Growth characteristics of basal cell carcinoma

Chapter 8

General discussion and summary

In this final chapter an attempt will be made to summarize and discuss the results presented in this thesis in a somewhat broader context than was aimed in the discussion of the individual chapters. Three main themes will be adressed. First, our findings in chapters 2 and 3 are discussed in relation to the possible role of specific cell-mediated immunity as an important mechanism in limiting BCC tumor spread. Second, the role of programmed cell death regarding BCC tumor growth, studied by *bcl-2*, will be discussed by reviewing chapters 4 and 5. Finally, clinical and histological features of BCCs, and their consequences upon optimal therapeutical care are discussed in the context of chapters 6 and 7.

Basal cell carcinomas (BCCs) are frequently associated with a peritumoral mononuclear cell infiltrate. The infiltrate surrounding BCC tumor islands largely consists of T cells with a predominance of the CD4+/T-helper (Th) over the CD8+/T-cytotoxic (Tc) subset (Th/Tc ratio of 1.9 ± 0.8), similar to that found in typical delayed hypersensitivity reactions. Natural killer cells (NK) and B cells are seen in much lower numbers.

In the last decade, several investigators have attempted to elucidate the function of this cellular inflammatory infiltrate surrounding these skin cancers and its possible role in controlling tumor growth.

The relatively benign tumor-behaviour, areas of spontaneous regression and the therapeutic response to intralesional administration of cytokines, such as IL-2 and IFN-α, are in favour of a specific anti-tumor immune response in BCC. On the other hand, the observations that the stromal inflammatory reaction surrounding BCC is usually mild, T cells do not infiltrate BCC tumor lobules and the frequency of BCC in immunosuppressed patients is only slightly increased compared with squamous cell carcinoma, argue against a specific anti-tumor response.

In **chapter 2**, we investigated if BCC tumor cells and endothelial cells of the peritumoral vasculature express adhesion molecules.

A tumor specific cellular immune response is considered to be associated with adhesion molecule expression on endothelial cells for recruitment of T cells into the tissue, and on tumor cells for lymphocyte-target cell interaction.

Recent interest has focussed upon three cytokine-inducible leukocyte adhesion molecules, designated intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.

ICAM-1, which is inducible by IFN-γ and TNF-α, is expressed by a wide variety of cells, including resting endothelial cells and binds virtually all circulating white cells. E-selectin and VCAM-1 expression correlates with endothelial cell activation and is both inducible by TNF-α and IL-1.

The expression and distribution of the adhesion molecules ICAM-1, VCAM-1 and E-selectin by microvascular endothelial cells and tumor cells, together with their leukocyte receptors LFA-1, VLA-4 and CLA respectively, were studied in 33 BCCs of different histological subtypes.

Endothelial ICAM-1 expression was only slightly increased compared to normal skin, whereas expression of endothelial VCAM-1 and E-selectin was low or absent in all BCCs examined. Peritumoral infiltrates contained mostly LFA-1 expressing lymphocytes, with minimal VLA-4 and CLA positivity.

In none of the cases studied adhesion molecule expression of BCC tumor cells was identified.

This observed failure to express adhesion molecules on tumor cells possibly enables malignant cells to escape immunosurveillance, by preventing binding of the T lymphocyte to the target cell.

The inability of BCC tumor cells to express adhesion molecules is not absolute. Taylor et al presented a study where they demonstrated induction of ICAM-1 expression on tumor cells after BCC tissue had been incubated *in vitro* with IFN-γ.[1]

The absence of adhesion molecules on BCC tumor cells as well on the overlying epidermal keratinocytes as demonstrated in this study, together with the moderate ICAM-1 and minimal VCAM-1 or E-selectin expression on the surrounding peritumoral endothelial cells could be explained by insufficient *in vivo* cytokine levels.

Two causal hypotheses of insufficient *in vivo* cytokine levels will be discussed.

First, activated T lymphocytes are an important source of cytokines, especially those T lymphocytes responding to tumor-antigen stimulation. Different stages of lymphocyte activation are characterized by the appearance of specific cell surface antigens, such as IL-2 receptor, transferrin receptor and HLA-DR expression. The presence of activated T cells surrounding BCC has been demonstrated; with up to 30% to 50% of cells expressing activation antigens on their surfaces.[2] Whether this only distinguishes them from naive T cells activated by general stimuli (for example epidermal ulceration, a characteristic feature of nodular type BCCs), or supports the concept of tumor-derived antigens initiating a cell-mediated host-specific response is as yet unclear.

Second, CD4+ T cells are recently subdivided into functional subsets (Th1 and Th2) based upon cytokine profiles. Th1 cells produce IL-2, IFN-γ and TNF-α, resulting in T cell proliferation and macrophage activation, characteristic of cell-

mediated immune responses. In contrast, Th2 cells produce IL-4, IL-5, and IL-10, cytokines that augment antibody responses. Recently, Yamamura et al demonstrated IL-4, IL-5, and IL-10 mRNAs in BCC lesions and concluded that a particular T cell population producing type-2 cytokines accumulates in BCC.[3] Obviously, if Th1 cells are indeed in the minority in BCCs, a low level of IFN-γ may be the cause of absence of ICAM-1 expression in BCC. Thus, BCC progression may be assisted by the activation of Th2 cells involved in antibody formation, and suppression of a population of Th1 cells that normally contribute to tumor stasis or elimination.
In conclusion, besides identifying the presence of adhesion molecules on both vascular endothelial cells and BCC tumor cells, as demonstrated in this study, information about the composition of the infiltrate also is essential.
We would especially be interested in new techniques differentiating Th1 from Th2 cells, and also in new monoclonal antibodies differentiating immune effector cells specifically activated by tumor cells.

As mentioned above, the cellular inflammatory infiltrate surrounding BCCs, largely consists of CD4+/T-helper cells. This predominance of Th-cells over Tc-cells is an interesting observation, since Tc- and NK- cells are the effector cells in major histocompatibility complex (MHC)-restricted and non-MHC-restricted cellular cytotoxicity, respectively. Especially, these cytotoxic reactions are thought to be important in the elimination of tumor cells. The mechanism by which Th-cells mediate tumor regression remains unknown.
Our objective of the study presented in **chapter 3** was to study the expression of granzyme B (grB) by cells present in immune infiltrates surrounding BCC, as an indicator of cytolytic activity against the tumor.
The presence of characteristic granules in the cytoplasm of cytotoxic T cells and natural killer cells correlates with activation and subsequent cytolytic potential of these cells *in vitro*. These cytoplasmic granules contain a pore-forming protein, perforin, and several homologous serine proteinases called granzymes. The action of granzymes results in the apoptotic death of the target cell.
We investigated the expression of grB in 10 cases of BCC, of which 8 were untreated and 2 had been treated once with intralesional interferon-α2b.
Despite the presence of significant numbers of CD3+ and CD8+ T cells, there was a striking scarcity of grB-positive cells in peritumoral BCC areas.
The small number of cells in the infiltrate that stained for grB appeared to be NK cells.
In the two cases of BCC treated with IFN-α2b, the number of grB-positive NK cells in perivascular areas appeared to be increased.

In contrast, the number of grB-positive Tc-cells in squamous cell carcinoma (n=5), melanoma (n=4), and seborrhoeic keratosis (n=3), was clearly increased compared to BCC.
To the best of our knowledge, our study represented the first report on expression of granzymes in immune infiltrates of human skin cancer. Recently, two new studies concerning perforin and granzymes were published. Deng et al studied perforin-expressing T cells in 40 BCCs.[4] Only in ten specimens, very small numbers of perforin-positive cells were detected. Shimizu et al showed CD8+ infiltrating cells to contain grB in lichen planus.[5] They suggested that these CD8+ T cells induce apoptosis of keratinocytes, resulting in the typical colloid bodies.
Besides the release of perforin and granzymes, cytotoxic T lymphocytes also are able to kill target cells by surface interaction between Fas ligand and Fas. This is a fascinating new field of research, especially since failure of BCC tumor cells to express the Fas antigen has been reported, thereby avoiding T cell killing by Fas ligand bearing cytotoxic T cells.[6]
In summary:
1) the resting state of peritumoral endothelial cells, 2) the absence of adhesion molecules on BCC tumor cells, and 3) the lack of grB expression of peritumoral Tc-cells, plead against a role of a specific anti-tumor response in BCC.
One could speculate that this is caused by an insufficient number of adequately activated T lymphocytes (with their corresponding cytokine profile), responding to a specific tumor-antigen stimulation.

BCCs are typically slow growing tumors, often taking months to years to reach significant proportions. Several explanations for this clinical behaviour have been proposed: 1) prolongation of the S-(DNA synthesis) phase of the cell cycle in tumor cells; 2) a small proliferation fraction in the tumor; 3) tumor regression in response to host immune factors; 4) prominent cell death by the phenomenon of apoptosis.
Option number three, tumor regression in response to host immune factors is already discussed above.
We will now focus on the critical balance between cell proliferation and cell death.
In **chapter 4**, we investigated the expression of *bcl-2* in BCC and also in SCC of the skin. The proto-oncogen *bcl-2* encodes an inner mitochondrial membrane protein which shows a functional role of blocking programmed cell death, referred to as apoptosis. The protein is expressed in basal cells in normal human epithelium, but not in more superficial differentiated cells. It has been suggested that this pattern of expression assists survival of stem cells while preventing overaccumulation of differentiated cells.
Immunohistochemical localization using a monoclonal anti-*bcl-2* antibody revealed positive *bcl-2* expression of all BCCs (15 patients). SCC's failed to express

bcl-2 (5 patients). The positive *bcl-2* staining of BCC tumor cells supports the hypothesis that BCCs originate from the basal layer of the epidermis.
The prominent *bcl-2* expression of BCC tumor cells also suggests that inhibition of programmed cell death occurs, and would argue against the suggestion that prominent cell death in BCCs accounts for their typically slow growth rate.
It should be realized, however, that apoptosis is a complex process, in which several molecules besides *bcl-2* are involved. This issue is further discussed in the following section.
To elucidate the combined role of apoptosis and proliferation in BCC, we analyzed 6 cases of BCC in a pilot study (unpublished data). For the detection of apoptosis we used the terminal deoxynucleotidyl transferase (TdT)-mediated digoxigenin-dUTP nick end labeling (TUNEL) assay, wich is based on the detection of the 3'-OH nicks produced by apoptotic DNA fragmentation. Proliferation was measured by staining with Ki-67, a monoclonal mouse anti-human antibody which reacts to a nuclear antigen present in all phases of the cell cycle except for the G_0 phase. In general, we observed very low percentages of TUNEL positive cells with a mean of 0.17% positive tumor cells. The mean proliferation index was 6.15%. Combining these two results, it appears that BCCs show a low proliferation index, in combination with a low apoptotic index, i.e., a low cell turnover rate. These results were supported by several other authors.[7,8] Moreover, in our group of 6 BCCs, we observed Ki-67 reactivity especially restricted to the nuclei of three to five rows of peripheral cells localized at the base of the tumor nests. Unfortunately, this is a tumor area which often remains behind in the case of incomplete excision.
In history, the relationship between life and death already has challenged many philosophers, poets, and theologians. It is now a subject of special interest to cell and molecular biologists as well.
For example, the wild type *p53* gene is located at chromosome 17p and functions as a tumor suppressor gene by acting like a brake on tumor growth to allow optimal repair of damaged DNA. It is suggested that if this repair is not succesfull the *p53* gene induces apoptosis. The mutant version of *p53* gene is possibly enhancing the *bcl-2* action by further impairment of apoptosis.[9] Interestingly, in BCC an aberrant expression of *p53* protein has been reported.[10]
David Norris stated in 1995: "I suspect that the issue of control of apoptosis induction in BCCs is more complex than is suggested by immunostaining for *bcl-2* alone.[11] Since then the following proteins were discovered. The protein *bax* is central to activation of the program of apoptosis.[12] Activation of *p53* induces the immediate up-regulation of *bax* and the rapid induction of apoptosis. *Bcl-2*, a homologue of *bax*, heterodimerizes *in vitro* with *bax* and decreases the induction of apoptosis by *bax* homodimeric complexes. Similar dimerization occurs between *bax* and *bcl-xL*, which also inhibits the formation of *bax* homodizers and the in-

duction of apoptosis. *Bad* is a newly described homologue of *bcl-2* that forms complexes with the inhibitory molecules *bcl-2* and *bcl-xL*.[13] By binding to the inhibitory proteins *bcl-2* and *bcl-x*, *bad* promotes the formation of *bax* homodimers and promotes apoptosis. Finally, *bak* is the most recent proapoptotic member of the ever-expanding *bcl-2* gene family. *Bak* has been primarily found to enhance apoptotic cell death following an appropiate stimulus. Tomkova et al showed in a recent study negative *bak* expression in 20 BCCs studied.[14]
In conclusion, the high expression of the apoptosis-suppressing protein *bcl-2* together with the small percentage of apoptotic and actively proliferating tumor cells, may indicate that lack of apoptosis and not increased cell proliferation is an important factor in tumor expansion in BCC.
This is an interesting speculation since some authors suggest that inhibition of programmed cell death may lead to neoplastic growth at slower rates than growth induced by oncogenes that act by stimulating cell proliferation. This could possibly be a new explanation for the typical slow growth rate of BCC.[15]
However, the role of *bax, bcl-x, bad* and *bak* in BCC should be studied in the future before any firm conclusions can be drawn.

Besides the considerations concerning possible biological implications of *bcl-2* staining of BCC tumor cells, the immunohistochemical expression of *bcl-2* also can be used as a diagnostic marker. In **chapter 5** we studied staining differences of *bcl-2*, transforming growth factor-beta (*TGF*-β), and *CD34* between BCC and trichoepithelioma (TE). TEs are benign tumors confined to the dermis and characterized by aggregates of neoplastic epithelial cells with limited follicular differentiation.
Both the clinical as well the histological distinction between TE and BCC can be a diagnostic dilemma, especially for the infundibulocystic type of BCC. This distinction is important because of the consequences for subsequent patient management. BCC displays a much more agressive tumor behaviour than TE, and should be excised completely.
Recently, *bcl-2* and *CD34* expression already had been reported to be of diagnostic help in differentiating between TE and BCC. In addition to *bcl-2* and *CD34*, we selected *TGF*-β (Transforming Growth Factor-β) because of its pleiotropic effects on cellular differentiation and growth. Seventeen cases of TE / BCC were studied and the results of the hematoxylin-eosin stained histological sections compared with immunohistochemical analysis of consecutive sections stained with *TGF*-β, *bcl-2* and *CD34*. The combination of staining for *TGF*-β and *bcl-2* seemed to be most valuable in differentiating TE from BCC. In general, TE displayed *TGF*-β positivity combined with negative *bcl-2* staining whereas BCC demonstrated the opposite. The *CD34* staining pattern was considerably less consistent.

TGF-β is a multifunctional regulator of both cell growth and differentiation and has been shown to inhibit the growth of epithelial cells, stimulate mesenchymal cells and promote epithelial cell differentiation.
Glick et al studied *TGF*-β1 and *TGF*-β2 expression in murine skin and skin tumors.[16] They showed that loss of expression of *TGF*-β from epidermal cells may be associated with increased proliferation and decreased apoptosis. If we extrapolate this to the human situation in BCC, loss of *TGF*-β expression could be a second mechanism responsible for extended cell survival besides expression of *bcl-2*. Moreover, the absence of *TGF*-β expression in BCC could also be correlated with the apparent failure of the tumor cells to differentiate.
One could speculate that the *bcl-2* positive/*TGF*-β negative cells in BCC are responsible for the biologically more agressive tumor behaviour of these tumors, compared to the *bcl-2* negative/*TGF*-β positive cells in TE. So, tumors with a *bcl-2* positive/*TGF*-β negative staining pattern possibly deserve a more agressive therapeutical approach.
In conclusion, the *TGF*-β staining pattern appears to be a helpful additional marker together with *bcl-2* in differentiating between TE and BCC. The demonstrated staining differences may relate to the distinct origin and biological behaviour of the two tumors and may therefore be of value in subsequent patient management.

BCC is the most common malignancy in man, predominantly found on sun-exposed areas. The incidence of BCC is rapidly increasing worldwide. In the Netherlands (15 million inhabitants), approximately 18.000 BCCs were diagnosed in 1994, with an expected increase of 2.700 BCCs yearly.
Most BCCs are small, slow growing, well defined lesions easily treated by various methods. On the other hand, there is also a group of aggressive BCCs characterized by extensive local invasion and disfigurement.
In **chapter 6**, possible risk factors, both clinically and histologically, were evaluated in a retrospective study of 72 recurrent extensive BCCs treated by Mohs' micrographic surgery (MMS). All selected cases required at least three surgical Mohs stages before complete tumor removal was achieved.
The mean preoperative tumor size in our study was 2.1 cm. All 72 tumors except one were located in the face, of which the nasal-perinasal area was the most common site (51%). In 33% of the cases the histologic pattern displayed a mixture of solid and infiltrative components in the same tumor, 38% demonstrated a purely nodular tumor growth, whereas 21% was of the infiltrative type.
Besides the documented aggressiveness of the morpheaform type BCC, we also demonstrated extensive tumor growth of both nodular and nodular-infiltrative

type BCC. Especially the perinasal area was characterized by extensive vertical tumor expansion of the nodular/nodular-infiltrative tumor type.
In chapter 7, guidelines for surgical margins for excision of primary and recurrent BCCs were formulated on the basis of a retrospective study of 309 tumors treated by MMS. In our group of primary BCCs selected for high-risk areas, large tumor size and/or infiltrative histologic subtype, a minimum margin of 6 mm was necessary for complete removal of the tumor in more than 85% of the cases. The group of recurrent BCCs required margins of at least 10mm to achieve a comparable tumor clearance.
Previous treatment (recurrent tumors) and increasing tumor diameter (larger than 2 cm), proved to be the two most important risk factors correlated with extensive subclinical tumor expansion.
First we will discuss reasonable cure rates, and how many BCCs require MMS. In this thesis surgical margins were calculated to achieve cure rates of 85%. Of course there will be a progressive improvement in cure rates as one takes wider and wider margins. If we took 15-to 30 mm margins with BCC, the cure rate would be nearly 100%, but we do not believe that the subsequent morbidity would justify such an agressive approach. Because of the asymmetry of subclinical tumor extensions, whose precise location cannot be predicted, a comprehensive amount of normal tissue is always sacrified when margins of this extent are taken. We believe that it might be reasonable to aim for a 85% to 90% cure rate of BCC. Surgeons getting a 99% cure rate for BCC are simply cutting out too much.
Overall, the percentage of nonmelanoma skin cancer treated by MMS amounts 30% in the United States. About 5% of Mohs surgeons believe that all BCCs require MMS, but most would agree that this is an excessive use of resources.[17]
In the Netherlands, MMS is quite controversial. Currently, only 1.6% of all BCCs are treated by MMS. Most of the criticism probably is based on the fact that people are not well informed. It has been said that "in its wonderful conceptual simplicity, MMS evokes a copying response from nearly every cutaneous surgeon who witnesses it".[18] Arguments of opponents of the MMS procedure are the following; time consuming, expensive and surgical overutilization. However, MMS can be an effective use of physician time, depending on the infrastructure of your practice. With MMS and fresh-frozen tissue analysis it is possible to schedule four patients for MMS in the morning, and routinely all procedures, including reconstruction, are completed by afternoon. Miller et al, emphasizes that MMS is not only cost-effective but also usually less expensive than other types of therapy.[19] Finally, a lot of dermatologists comment that Mohs surgeons use a bazooka to "cure" a flea. Yet, because some skin cancers have invisible extensions, huge surgical defects are sometimes necessary, even when the clinically apparent lesion may have been small. In other words, one of the reasons for a high cure rate with MMS, besides the meticulous histologic evaluation of margins, is the simple fact that

Mohs surgeons make bigger holes than the average dermatologist. Of course, some of these big holes are the consequence of previous inadequate treatment by others. In our opinion, MMS is the preferred technique for treatment of high risk BCCs as described in chapter 6 and 7, because of the routine methodic accuracy for evaluation 100% of the surgical margins of the specimen, the subsequent high rate of oncologic cure, and the tissue sparing quality of the procedure. Since the incidence of BCCs is rising, hospitals in the Netherlands should provide more MMS settings in order to manage the selected difficult cases.

Another point of discussion concerns the management of tumor-positive surgical margins. Consequently, even when performing the recommended wide margins as suggested in our study, still 15% of the cases will be excised incompletely. Bieley et al, reviewed 78 tumors treated by MMS because of margin involvement after primary excision of BCC to detect the presence or absence of residual tumor.[20] Residual tumor was found in 55% of the cases as defined by the need for two or more stages of MMS to achieve a tumor-free plane. Reported recurrence rates for incompletely excised BCCs vary between 33%-39%.[21] Because of these figures, some authors advocate a conservative policy of close observation for clinical recurrence. One study even concluded that it was cost-effective to wait and see if a recurrence appears.[22] Others believe in a selective retreating approach. They feel that features like anatomic location and histologic subtype should determine if further surgery is necessary or not. In this thesis, we want to state that disease-positive surgical margins should not be ignored. With a likelihood of tumor recurrence ranging from 33%-39%, wait and see is unacceptable, and therefore only in very limited situations should watchful waiting be encouraged. As Salasche stated, "leaving tumor behind expectantly is a mix of wishful thinking and Russian roulette".[23] Patients do not always return for follow up, which leads to delays in diagnosis, and when recurrences do develop, they are often deep and destructive.

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Samenvatting

Hoofdstuk 1 geeft een overzicht van de diverse aspecten van het basaalcelcarcinoom (BCC) en beschrijft het doel van het onderzoek dat in dit proefschrift wordt beschreven.
Het BCC is de meest gediagnostiseerde maligniteit in Nederland, met ongeveer 29.000 nieuwe gevallen per jaar. Het veranderde zon- en reisgedrag in de laatste decennia, de vergrijzing van de bevolking en de afname van de ozonlaag spelen mogelijk een rol bij de sterke toename van de BCC-incidentie. Het BCC komt vooral voor bij de blanke oudere mens op de aan zon blootgestelde huid.
Het BCC is een langzaam groeiende tumor, die slechts zelden metastaseert. Wanneer het BCC echter niet afdoende behandeld wordt, kan het lokaal een destructief en invasief karakter hebben en op die wijze veel schade aanrichten, zowel in functionele als in cosmetische zin.
Dit proefschrift is onder te verdelen in drie verschillende onderzoekslijnen waarbij met name aandacht wordt besteed aan zowel histopathologische als ook klinische aspecten betreffende de karakteristieke langzame groeiwijze van het BCC (1-6 mm groei per jaar).
Wij hebben onderzocht in hoeverre een specifieke cellulaire immuunrespons (hoofdstuk 2,3) en een omvangrijke celdood (hoofdstuk 4,5) mede verantwoordelijk zouden kunnen zijn voor deze langzame groeiwijze.
Tot slot zijn wij op zoek gegaan naar klinisch relevante risicofactoren van het BCC, en hebben wij een poging ondernomen tot het opstellen van therapeutische excisiemarges (hoofdstuk 6,7).
Het doel van het onderzoek dat in **hoofdstuk 2** beschreven wordt, was om de mogelijke rol van het ontstekingsinfiltraat gelocaliseerd rondom de BCC tumorvelden op waarde te schatten. Menig auteur houdt dit ontstekinginfiltraat verantwoordelijk voor de langzame groeiwijze van het BCC, ondanks het feit dat de ontstekingscellen vaak op afstand van de tumorvelden gelocaliseerd zijn en niet de tumor binnendringen. Voor een effectieve interactie tussen ontstekingscellen (met name T cellen) en tumorcellen, is de expressie van verschillende bindingsmoleculen noodzakelijk. In dit hoofdstuk hebben wij de aanwezigheid van de bindingsmoleculen *ICAM-1*, *VCAM-1* en *E-selectin* op de omringende bloedvaten alsook op de tumorcellen onderzocht. Behoudens een lichte aankleuring van *ICAM-1* op de bloedvaten (endotheel), toonden met name de tumorcellen geen aankleuring.
Naast het onderzoeken van aan/afwezigheid van bindingsmoleculen, is het ook van belang geïnformeerd te zijn over functionele mogelijkheden van de ontstekingscellen. Zijn de ontstekingscellen ook in staat om de tumorcel te doden?

In **hoofdstuk 3** hebben wij de aanwezigheid van *granzyme B* onderzocht bij T cellen. Alleen wanneer deze T cellen op adequate wijze door tumorcellen zijn geactiveerd, brengen deze T cellen *granzyme B* tot expressie, en zijn zij in staat de tumorcel te doden. Uit ons onderzoek kwam naar voren dat slechts enkele T cellen *granzyme B* positief waren.
De aangetoonde afwezigheid van bindingsmoleculen op BCC tumorcellen, samen met de minimale expressie van *granzyme B* op de omliggende T cellen, pleiten niet voor een belangrijke rol van het ontstekingsinfiltraat in het BCC ten aanzien van een specifieke tumorafweer.
Naast de cellulaire immuunrespons wordt ook celverlies in de vorm van geprogrammeerde celdood (apoptosis) als oorzaak voor de langzame groeiwijze van BCCs genoemd. Tumorgroei weerspiegelt een verstoring van het evenwicht tussen celaanmaak en celafbraak. In voorafgaande studies was de aandacht met name gericht op toename van celaanmaak (proliferatie). Momenteel gaat de interesse in de wereldliteratuur echter vooral uit naar celaccumulatie ten gevolge van verminderde celdood. In **hoofdstuk 4** hebben wij de aanwezigheid van *bcl-2* onderzocht in BCC. *Bcl-2* is een oncogen dat cellen beschermt tegen geprogrammeerde celdood. Dit oncogen komt onder andere tot expressie in de basale cellen van de opperhuid. In de BCCs door ons onderzocht werd een duidelijke positiviteit voor *bcl-2* gevonden. Daarnaast bleek slechts 6.15 % van de tumorcellen proliferatief actief te zijn (Ki-67 kleuring). Deze resultaten wijzen er mogelijk op dat de groei van het BCC met name wordt veroorzaakt door verminderde celdood en niet als gevolg van celproliferatie. Dit is van belang omdat tumorgroei als gevolg van verminderde celdood geassocieerd zou zijn met een minder agressief tumorgedrag.
In **hoofdstuk 5** blijkt *bcl-2* samen met *TGF*-β van diagnostische waarde te zijn in het onderscheid tussen BCC en trichoepithelioma. Beide huidtumoren zijn vaak op klinische gronden niet te differentiëren, echter het BCC is een kwaadaardige tumor terwijl het trichoepithelioom een goedaardige groeiwijze vertoont.
In **hoofdstuk 6** worden mogelijke risicofactoren van het BCC beschreven. Uit onze patiëntenpopulatie die werd behandeld door middel van Mohs' micrografische chirurgie werden die tumoren geselecteerd waarbij sprake was van een recidief na eerdere behandeling, en waarbij tenminste drie Mohs-ronden noodzakelijk waren voordat het operatiegebied als tumorvrij werd afgegeven. De essentie van Mohs' micrografische chirurgie bestaat uit een goede grafische voorstelling van de tumorlokalisatie en het laagsgewijs excideren van de tumor. Van iedere Mohs snede wordt het gehele resectievlak, door middel van horizontale vriescoupes, gecontroleerd. Het basisprincipe is maximale curatie bij minimale weefselbeschadiging. Belangrijkste risicofactoren waren: 1) grootte van de tumor, met name groter dan 2 cm, 2) lokalisatie, waarbij met name de tumoren gelokaliseerd ter hoogte van de nasolabiaalplooi gekenmerkt werden door uitgebreide verticale diepte-groei, en 3)

histologische groeiwijze, waarbij naast de sprieterige groeiwijze, ook bij het nodulaire type uitgebreide subklinische tumorgroei werd waargenomen.
In **hoofdstuk** 7 worden richtlijnen geformuleerd betreffende excisie-marges voor primaire- en recidief BCCs, op basis van een retrospectieve studie van 309 BCCs door ons behandeld door middel van Mohs' micrografische chirurgie. Bij de groep primaire BCCs, door ons geselecteerd voor Mohs' micrografische chirurgie in verband met grootte van de tumor (> 2 cm), sprieterige histologische groeiwijze en/of lokalisatie in het centrum van het gelaat, werd een minimale excisie-marge van 6 mm berekend, om een radicaliteitspercentage van 85 % te bereiken. Bij de groep van recidief tumoren betrof dit 10 mm.
Tot slot kan geconcludeerd worden dat bij deze geselecteerde groep BCCs met deze subklinische uitbreiding, behandeling door middel van Mohs' micrografische chirurgie de voorkeur verdient.