UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

PhD THESIS

STUDY OF CLINICAL-MORPHOLOGICAL PARAMETERS AND IMMUNOHISTOCHEMICAL MARKERS WITH TUMOURIGENIC, PROGNOSTIC AND THERAPEUTIC IMPLICATIONS IN CUTANEOUS MALIGNANT MELANOMA

ABSTRACT

PhD COORDINATOR:
PROF. UNIV. DR. ANICA DRICU

PhD STUDENT:
BLENDEA ANTONIA

CRAIOVA
2017

SUMMARY

KEYWORDS: cutaneous malignant melanoma, carcinogenesis, risk factors, molecular pathways, oncogenes, biomarkers, immunohistochemistry, prognostic parameters, oxidative stress, UV radiation.

INTRODUCTION

The significant increase in cancer incidence over the last three decades has become a reality for almost all countries of the world, confirmed by undisputed clinical and statistical research.

Of the cutaneous malignant tumours, the most frequent are the epitheliomas, representing 90-95% of the total, but also the least severe. Malignant melanomas, although much more rare (5%), are very serious, being responsible for most deaths from cutaneous cancer.

World Health Organization statistics show an increased incidence of cutaneous malignant melanoma over the past decade, with 132,000 new cases globally reported each year. As ozone levels are steadily decreasing and the atmosphere is increasingly losing its protective filter function, more and more solar UV radiation reaches the surface of the Earth. It has been estimated that a 10% decrease in ozone may result in 4,500 cutaneous malignant melanomas.

Although it is not an exclusive tumour of the skin, cutaneous malignant melanoma is 95% of the total melanoma, posing the most serious prognostic problems. Clinical studies show an early occurrence of this tumour, which evolves rapidly and with the highest mortality, thus enrolling melanoma on first rank in terms of aggression and unfavourable development.

Due to the biological complexity of the malignant melanoma, the main problem remains how to translate these clinical-morphological and immunohistochemical markers of high relevance to the benefit of patients with malignant melanoma. The solution lies in identifying the true biological "Achilles' heel" for each subtype of melanoma. Most likely, approaches to malignant melanoma, using 21st century genetic profiling technology, will yield interesting results.

STAGE OF KNOWLEDGE

Cancer is a complex condition triggered by both intrinsic factors (genetic, immunological, hormonal, produced by the malignant tumour itself) as well as extrinsic factors (physical, chemical, viral, smoking, trauma) that cause the emergence of some mutations in certain genes.

In general, it should not be neglected that repeated exposures, sometimes exaggerated to UV rays, represent a real risk factor because of the cumulative effect. Sun exposure in childhood, sometimes excessive, explains the occurrence of large-scale malignant melanomas (MM) in young people. It has been recognized that the most important factor in the genesis of this tumour is solar radiation.

Nowadays, the many molecular events that can lead to the development of this tumour are explored in detail. The immense number of molecular reactions that occur in cascade and the alterations associated with the malignant transformation into melanoma have made the understanding of the biology of this type of cancer so complicated.

The primary event that initiates the transformation of melanocytes consists of clonal inherited cellular modifications that contribute to malignancy. These are the result of oncogenic activation by genetic modifications (gene mutations, deletions, amplifications or translocations) or by epigenetic events (hereditary modifications other than those affecting the DNA sequence, transcriptional modulation by DNA methylation or chromatin alterations by histone modification). This type of modification can produce melanocytic clones with proliferative advantage over neighbouring cells. In primary clonal alterations, several pathways, such as those that induce cell proliferation (the proliferative pathway), or pathways that avoid cellular senescence (the cellular senescence pathway) have been identified. Apoptosis can be a very selective process, or even necessary for the development of advanced MM (the apoptotic pathway).

The study of immune response in cutaneous MM comprises two main categories of research: immune cells and humoral factors, which quantifies the effectiveness of the antitumoural response. The study of immune biomarkers in MM is interesting because of the dual involvement of the immune system in tumour development. Immune markers can be used on one hand to quantify the antitumoural immune response and, on the other hand, due to the demonstrated immune suppression, may be therapeutic targets.

In the case of MM occurrence, immunosuppression mechanisms are triggered by the neoplastic transformed melanocyte and/or by the immune factors that block the immune response of antitumour surveillance at skin level.

Neoplastic transformed cells migrate to different tissues, developing metastases, primarily in the regional lymph nodes, as satellite or transit lesions, and then in other organs and tissues in advanced forms, through lymphatic circulation and chemotaxis mechanisms.

Tissue and circulatory immune markers correlate with the evolutionary stages of the tumoural process: initiation of the tumour process; angiogenesis; metastasis; antitumoural immune response.

From the multitude of immune biomarkers studied over time, those predictive markers have not yet been found, of a pressing necessity for detecting therapeutic efficacy in cutaneous MM.

Recently gained information on the functional importance of mutant genes in a large proportion in MM has fundamentally changed the diagnostic and therapeutic approach. In

terms of focusing on BRAF, N-RAS, KIT and PTEN, four key genomic alterations and their corresponding pathways expanded the understanding of the astounding biological complexity of MM.

While MM carcinogenesis is still too little understood in its complexity to predict when, how and what kind of mutation develops a metastatic or invasive tumour, analysis of the primary or metastatic tumour genome will undoubtedly change future classifications and, as a result, treatment schemes.

In the primary assessment of cutaneous MM, there is a hierarchy of clinical and paraclinical methods, which should be used to establish a more accurate, complete and early diagnosis, which is extremely important for the most correct therapeutic management option.

The list of mandatory histopathological approaches contains elements which, for the most part, are either criteria for determining the tumour stage (thickness, ulceration, presence of secondary nodules, lymph node or transit metastases), or prognostic factors (incomplete resection or resection with insufficient oncological safety margins, ulceration, mitotic index, regression, vascular invasion, etc.).

The immunohistochemical study involves the use of many generic cellular markers (vimentin, S100 protein), cell proliferation markers (Ki-67, PCNA), cell cycle regulation markers (D1 cyclin), cell adhesion molecules and melanocytic markers (HMB45, Melan-A, Tyrosinase, MITF, TRP2).

Due to the melanocytic markers non-specificity, pan-melanoma cocktails, comprising HMB45 + Melan-A/MART-1 + Tyrosinase or Melan-A/MART-1 + Tyrosinase have been made. Because of the promising results, TRP2 is to be introduced into a new pan-melanoma cocktail needed to highlight the intraepidermal invasion in amelanotic MM.

Dermoscopy makes it possible to identify specific MM structures that allow us to differentiate it from other tumours by targeting the diagnosis. Confocal Reflection Microscopy is a modern *in vivo* skin visualization technique that makes it possible to non-invasively examine the cutaneous tumour morphology at a resolution similar to optical microscopy.

Despite all the therapeutic and preventive efforts, MM remains, however, the most aggressive and deadly human skin cancer, of a real complexity. Primary prevention campaigns have already, to a certain extent, achieved an earlier diagnosis of tumours with a better prognosis. However, the rate of MM incidence is rising worldwide, mainly due to the imprudent habit of sun exposure, especially among young adults.

Once diagnosed, prognosis and therapy are bummed by a series of clinical-pathological risk factors, such as: tumour thickness, sentinel lymph node condition, ulceration and mitotic rate.

From the first publications made by Breslow and Clark in the 1960s and 1970s, knowledge of prognostic factors in MM has increased dramatically, with the most relevant data emerging mainly in the last decade. Although the high mortality rate still associated with MM may suggest that progress has been lacking, yet an effective therapeutic objective is much closer than before. Such progress is commendable in view of the almost complete absence of any effective treatment until recently.

Despite some new promising agents, there are still no therapeutic strategies embracing the complexity of the pathways leading to increased virulence in MM. Taking into account several multimarker tests, using *in vivo* samples and MM primary and metastatic cell cultures all together, MM development involves several hundred genes that are too

numerous to be individualized for a single-patient targeted therapy, even if new genetic markers have been identified and are currently being tested.

The emergence of resistance, undesirable side effects and rapid tumour progression after a treatment-positive initial response increase the importance of a rigorous genotypic classification and focused synergic therapy. Therefore, it is necessary to develop a hierarchy of oncogenes, differentiating between the main and the secondary ones.

PERSONAL CONTRIBUTIONS

1. MOTIVATION, PURPOSE AND OBJECTIVES OF THE RESEARCH

In this paper, we have proposed a detailed clinical, histological and immunohistochemical retrospective study of 110 patients admitted to Craiova County Emergency Clinical Hospital with the diagnosis of cutaneous malignant melanoma for a period of five years (2012-2016).

The **purpose** of this study was to highlight the importance of clinical and morphological parameters and immunohistochemical markers (CD 117, 4-HNE and Ki-67) with tumourigenic, prognostic and therapeutic implications in cutaneous malignant melanoma.

In terms of cancer in general, reactive oxygen species have been extensively studied in a wide range of human cancers, but are poorly analyzed in cutaneous malignant melanomas, especially in terms of the 4-hydroxynonenal (4-HNE) produced by lipid peroxidation, which is why we chose to introduce this marker in this paper, as a **novelty element** in these neoplasia, as well as in our study. In addition, comparative analyzes of 4-HNE expression in human benign and malignant (primary or metastatic) melanocytic lesions have not been conducted until the present research.

2. CLINICAL STUDY OF CUTANEOUS MALIGNANT MELANOMA

It took into account the following **objectives**:

1. The determination of the distribution of cases studied according to clinical prognostic factors, such as sex, age, patient's origin, location and oldness of cutaneous lesions, and the correlations between them;
2. The prevalence of clinical forms of malignant melanoma in our geographical region and their correlation with the clinical parameters;
3. The rate of exposure to UV radiation - as a key risk factor in triggering the malignant process and the distribution of cases according to the patients' phototype;
4. Demonstrating the importance of traumatizing the lesion in malignant transformation and the presence of the nebulous structures as precursors to the occurrence of melanoma;
5. The correlation of metastasis with clinical parameters such as sex, age and location of the lesion.

Material and methods. For each of the 110 cases studied, an individual sheet was drawn up, mentioning, besides the personal data, namely the age, sex, profession, the environment of origin, also a series of other observations regarding: clinical diagnosis, MM location, oldness of the lesion, size, phototype, exposure to UV radiation, MM developed on pre-existing naevus, the presence of trauma as a risk factor, the association with other cutaneous lesions, dermoscopic aspects.

Data was processed statistically using Microsoft Excel for descriptive statistics and SPSS for inferential statistics.

The dermoscopic examination was performed with a PhotoFinder dermoscope and another MoleMax HD 1.5 dermoscope in 19 patients, of which 10 cases from the study sample and 9 cases from a comparative sample.

Results and discussions. Most cases were encountered in 2012, 27%, which could be correlated with excessive UV radiation due to solar explosions at that time. Most of the cases studied were classified in phototype II (56%) and admitted the prior excessive exposure to UV radiation (67%), the main risk factor in the occurrence of MM. Another risk factor associated with exposure to UV was traumatizing the lesions, particularly the naevi (58%).

69% of all patients came from urban areas, as patient addressability and accessibility of medical services is significantly higher than in rural ones.

It is worth pointing out that most malignant melanomas (75%) developed *de novo* and not on pre-existing nevi, according to specialist statistics.

In the studied cases, we have encountered the following clinical subtypes of cutaneous MM, namely: superficial spreading melanoma (49%), nodular melanoma (35%), acral lentiginous melanoma (6%), amelanotic melanoma (6%), lentigo maligna melanoma (3%) and a particular case of melanoma on blue naevus (1%).

The average age of cutaneous MM in the group we studied was 62 years, with the observation that 22% of patients were under the age of 50, and until the age of 30 only women were affected (3%). This finding could justify ozone depletion, increased UV rays harmfulness, and young people's tendency to excessively exposing themselves to the sun or artificial tanning booths.

We found that superficial spreading melanoma was more frequent in both women and men in the 51-60 years age group. Regarding nodular melanoma, it was more frequent in the 71-80 years age group in males, while in females, the age group where we encountered most cases of nodular melanoma was 51-60 years. The two clinical forms, namely lentigo maligna melanoma and acral lentiginous melanoma, did not occur until the age of 50.

As for the cutaneous MM sex distribution, our data show an approximately equal incidence, with a discrete predominance of female sex, i.e. 52% versus 48% for male sex. In terms of clinical subtypes of melanoma, superficial spreading melanoma predominated in females, while nodular melanoma had an equal prevalence.

Most MM were localized in the trunk area (50%), followed by lower limbs (23%) and head region (21%). These locations were closely correlated with the patients' profession, which involved prolonged exposure to sunlight (head and trunk) as well as repeated trauma (lower limbs).

The studied melanomas were most frequently associated with lesions such as dysplastic nevi (12%) in nodular melanomas and facial actinic keratoses (10%) in superficial spreading melanomas. We also noticed that the majority of lesions were up to 1 year old and smaller than 1 cm^2, indicating that more than half of the patients came to the doctor shortly after the tumour occurrence.

From the metastasis correlation with clinical parameters, we found that in the 61-70 years age group there is the highest risk of metastasis, with the male sex and the trunk-localized MM being more affected.

In our study, the dermoscopic examination was performed in 19 patients, of which 10 cases of primary cutaneous MM were part of the study group. The other 9 cases, 8 nevi (nevocellular, congenital, dysplastic, blue) and an *in situ* melanoma, constituted for this study a comparative sample, in order to highlight the characteristic dermoscopic features of cutaneous benign pigmented lesions compared to malignant ones, dermoscopy having an essential role in the early detection of melanocytic lesions with malignant transformation potential.

3. HISTOPATHOLOGICAL STUDY AND MORPHOCLINICAL CORRELATIONS

Objectives. This study aimed at establishing the correlations between a series of histopathological parameters with prognostic implications in patients with melanoma:

1. histopathological subtype of melanoma;
2. Clark's level of invasion;
3. Breslow's thickness;
4. pTNM stage at the time of diagnosis;
5. presence/absence of ulceration;
6. presence/absence of tumour necrosis;
7. intensity of inflammatory infiltrate in intra- and peritumoural area;
8. local-regional or distant metastases occurring during the course of the study.

Material and methods. Of the 110 cases of MM analysed, 20 cases (18.18%) presented local-regional or distant metastases, either at the time of the diagnosis, or occurred within the 5-year period (2012-2016) in which this study was conducted. All of these metastases were confirmed by histopathological examination and included in the study.

Tissues from primary tumours and metastases were processed using the usual histological paraffin embedding technique and standard Hemalaum-Eosin staining.

The statistical analysis method used average values and confidence intervals, as well as comparative tests for the formed groups, using the SPSS10 software. The Chi square test was used to interpret incidence tables.

Results and discussions. The main four histopathological subtypes of cutaneous MM respect the standard prevalence of these tumours in the caucasian population with the exception of acral lentiginous melanoma, an aggressive form of cutaneous MM, which was more frequent, compared to the lentigo maligna melanoma, and has metastasized in over half of the cases (57.4% of cases).

Cutaneous MM metastases are more frequent in the regional lymph nodes (75%), followed by the distant cutaneous metastases (15%) and then by the satellite and visceral ones.

The histopathological subtype of nodular MM correlates significantly with metastases (p=0.0032), probably due to the higher thickness index than in any other histopathological subtype.

The overwhelming majority of patients were diagnosed with a Clark's level of invasion over III (98 cases, 89.1%) and a Breslow's thickness over 2 mm (74 cases, 62.27%), most likely due to the delayed visit at the specialist physician.

There are no significant correlations of Clark's level of invasion with cutaneous MM metastases (p=0.303), but metastases are more frequent as this level of invasion increases. Instead, the Breslow's thickness can accurately predict the risk of lymph node metastasis,

melanomas with Breslow's thickness over 2 mm correlating statistically significantly (p=0.0034) with the development of metastases.

Metastases occurred more frequently in cases of ulcerated tumours (70% of metastases developed in cases of ulcerative tumours) compared to those without ulceration (30%), the difference was not statistically significant (p=0.922), but ulceration in MM could reflect rapid tumour growth.

The occurrence of metastases correlated highly significantly statistically (p=0.00412) with the existence of tumour necrosis areas in primary tumours, the metastases being 4.8 times more frequent in patients with tumour necrosis in primary MM (OR=4.846) compared to those who have not been identified with necrosis outbreaks. Thus, it seems that necrosis may have a greater influence on prognosis compared to ulceration.

The analysis of the prevalence of metastases depending on the intensity of the peri- and intratumoural infiltrate reveals that the higher the intensity of the inflammatory infiltrate, the lower the prevalence of metastases, without a statistically significant correlation (p=0.269), suggesting that the way in which the host generates a defence reaction (inflammatory infiltrate) in the primary tumour could be investigated and used to develop metastatic melanoma therapies.

Metastases are significantly associated with stages ≥ II (p=0.00594) suggesting the major importance of diagnosing melanoma in incipient stages, 0 or I.

4. IMMUNOHISTOCHEMICAL STUDY

Objectives. The study of immunohistochemical markers (CD 117, 4-HNE, Ki-67) that assess tumour progression and prognosis. Comparative analyses of the lipid peroxidation product's expression, 4-HNE, in benign and malignant (primary or metastatic) melanocytic lesions have not been performed so far, constituting the **element of originality** of the paper.

Material and methods. The immunohistochemical study was performed on a number of 55 cases, represented by a control group that included 5 cases of simple nevi and 5 cases of nevi with dysplastic lesions, as well as a study group consisting of 35 cases of primary MM and 10 metastases (one intestinal, 3 cutaneous - one satellite and two remote, as well as 6 in the lymph nodes). The study group was selected from the 110 cases of MM, clinically and morphologically analyzed, and included 15 cases of superficial spreading melanoma, 10 cases of nodular MM, 3 cases of lentigo maligna melanoma, 3 cases of acral lentiginous melanoma and 4 cases of amelanotic MM. The immunohistochemical study method used to identify the epitopes of interest was two-time, polymer-specific, with high sensitivity, high specificity and high affinity.

The methods of statistical analysis used averages and confidence intervals, as well as comparative tests (Chi square) for the formed groups, using the SPSS10 software.

Results and discussions. CD 117 (c-kit) is strongly involved in the cutaneous MM tumourigenesis process, being immunohistochemically undetectable in benign nevi lesions, but intensely expressed in dysplastic lesions (dysplastic nevi) and the melanoma *in situ* areas.

In invasive cutaneous MM, CD 117 expression tends to decrease as neoplasia progresses and passes into the tumourigenic, vertical growth phase, being more reduced in the profound dermal component of tumours and in nodular MM.

To get rid of the epidermal barriers and gain a proliferative advantage to allow the vertical phase of growth, it seems that malignant melanoma should lose the c-kit expression.

Cutaneous MM metastases express CD 117 at a level comparable to their primary tumours, suggesting that other mechanisms directly interfere with the metastasis process and not the loss of the c-kit expression by itself.

The CD 117 overexpression in cutaneous melanocytic lesions (≥10% of tumour cells) correlates significantly with an increased intensity of the immunostaining (+2/+3), suggesting that the immunohistochemical assessment of CD 117 may be a good method for the screening of patients, which would benefit from a personalized therapy with tyrosine kinase inhibitors.

The 4-HNE expression, a lipid peroxidation product, due to oxidative stress, is significantly increased in dysplastic nevi compared to common (benign) nevi ($p<0.05$) and is maintained at a level comparable to that of the dysplastic lesions in cutaneous MM ($p>0.05$).

4-HNE occurs early in the process of tumourigenesis of cutaneous MM, overexpressed strongly, once melanocytes have undergone dysplastic modifications. Metastases lose the 4-HNE lipid peroxidation product, correlated with increased proliferative activity detected in cutaneous MM metastases.

There are significant differences in the proliferative rate, determined immunohistochemically by ki-67, between common and dysplastic nevi, as well as between dysplastic cutaneous nevi and malignant melanomas, the growth fraction being an indicator of neoplastic progression in cutaneous melanocytic lesions. At the same time, the immunostaining of ki-67 plays a major diagnosis role in cutaneous melanocytic lesions, contributing significantly to the distinction between benign, dysplastic and malignant lesions.

The pre-therapeutic assessment of the Ki-67 expression is becoming increasingly important in assessing tumour aggressiveness and establishing appropriate treatment, ki-67 being a robust marker and a strong prognostic marker in cutaneous MM that could allow selection of patients with high tumour proliferating rate which require a differentiated therapy.

In addition to its diagnostic and prognostic role, Ki-67 is also a promising candidate for new cutaneous MM therapies to possibly involve antisense oligonucleotide (ki-67-ASO) systems, anti-ki-67 peptide nucleic acids (anti-ki 67 PNA), oncolytic-RNA adenovirus systems or anti-ki-67 antibodies that have already been studied and applied to other types of human cancer.

FINAL CONCLUSIONS

1. The main risk factor in the occurrence of malignant melanoma in the group we studied was the excessive exposure to **UV radiation** in the past; until the age of 30, only women were affected as a result of the tendency to use artificial tanning booths; another risk factor associated with UV exposure was traumatizing the lesions, especially the nevi;
2. Most cases were malignant melanoma arising *de novo*, affecting in particular **women** at an average age of **62 years**, and the most frequent clinical subtype was the **superficial spreading melanoma**, predominantly located on the **trunk**;
3. As a negative prognostic factor in malignant melanoma, **metastases** occupy the first place, the most frequent being in regional lymph nodes. The subtype of nodular malignant melanoma is significantly correlated with metastases;
4. The prognosis of primary cutaneous malignant melanoma and, consequently, the therapeutic conduct, are marked by a series of **morphoclinical parameters**, among

which the most important are: the Breslow's thickness, the presence of tumour necrosis, the stage of melanoma at the time of the diagnosis and the histopathological subtype;

5. The **oxidative stress** is involved in the initiation and progression of cutaneous melanomas through lipid peroxidation products, such as bioactive alkenes, like 4-hydroxynonenal (4-HNE) generated at mitochondrial level, which causes the metabolic reprogramming of cells. Handling the generation of reactive oxygen species can be a viable approach to prevent and treat malignant melanoma in the future.
6. **CD 117** (c-kit), **4-HNE** and **Ki-67** are strongly involved markers in the tumourigenesis, diagnosis and prognosis of cutaneous malignant melanoma, and may be a significant new generation of therapeutic agents.

ACKNOWLEDGEMENT

This paper was published under the frame of the project "Excellence programme for the multidisciplinary doctoral and postdoctoral research in chronic diseases", grant no. POSDRU/159/1.5/S/133377, co-financed from the European Social Fund through the Sectorial Operational Programme for Human Resources Development 2007-2013.

SELECTIVE REFERENCES

1. Aldini G., Carini M., Yeum K.-J., Vistoli G., Novel Molecular Approaches for Improving Enzymatic and Nonenzymatic Detoxification of 4-Hydroxynonenal: Toward the Discovery of a Novel Class of Bioactive Compounds, Free Radical Biology and Medicine, 69(0), 145–156, 2014
2. Alecu M., Coman G., Coman A.O. et al., Biomarkeri pentru aprecierea prognosticului în melanomul malign [Biomarkers for the Assessment of Prognosis in Malignant Melanoma], Dermatovenerol., Bucharest, 57, 155-168, 2012
3. Bailey E.C., Sober A.J., Tsao H., Chapter 124. „Cutaneous Melanoma" in Goldsmith L.A., Katz S.I., Gilchrest B.A. et al., Fitzpatrick's Dermatology in General Medicine, 8th Edition, volume 1, McGrawHill Medical, 2012
4. Baroudjian B., Pagès C., Lebbé C., Melanoma, from Diagnosis to Treatment, Rev. Infirm., (219):16-8, 2016
5. Barrera G., Pizzimenti S., Ciamporcero E.S., Daga M., Ullio C., Arcaro A. et al., Role of 4-Hydroxynonenal-Protein Adducts in Human Diseases, Antioxidants & Redox Signaling, 2014
6. Bellot G.L., Liu D., Pervaiz S., ROS, Autophagy, Mitochondria and Cancer: Ras, the Hidden Master?, Mitochondrion, 13(3), 155–162, 2013
7. Berwick M., Buller D.B., Cust A. et al., Melanoma Epidemiology and Prevention, Cancer Treat. Res., 167:17-49, 2016
8. Borgenvik T.L., Karlsvik T.M., Ray S. et al., Blue Nevus-Like and Blue Nevus-Associated Melanoma: A Comprehensive Review of the Literature, ANZ J. Surg., 87(5):345-349, 2017
9. Cosgarea R., Stăniceanu F., Zurac S., Baican C.I. et al., Chapter 12 "Melanomul [Melanoma]" in Boda D. (coord.), Dermato-Oncology Treaty, Carol Davila Universitz Publishing House, Bucharest, 593-696, 2012
10. Fernandez-Flores A., Prognostic Factors for Melanoma Progression and Metastasis: from Hematoxylin-Eosin to Genetics, Rom. J. Morphol. Embriol., 53(3), 449-459, 2012

11. Ilie M.A., Boda D., Zurac S.A. et al., Microscopia confocală in vivo – o fereastră deschisă spre microstructura pielii [In Vivo Confocal Microscopy - An Open Window to the Microstructure of the Skin], in Brănişteanu D.E., Patologia Dermatologică la graniţa dintre specialităţi [Dermatological Pathology at the Border Between Specialties], Junimea Publishing House, Iaşi, 26-34, 2017
12. Kruijff S., Hoekstra H.J., The Current Status of S-100B as a Biomarker in Melanoma, Eur. J. Surg. Oncol., 38(4), 281-285, 2012
13. Ladstein R.G., Bachmann I.M., Straume O. et al., Tumour Necrosis is a Prognostic Factor in Thick Cutaneous Melanoma, American Journal of Surgical Pathology, 36(10), 1477–1482, 2012
14. Li L.T., Jiang G., Chen Q., Zheng J.N., Ki-67 is a Promising Molecular Target in the Diagnosis of Cancer (Review), Molecular Medicine Reports, 11:1566-1572, 2015
15. Maranduca M.A., Şerban I.L., "Fiziologia pielii [Skin Physiology]" in Brănişteanu D.E., Patologia Dermatologică la graniţa dintre specialităţi [Dermatological Pathology at the Border Between Specialties], Junimea Publishing House, Iaşi, 1-16, 2017
16. Minocha R., Damian D.L., Halliday D.M., Melanoma and Non-Melanoma Skin Cancer Chemoprevention: A Role for Nicotinamide?, Photodermatol. Photoimmunol. Photomed., 1-8, 2017
17. Nandy A., Sumit K.D., Sujata D. et al., Gold (I) N-Heterocyclic Carbene Complex Inhibits Mouse Melanoma Growth by p53 Upregulation, Molecular Cancer, 13(57), 1-14, 2014
18. Nichols E.E., Richmond A., Daniels A.B., Tumour Characteristics, Genetics, Management, and the Risk of Metastasis in Uveal Melanoma, Semin. Ophthalmol., 31(4):304-9, 2016
19. Nielsen P.S., Riber‑Hansen R., Jensen T.O. et al., Proliferation Indices of Phosphor-Histone H3 and Ki-67: Strong Prognostic Markers in a Consecutive Cohort with Stage I/II Melanoma, Mod. Pathol., 26(3):404-13, 2013
20. Pierard G.E., Cell Proliferation in Cutaneous Malignant Melanoma: Relationship with Neoplastic Progression, International Scholarly Research Network, ISRN Dermatology, 2012:1-12, 2012
21. Pierard G.E., Piérard-Franchimont C., Delvenne P., Simulants of Malignant Melanoma, Oncology Reviews, vol. 9:278, 2015
22. Pizzimenti S., Ciamporcero E., Pettazzoni P. et al., The Inclusion Complex of 4-Hydroxynonenal with a Polymeric Derivative of β-Cyclodextrin Enhances the Antitumoural Efficacy of the Aldehyde in Several Tumour Cell Lines and in a Three-Dimensional Human Melanoma Model, Free Radic. Biol. Med., 65:765-77, 2013
23. Pung P.P., Sarlomo-Rikala M., La Sota J. et al., Immunohistochemical Analysis of 1563 Tumours Including Metastatic, Desmoplastic and Mucosal Melanomas and their Mimics. Am.J.of Surg. Pathol., 36(2), 265-292, 2012
24. Reginster M.A., Pierard-Franchimont C., Pierard G.E. et al., Molecular Dermatopathology in Malignant Melanoma, Dermatology Research and Practice, Article ID 684032, page 6, 2012
25. Ribero S., Longo C., Glass D. et al., What Is New in Melanoma Genetics and Treatment?, Dermatology; 232:259–264, 2016
26. Shoeb M., Ansari N.H., Srivastava S.K. et al., 4-Hydroxynonenal in the Pathogenesis and Progression of Human Diseases, Curr. Med. Chem., 21(2), 230–237, 2014
27. Thomas L., Prevention, Early Detection and Monitoring of Cutaneous Melanoma, Rev. Infirm., (219):19-22, 2016

28. Vogt N., Klapper W., Variability in Morphology and Cell Proliferation in Sequential Biopsies of Mantle Cell Lymphoma at Diagnosis and Relapse: Clinical Correlation and Insights Into Disease Progression, Histopathology, 62:334-342, 2013
29. Xavier J.C.C., Munhoz Souza V., de Campos E.B.P. et al., Focal Invasiveness in Complete Histological Analyses of a Large Acral Lentiginous Melanoma, Diagnostic Pathology, 10:73-77, 2015
30. Zhong H., Yin H., Role of Lipid Peroxidation Derived 4-Hydroxynonenal (4-HNE) in Cancer: Focusing on Mitochondria, Redox Biol., 4, 193–199, 2015