Clinical and molecular genetic studies in hereditary syndromes featuring skin appendage tumors

Chapter 9

Discussion and valorisation

Skin adnexal tumors show a broad range of phenotypic variability and usually distinct, but sometimes also overlapping histopathological characteristics. To date, different genes and signaling routes have been implicated in the development and growth of these tumors, including the hedgehog and the NF-kB signaling pathway, as in BDCS and BSS, respectively. A better understanding of the specific molecular defects giving rise to rare hereditary disorders associated with cutaneous adnexal tumors might pave the way to the development of targeted topical and systemic therapies for these tumors when occurring as sporadic and solitary lesions or in a non-syndromic setting.

Both BSS and BDCS might be underdiagnosed or can even go undiagnosed for years. This is mainly due to the reason that i) both disorders are rare or, in the case of BDCS, even very rare and, thus, possibly unfamiliar to most physicians, ii) their clinical symptoms may be mild and subtle, iii) disease onset can occur at different age, and iv) patients and families with both BSS and BDCS show inter- and intrafamilial phenotypic variability.

Here, we sought to delineate more comprehensively different aspects of these cutaneous tumor disorders, in particular the variable clinical manifestation of apparently allelic diseases, the molecular genetic background and mutational spectrum of BSS and BDCS, as well as established and novel treatment options.

Brooke-Spiegler syndrome

Clinical spectrum

In BSS, the extent and size of individual tumors may vary from a few, subtle and barely noticeable translucent to skin-colored facial papules of 2-3 mm in diameter to the formation of an enormous tumor mass that can cover the entire circumference of the scalp, a so-called turban tumor. Such phenotypic differences can even be observed within the same family with an identical *CYLD* mutation.[1] Albeit the full-blown picture of turban tumor is very rare, this severe and most dramatic clinical manifestation of BSS is associated more frequently with complications such as secondary infection, fetor, and ulceration. In such cases, the psychological burden impaled on affected individuals should not be underestimated. In about 6% of BSS patients, cylindromas can grow into the external auditory channel, thereby leading to hearing impairment or even loss due to obstruction and occlusion of the auditory channel, as in the individual described in chapter 2. Apart from hearing impairment, also calvarian defects and intracranial invasion have been reported.[2-4]

Intriguingly, some patients with BSS may manifest cylindromas or trichoepitheliomas only. These disorders, referred to as familial cylindromatosis and multiple familial trichoepithelioma, respectively, are allelic with BSS. This can be proven by molecular genetic diagnosis and identification of an underlying *CYLD* mutation. However, there is

evidence that the occurrence of familial trichoepitheliomas can also be due to an as of yet unknown genetic defect located on chromosome 9p21, as shown by Harada and colleagues in 1996.[5]

Molecular genetic background

Currently, more than 100 *CYLD* mutations have been detected. The spectrum of *CYLD* mutations reported to date comprises missense (12%), nonsense (30%), frameshift (50%), splice site (15%) mutations, and large genomic deletions (3%). Through molecular studies we added 9 novel and 2 recurrent *CYLD* germline mutations in Dutch, German and Turkish patients and families, as demonstrated in chapters 3.1 and 3.2.

Until recently, there were no studies on the structural and functional consequences of *CYLD* splice site mutations. Therefore, we evaluated the effects of three splice site mutations on the cellular and RNA genetic level, as shown in chapter 3.1.

First, we demonstrated equal CYLD expression in normal human epidermal keratinocytes, leukocytes and peripheral blood mononuclear cells. Knowledge on the cell-type specific expression of CYLD should facilitate the functional assessment of splice site mutations in the future.

Generally, splice site mutations can cause intron retention, exon skipping, cryptic splicing and combinations thereof, resulting in in-frame or out-of-frame translation. Out-of-frame translation and nonsense mutations generally lead to the occurrence of a premature stop codon (PTC).[6] Such PTCs can either result in the formation of a truncated protein[7] or lead to degradation and elimination of mutated transcripts by nonsense-mediated mRNA decay.[6] By reverse transcriptase (RT-)PCR, we ascertained that two of the splice site mutations (c.2108+1G>A and c.2242-2A>G) result in skipping of exons 15 and 17, respectively, leading to putative out-of-frame translation and generation of a PTC. By contrast, splice site mutation c.2109-2A>C did not show an aberrant splicing pattern. This finding suggested that the transcript of the mutated allele is subject to nonsense-mediated mRNA decay. CYLD mRNA expression was measured in peripheral blood leukocytes by quantitative RT-PCR. This confirmed the outcome of our RT-PCR, since only mutation c.2109-2A>C is associated with evident mRNA decay. Clinically, the patient carrying mutation c.2109-2A>C presented with cylindromas only. As opposed to this, mutations c.2108+1G>A and c.2242-2A>G only lead to negligible mRNA decay, indicating that transcription from these mutant alleles might result in the formation of truncated and dysfunctional proteins. Clinically, both patients carrying these mutations showed cylindromas and trichoepitheliomas. Although these data could indicate a certain genotype-phenotype-correlation, the residual amounts of mRNA seem to be unrelated to the distinct phenotypes in patients with *CYLD* splice site mutations, because mutation c.2109-2A>C has already been described in a patient with trichoepithelioma only.[8] This finding currently largely excludes a genotype-phenotype correlation in BSS.

Since a single *CYLD* mutation can give rise to different phenotypes, even within one family, the question arises whether other mechanisms are involved in the development of BSS, multiple familial trichoepithelioma and familial cylindromatosis. Such factors may include as of yet unknown environmental factors, modifier genes or epigenetic events.[9]

Mosaicism in BSS

A segmental or linear presentation of cylindromas, trichoepitheliomas or spiradenomas has been described on few occasions only.[10-17]

Here, we studied a female patient with trichoepitheliomas in a segmental distribution on her shoulder, as described in **chapter 3.3**. Clinically, we considered a type 1 segmental mosaicism since i) the tumors occurred at the same age as one would expect the common and diffuse phenotype to manifest, and ii) disease severity was equal as in the symmetric phenotype caused by a heterozygous germline mutation. After extracting DNA from the segmentally aligned trichoepitheliomas, direct sequencing of the *CYLD* gene did not reveal a mutation. This, however, does not completely rule out the possibility of a postzygotic somatic *CYLD* mutation as underlying genetic cause. For instance, it would be conceivable that we missed a *CYLD* mutation located in the promoter or deep intronic regions. Likewise, epigenetic events could be responsible for the phenotype, as could be the involvement of other, hitherto unknown genes. As to the latter, one possible candidate gene could be located within the linkage interval for multiple familial trichoepitheliomas on chromosome 9p21, as previously reported.[5] In this context, it would be challenging and interesting to study more patients with mosaic manifestation of cylindromas, trichoepitheliomas or spiradenomas, to elucidate the underlying pathogenetic mechanisms.

Treatment

To date, no curative therapy is available for BSS. The treatment of first choice in affected patients with BSS is surgical excision of the tumors. However, in individuals with a turban tumor this can be challenging, mainly due to two reasons. First, the possible extent of the tumors masses; and second, due to the fact that cylindromas and trichoepitheliomas are hair follicle tumors, which results in the necessity to excise the entire skin to prevent tumor recurrence. Furthermore, the functional outcome of a subsequent reconstruction is very important because affected individuals often want to wear a wig post surgery. The use of artificial dermis to establish a solid outcome is well established in the management of acute burns and oncological resections with full-thickness calvarial defects.[18,19] In cooperation with our colleagues from the Department of Plastic and Reconstructive Surgery of the Maastricht University Medical Center+ we for the first time treated a female patient with extensive tumor manifestation on the scalp with this therapeutic regimen, as described in **chapter 4**.

Since the treatment of turban tumors is very complex, we generally recommend a multidisciplinary approach in which dermatologists, plastic surgeons, psychologists, and otorhinolaryngologists can bundle their expertise.

Theoretically, a targeted, pathway-based therapy would be desirable. This could be achieved, e.g. by interference with the NF-κB signaling route. Although Brummelkamp and colleagues[20] as well as other groups have explored such therapeutic approaches, they altogether have been unsuccessful, as of yet. Still, such causal therapeutic attempts could eventually lead to improved treatment options for affected individuals. Salicylic acid inhibits IKK-gamma, and thereby it interferes with the canonical NF-κB pathway. However, its inhibitory potential is weak and, thus, a possible solution could be the use of more potent pathway inhibitors. We also applied this treatment to the patient described in chapter 2. Unfortunately though, this therapy likewise was not successful. Therefore, more insights into the underlying genetic mechanisms governing tumor formation will be needed to improve targeted therapeutic approaches in BSS.

Part 2. Bazex-Dupré-Christol syndrome

Clinical spectrum

Besides the characteristic clinical triad of hypotrichosis, follicular atrophoderma and basal cell carcinomas that was described in the original report of Bazex, Dupré and Christol,[21] other symptoms can be encountered less frequently. These symptoms features comprise milia, hypohidrosis, hair shaft abnormalities, such as pili torti and trichorrhexis nodosa, facial hyperpigmentation, pinched nose with hypoplastic nasal alae and prominent columella, and trichoepitheliomas.

In an attempt to delineate more precisely the frequency of milia, we revisited all reports on BDCS. Thereby, we found that milia occur more often than, e.g. follicular atrophoderma, namely in 81% of patients and families with BDCS, as described in chapter 7.[22-24] Therefore, we suggest that milia should be added to the triad of hypotrichosis, follicular atrophoderma and basal cell carcinomas, as a frequent clinical manifestation of the disorder.

To date, the manifestation of basal cell carcinomas in BDCS was described from the second decade onwards. In chapter 7, however, we show for the first time these tumors may also occur in children at the age of three and five years, respectively. Whenever basal cell carcinomas occur in young individuals, an underlying genetic disorder, such as BDCS, should be excluded. Other hereditary tumor disorders to be excluded in such cases include basal cell nevus syndrome, Rombo syndrome, Oley syndrome and Xeroderma pigmentosum, as described in chapter 5.

The differential diagnosis between BDCS and these other disorders may be difficult since there are overlapping clinical features. Patients with basal cell nevus syndrome

develop basal cell carcinomas and facial milia, but, most importantly, also extracutaneous manifestations such as macrocephaly, jaw cysts and skeletal abnormalities, which are not encountered in BDCS. Rombo syndrome is an autosomal dominant disorder characterized by vermiculate atrophoderma on the elbows and cheeks, development of basal cell carcinomas, hypotrichosis, trichoepitheliomas and reddening of the skin. Both vermiculate atrophoderma on the elbows and cheeks as well as peripheral vasodilatation have not yet been described in BDCS. Oley syndrome manifests with congenital hypotrichosis, milia and basal cell carcinomas. However, it is not clear if it reflects an own entity or merely a milder variant of BDCS. Individuals with Xeroderma pigmentosum can also develop basal cell carcinomas. However, this disorder can also manifest with other malignant skin tumors, e.g. malignant melanoma, and is characterized by severe early-onset photosensitivity, premature skin ageing, photophobia, and poikiloderma.

In approximately one third of families with BDCS, trichoepitheliomas are observed.[22,25-28] Both clinically and histologically, it can be difficult to differentiate between basal cell carcinomas and trichoepitheliomas. The predilection sites of basal cell carcinomas and trichoepitheliomas are similar and include the head and neck region as well as the trunk. Histologically, both trichoepitheliomas and basal cell carcinomas show nests of basaloid cells with follicular differentiation. Trichoepitheliomas show trabecular nests surrounded by stroma containing fibroblasts, while basal cell carcinomas manifest peripheral palisading of basaloid keratinocytes, cleft formation between tumor nests and stroma, ulceration and mitotic figures. However, not all characteristics may be present, and therefore it can be challenging to distinct a trichoepithelioma from a basal cell carcinoma histologically. Additional immunohistochemistry can offer diagnostic support in these cases. In previous studies, several immunohistochemical markers have been applied, albeit with ambiguous results. Here, we studied the potential of androgen receptor and TGF-β as histological biomarkers for the differentiation between these two tumors, as described in chapter 6.

Androgen receptor is a nuclear ligand-dependent transcription factor that is activated by binding to androgens, testosterone, of dihydrotestosterone. It is expressed in 80% of basal cell carcinomas, but not in trichoepitheliomas, as confirmed by Mostafa et al. and Arits et al.[29,30] Whereas a positive staining confirms the diagnosis of basal cell carcinoma, a negative staining does not exclude it.

TGF-β plays a key role in controlling cell proliferation and differentiation. TGF-β shows a positive staining in trichoepitheliomas, but is negative in basal cell carcinomas. Other useful markers to differentiate between basal cell carcinomas and trichoepitheliomas include CD10, Ki-67, and PHLDA1.[29]

Molecular genetic background

Until recently, the underlying genetic defect of BDCS was unknown. An X-linked inheritance pattern was suspected since none of the affected families showed male-to-male transmission. In 1995, Vabres and colleagues mapped the gene of BDCS to chromosome Xq24-27.1.[31] In chapter 8, we studied a German family with BDCS and performed haplotype analysis. We were able to refine the candidate region to an 11.4 Mb interval on chromosome Xq25-27.1, which contained 101 genes. Since we hypothesized that the causing gene would play a role in hair follicle differentiation and regulating cell proliferation, we selected 12 candidate genes: *ACTRT1, AIFM1, RAB33A, SUHW3/ZNF280C, ENOX2, MST4, RAP2C/TFPD3, HS6ST2, ZNF449, ZNF75D, GPR112,* and *ARHGEF6.* We performed mutation analysis of the coding regions and adjacent splice sites, but were not able to detect a pathogenic mutation. Interestingly, Bal and colleagues reported in 2017 that they found mutations in the *ACTRT1* gene in two families with BDCS, and germline mutations in transcribed sequences encoding enhancer RNAs (eRNAs), located in the non-coding sequences surrounding the *ACTRT1* gene in 4 other families with BDCS.[32] Intriguingly, the *ACTRT1* gene is located outside our candidate region in a 2.6 Mb gene desert, and we did not detect a mutation in the *ACTRT1* gene in our family. One explanation could be that the underlying genetic defect in our family is located in non-coding sequences of the *ACTRT1* gene that we did not sequence. Other possible explanations for not detecting disease-causing sequence alterations in the *ACTRT1* gene in our family could be either mutations in the promoter region or eRNAs; or the involvement of other hitherto unknown genes in disease pathogenesis. Likewise, epigenetic events could silence the function of *ACTRT1*, e.g. DNA methylation or histone modification.

The *ACTRT1* gene encodes for actin-related protein T1 (ARP-T1), which plays a role in cell growth and/or maintenance. ARP-T1 inhibits the transcription factor GLI1 expression by binding to the GLI1 promoter, which leads to activation of the Hedgehog signaling pathway. The hedgehog signaling pathway is also involved in the development of sporadic basal cell carcinomas and basal cell nevus syndrome.[33] Further studies will be required to elucidate possible other pathogenetic mechanisms involved in BDCS.

In summary, our studies on BSS and BDCS show that several facets of these disorders are still not well understood and require in depth molecular studies to better understand the underlying pathogenetic mechanisms. This, eventually, should also lead to the development of better treatment modalities with less necessity for repeated surgical interventions and, thus, to an increased quality of life for affected individuals.

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