, Rogers M. Review of neurological manifestations in 196 patients with sebaceous naevi. Australas J Dermatol 2002; 43:20-3; PMID:11869203; http://dx.doi.org/10.1046/j.1440-0960.2002.00546.x
28. Rijntjes-Jacobs EG, Lopriore E, Steggerda SJ, Kant SG, Walther FJ. Discordance for Schimmelpenning-Feuerstein-Mims syndrome in monochorionic twins supports the concept of a postzygotic mutation. Am J Med Genet A 2010; 152A:2816-9; PMID:20949522; http://dx.doi.org/10.1002/ajmg.a.33635
29. Zutt M, Strutz F, Happle R, Habenicht EM, Emmert S, Haenssle HA, et al. Schimmelpenning-Feuerstein-Mims syndrome with hypophosphatemic rickets. Dermatology 2003; 207:72-6; PMID:12835555; http://dx.doi.org/10.1159/000070948
30. Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, et al. Germline mutations in HRAS proto-oncogene cause Costello syndrome. Nat Genet 2005; 37:1038-40; PMID:16170316; http://dx.doi.org/10.1038/ng1641
31. Gripp KW, Lin AE. Costello syndrome: a Ras/mitogen activated protein kinase pathway syndrome (rasopathy) resulting from HRAS germline mutations. Genet Med 2012; 14:285-92; PMID:22261753; http://dx.doi.org/10.1038/gim.0b013e31822dd91f
32. Gripp KW, Stabley DL, Nicholson L, Hoffman JD, Sol-Church K. Somatic mosaicism for an HRAS mutation causes Costello syndrome. Am J Med Genet A 2006; 140:2163-9; PMID:16969868; http://dx.doi.org/10.1002/ajmg.a.31456
33. Sol-Church K, Stabley DL, Demmer LA, Agbulos A, Lin AE, Smoot L, et al. Male-to-male transmission of Costello syndrome: G12S HRAS germline mutation inherited from a father with somatic mosaicism. Am J Med Genet A 2009; 149A:315-21; PMID:19206176; http://dx.doi.org/10.1002/ajmg.a.32639
34. Williams VC, Lucas J, Babcock MA, Gutmann DH, Korf B, Maria BL. Neurofibromatosis type 1 revisited. Pediatrics 2009; 123:124-33; PMID:19117870; http://dx.doi.org/10.1542/peds.2007-3204
35. Wiest V, Eisenbarth I, Schmegner C, Krone W, Assum G. Somatic NF1 mutation spectra in a family with neurofibromatosis type 1: toward a theory of genetic modifiers. Hum Mutat 2003; 22:423-7; PMID:14635100; http://dx.doi.org/10.1002/humu.10272
36. Serra E, Ars E, Ravella A, Sánchez A, Puig S, Rosenbaum T, et al. Somatic NF1 mutational spectrum in benign neurofibromas: mRNA splice defects are common among point mutations. Hum Genet 2001; 108:416-29; PMID:11409870; http://dx.doi.org/10.1007/s004390100514
37. Serra E, Rosenbaum T, Winner U, Aledo R, Ars E, Estivill X, et al. Schwann cells harbor the somatic NF1 mutation in neurofibromas: evidence of two different Schwann cell subpopulations. Hum Mol Genet 2000; 9:3055-64; PMID:11115850; http://dx.doi.org/10.1093/hmg/9.20.3055
38. Colman SD, Rasmussen SA, Ho VT, Abernathy CR, Wallace MR. Somatic mosaicism in a patient with neurofibromatosis type 1. Am J Hum Genet 1996; 58:484-90; PMID:8644707
39. Vandenbroucke I, van Doorn R, Callens T, Cobben JM, Starink TM, Messiaen L. Genetic and clinical mosaicism in a patient with neurofibromatosis type 1. Hum Genet 2004; 114:284-90; PMID:14605872; http://dx.doi.org/10.1007/s00439-003-1047-9
40. Redlick FP, Shaw JC. Segmental neurofibromatosis follows blaschko's lines or dermatomes depending on the cell line affected: case report and literature review. J Cutan Med Surg 2004; 8:353-6; PMID:15868313; http://dx.doi.org/10.1007/s10227-005-0029-z
41. Wolkenstein P, Mahmoudi A, Zeller J, Revuz J. More on the frequency of segmental neurofibromatosis. Arch Dermatol 1995; 131:1465; PMID:7492147; http://dx.doi.org/10.1001/archderm.1995.01690240131030
42. Tinschert S, Naumann I, Stegmann E, Buske A, Kaufmann D, Thiel G, et al. Segmental neurofibromatosis is caused by somatic mutation of the neurofibromatosis type 1 (NF1) gene. Eur J Hum Genet 2000; 8:455-9; PMID:10878667; http://dx.doi.org/10.1038/sj.ejhg.5200493
43. Writzl K, Hoovers J, Sistermans EA, Hennekam RC. LEOPARD syndrome with partly normal skin and sex chromosome mosaicism. Am J Med Genet A 2007; 143A:2612-5; PMID:17935252; http://dx.doi.org/10.1002/ajmg.a.31991
44. Bernard-Pierrot I, Brams A, Dunois-Lardé C, Caillault A, Diez de Medina SG, Cappellen D, et al. Oncogenic properties of the mutated forms of fibroblast growth factor receptor 3b. Carcinogenesis 2006; 27:740-7; PMID:16338952; http://dx.doi.org/10.1093/carcin/bgi290
45. Hernández S, Toll A, Baselga E, Ribé A, Azua-Romeo J, Pujol RM, et al. Fibroblast growth factor receptor 3 mutations in epidermal nevi and associated low grade bladder tumors. J Invest Dermatol 2007; 127:1664-6; PMID:17255960
46. Hafner C, Vogt T, Hartmann A. FGFR3 mutations in benign skin tumors. Cell Cycle 2006; 5:2723-8; PMID:17172848; http://dx.doi.org/10.4161/cc.5.23.3509
47. Hafner C, Hartmann A, Real FX, Hofstaedter F, Landthaler M, Vogt T. Spectrum of FGFR3 mutations in multiple intraindividual seborrheic keratoses. J Invest Dermatol 2007; 127:1883-5; PMID:17392824; http://dx.doi.org/10.1038/sj.jid.5700804
48. Hafner C, Toll A, Fernández-Casado A, Earl J, Marqués M, Acquadro F, et al. Multiple oncogenic mutations and clonal relationship in spatially distinct benign human epidermal tumors. Proc Natl Acad Sci USA 2010; 107:20780-5; PMID:21078999; http://dx.doi.org/10.1073/pnas.1008365107
49. Hafner C, van Oers JM, Hartmann A, Landthaler M, Stoehr R, Blaszyk H, et al. High frequency of FGFR3 mutations in adenoid seborrheic keratoses. J Invest Dermatol 2006; 126:2404-7; PMID:16778799; http://dx.doi.org/10.1038/sj.jid.5700422
50. Siegel DH, Mann JA, Krol AL, Rauen KA. Dermatological phenotype in Costello syndrome: consequences of Ras dysregulation in development. Br J Dermatol 2012; 166:601-7; PMID:22098123; http://dx.doi.org/10.1111/j.1365-2133.2011.10744.x
51. Downward J. Signal transduction. Prelude to an anniversary for the RAS oncogene. Science 2006; 314:433-4; PMID:17053139; http://dx.doi.org/10.1126/science.1134727
52. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417:949-54; PMID:12068308; http://dx.doi.org/10.1038/nature00766
53. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, et al. High frequency of BRAF mutations in nevi. Nat Genet 2003; 33:19-20; PMID:12447372; http://dx.doi.org/10.1038/ng1054
54. Kratz CP, Rapisuwon S, Reed H, Hasle H, Rosenberg PS. Cancer in Noonan, Costello, cardiofaciocutaneous and LEOPARD syndromes. Am J Med Genet C Semin Med Genet 2011; 157:83-9; PMID:21500339; http://dx.doi.org/10.1002/ajmg.c.30300
55. Side L, Taylor B, Cayouette M, Conner E, Thompson P, Luce M, et al. Homozygous inactivation of the NF1 gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl J Med 1997; 336:1713-20; PMID:9180088; http://dx.doi.org/10.1056/NEJM199706123362404
56. Leroy K, Dumas V, Martin-Garcia N, Falzone MC, Voisin MC, Wechsler J, et al. Malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1: a clinicopathologic and molecular study of 17 patients. Arch Dermatol 2001; 137:908-13; PMID:11453810
57. Thiagalingam S, Flaherty M, Billson F, North K. Neurofibromatosis type 1 and optic pathway gliomas: follow-up of 54 patients. Ophthalmology 2004; 111:568-77; PMID:15019338; http://dx.doi.org/10.1016/j.ophtha.2003.06.008
58. Bausch B, Borozdin W, Neumann HP; European-American Pheochromocytoma Study Group. Clinical and genetic characteristics of patients with neurofibromatosis type 1 and pheochromocytoma. N Engl J Med 2006; 354:2729-31; PMID:16790714; http://dx.doi.org/10.1056/NEJMc066006

59. Martinelli S, McDowell HP, Vigne SD, Kokai G, Uccini S, Tartaglia M, et al. RAS signaling dysregulation in human embryonal Rhabdomyosarcoma. Genes Chromosomes Cancer 2009; 48:975-82; PMID:19681119; http://dx.doi.org/10.1002/gcc.20702
60. Flosadóttir E, Bjarnason B. A non-epidermolytic epidermal naevus of a soft, papillomatous type with transitional cell cancer of the bladder: a case report and a review of non-cutaneous cancers associated with the epidermal naevi. Acta Derm Venereol 2008; 88:173-5; PMID:18311453; http://dx.doi.org/10.2340/00015555-0365
61. Vidaurri-de la Cruz H, Tamayo-Sánchez L, Durán-McKinster C, de la Luz Orozco-Covarrubias M, Ruiz-Maldonado R. Epidermal nevus syndromes: clinical findings in 35 patients. Pediatr Dermatol 2004; 21:432-9; PMID:15283784; http://dx.doi.org/10.1111/j.0736-8046.2004.21402.x
62. García de Jalón A, Azúa-Romeo J, Trivez MA, Pascual D, Blas M, Rioja LA. Epidermal naevus syndrome (Solomon's syndrome) associated with bladder cancer in a 20-year-old female. Scand J Urol Nephrol 2004; 38:85-7; PMID:15204433; http://dx.doi.org/10.1080/00365590310017316
63. Rongioletti F, Rebora A. Epidermal nevus with transitional cell carcinomas of the urinary tract. J Am Acad Dermatol 1991; 25:856-8; PMID:1802914; http://dx.doi.org/10.1016/S0190-9622(08)80987-5
64. Rosenthal D, Fretzin DF. Epidermal nevus syndrome: report of association with transitional cell carcinoma of the bladder. Pediatr Dermatol 1986; 3:455-8; PMID:3562359; http://dx.doi.org/10.1111/j.1525-1470.1986.tb00650.x
65. Ball EA, Hussain M, Moss AL. Squamous cell carcinoma and basal cell carcinoma arising in a naevus sebaceous of Jadassohn: case report and literature review. Clin Exp Dermatol 2005; 30:259-60; PMID:15807685; http://dx.doi.org/10.1111/j.1365-2230.2005.01744.x
66. Chou CY, Chen WY, Wang KH, Chen TJ. Carcinosarcoma derived from nevus sebaceus. J Clin Oncol 2011; 29:e719-21; PMID:21788565; http://dx.doi.org/10.1200/JCO.2011.35.8093
67. Izumi M, Tang X, Chiu CS, Nagai T, Matsubayashi J, Iwaya K, et al. Ten cases of sebaceous carcinoma arising in nevus sebaceus. J Dermatol 2008; 35:704-11; PMID:19120764; http://dx.doi.org/10.1111/j.1346-8138.2008.00550.x
68. Mauerer A, Herschberger E, Dietmaier W, Landthaler M, Hafner C. Low incidence of EGFR and HRAS mutations in cutaneous squamous cell carcinomas of a German cohort. Exp Dermatol 2011; 20:848-50; PMID:21771097; http://dx.doi.org/10.1111/j.1600-0625.2011.01334.x
69. Oberholzer PA, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, et al. RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. J Clin Oncol 2012; 30:316-21; PMID:22067401; http://dx.doi.org/10.1200/JCO.2011.36.7680
70. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012; 366:207-15; PMID:22256804; http://dx.doi.org/10.1056/NEJMoa1105358
71. Hafner C, Klein A, Landthaler M, Vogt T. Clonality of basal cell carcinoma arising in an epidermal nevus. New insights provided by molecular analysis. Dermatology 2009; 218:278-81; PMID:19122450; http://dx.doi.org/10.1159/000189209
72. Tavormina PL, Shiang R, Thompson LM, Zhu YZ, Wilkin DJ, Lachman RS, et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. Nat Genet 1995; 9:321-8; PMID:7773297; http://dx.doi.org/10.1038/ng0395-321