Review Article
From Melanocyte to Metastatic Malignant Melanoma

Bizhan Bandarchi,1 Linglei Ma,2 Roya Navab,1 Arun Seth,3 and Golnar Rasty4

1 Department of Applied Molecular Oncology, Princess Margaret Hospital, Ontario Cancer Institute, University of Toronto, 7 Yorkview Drive, Toronto, ON, Canada M2N 2R9
2 Department of Pathology, University of Michigan Hospital, University of Michigan, Michigan 48109-0602, USA
3 Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada M4N 3M5
4 Department of Laboratory Medicine and Pathobiology, University Health Network, University of Toronto, Toronto, Canada MSG 2C4

Correspondence should be addressed to Bizhan Bandarchi, bizhanb@yahoo.com

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Malignant melanoma is one of the most aggressive malignancies in human and is responsible for almost 60% of lethal skin tumors. Its incidence has been increasing in white population in the past two decades. There is a complex interaction of environmental (exogenous) and endogenous, including genetic, risk factors in developing malignant melanoma. 8–12% of familial melanomas occur in a familial setting related to mutation of the CDKN2A gene that encodes p16. The aim of this is to briefly review the microanatomy and physiology of the melanocytes, epidemiology, risk factors, clinical presentation, historical classification and histopathology and, more in details, the most recent discoveries in biology and genetics of malignant melanoma. At the end, the final version of 2009 AJCC malignant melanoma staging and classification is presented.

1. Introduction

The epidermis contains two types of dendritic cells, besides keratinocytes, that could mimic each other: Langerhans’ cells and melanocytes. Langerhans’ cells are dendritic antigen processing cells and hence play a primary role in cellular response to tumor antigens, skin graft rejection, and microorganism [1, 2]. Langerhans’ cells are located in the suprabasal layer of the epidermis, a feature differentiating them from melanocytes on routine H&E stain. Melanocytes originate from the neural crest and in contrast to Langerhans’ cells are located amongst the basal layer of the epidermis, hair bulb, eyes, ears, and meninges [3–5]. Melanocyte migration to the epidermis, function, and its survival are all dependant on expression of the tyrosine kinase receptor c-kit gene [4, 6]. The pigmentedary system of the skin is a complex set of reactions with many potential sites for dysfunction [7]. Melanin pigment is produced by melanocytes in their specific cytoplasmic organelles called melanosomes. Melanosomes may represent a variant of lysosome [8], in which tyrosinase acts on the substrate tyrosine, resulting in dopa and dopaquinone formation [9, 10]. Melanin pigment synthesized by each melanocyte is transferred to an average of 36 keratinocytes. PAR-2 on the keratinocyte surface is a key receptor in this transfer [11, 12]. The transferred melanin then forms a cap at the top of nucleus of mitotically active basal cells and prevents the ultraviolet injurious effects on nucleus. Melanin stains with Masson-Fontana silver method, based on a positive argentaffin reaction, in which means the melanocytes take up silver and then reduce it to a visible metallic state, without the aid of a reducing agent.

2. Epidemiology

The incidence of malignant melanoma has been increasing in white populations [13–15]. Although malignant melanoma comprises less than 5% of malignant skin tumors; however, it is responsible for almost 60% of lethal skin neoplasia [16]. One of the highest incidence rates is in Queensland, Australia [17]. The incidence of malignant melanoma appears to be lower and stable in darkskin individuals (Africans, Native Americans, Asians, and Hispanics). Decreased incidence reported from some countries is probably partly due to
an influx of low risk immigrants [18–21]. With increased life expectancy of the elderly population, melanoma will be a public health challenge [22]. Increased incidence of melanoma is partly due to early detection (thin melanomas) and partly due to true increase of incidence. Despite the increase in the incidence of melanoma, the prognosis has been improving due to earlier diagnosis of thin melanomas and hence in a curable stage [23–25]. The incidence of melanoma is equal in men and women and uncommon in children although there are reports that the incidence may be higher in women. A typical patient is usually a Caucasian adult in the 4th decade of life with lesion on the back and leg in male and female, respectively. One typical study revealed that the most common sites in decreasing order are the trunk (43.5%), extremities (33.9%), acral sites (11.9%), and head and neck (10.7%) [16].

3. Risk Factors

There is a complex interaction of environmental (exogenous) and endogenous factors. Up to 65% of malignant melanomas are sun-related [26–28]. The role of chronic sun exposure is controversial. Some studies suggested that total accumulated exposure to sun is a very important factor whereas long-term occupational exposure actually may be protective [29, 30]. In either case the general acceptance is that intermittent sun exposure is the most important factor. The list of risk factors in developing malignant melanoma is long and includes pale skin, blond or red hair, numerous freckles and tendency to burn and tan poorly (predominantly skin phototype 1–3) [26–28, 31], presence of more than 50 acquired (common, banal) nevi [32], more than five dysplastic (atypical, Clark’s) nevi, large congenital nevi [33, 34], nevi larger than 6 mm [35], PUVA therapy, tendency to sunburn and tan poorly, use of tanning salons, Xeroderma pigmentosum, immunosuppression, chemical exposures, scars, Marjolin’s ulcer [36–39], and genetic factors. In fact 8%–12% of malignant melanomas occur in a familial setting which may be related to mutations of the CDKN2A gene that encodes p16 and is linked to chromosome 9p21 [40, 41].

4. Clinical Presentation

Typical malignant melanomas usually present as “Malignant Melanoma ABCD”: asymmetry, border irregularity, color variegation, diameter more than 6 mm. However, many exceptions may occur as they may do in other medical disciplines.

5. Diagnosis

Any suspicious pigmented lesions must be biopsied to rule out or rule in melanoma. Even though dermoscopy, even in the hands of a relatively inexpert practitioner, may show high diagnostic accuracy [42] and boost the clinical suspicion in diagnosing malignant melanoma; however, the definitive diagnosis is confirmed done by biopsy.

6. Classification and Histopathology

Historically, malignant melanoma was classified by Wallace Clark and coworkers into superficial spreading type, lentigo malignant type, and nodular type [43, 44]. Later on Dr. Richard Reed added a fourth type called acral lentiginous malignant melanoma [45]. Since then the classification of malignant melanoma with their relative incidences has been as follows: superficial spreading melanoma (50%–75%), nodular melanoma (15%–35%), lentigo maligna melanoma (5%–15%), acral lentiginous melanoma (5%–10%), desmoplastic melanoma (uncommon), miscellaneous group (Rare).

Melanoma presents three clinically and histomorphologically discernable steps in tumor progression [46].

(1) Malignant Melanoma confined to the epidermis (melanoma in situ), which is called Radial Growth Phase- (RPG-) confined melanoma.

(2) Radial Growth Phase (RGP)-confined microinvasive, which shows some malignant cells present in superficial papillary dermis.

(3) Vertical Growth Phase (VGP), which means melanoma, has entered the tumorigenic and/or mitogenic phase (usually Clark’s level II and occasionally Clark’s level III).

The importance of RPG is best demonstrated by the Taran and Heenan study, which showed development of metastatic melanoma in only 5 of 1716 patients with level 2 melanomas (= 1 mm thick) in 7 to 14 years followup [47]. Those 5 cases with metastatic melanomas revealed regression.

6.1. Superficial Spreading Melanoma (SSM). SSM is the most common melanoma that can occur at any site and at any age [48]. About 75% of SSMs occur de novo. The classic lesions show variation in pigmentation and pagetoid spread of melanoma cell in epidermis.

6.2. Nodular Melanoma (NM). NM melanoma by definition has no radial growth phase and could be nodular, polyoid, or pedunculated [49, 50].

6.3. Lentigo Maligna Melanoma (LMM). This variant occurs on the sun-exposed skin, face, and upper extremities of elderly patients [51]. Lentigo maligna (also called Hutchinson freckle) is basically in situ melanoma and is characterized by epidermal atrophy, extensive solar, lentiginous, and back-to-back proliferation of melanoma cells with nest formation with extension into cutaneous adnexa. Only 5% of patients with lentigo maligna progress to lentigo maligna melanoma, and it usually takes several years [52]. Several methods of therapy can be used to treat lentigo maligna including cryotherapy, superficial radiation, and surgical excision with mapping and modified Mohs’ surgery [53–55].

6.4. Acral Lentiginous Melanoma (ALM). ALM is common on palmar, plantar, and ungual skin of Black and Japanese
people [56]. Ulcerate and melanonychia striata may occur. Although this type of melanoma is common in the above-mentioned locations, other types of malignant melanoma may still develop at the same location [57]. Most of the mucosal melanomas including oral cavity, vulva vagina, and cervix uteri follow the histological features of acral lentiginous melanomas [58, 59].

7. Rare Variants

There are rare variants of malignant melanoma that do not show the typical classical histopathology. Amongst these variants, Desmoplastic Melanoma (DM)/Neurotropic Melanoma is worth mentioning more in detail since it could easily be misdiagnosed as fibroblastic proliferation and scar. This variant usually presents as indurated plaque or bulky tumor on the head and neck location [60] and is characterized by paucicellular proliferation of atypical dermal spindle melanocytes, dermal collections of lymphocytes with overlying epidermis commonly showing lentigo maligna [61]. This variant also commonly shows neurotropism. The dermal component of desmoplastic melanoma is usually negative for Melan A (or Mart1) and HMB45. These two immunostains, however, highlight the presence of Lentigo maligna in situ. Both the epidermal component and dermal atypical spindle melanocytes are positive for S100 immunostain. Other rare variants include nevoid melanoma [62, 63], verrucous melanoma, small cell melanoma, signet ring melanoma, myxoid melanoma, osteogenic melanoma of the finger, animal (Equine/pigment synthesizing) melanoma, myxoid melanoma, excongenital nevus, and minimal deviation malignant melanoma. Minimal deviation subtype is characterized by uniform proliferation of melanocytes that show minimal cytomorphological atypia [64].

8. Prognostic Factors in Melanoma

There are three classes of adverse prognostic factors in melanoma: pathological, clinical, and other factors including genetic alteration. The first group includes increasing the Breslow thickness [65], ulceration, mitotic rate, Clark level [44], absent or nonbrisk tumor infiltrating lymphocytes [66], regression [67, 68], microscopic satellites [69], lymphovascular invasion [70], angiotropism, tumor volume, neurotropism, cell type, local recurrence, histopathologic subtype, and presence of vertical growth phase. Clinical adverse factors include increasing age, male, location of the lesion, and metastasis.

9. The Biology and Genetics of Malignant Melanoma

Two genes have been discovered in melanoma families: CDKN2A (p16) on chromosome 9p21 and CDK4 on chromosome 12 [70, 71]. The CDKN2A gene acts as a tumor suppressor gene and plays a crucial role in cell cycle regulation and senescence. Mutations of the CDKN2A gene confer susceptibility to familial melanoma. Partial or complete loss of p16 expression has also been identified in sporadic melanomas. Other genes, such as MC1R (Melanocortin 1 Receptor) and DNA repair genes, are likely to be more important in determining susceptibility for melanoma in the general population [72]. Although nevi and melanomas share initiating genetic alterations such as oncogenic mutations in BRAF and NRAS, melanomas often show recurrent patterns of chromosomal aberrations such as losses of chromosomes 6q, 8p, 9p, and 10q along with gains of chromosomes 1q, 6p, 7, 8q, 17q, and 20q, while benign nevi tend to have no detectable chromosomal aberrations by comparative genomic hybridization (CGH) or karyotyping [73–75]. Recently, a fluorescence in situ hybridization- (FISH-) based test, using a combination of 4 FISH probes targeting 3 loci on chromosome 6 (RREB1 and MYB genes) and 1 on chromosome 11 (Cyclin D1 gene), was developed [76, 77]. The method is applicable to formalin-fixed paraffin-embedded tissue and has the most powerful discriminatory ability between melanoma and nevi.

Metastatic melanoma is an incurable disease with high mortality rate. Patients with metastatic disease have an average survival of <1 year. This high mortality rate is largely the result of the resistance to chemotherapy and radiotherapy [78, 79]. Transformation of melanocytes to melanoma cells is still largely unclear [78]. A combination of up- or down-regulation of various effectors acting on different molecular pathways appears to be involved in progression of normal melanocyte to metastatic malignant cells [80]. Numerous studies using tissue specimens, cell lines, and xenografts to discover the mechanism(s) behind this transformation, invasiveness, and metastasis are in progress.

Alteration of cell cycle proteins (e.g., cyclin D1, pRb, and p16) has a role in transformation and progression in melanocytic tumors. It has been shown that progressive loss of p16 can be seen in transformation of benign nevi to melanoma and to metastatic melanoma. Progressive increase in expression of cyclin D1 and pRb is associated with progression to melanoma cells; however, cyclin D1 and pRb show relative decrease in thick melanoma and metastatic melanoma [81].

Higher expression of PAR-1 (protease-activated receptor-1) is seen in melanoma cell lines and tissue specimens. Upregulation of PAR-1 mediates high levels of Cx-43 (gap junctional intracellular communication molecule connexin) expression. This molecule is involved in tumor cell diapedesis and attachment to endothelial cells [82]. Type I collagenase and PAR-1 activating functions of MMP-1 (matrix metalloproteinase-1) are required for melanoma progression. Highly expressed MMP-1 is suggested to be involved in progression of noninvasive melanoma to invasive vertical growth phase by degrading type I collagen of skin [83].

Protein Kinase C (PKC) mediates signals for cell growth and is a target of tumor-promoting phorbol esters in malignant transformation [84].

Downregulation of E-cadherin and upregulation of N-cadherin may be seen in melanoma cells. Such shift of cadherin profile may have a role in uncontrolled proliferation, invasion, and migration [85].
S100A1, S100B, Bcl-2, and CD44 have been described in transformation of melanocytes to melanoma cells. S100A1 expression is increased in contrast to S100B, which shows higher expression in benign nevi. The Sviatoha et al. demonstrated studies of a higher expression of CD44 antigen in melanomas with known metastases than in those without metastases, but this difference was not statistically significant [86]. Interaction of the transcription factor E2F-1 with RGFR can act as driving force in melanoma progression [87].

The studies of Mehnert et al., based on the fact that angiogenesis is one of the factors required for progression and melanoma metastasis, demonstrated that vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1, VEGF-R2, and VEGF-R3) are higher in melanomas and advanced melanomas than in benign nevi. VEGF-R2 shows higher expression of VEGF-R2 in metastatic melanomas than in primary melanoma [88].

Using immunohistochemistry on human tissue, it has been shown that there is significantly higher cortactin (a multidomain actin-binding protein important for the function of cytoskeleton) expression in melanomas than in nevi and higher expression in metastatic melanoma than in invasive primary melanomas [89]. MHC (major histocompatibility complex) molecule overexpression in earlier stages of melanoma and downregulation in metastatic malignant melanoma have been observed [90].

PEDF (pigment epithelium-derived factor) loss appears to be associated with invasive phenotype and malignant progression [91]. Deregulation of microRNAs (miRNAs) using cell lines from primary or metastatic melanoma contributes in formation and progression of melanoma [92]. Melanoma chondroitin sulfate proteoglycan (MCSP) facilitates the growth, motility, and invasiveness of tumor cells. MCSP expression is associated with increased expression of c-Met and HGF. c-Met inhibition limits growth and motility of melanoma cell lines [93]. Up-regulated expression of C-Raf is seen in a subset of melanomas [94].

ATP-binding cassette (ABC) transporters regulate the transport of physiologic substrates. ABC-transporter mRNA expression profile may have some roles in melanoma tumorigenesis [95]. It has been suggested that there is transient upregulation of cDNA clone pCma1 in neoplastic progression of melanocytes [96].

It has been shown that angiogenesis and metastasis can be inhibited by heparin and its derivatives. The study conducted by Kenessey et al. revealed that fragments of heparin, not involved in its haemostatic effect, may have a role in antimigratory and antimitostatic processes [97].

10. Treatment of Malignant Melanoma and Followup

Avoiding sunlight if possible, frequent use of sunscreen and routine checkups in high risk patients are important preventive measures. Sometimes the use of sunscreen is associated with higher incidence of malignant melanoma but in fact this is due to modified sun-exposure behavior [98, 99]. Adequate clear resection margins handling of the reexcision specimens and sentinel lymph node biopsies are important factors in management of malignant melanoma. A variety of protocols for excision of primary cutaneous melanomas exist. One commonly used protocol [100–104] is in situ melanoma: 0.5 cm clear margin, <1 mm: 1 cm clear margin 1–2 mm 1–2 cm clear margin (depending on the location), 2–4 mm 2 cm clear margin, >4 mm 3 cm clear margin. The sentinel lymph node is the first node in the basin of the regional lymph node that picks up the 99mTc and/or blue dye. Sentinel node biopsy has had a significant impact on managing patients of melanoma [105].

Surgical excision, interferon therapy, hypothermic isolated limb perfusion with melphalan, CO2 laser ablation, and intralesional BCG have been used for treatment of in-transit melanoma. In-transit melanoma metastasis is defined by in-transit cutaneous malignant melanoma deposits between the site of excision and the draining lymph nodes [106] more than 2 cm from primary melanoma, which is different from satellite metastasis defined by lesions less than 2 cm from the primary melanoma. Despite of different definitions the biologic behaviour of both is similar and is categorized as intralymphatic metastasis as another criterion in the N category regardless of the number of lesions based on the final version of the 2009 AJCC melanoma staging and classification [107, 108]. Patients with positive nodes or node-negative melanomas thicker than 4 mm, ulceration, or Clark’s level IV or V may benefit of adjuvant therapy. Interferon-alpha 2b is the most commonly used FDA-approved adjuvant therapy [109]. There is no definite proof that longevity of patients is affected by routine laboratory tests such as lactate dehydrogenase (LDH) and/or imaging studies such as CT scan, MRI, and PET scan. There are several guidelines, which recommend limited use of laboratory test and imaging based on the disease stage. Low yield, high rate of false-positive tests, and lack of significant impact of early detection of metastases on survival argue that chest X-ray and serum LDH should probably not be accepted into routine clinical practice in clinically localized melanoma in the absence of data supporting their use [110, 111]. However, patients with higher stage may benefit from these tests.

11. Final Version of 2009 AJCC Melanoma Staging and Classification

In the final version of 2009 AJCC, the 7th edition [107], the mitotic rate per mm2 has been added to staging of melanoma. Mitosis = 1 per mm2 is included in primary criterion for defining T1b melanoma. Immunohistochemical detection of nodal metastasis has also been incorporated and must include at least one melanoma-associated marker (e.g., HMB45, Melan-A, and Mart-1) unless diagnostic cellular morphology is present. In addition there is no lower threshold of staging N disease [107]. The new Melanoma Staging Database clearly demonstrates that an elevated LDH is an independent and highly significant predictor of survival or outcome of stage IV malignant melanoma. LDH is amongst the most predictive independent factors.
of diminished survival when is analyzed in a multivariate analysis [107, 112, 113].

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References

[1] I. R. Williams and T. S. Kupper, “Immunity at the surface: homeostatic mechanisms of the skin immune system,” Life Sciences, vol. 58, no. 18, pp. 1485–1507, 1996.

[2] J. W. Streilein, G. B. Toews, and P. R. Bergstresser, “Corneal allografts fail to express Ia antigens,” Nature, vol. 282, no. 5736, pp. 326–327, 1979.

[3] T. B. Fitzpatrick, “The biology of pigmentation,” Birth Defects Original Article Series, vol. 7, no. 8, pp. 5–12, 1971.

[4] J. J. Nordlund and R. E. Boissy, “The biology of Melanosomes,” in The Biology of the Skin, R. K. Freinkel and D. T. Woodley, Eds., pp. 113–131, Parthenon Publishing, New York, NY, USA, 2001.

[5] A. Haaka and G. A. Scott, “Structure and function of the skin: overview of the epidermis and dermis,” in The Biology of the Skin, R. K. Freinkel and D. T. Woodley, Eds., pp. 19–46, Parthenon Publishing, New York, NY, USA, 2001.

[6] R. A. Fleischman, D. L. Saltman, V. Stastny, and S. Zneimer, “Detection of the c-kit protooncogene in the human developmental defect piebald trait,” Proceedings of the National Academy of Sciences of the United States of America, vol. 88, no. 23, pp. 10885–10889, 1991.

[7] J. M. Grichnik, J. A. Burch, J. Burchette, and C. R. Shea, “The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis,” Journal of Investigative Dermatology, vol. 111, no. 2, pp. 233–238, 1998.

[8] S. J. Orlow, “Melanosomes are specialized members of the lysosomal lineage of organelles,” Journal of Investigative Dermatology, vol. 105, no. 1, pp. 3–7, 1995.

[9] V. J. Hearing and M. Jiménez, “Analysis of mammalian pigmentation at the molecular level,” Pigment Cell Research, vol. 2, no. 2, pp. 75–85, 1989.

[10] J. M. Naeyaert, M. Eller, P. R. Gordon, H.-Y. Park, and B. A. Gilchrest, “Pigment content of cultured human melanocytes does not correlate with tyrosinase message level,” British Journal of Dermatology, vol. 125, no. 4, pp. 297–303, 1991.

[11] J. L. Bolognia and J. M. Pawelek, “Biology of hypopigmentation,” Journal of the American Academy of Dermatology, vol. 19, no. 2, pp. 217–258, 1988.

[12] J. F. Hermans, L. Petit, O. Martalo, C. Piérard-Franchimont, G. Cauwenbergh, and G. E. Piérard, “Unraveling the patterns of subclinical phaeomelanin-enriched facial hyperpigmentation: effect of depigmenting agents,” Dermatology, vol. 201, no. 2, pp. 118–122, 2000.

[13] T.-Y. Chuang, J. Charles, G. T. Reizner, D. J. Elpern, and E. R. Farmer, “Melanoma in Kauai, Hawaii: 1981–1990: the significance of in situ melanoma and the incidence trend,” International Journal of Dermatology, vol. 38, no. 2, pp. 101–107, 1999.

[14] H. I. Hall, D. R. Miller, J. D. Rogers, and B. Beversee, “Update on the incidence and mortality from melanoma in the United States,” Journal of the American Academy of Dermatology, vol. 40, no. 1, pp. 35–42, 1999.

[15] H. J. van der Rhee, L. M. T. van der Spek-Keijser, R. van Westering, and J. W. W. Coebergh, “Increase in and stabilization of incidence and mortality of primary cutaneous malignant melanoma in western Netherlands, 1980–1995,” British Journal of Dermatology, vol. 140, no. 3, pp. 463–467, 1999.

[16] V. Radović-Kovacević, T. Pekmezović, B. Adanja, M. Jarebinski, J. Marinković, and R. Tomin, “Survival analysis in patients with cutaneous malignant melanoma,” Srpski Arhiv Za Celokupno Lekarstvo, vol. 125, no. 5–6, pp. 132–137, 1997.

[17] R. MacLennan, A. C. Green, G. R. C. McLeod, and N. G. Martin, “Increasing incidence of cutaneous melanoma in Queensland, Australia,” Journal of the National Cancer Institute, vol. 84, no. 18, pp. 1427–1432, 1992.

[18] L. K. Dennis, “Analysis of the melanoma epidemic, both apparent and real: data from the 1973 through 1994 surveillance, epidemiology, and end results program registry,” Archives of Dermatology, vol. 135, no. 3, pp. 275–280, 1999.

[19] R. A. Swerlick and S. Chen, “The melanoma epidemic: is increased surveillance the solution or the problem?” Archives of Dermatology, vol. 132, no. 8, pp. 881–884, 1996.

[20] R. A. Swerlick, “The melanoma epidemic: more apparent than real?” Mayo Clinic Proceedings, vol. 72, no. 6, pp. 559–564, 1997.

[21] D. Czarnecki and C. J. Meehan, “Is the incidence of malignant melanoma decreasing in young Australians?” Journal of the American Academy of Dermatology, vol. 42, no. 4, pp. 672–674, 2000.

[22] J. W. Kelly, “Melanoma in the elderly. A neglected public health challenge,” Medical Journal of Australia, vol. 169, no. 8, pp. 403–404, 1998.

[23] R. Shafir, J. Hiss, H. Tsur, and J. J. Bubis, “The thin malignant melanoma. Changing patterns of epidemiology and treatment,” Cancer, vol. 50, no. 4, pp. 817–819, 1982.

[24] R. M. MacKie, “Melanoma and the dermatologist in the third millennium,” Archives of Dermatology, vol. 136, no. 1, pp. 71–73, 2000.

[25] S. M. Richert, F. D’Amico, and A. R. Rhodes, “Cutaneous melanoma: patient surveillance and tumor progression,” Journal of the American Academy of Dermatology, vol. 39, no. 4, pp. 571–577, 1998.

[26] A. Katsambas and E. Nicolaidou, “Cutaneous malignant melanoma and sun exposure: recent developments in epidemiology,” Archives of Dermatology, vol. 132, no. 4, pp. 444–450, 1996.

[27] V. Beral, S. Evans, H. Shaw, and G. Milton, “Cutaneous factors related to the risk of malignant melanoma,” British Journal of Dermatology, vol. 109, no. 2, pp. 165–172, 1983.

[28] D. C. Whiteman and A. C. Green, “Melanoma and sun exposure: where are we now?” International Journal of Dermatology, vol. 38, no. 7, pp. 481–489, 1999.

[29] M. M. Schreiber, T. E. Moon, and P. D. Bozzo, “Chronic solar ultraviolet damage associated with malignant melanoma of the skin,” Journal of the American Academy of Dermatology, vol. 10, no. 5, pp. 755–759, 1984.

[30] A. Green, R. MacLennan, P. Youl, and N. Martin, “Site distribution of cutaneous melanoma in Queensland,” International Journal of Cancer, vol. 53, no. 2, pp. 232–236, 1993.

[31] H. Beitner, U. Ringborg, G. Wennersten, and B. Lagerlof, “Further evidence for increased light sensitivity in patients with malignant melanoma,” British Journal of Dermatology, vol. 104, no. 3, pp. 289–294, 1981.
[32] R. Mikkilineni and M. A. Weinstock, “Is the self-counting of moles a valid method of assessing melanoma risk?” *Archives of Dermatology*, vol. 136, no. 12, pp. 1550–1551, 2000.

[33] J. S. Schneider, D. H. Moore II, and R. W. Sagebiel, “Risk factors for melanoma incidence in prospective follow-up: the importance of atypical (dysplastic) nevi,” *Archives of Dermatology*, vol. 130, no. 8, pp. 1002–1007, 1994.

[34] J. A. Newton, V. Bataille, K. Griffiths et al., “How common is the atypical mole syndrome phenotype in apparently sporadic melanoma?” *Journal of the American Academy of Dermatology*, vol. 29, no. 6, pp. 989–996, 1993.

[35] L. Naldi, G. L. Imberti, F. Parazzini et al., “ Pigmentary traits, modalities of sun reaction, history of sunburns, and melanocytic nevi as risk factors for cutaneous malignant melanoma in the Italian population: results of a collaborative case-control study,” *Cancer*, vol. 88, no. 12, pp. 2703–2710, 2000.

[36] J. Y.-Y. Lee, S. B. Kapadia, R. H. Musgrave, and W. J. Futrell, “Neutrotropic malignant melanoma occurring in a stable burn scar,” *Journal of Cutaneous Pathology*, vol. 19, no. 2, pp. 145–150, 1992.

[37] P. F. Rockley, N. Triefff, R. F. Wagner Jr., and S. K. Tyring, “Nonsunlight risk factors for malignant melanoma part I: chemical agents, physical conditions, and occupation,” *International Journal of Dermatology*, vol. 33, no. 6, pp. 398–406, 1994.

[38] B. S. Gan, R. G. Colcleugh, C. G. Scilley, and I. D. Craig, “Melanoma arising in a chronic (Marjolin’s) ulcer,” *Journal of the American Academy of Dermatology*, vol. 32, no. 6, pp. 1058–1059, 1995.

[39] T. Merkle, M. Landthaler, F. Eckert, and O. Braun-Falco, “Acral verrucous malignant melanoma in an immunosuppressed patient after kidney transplantation,” *Journal of the American Academy of Dermatology*, vol. 24, no. 3, pp. 505–506, 1991.

[40] F. Grange, A. Chompret, M. Guilloud-Bataille et al., “Comparison between familial and nonfamilial melanoma in France,” *Archives of Dermatology*, vol. 131, no. 10, pp. 1154–1159, 1995.

[41] M. H. Greene, L. R. Goldin, and W. H. Clark Jr., “Familial cutaneous malignant melanoma: autosomal dominant trait possibly linked to the Rh locus,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 80, no. 19, pp. 6071–6075, 1983.

[42] C. Doliaditis, J. Kelly, R. Wolfe, and P. Simpson, “Comparative performance of 4 dermoscopic algorithms by nonexperts for the diagnosis of melanocytic lesions,” *Archives of Dermatology*, vol. 141, no. 8, pp. 1008–1014, 2005.

[43] W. H. Clark Jr., “A classification of malignant melanoma in man correlated with histogenesis and biological behaviour,” in *Advances in the Biology of the Skin*, Vol III, W. Montagna and E. Hu, Eds., pp. 621–647, Pergamon, New York, NY, USA, 1967.

[44] W. H. Clark Jr., L. From, E. A. Bernardino, and M. C. Mihm, “The histogenesis and biologic behavior of primary human malignant melanomas of the skin,” *Cancer Research*, vol. 29, no. 3, pp. 705–727, 1969.

[45] R. J. Reed, “Acral lentiginous melanoma,” in *New Concepts in Surgical Pathology of the Skin*, pp. 89–90, Wiley, New York, NY, USA, 1976.

[46] D.-P. Guerry IV, M. Synnestvedt, D. E. Elder, and D. Schultz, “Lessons from tumor progression: the invasive radial growth phase of melanoma is common, incapable of metastasis, and indolent,” *Journal of Investigative Dermatology*, vol. 100, no. 3, pp. 3428–3455, 1993.

[47] J. M. Taran and P. J. Heenan, “Clinical and histologic features of level 2 cutaneous malignant melanoma associated with metastasis,” *Cancer*, vol. 91, no. 9, pp. 1822–1825, 2001.

[48] T. Demitsu, H. Nagato, K. Nishimaki et al., “Melanoma in situ of the penis,” *Journal of the American Academy of Dermatology*, vol. 42, no. 2, pp. 386–388, 2000.

[49] H. Plotnick, N. Rachmaninoff, and H. J. VandenBerg Jr., “Polypoid melanoma: a virulent variant of nodular melanoma,” *Journal of the American Academy of Dermatology*, vol. 23, no. 5, pp. 880–884, 1990.

[50] P. Kiene, C. Petres-Dunsche, and R. Fölster-Holst, “Pigmented pedunculated malignant melanoma. A rare variant of nodular melanoma,” *British Journal of Dermatology*, vol. 133, no. 2, pp. 300–302, 1995.

[51] L. M. Cohen, “Lentigo maligna and lentigo maligna melanoma,” *Journal of the American Academy of Dermatology*, vol. 33, no. 6, pp. 923–939, 1995.

[52] M. A. Weinstock and A. J. Sober, “The risk of progression of lentigo maligna to lentigo maligna melanoma,” *British Journal of Dermatology*, vol. 116, no. 3, pp. 303–310, 1987.

[53] D. C. Hill and A. A. Gramp, “Surgical treatment of lentigo maligna and lentigo maligna melanoma,” *Australian Journal of Dermatology*, vol. 40, no. 1, pp. 25–30, 1999.

[54] T. M. Johnson, J. T. Headington, S. R. Baker, and L. Lowe, “Usefulness of the staged excision for lentigo maligna and lentigo maligna melanoma: the “square” procedure,” *Journal of the American Academy of Dermatology*, vol. 37, no. 5, pp. 758–764, 1997.

[55] H. Breuninger, B. Schiagenvauff, W. Stroebel, G. Schaumburg-Lever, and G. Rassner, “Patterns of local horizontal spread of melanomas: consequences for surgery and histopathologic investigation,” *American Journal of Surgical Pathology*, vol. 23, no. 12, pp. 1493–1498, 1999.

[56] Y.-J. Chen, C.-Y. Wu, J.-T. Chen, J.-L. Shen, C.-C. Chen, and H.-C. Wang, “Clinicopathologic analysis of malignant melanoma in Taiwan,” *Journal of the American Academy of Dermatology*, vol. 41, no. 6, pp. 945–949, 1999.

[57] C. Kuchelmeister, G. Schaumburg-Lever, and C. Garbe, “Acral cutaneous melanoma in Caucasians: clinical features, histopathology and prognosis in 112 patients,” *British Journal of Dermatology*, vol. 143, no. 2, pp. 275–280, 2000.

[58] S. G. Ronan, A. M. Eng, H. A. Briele, M. J. Walker, and T. K. Das Gupta, “Malignant melanoma of the female genitalia,” *Journal of the American Academy of Dermatology*, vol. 22, no. 3, pp. 428–435, 1990.

[59] J. G. Batsakis and P. Suarez, “Mucosal melanomas: a review,” *Advances in Anatomic Pathology*, vol. 7, no. 3, pp. 167–180, 2000.

[60] D. C. Whitaker, Z. Argenyi, and A. C. Smith, “Desmoplastic malignant melanoma: rare and difficult to diagnose,” *Journal of the American Academy of Dermatology*, vol. 26, no. 5, pp. 704–709, 1992.

[61] S. Jain and P. W. Allen, “Desmoplastic malignant melanoma and its variants. A study of 45 cases,” *American Journal of Surgical Pathology*, vol. 13, no. 5, pp. 358–373, 1989.

[62] T.-Y. Wong, S. Suster, L. M. Duncan, and M. C. Mihm Jr., “Nevoid melanoma: a clinicopathological study of seven
cases of malignant melanoma mimicking spindle and epithelioid cell nevus and verrucous dermal nevus,” Human Pathology, vol. 26, no. 2, pp. 171–179, 1995.

[63] C. Schmeoeckel, C. E. Castro, and O. Braun-Falco, “Nevoid malignant melanoma,” Archives of Dermatological Research, vol. 277, no. 5, pp. 362–369, 1985.

[64] R. I. Reed, “Minimal deviation melanoma,” Human Pathology, vol. 21, no. 12, pp. 1206–1211, 1990.

[65] A. Breslow, “Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma,” Annals of Surgery, vol. 172, no. 5, pp. 902–908, 1970.

[66] C. G. Clemente, M. C. Mihm Jr., R. Bufalino, S. Zurrida, P. Collini, and N. Cascinelli, “Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma,” Cancer, vol. 77, no. 7, pp. 1303–1310, 1996.

[67] S. G. Ronan, A. M. Eng, H. A. Briele, N. N. Shioura, and T. K. Das Gupta, “Thin malignant melanomas with regression and metastases,” Archives of Dermatology, vol. 123, no. 10, pp. 1326–1330, 1987.

[68] H. R. Byers and J. Bhawan, “Pathologic parameters in the diagnosis and prognosis of primary cutaneous melanoma,” Hematology/Oncology Clinics of North America, vol. 12, no. 4, pp. 717–735, 1998.

[69] S. Mraz-Gernhard, R. W. Sagebiel, M. Kashani-Sabet, J. R. Miller III, and S. P. L. Leong, “Prediction of sentinel lymph node micrometastasis by histological features in primary cutaneous malignant melanoma,” Archives of Dermatology, vol. 134, no. 8, pp. 983–987, 1998.

[70] V. Bataille, “Genetics of familial and sporadic melanoma,” Clinical and Experimental Dermatology, vol. 25, no. 6, pp. 464–470, 2000.

[71] E. Nagore, J. Climenti, M. D. Panelles et al., “Analysis of the CDKN2A and CDK4 genes and HLA-DR and HLA-DQ alleles in two Spanish familial melanoma kindreds,” Acta Dermato-Venereologica, vol. 80, no. 6, pp. 440–442, 2000.

[72] V. Bataille, “Genetic epidemiology of melanoma,” European Journal of Cancer, vol. 39, no. 10, pp. 1341–1347, 2003.

[73] K. M. Greulich, J. Utikal, R.-U. Peter, and G. Krähn, “c-MYC and nodular malignant melanoma: a case report,” Cancer, vol. 89, no. 1, pp. 97–103, 2000.

[74] B. C. Bastian, A. B. Olshen, P. E. LeBoit, and D. Pinkel, “Classifying melanocytic tumors based on DNA copy number changes,” American Journal of Pathology, vol. 163, no. 5, pp. 1765–1770, 2003.

[75] J. M. Cowan, R. Halaban, and U. Francke, “Cytogenetic analysis of melanocytes from premalignant nevi and melanomas,” Journal of the National Cancer Institute, vol. 80, no. 14, pp. 1159–164, 1988.

[76] P. Gerami, S. S. Jewell, L. E. Morrison et al., “Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma,” American Journal of Surgical Pathology, vol. 33, no. 8, pp. 1146–1156, 2009.

[77] A. L. Morey, R. Murali, S. W. McCarthy, G. J. Mann, and R. A. Scolyer, “Diagnosis of cutaneous melanocytic tumours by four-colour fluorescence in situ hybridisation,” Pathology, vol. 41, no. 4, pp. 383–387, 2009.

[78] A. Uong and L. I. Zon, “Melanocytes in development and cancer,” Journal of Cellular Physiology, vol. 222, no. 1, pp. 38–41, 2010.

[79] R. M. Eager, C. C. Casey, N. N. Senzer et al., “Phase II assessment of talbotstat and cisplatin in second-line stage IV melanoma,” BMC Cancer, vol. 9, article 263, 2009.

[80] G. Palmieri, M. Capone, M. L. Ascierto et al., “Main roads to melanoma,” Journal of Translational Medicine, vol. 7, article 86, 2009.

[81] R. Z. Karim, W. Li, A. Sanki et al., “Reduced p16 and increased cyclin D1 and pRB expression are correlated with progression in cutaneous melanocytic tumors,” International Journal of Surgical Pathology, vol. 17, no. 5, pp. 361–367, 2009.

[82] G. J. Villares, A. S. Dobroff, H. Wang et al., “Overexpression of protease-activated receptor-1 contributes to melanoma metastasis via regulation of connexin 43,” Cancer Research, vol. 69, no. 16, pp. 6730–6737, 2009.

[83] J. S. Blackburn, I. Liu, C. I. Coon, and C. E. Brinckerhoff, “A matrix metalloproteinase-1/protease activated receptor-1 signaling axis promotes melanoma invasion and metastasis,” Oncogene, vol. 28, no. 48, pp. 4237–4248, 2009.

[84] M. Oka and U. Kikkawa, “Protein kinase C in melanoma,” Cancer and Metastasis Reviews, vol. 24, no. 2, pp. 287–300, 2005.

[85] M.-Y. Hsu, “Shifts in cadherin profiles between human normal melanocytes and melanomas,” Journal of Investigative Dermatology, vol. 1, no. 2, pp. 188–194, 1996.

[86] V. Sviatoha, E. Tani, M. Sperga, and L. Skoog, “Immunohistochemical analysis of the S100A1, S100B, CD44 and Bcl-2 antigens and the rate of cell proliferation assessed by Ki-67 antibody in benign and malignant melanocytic tumours,” Melanoma Research, vol. 20, no. 2, pp. 118–125, 2010.

[87] V. Alla, D. Engelmann, A. Nietzelt et al., “E2F1 in melanoma progression and metastasis,” Journal of the National Cancer Institute, vol. 102, no. 2, pp. 127–133, 2010.

[88] J. M. Mehnert, M. M. McCarthy, L. Jilaveanu et al., “Quantitative expression of VEGF, VEGF-R1, VEGF-R2, and VEGF-R3 in melanoma tissue microarrays,” Human Pathology, vol. 41, no. 3, pp. 375–384, 2010.

[89] X.-Z. Xu, M. V. Garcia, T.-Y. Li et al., “Cytoskeleton alterations in melanoma: aberrant expression of cortactin, an actin-binding adapter protein, correlates with melanocytic tumor progression,” Modern Pathology, vol. 23, no. 2, pp. 187–196, 2010.

[90] Y. Degenhardt, J. Huang, J. Greshock et al., “Distinct MHC gene expression patterns during progression of melanoma,” Genes Chromosomes and Cancer, vol. 49, no. 2, pp. 144–154, 2010.

[91] J. L. Orgaz, O. Ladhani, N. S. Hoek et al., “Loss of pigment epithelium-derived factor enables migration, invasion and metastatic spread of human melanoma,” Oncogene, vol. 28, no. 47, pp. 4147–4161, 2009.

[92] D. W. Mueller, M. Rehli, and A. K. Bosserhoff, “MiRNA expression profiling in melanocytes and melanoma cell lines reveals miRNAs associated with formation and progression of malignant melanoma,” Journal of Investigative Dermatology, vol. 129, no. 7, pp. 1740–1751, 2009.

[93] J. Yang, M. A. Price, Y. L. Gui et al., “Melanoma proteoglycan modifies gene expression to stimulate tumor cell motility, growth, and epithelial-to-mesenchymal transition,” Cancer Research, vol. 69, no. 19, pp. 7538–7547, 2009.

[94] L. B. Jilaveanu, C. R. Zito, S. A. Aziz et al., “C-Raf is associated with disease progression and cell proliferation in a subset of melanomas,” Clinical Cancer Research, vol. 15, no. 18, pp. 5704–5713, 2009.
[95] S. Heimerl, A. K. Bosserhoff, T. Langmann, J. Ecker, and G. Schmitz, “Mapping ATP-binding cassette transporter gene expression profiles in melanocytes and melanoma cells,” *Melanoma Research*, vol. 17, no. 3, pp. 265–273, 2007.

[96] C. B. Meije, P. K. Das, M. M. E. Jans et al., “Multiple complementary transcripts of pCMaI, a novel gene located at chromosome 11p15.1–2, and melanocytic cell transformation,” *Journal of Pathology*, vol. 197, no. 5, pp. 668–676, 2002.

[97] I. Kenessey, E. Simon, K. Futosi, et al., “Antimigratory and antimetastatic effect of heparin-derived 4–18 unit oligosaccharides in a preclinical human melanoma metastasis model,” *Thrombosis and Haemostasis*, vol. 102, no. 6, pp. 1265–1273, 2009.

[98] R. S. Rogers III, “Malignant melanoma in the 21st century,” *International Journal of Dermatology*, vol. 39, no. 3, pp. 178–179, 2000.

[99] R. Marks, “Epidemiology of melanoma,” *Clinical and Experimental Dermatology*, vol. 25, no. 6, pp. 459–463, 2000.

[100] T. M. Johnson, J. W. Smith II, B. R. Nelson, and A. Chang, “Current therapy for cutaneous melanoma,” *Journal of the American Academy of Dermatology*, vol. 32, no. 5, pp. 689–707, 1995.

[101] M. S. Green, “The changing controversy over surgical resection margins for stage I cutaneous melanoma,” *Mount Sinai Journal of Medicine*, vol. 58, no. 4, pp. 341–346, 1991.

[102] M. G. E. O’Rourke and C. R. Altmann, “Melanoma recurrence after excision: is a wide margin justified?” *Annals of Surgery*, vol. 217, no. 1, pp. 2–5, 1993.

[103] C. M. Balch, M. M. Urist, C. P. Karakousis et al., “Efficacy of 2-cm surgical margins for intermediate-thickness melanomas (1 to 4 mm): results of a multi-institutional randomized surgical trial,” *Annals of Surgery*, vol. 218, no. 3, pp. 262–269, 1993.

[104] U. Ringborg, R. Andersson, J. Eldh et al., “Resection margins of 2 versus 5 cm for cutaneous malignant melanoma with a tumor thickness of 0.8 to 2.0 mm: a randomized study by the Swedish Melanoma Study Group,” *Cancer*, vol. 77, no. 9, pp. 1809–1814, 1996.

[105] P. Walsh, P. Gibbs, and R. Gonzalez, “Newer strategies for effective evaluation of primary melanoma and treatment of stage III and IV disease,” *Journal of the American Academy of Dermatology*, vol. 42, no. 3, pp. 480–489, 2000.

[106] N. Kandamany and P. Mahaffey, “Carbon dioxide laser ablation as first-line management of in-transit cutaneous malignant melanoma metastases,” *Lasers in Medical Science*, vol. 24, no. 3, pp. 411–414, 2009.

[107] C. M. Balch, J. E. Gershenwald, S.-J. Soong et al., “Final version of 2009 AJCC melanoma staging and classification,” *Journal of Clinical Oncology*, vol. 27, no. 36, pp. 6199–6206, 2009.

[108] A. C. Buzaid, M. I. Ross, C. M. Balch et al., “Critical analysis of the current American Joint Committee on Cancer staging system for cutaneous melanoma and proposal of a new staging system,” *Journal of Clinical Oncology*, vol. 15, no. 3, pp. 1039–1051, 1997.

[109] R. F. Kefford, “Adjuvant therapy of cutaneous melanoma: the interferon debate,” *Annals of Oncology*, vol. 14, no. 3, pp. 358–365, 2003.

[110] T. S. Wang, T. M. Johnson, P. N. Cascade, B. G. Redman, V. K. Sondak, and J. L. Schwartz, “Evaluation of staging chest radiographs and serum lactate dehydrogenase for localized melanoma,” *Journal of the American Academy of Dermatology*, vol. 51, no. 3, pp. 399–405, 2004.

[111] T. M. Johnson, C. R. Bradford, S. B. Gruber, V. K. Sondak, and J. L. Schwartz, “Staging workup, sentinel node biopsy, and follow-up tests for melanoma: update of current concepts,” *Archives of Dermatology*, vol. 140, no. 1, pp. 107–113, 2004.

[112] H. B. Neuman, A. Patel, N. Ishill et al., “A single-institution validation of the AJCC staging system for stage IV melanoma,” *Annals of Surgical Oncology*, vol. 15, no. 7, pp. 2034–2041, 2008.

[113] A. Y. Bedikian, M. M. Johnson, C. L. Warner et al., “Prognostic factors that determine the long-term survival of patients with resectable metastatic melanoma,” *Cancer Investigation*, vol. 26, no. 6, pp. 624–633, 2008.