Histopathological aspects of unusual skin tumors with clinical correlations

Summary of the Ph.D.Thesis

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1. Publications related to the subject of the thesis

Journal articles

I. Korom I, Oláh J, Gyulai R, **Varga E:** Szokatlan megjelenésű melanomákról. Bőrgyógy. Vener. Szle. 75: 133-136, 1999.

II. Oláh J, Gyulai R, Varga J, Mohos G, Kapitány K, Papos M, Pávics L, **Varga E**, Korom I, Dobozy A: Őrszemnyirokcsomó-biopsziával szerzett tapasztalataink melanoma malignumban. LAM 11: 536-41, 2001.

III. Oláh J, Gyulai R, Korom I, **Varga E**, Dobozy A: Tumour regression predicts higher risk of sentinel node involvement in thin cutaneous melanoma. Br J Dermatol 149: 662-663, 2003. **IF: 2,659**

IV. Korom I, Oláh J, **Varga E**, Kapitány K: Melanoma malignum gyermekkorban. Bőrgyógy Vener Szle 80: 275-278, 2004

V. Korom I, Oláh J, **Varga E**, Földes M, Kemény L: Ritka melanocytás naevusok: clonalis naevusok. Rare melanocytic nevi: clonal nevi. Bőrgyógy Vener Szle 82: 96-99, 2006

VI. **Varga E,** Kiss M, Szabó K, Kemény L: Detection of Merkel cell polyoma virus DNA in Merkel cell carcinomas. Br J Dermatol 161 :930-2, 2009 **IF: 3,489**

VII. **Varga E,** Korom I, Varga J, Kohán J, Kemény L, Oláh J: Malignant melanoma and dysplastic nevi in decorative tattoos: case reports. *J Cutan Pathol* – in press **IF**: **1.486**

VIII. **Varga E**, Korom I, Raskó Z, Kis E, Varga J, Oláh J, Kemény L: Neglected basal cell carcinomas of the 21st century. *Journal of Skin Cancer* – in press

2. Introduction

2.1 Rare melanocytic tumors

Apart from the conventional, classic forms of melanocytic tumors (junctional, intradermal, compound) the recognition of different rare variants are very important. The knowledge of their clinical and histopathological features is essential in the differential diagnosis of the various benign forms and for the differentiation from malignant melanoma.

2.1.1. Clonal nevi

Ball and Golitz in 1994 presented a new and unusual variant of melanocytic nevus called clonal nevus. Clonal nevi represent melanocytic nevi with dermal foci of heavily pigmented, epithelioid melanocytes within a banal melanocytic nevus. The typical clinical history in clonal nevus is a recently presenting dark area in a preexisting nevus. The histopathologically well circumscribed, large nests of epithelioid melanocytes could be found in the upper dermal part of a banal compound or dermal melanocytic nevus. The cells contain dust-like melanin pigment without significant atypia. Mitotic figures are absent or very rare. The nests are surrounded by melanophages. Clonal nevi can be clinically and sometimes histopathologically resemble to deep penetrating nevus, combined nevus, cellular blue nevus or plexiform spindle cell nevus. Sometimes it was misdiagnosed clinically or histopathologically as malignant melanoma. The biological behavior of clonal nevi biologically is benign.

2.1.2 Childhood melanoma

Approximately 1-3% of all childhood malignant tumors are melanomas and only 0.3-0.4% of all malignant melanomas arises in children under the age of 14. The precise incidence and prognosis of childhood malignant melanomas are not known although there are increasing numbers of literature data about this subject in the recent years.

Richardson and co-workers classified the prepubertal melanomas in three groups:

1. congenital melanoma: the melanoma develops in the intrauterine life and is present at birth
2. infantile melanoma: the tumor appear in the first year of life
3. childhood melanoma: melanomas occurring from birth to 12 years of age

Statistical data also shows exponential increase in the incidence of melanoma around the age of 12.

The different risk factors in childhood melanomas are: giant congenital nevi, dysplastic nevi, xeroderma pigmentosum, genetic immunodeficiency, or secondary immunosuppression (organ transplantation or HIV infection).

Congenital melanoma can develop through transplacental transmission and can also arise as a dermal-subcutaneous node on the basis of a giant congenital nevus however de novo cases could occur sometimes.

One third of childhood melanomas develops also on the basis of a giant congenital nevus. Melanomas developing in small congenital nevi are mainly occurs after puberty. Approximately half of childhood melanomas develop de novo and their clinical and histopathological appearance is similar to their adult counterparts.

2.1.3. Nevi and melanoma in tattoos

Although the incidence of melanoma and the number of decorative tattoos have recently been increasing, tattoos are generally not considered as risk factors for melanoma formation.

The co-incidence of various dermatological diseases and malignant skin tumors with a tattoo have been documented with some frequency, but reports on melanoma are exceedingly rare.

To date, only 13 cases have been documented in the English literature. As the methods and materials of tattooing differ, it is impossible to prove a direct connection between tattooing and the malignant process. Mere coincidence can be the possible explanation, however according to the previous reports other associations could be considered. Ultraviolet light, photoallergic effect, inflammatory reaction or trauma

may promote the malignant transformation. The tattooing ink applied to denote a radiation field during the radiotherapy and the irradiation itself emerged to be co-carcinogens.
The possibility of the association and the consequences of tattoos as concerns melanocytic nevi have not been studied yet. Furthermore one must also consider that tattooing causes problems in the assessment of a sentinel node biopsy in a patient with malignant melanoma.

2.2. Rare non-melanocytic skin tumors

2.2.1. Merkel cell carcinoma

Merkel cell carcinoma (MCC) (a neuroendocrine carcinoma of the skin, trabecular carcinoma) is a rare but aggressive tumor that affects mostly elderly individuals of Caucasian origin.

Feng et al. recently isolated a new human polyomavirus which they named Merkel cell polyomavirus (MCV) from MCC by applying the digital transcriptome subtraction methodology; they obtained viral DNA by the digital transcriptome subtraction of RNA from MCC, and detected MCV sequences in 8 out of 10 MCCs. Accordingly they suggested that MCV has a role in the pathogenesis of MCC.

2.2.2. Neglected basal cell carcinomas

Tumors on the surface of the skin are generally visible and considered to be easily recognizable both for health-care professionals and for the patients themselves. However people with neglected advanced skin neoplasms are still encountered in dermatological practice in the 21st century. There can be numerous causes of the delay in the diagnosis: the person may fear the diagnosis and the treatment or become accustomed to the usually slowly-growing tumor. Old age, a low social milieu and an inadequate hygienic culture may also be factors explaining why some people are not aware of the significance of a delayed diagnosis.
Basal cell carcinoma (BCC) is the most common cutaneous tumor. Most of these tumors arise in the head and neck area, particularly in the

elderly, and usually grow slowly. The metastatic potential is very low and is mainly detected in association with aggressive or long-standing, large neglected tumors. The characteristics of BCCs suggest that they might well be the “ideal candidates” for neglected tumors.

3. Aims

3.1. To collect the unusual, rare melanocytic tumors and non-melanoma skin cancers:

- Clonal nevi
- Childhood melanoma
- Nevi and melanomas in tattoos
- Merkel cell carcinoma
- Neglected basal cell carcinomas

3.2. Characterize the clinical and the histopathological features and the prevalence of clonal nevi with the retrospective analysis of the histopathological records.

3.3. To describe the prevalence, the clinical and the histopathological features, the treatment modalities and the follow-up data of childhood melanoma with the retrospective analysis of the last 30 years’ histopathological records.

3.4. To evaluate the clinical and histopathological characteristics of nevi and melanomas arising in decorative tattoos.

3.5. To examine the prevalence of Merkel cell polyomavirus in the Merkel cell carcinomas of the Hungarian population.

3.6. To summarize the background, the histopathological features and treatment possibilities in neglected basal cell carcinomas.

4. Patients and methods

We examined retrospectively and prospectively the routine histopathological material of the Department of Dermatology and Allergology, Albert Szent-Györgyi Clinical Center, University of Szeged.

4.1. Melanocytic tumors

4.1.1. Clonal nevi

With the systematic search of more than 4.000 surgical biopsies of year 2006 we found 8 clonal nevi among 1267 melanocytic nevi.

4.1.2. Childhood melanoma

We collected the childhood melanoma cases which have been diagnosed during the last 30 years. We decided to consider a melanoma as a childhood one if the patient was between birth and 16 years of age.

4.1.3. Nevi and melanoma in tattoos

We managed to diagnose two cases of dysplastic nevi and one of malignant melanoma in decorative tattoos. We also detected another melanoma case where we found tattoo pigment in the sentinel lymph node.

4.2. Rare non-melanocytic skin tumors

4.2.1. Merkel cell carcinoma

We have examined 12 tumors from 10 patients with MCC, selected retrospectively from the period between 2001 and 2010. We analyzed the biopsies using routine hematoxilin-eosin staining, immunohistochemical methods. Randomly selected paraffin-embedded samples of 13 squamous cell carcinomas, 10 basal cell carcinomas, 3 baso-squamous carcinomas and 3 malignant melanomas were used

for comparative purposes. From formalin-fixed paraffin-embedded tissue biopsy specimens from the patients the DNA was extracted. The presence of MCV was detected by primer-directed amplification with PCR. Specific primer pairs were designed to detect the viral large T protein (LT1 and LT3), and the viral capsid protein (VP1). Amplification products were separated by electrophoresis in 1.5% agarose gel stained with GelRed (Biotium). The purified PCR products were subjected by direct sequencing.

4.2.2. Neglected basal cell carcinomas

During the first 10 years in the 21st century 5 neglected and advanced cases of basal cell carcinoma had been diagnosed in our institute (Table 1).

Table 1.
The main clinical data of the neglected BCC patients and their treatment

Patient	Age/sex	Living place	Duration, clinical history	Diagnosis	Treatment
1.	44 y/o, male	farm	injury 2 years ago, growing lesion for 10 months	BCC	surgical removal
2.	96 y/o, female	town	more than two years	BCC	radiotherapy
3.	63 y/o, male	town	several years, rapid growing for months	BCC with suppurative inflammation	surgical removal
4.	84 y/o, male	village	1 year	BCC	surgical removal
5.	68 y/o, male	village	1 year, immunosuppressed (renal transplant) patient	neck: BCC face, ear: SCC	surgical removal

5. Results and discussion

5.1. Rare melanocytic tumors

5.1.1. Clonal nevi

Histopathologically a compound melanocytic nevus could be seen with maturing nevus cell nests. Large epithelioid groups of melanocytes could be detected in the upper dermal part of the nevi. Sometimes the cells showed vacuolization. There was no considerable atypia or mitotic activity. Melanophages were situated around these foci. With immunohistochemical analysis the nevi showed overall S100 protein positivity. The junctional nevus cells and the epithelioid dermal melanocytes stained positively with HMB45.

5.1.2. Childhood melanoma

We analyzed the data of 14 melanoma patients younger than 16 years of age. There was female predominance, the female:male ratio was 11:3. Three patients were newborns, according to Richardson's classification and they had congenital melanoma. All these cases developed in giant congenital nevus.

All of the other 11 patients had childhood melanoma. 8 of these neoplasms developed on the basis of a preexisting melanocytic lesion and 3 tumors appeared de novo. One girl was 5 years old at the time of the diagnosis, the other patients were older than 10 years of age. One tumor developed on the scalp, 7 on the back 3 on the upper and 1 on the lower extremity. Histopathology revealed thick nodular melanoma in 7 cases. Patient 8 with dysplastic nevus syndrome had double melanoma on the basis of dysplastic nevi. During the follow up periods which lasts from months to several years two of our patient deceased due to the melanoma. There was no local recurrent disease but regional lymph node involvement could be detected in 2 cases. Two patients received interferon treatment. Three other patients were lost for follow-up.

5.1.3. Nevi and melanoma in tattoos

Patient 1: A 28-year-old Caucasian male had had a mole on his left upper arm since childhood within a non-figurative tattoo was placed on that site approximately 5 years earlier. After an injury the lesion has gradually become larger and crusted. The clinical diagnosis was malignant melanoma and the histopathology showed a melanoma, superficial spreading type with a small ulceration and with a remnant of the previous nevus. The Breslow thickness was 0.99 mm, and invasion to Clark level II was detected (pT1b). There was no metastatic lesion in the lymph node. However, the lymph node contained a large amount of black tattoo pigment.

Patient 2: A 34-year-old Caucasian male had an injured mole on his right upper arm in a decorative non-figurative black tattoo. The mole enlarged within the last two years. The clinical diagnosis was "injured melanocytic nevus". Histopathology revealed a severely atypical dysplastic junctional melanocytic nevus.

Patient 3: A 23-year-old Caucasian female presented with a large, artistic, multicolored decorative tattoo on her lumbar region. She had previously had a few moles in that area and on clinical examination one of these moles raised the suspicion of malignant melanoma. The histopathology revealed a lentiginous moderately dysplastic compound melanocytic nevus.

Patient 4: A 22-year-old Caucasian female had had a black enlarging, changing mole on her back. The clinical examination raised the possibility of malignant melanoma and the histopathology revealed an exophytic, spitzoid ulcerated nodular malignant melanoma (pT2b). Sentinel lymph node biopsy showed black pigment and a few tumor cells in the lymph node. As the black pigment histopathologically looked as an exogenous one the possibility of tattoo pigment had been suggested. The clinical examination confirmed the presence of a yellow-black tattoo on the patient's wrist.

At present, the pathogenesis of melanoma developing in a tattoo is unknown. We diagnosed two cases of dysplastic nevi and one of malignant melanoma in decorative tattoos. With this we present the

14th case of a melanoma developing in a preexisting tattoo in the English literature.
As the 14th melanoma reported in a tattoo, our case exhibits numerous similarities with the previous ones. There had been a previous nevus at the site, and thus the possible effect of injury cannot be excluded. In our presented case, the tumor developed some years after the tattooing, though, the time interval was relatively short (5 years). In the previous cases reported in the literature melanoma developed 2 to 40 years after tattooing and 8 cases emerged even after 10 years or more (median interval 15,6 years). Moreover dermatologists and histopathologists must be aware that the tattoo pigment can disguise changes in preexisting nevi when making clinical and histopathological diagnosis in these cases. Furthermore one must also consider that tattooing causes problems in the assessment of a sentinel node biopsy in a patient with malignant melanoma. As the tattoo pigment can mimic metastatic disease, it is very important for surgeons and pathologists to be informed in advance, as to whether the patient has a previous history of tattooing or tattoo removal.

5.2. Rare non-melanocytic skin tumors

5.2.1. Merkel cell carcinoma

Among the analyzed 10 cases the men:women ratio was 5:5, and the mean age at the time of the diagnosis of the primary tumor was 75,2 years. The youngest patient was 57 and the oldest was 87 years old; both were women. 7 tumors were primary and 5 were locally recurrent lesions. Of the 12 MCC tumors, 10 tested positive for MCV sequences by PCR. Ten tumors were positive for LT1 and/or LT3 amplification, whereas only 3 tumors were positive for VP1. The amplified PCR products were retrieved from the bands and subjected to DNA sequence analysis. The results proved that the sequences of the amplimers were exactly the same as determined by each of the primer pairs in the MCV genome (Accession No. EU375803). We could not detect the presence of MCV DNA in our reference samples by using LT1, LT3 and VP1 primer pairs. Thus, with MCV specific primers, we

demonstrated the presence of viral T antigen and/or viral capsid DNA sequences in 10 of the 12 MCC lesions both in primary tumors and in recurrent lesions. Our results are very similar to those of Feng et al. Our findings strongly support the hypothesis that MCV plays a role in the pathogenesis of MCC.

5.2.2. Neglected basal cell carcinomas

There can be numerous reasons for delay in seeking medical advice. The factors are best documented in malignant melanoma cases. A low social milieu, inadequate hygienic culture associated with poverty and a low level of knowledge about skin tumors may be the explanation in some cases. Patients in these circumstances may not be aware of the possible significance of their growing lesion, though most of our patients live in towns or villages where family members, family doctors or neighbors are easily accessible and media campaigns can reach them. Old age and a slowly-growing, not painful neoplasm may also result in a delay in seeking medical advice. The patients may not see properly or not realize the changing and extremely unpleasant clinical picture or they might accept the slowly, but continuously progressing situation. Finally a delay may be caused by an incorrect initial diagnosis, although this occurs mainly with melanocytic tumors. The organ transplanted and/or immunocompromised individuals comprise a special group. In consequence of the immunosuppression, skin tumors are more frequent and more rapidly growing in these individuals.

Another challenging question is the treatment of these advanced neoplasms. The therapy of neglected cases, however, demands an individual multidisciplinary approach and teamwork.

The treatment of choice is often surgery (plastic, cranio-facial or neurosurgery) alone or combined with radiotherapy, with the help of imaging techniques (CT, MRI and angiography). Reconstruction and a long-term follow-up are usually needed, with the cooperation of medical experts.

6. Conclusions

6.1.1. Clonal nevi

The knowledge of the clinical and histopathological characteristics of clonal nevi is very important for the proper diagnosis of these nevi. It prevents patients, clinicians and histopathologists from the consequences of the misdiagnosis of malignant melanoma.

6.1.2 Childhood melanoma

The diagnosis of melanoma in a child is difficult both for the clinician and the histopathologist. Very careful analysis of the histopathology material, consultation with experienced pathologists is always necessary in order to differentiate the lesion from other childhood melanocytic lesions such as Spitz's nevus or proliferative nodule.

In questionable cases the re-examination of the slides and long-term follow-up of the patient is needed. As these tumors are very rare the collection and analysis of sufficient data for prognostic purposes takes decades. However these data are essential for improving treatment modalities.

6.1.3. Nevi and melanoma in tattoos

At present, the pathogenesis of melanoma developing in a tattoo is unknown. Mere coincidence cannot be ruled out however trauma, ultraviolet light, a photoallergic effect, or an inflammatory reaction may promote the malignant transformation. Clinicians and histopathologists should be aware of the clinical and histological features if they are to make a correct diagnosis.

6.2.1. Merkel cell carcinoma

The presence of viral T antigen and/or viral capsid DNA sequences was demonstrated in 10 of the 12 MCC lesions. None of the comparative samples contained MCV DNA.

Our findings strongly support the hypothesis that MCV infection may well be specific for MCC, and MCV may play a role in the pathogenesis of MCC.

6.2.2. Neglected basal cell carcinomas

Neglected advanced skin tumors can be encountered even in the 21st century. There can be numerous causes of the delay in the diagnosis: the person may fear the diagnosis and the treatment or become accustomed to the usually slowly-growing tumor. Old age, a low social milieu and an inadequate hygienic culture may also be factors explaining why some people are not aware of the significance of a delayed diagnosis.

BCC, the most common cutaneous tumor, usually develops in the elderly, grows slowly and has an extremely low metastatic potential making it an “ideal candidate” for a neglected tumor. Although there are many possibilities for the treatment of BCCs, the therapy of such neglected cases always demands an individual and multidisciplinary approach and teamwork.

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