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The hedgehog pathway in basal cell carcinoma

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Chapter 10

Summary, discussion, future perspectives and valorisation

Part one
Basal cell naevus syndrome

Basal cell naevus syndrome (BNCS), also known as Gorlin syndrome, is an autosomal dominant disorder, characterized by multiple basal cell carcinomas (BCCs), odontogenic keratocysts, cerebral calcifications and palmoplantar pits.[1,2] The syndrome is associated with germline mutations in the *Drosophila homolog of patched-1 (PTCH1)* gene that encodes the PTCH1 protein. PTCH1 is part of the hedgehog (Hh) signalling pathway, which plays an important role in the regulation of cell growth and differentiation. BCNS belongs to the group of hereditary tumour syndromes, in which mutations in one or more genes predispose to the development of cancer. The development of sometimes hundreds of BCCs is a major problem in this group of patients.

Genetic basis of BCNS

In 1996 the *patched-1* (*PTCH1*) gene (MIM#601309) was first reported as a candidate gene for BCNS. Two different heterozygous mutations in the *PTCH1* gene were identified in two patients with BCNS.[3] *PTCH1* is the human homolog of the *Drosophila patched-1* gene and is located on chromosome 9q22.3. With DNA sequencing analysis of the *PTCH1* gene, mutation detection frequency ranges from 40% to 85% in individuals with typical findings of BCNS.[4–8] In **chapter 2** we describe a new database containing all previously published and newly identified *PTCH1* mutations. We established a database for *PTCH1* using the Leiden Open Variation Database (LOVD) version 3.0.[9] The purpose of this database is to assemble molecular variants of the *PTCH1* gene in a standardised format. The database provides an open collection for both clinicians and researchers containing published and unpublished *PTCH1* mutations. To accomplish this, we listed the results from all blood samples sent to the two Diagnostic DNA Laboratories in the Netherlands for *PTCH1* mutation analysis between 1999 and 2015. In **chapter 3** we investigate whether there is a genotype-phenotype correlation in this population by collecting clinical symptoms from patients' medical records. In this research, we only looked at the patients who carried a *PTCH1* mutation in the blood. In the investigated Dutch cohort, we did not find a genotype-phenotype correlation based either on the location or on the type of *PTCH1* mutation. Our findings are consistent with the results of previous studies and are in line with the phenotype variability within families found in this study.[4,10–13] The presence of a germline mutation in SUFU increases the risk of developing medulloblastoma in childhood.[6] The presence of genetic variants (polymorphisms (SNPs), modifiers) that increase the risks of developing sporadic BCC, like MC1R and TERT-CLPTM1L, is associated with an earlier age of onset for BCCs in BCNS.[14] The mutation detection percentage in our population is low (21%). Probably, relatively many patients with only minimal clinical symptoms of BCNS were tested. Another explanation can be that for many patients with clinical suspicion of BCNS, standard genetic tests are not yet suitable to detect the underlying genetic cause of the symptoms.

Postzygotic mosaicism in BCNS

In a substantial number of BCNS patients no *PTCH1* mutation is found in DNA extracted from blood. It is known that genomic rearrangments in *PTCH1 or* deep intronic variants causing cryptic splicing cannot be detected with generally used genetic diagnostic tests.[15] Also, mutations could be located in other genes involved in the Hh pathway (or yet unknown pathways), for example *SUFU* or *PTCH2*.[6,16–18] Another explanation is the presence of genetic mosaicism. Low-grade mosaicism can be difficult or even impossible to detect in DNA extracted from blood. With standard techniques, e.g. Sanger sequencing, mutation loads under 5% are not detectable. Next generation sequencing (NGS) of target genes (i.e. *PTCH1*) in blood is more sensitive and has sufficient sequence read depth to not only reliably detect, but also quantify the degree of mosaicism[19]. Sometimes though, a mutation is only present in the skin and cannot be found in blood. When the presence of a mutation causes visible skin lesions, as in mosaic ichthyosis, the segmentally appearing skin disorder is an evident clue for the existence of segmental mosaicism caused by dominant mutations.[20] Mosaic skin diseases may present different patterns as described in **chapter 1**. Lesions can for example follow the lines of Blaschko, in broad or narrow bands, or appear in a checkerboard pattern.[21] In BCNS, but also in other hereditary tumour syndromes, skin with only one affected allele has a normal appearance. Only a second hit of the other allele, through loss-of-heterozygosity (LOH) or somatic gene mutation, initiates the development of a BCC. As the presence of a BCC is a marker for affected skin, genetic mosaicism in the patient can be demonstrated when different BCCs share the same *PTCH1* mutation. In **chapter 4** and **5** we described three patients with BCNS caused by *PTCH1* mosaicism. Only in one patient there is a clear segmental distribution of skin lesions as they are limited to only one side of the body. This emphasizes the difficulty of recognizing a mosaic presentation of BCNS. To demonstrate a shared mutation in the different BCCs of these patients we used NGS in combination with a gene panel, consisting of a selection of genes participating in the Hh pathway including *PTCH1*, *PTCH2*, *SUFU and SMO*. Mosaic mutations in the latter has been described in Happle-Tinschert syndrome, that should be distinguished from mosaic BCNS[22,23]

Mosaic manifestations of other hereditary tumour syndromes have been described as well, for example neurofibromatosis type 1.[24] In BCNS *PTCH1* postzygotic mosaicism could actually be more common than previously assumed. It is plausible that patients do not always fulfil the diagnostic criteria of BCNS and may even present only with multiple BCCs at a young age. Of note, low-grade mosaicism can affect the gonadal cells as well. If this is the case, patients have a chance, although probably very small, to give birth to a child with a germline mutation of the disease. Unfortunately, the degree of involvement of different organ systems is difficult to predict. Therefore, surveillance programs and treatment of BCCs should have the same approach as in patients with a germline mutation. So, in patients suspected of having BCNS and a negative mutation analysis on blood, even with NGS techniques, it is worthwhile to search for a shared mutation in their BCCs. In case of mosaic disease, genetic counselling can be adjusted.

Care pathways and patient customized health care

Currently, our department is developing an integrated care pathway for patients with BCNS. This project is a collaboration between different specialties: paediatrics, clinical genetics, maxillary surgery, (paediatric) neurology, ophthalmology, cardiology, gynaecology, psychology and medical oncology, with the dermatologist as the initiator of the project. The purpose of this plan is to standardize the health care for this group of patients based on best available evidence. The dermatologist has a central position in this care pathway, as the management of the multiple BCCs is a difficult and ongoing problem. Information about sun protective measures are very important in patients with BCNS, as individuals with a low tumour burden have significantly fewer UV signature somatic mutations.[25] Our dermatology department offers specialized consultations for patients with BCNS. Treatment of multiple BCCs can be very complex and has a high psychological impact on patients. Different treatment modalities are available, and choices are preferably made combining evidence-based medicine with shared decision-making. Maintenance therapy with Hh pathway inhibitors, such as vismodegib, can be considered in selected patients as I will discuss in more detail in part two of this chapter.

Future perspectives

Insight into the genetic cause of BCNS is not only helpful to understand the pathogenesis of this syndrome. For the individual patient, it is just as important, because it allows us to diagnose the disease at an early age and to offer patients correct screening programs and adequate genetic counselling.

Whole Exome Sequencing (WES) is increasingly used, especially in syndromes with no clearly defined candidate gene or in very heterogeneous diseases, because it enables the sequencing of all genes in one go. The method is however still costly and moreover it has a limited sequencing depth.[19] In case of a high clinical suspicion of BCNS it is more efficient to look specifically at participants of the Hh pathway. We designed an NGS-based gene panel that includes *PTCH1*, *PTCH2*, *SMO* and *SUFU*. Its implementation in daily practice will provide a more effective, faster and less expensive diagnostic screening method for BCNS patients. Because of the higher sequencing depth, mosaic disease is more likely to be discovered. In patients with a high suspicion of BCNS in whom no PTCH1 mutation can be detected in blood with regular Sanger sequencing, it is worthwile to implement the gene panel, in order to analyze other genes involved in the Hh pathway, but also to reach a sequencing depth sufficient to detect low-grade mosaicism. To gain more information about the prevalence of mutations in other genes and the presence of mosaic disease in BCNS, it is of interest to look at the patients in our initial cohort that had no mutation in blood.

Part two
Treatment of locally advanced and metastasised basal cell carcinoma with vismodegib

The development of Hh pathway inhibitors provided a new treatment option for patients with metastasized or locally advanced BCC ineligible for surgery or radiotherapy. In **chapter 6** we described the first Dutch patient who was treated with vismodegib for a locally advanced BCC with locoregional metastasis of her BCC. An interesting detail is the presence of BCNS in this patient, already giving a preview of the effects of vismodegib on the multiple small BCCs in BCNS. Since the registration of the Hh pathway inhibitor vismodegib in the Netherlands in 2014, it is possible to treat patients outside the clinical trial setting. However, it is important to realise that only very few patients with BCC are candidates for this systemic therapy. In 2015 only 25 patients were treated for this indication in the Netherlands. Although treatment with Hh pathway inhibitors is initially effective, development of tumour resistance is common and adverse events frequently lead to discontinuation of therapy. Some patients with BCNS benefit from treatment with oral Hh pathway inhibitors, but especially in this group side effects are an important reason to discontinue therapy. An overview of treatment of BCC with Hh pathway inhibitors is given in **chapter 7** and some aspects of this treatment are discussed in more detail below. We published the topics mentioned above in a national journal, as we believe it is important to share experiences on new treatment options.

Efficacy and side effects

Vismodegib is a small molecule inhibitor targeting the Hh pathway. Binding of vismodegib to SMO leads to inactivation of this pathway.[26] Because most BCCs carry mutations in *PTCH1* (the inhibitor of SMO) resulting in upregulation of the Hh pathway,[25] vismodegib is initially effective in the vast majority of patients. The STEVIE trial assessed the safety and efficacy of vismodegib in patients with advanced BCC.[27,28] In this trial 1215 patients (1119 laBCC, 96 mBCC) were treated with vismodegib with a mean follow-up of 18 months. The best measured response rate in patients with laBCC was a complete response in 33% and a partial response in 35%, stable disease in 25% and progressive disease in 2% of patients. The median duration of response was 23 months. In metastatic BCC the efficacy was lower. In only 5% of patients the best measured response was a complete response, in 32% a partial response, in 46% stable disease and progressive disease in 11%. The median duration of response was 12 months. At the clinical cut-off of the study, only 12% of patients were still receiving treatment with vismodegib. Main reasons to discontinue treatment were side effects and progressive disease. The presence of BCNS did not affect the efficacy of vismodegib.[28]

In general, 30 to 40% of patients discontinued therapy because of side effects.[28] In patients treated because of BCNS-related multiple small BCCs even more than 50% could not continue therapy because of drug toxicity.[29,30] Most frequently reported side effects, in about half of patients, are muscle cramps, dysgeusia and alopecia. Weight loss is reported in 33% of patients, possibly related with dysgeusia, but probably also due to changes in fat metabolism caused by the medication.[28] Diarrhoea, nausea and fatigue are other common side effects. The development of cutaneous squamous cell carcinoma (SCC) during vismodegib therapy has been reported as well.[31] The actual clinical impact of this observation is not clear, as later studies did not find a higher incidence of SCC during treatment.[32]

Proper patient support and consultation of a dietician during treatment are essential to prevent weight loss. Intermittent treatment is already implemented in clinical practice to reduce toxicity. Unfortunately, studies on intermittent dosing regimens only show reduction in toxicity to a certain level.[33]

Drug resistance

A major problem in the treatment of BCC with vismodegib is the development of drug resistance. The majority of tumours initially show a complete or partial response to treatment with vismodegib. Only few BCCs do not respond at all, so called non-responders. The mean duration of response to therapy is 24 months.[27] It is thought that about 15-20% of patients develop resistance, the exact percentage is unknown.[27,43] The number of resistant cases is probably underestimated because of the short follow-up and the slow growth of BCC. In **chapter 8** we describe two patients with locally advanced BCC, who are resistant to vismodegib. These cases illustrate how important it is to monitor the tumour area very carefully and perform sequential biopsies when needed.

Genetically, BCC is one of the most heterogenic forms of cancer.[25] However, for their growth BCCs are almost completely dependent on the Hh pathway. Genetic alterations that maintain Hh signalling in the presence of vismodegib are the primary mechanism of resistance. In **chapter 9** we describe a patient with acquired resistance to vismodegib due to *SMO* mutations. At the same time, it was found that drug resistance to vismodegib in the treatment of BCC is predominantly caused by *SMO* mutations that either directly impair drug binding or activate *SMO* to varying levels.[35,36] The question is whether responsible mutations are acquired during treatment, or that they already existed before treatment. In the latter case, the minor clones may have benefited from a positive selection effect when other cells were killed by vismodegib. In the future, combination treatments should be developed to overcome or slow down the development of resistance. Drugs should inhibit SMO through different mechanisms or target the Hh pathway at points downstream of SMO. Several drug candidates are suggested in literature, for example itraconazol, bis-amides, arsenic oxide, protein-kinase inhibitors and mTOR inhibitors.[37-40] There

are indications that imiquimod inhibits the Hh pathway as well.[41] Furthermore, GLI-antagonists are very promising as they target the GLI transcription factors that are most downstream in the Hh pathway.[42] Further research is needed to explore possible clinical applications of these drugs, especially in respect to pharmacokinetics and toxicity.

Hh inhibitors in basal cell naevus syndrome

Vismodegib has proven to be effective in the treatment of BCNS related BCCs. Existing BCCs disappear and development of new tumours is delayed.[29,30] Unfortunately, discontinuation of treatment is common, mainly because of the side effects. Development of tumour resistance also occurs in patients with BCNS when it comes to laBCC or mBCC, but maybe to a more limited extent then in patients with sporadic BCC. Resistance is caused by the same underlying mechanism as in sporadic BCCs, as acquired *SMO* mutations are found in resistant tumour tissue.[30] However, the BCNS-related small BCCs seem to develop resistance less frequently.[30] This is probably because small BCCs are less heterogenic then the large laBCCs.[25] There is also evidence that syndromic BCCs have a lower mutational load and better genomic stability compared to sporadic BCCs, irrespective of the size of the tumour.[43] Studies on intermittent treatment dosing regimens in patients with multiple BCCs show that interruption of treatment does not compromise activity of vismodegib, but only leads to some reduction in toxicity.[33] Optimal treatment regimens should be personalized and aim to strike a balance between sufficient activity and acceptable side effects as these patients need long-term treatment. At this moment vismodegib is not registered for treatment of multiple BCNS-related small BCCs. However, surgical treatment of the high number of BCCs may have unacceptable high morbidity in some patients. Radiotherapy is contraindicated in BCNS because of the induction of new tumours. Therefore, in selected patients with BCNS there is an indication for maintenance therapy with Hh pathway inhibitors.

Neoadjuvant therapy

One should be very cautious in using vismodegib as a neoadjuvant therapy in clinical practice. Treatment with vismodegib can cause discontinuous growth of the tumour, leading to an unreliable histological margin assessment.[44] There is strong evidence that tumour cells remain present and become clinically manifest after treatment discontinuation.[35,45] This means that to achieve a complete surgical resection the whole initially affected area should be removed anyway, so there is no benefit in reducing the tumour size before surgery. An interesting treatment option for non-syndromic locally advanced BCC is the combination of radiotherapy and vismodegib. This seems to be an effective and tolerable combination therapy, with a possible synergistic effect, but the results of a phase 2 trial are not yet available.[46-48]

Treatment settings and personalized medicine

A multidisciplinary oncological team in which a dermatologist, head and neck surgeon, oncological surgeon, radiotherapist, plastic surgeon and medical oncologist are represented, is the preferred setting for treatment of locally advanced BCC with Hh inhibitors.[49] Only then all treatment options can be considered and experiences shared. Not only tumour characteristics are important in deciding which treatment is best for the patient, also patient characteristics are of great significance. The majority of skin cancer occurs in the elderly. In the ageing population, frailty is an important issue. The term frailty refers to a state of vulnerability to poor resolution of homoeostasis after a stressor event and is a consequence of cumulative decline in many physiological systems during a lifetime.[50] In frail elderly, a minor stressor can trigger disproportionate changes in health status. In frail elderly with advanced BCC, surgery may be technically achievable, but associated with a high morbidity when complicated by for example delirium or hospital acquired pneumonia. Treatment with surgery or radiotherapy may lead to an impaired quality of life, for example when the BCC involves the orbita and treatment requires an enucleation or exenteration of the eye or leads to vision loss by radiation-induced toxicity. In those patients, palliative treatment with vismodegib may be an option, but it is important to realise that side effects, like muscle cramps and dysgeusia, can also significantly influence quality of life. Involvement of a geriatrist in making treatment decisions can be helpful in these cases.[51]

Future perspectives on Hh pathway inhibitors for BCC

In the past decades, there has been a steep rise in the incidence of BCC. As BCC in general is a very slow growing tumour, it will take many years for it to become inoperable. As a logical consequence, the increase of neglected locally advanced tumours will lag many years behind the rise in incidence of BCC in general. As a direct result, in the nearby future an increasing number of patients with locally advanced or metastatic BCC that is ineligible for surgery or radiotherapy is to be expected. This emphasizes the need to optimize systemic treatment options for this group of patients.

Rapid advancement in our understanding of Hh signalling in BCC has provided a new systemic treatment option for this common form of skin cancer: Hh pathway inhibitors. We already have insight into the mechanism of the development of resistance to Hh inhibitors in the treatment of BCC. In a next stage, treatment strategies to overcome the development of resistance need to be investigated. Combination therapy probably provides the best perspective. Different drug candidates are proposed in literature. However, development of combination therapy still has a long way to go before it can be used in clinical practice.

For the small BCNS related BCCs topical Hh inhibitors are a promising treatment option. The topical SMO inhibitor patidegib has been developed for this indication.[52] Results from a phase II trial in which patients with BCNS are treated with patidegib have not yet been published. A multicentre phase 3 trial will start this year, with the MUMC as participating centre in the Netherlands.

The past years have seen many significant achievements in the field of cancer genetics, driven by rapidly evolving technologies and decreasing costs of next-generation sequencing (NGS). NGS is already used in clinical practice in several forms of cancer to predict responsiveness to therapy. The term 'precision oncology' was introduced, referring to the molecular profiling of tumours to identify targetable alterations and to predict response to therapies. Precision oncology simply means to give the right drugs to the right patients.[19] In BCC, an NGS targeted panel consisting of genes participating in the Hh pathway allows us to analyse tumour tissue relatively cheaply and fast. With this technique, it is also possible to analyse paraffin embedded tissue. In the future, NGS will probably contribute to making treatment decisions for locally advanced or metastatic BCC.

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