Recent advancements in the research of malignant melanoma are reviewed. Among many gene alterations detected in human melanoma, defect of CDKN2A located at chromosome 9p21 seems to be most important in the earlier developmental phase, though significance of this gene in the evolution of melanoma in situ has not been confirmed yet. Deletions of PTEN/MMAC1 on 10q23.3 and AIM1 on 6q21 as well as mutations of ras gene are involved in the later progression stages of melanoma. Adhesion molecules relevant to development and progression of melanoma have been intensely investigated in recent years, revealing crucial roles of cadherins and αvβ3 integrin in the biologic behaviors of melanoma cells. Melanoma is characterized by extremely high potential of developing metastases. Dynamic changes of matrix metalloproteinase activity during invasion and movement of melanoma cells may be a major concern in this field. Fragility of blood vessels in melanoma lesions is another important point related to hematogeneous metastases. Acral lentiginous melanoma is a unique subtype of melanoma, because, in contrast to other subtypes, ultraviolet irradiation is not a major factor in its development. Investigation of pathogenesis of acral lentiginous melanoma surely provides us with new information about mechanism of melanocyte transformation.

Recent advances in the management of malignant melanoma are also briefly reviewed, such as biochemotherapy, immunotherapy, and gene therapy. Finally, the concept of molecular classification of melanoma by gene expression profile is introduced, which possibly enables us to give the tailor-made therapy for each melanoma patient in the near future. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Malignant melanoma; Pathogenesis; Gene alterations; Molecular events; New treatment

1. Introduction

Incidence of malignant melanoma is rapidly increasing particularly in white populations. Also in Japan, number of melanoma patients has obviously increased in recent years. Major advances have been made not only in basic research but also in clinical management of this highly malignant neoplasm. In this article, the author reviews the advancement in melanoma research, giving some personal comments on each topic.
2. Molecular events in melanoma development and progression

Recently, great advances have been made in the research of molecular biology of malignant melanoma [1–5]. These advances surely lead us to better understanding of the mechanisms involved in the development and progression of this neoplasm. Moreover, based on these advances, more rational management of patients with melanoma will be invented.

2.1. Genetic alterations in malignant melanoma

One of major recent advances in melanoma research is identification of cytogenetic and genetic alterations responsible for its development and progression. Disruptions of many genes have been detected on a variety of chromosomes. The followings are updated information about the main genes relevant to pathogenesis of melanoma.

2.1.1. CDKN2A (P16)

Linkage analysis of familial melanoma suggested presence of a melanoma susceptibility gene on chromosome 9p21[6,7]. Loss of heterozygosity (LOH) at 9p21 regions was also detected frequently in cultured human melanoma cells [8]. In addition, a non-familial melanoma patient who carried somatic germline defect at 9p21 developed multiple melanomas [9]. Soon later, CDKN2A (cyclin-dependent kinase inhibitor 2A) gene, also called MTS1, residing on this locus was suspected to be the melanoma susceptibility gene [10,11]. This gene encodes p16 or INK4a (inhibitor of kinase 4a), which inactivates CDK4 (cyclin-dependent kinase 4) through competing with cyclin D1 for binding of CDK4 [12]. Cycline D1/CDK4 complexes phosphorylate and thus inactivate RB (retinoblastoma gene product), resulting in the dissociation of the E2F factors, transcription factors required for expression of S phase genes. Therefore, CDKN2A plays an important role at the G1 checkpoint of the cell cycle and thus, same as RB, CDKN2A is considered as a tumor suppressor gene [2,3,7].

Germline mutations in CDKN2A are found in approximately 50% of 9p-linked melanoma-prone families [7]. Most of the mutations are found on exons 1a and 2; frame shift, nonsense or missense mutations, inframe deletions, and insertions resulting in production of non-functional proteins [2]. Some of the family members suffer also from pancreatic carcinoma [13]. Mutation or deletion at the CDKN2A locus is detected in ~70% of cultured melanoma cell lines and in ~35% of uncultured melanoma cells. Methylation of the promoter region of the gene is considered as another suppression mechanism of CDKN2A [14]. These data indicate involvement of CDKN2A in melanoma development. LOH analysis suggested that deletion of CDKN2A was one of the earliest events in melanoma development, as the deletion was detected in invasive primary lesions with thin tumor thickness [15]. Related to the mutations of CDKN2A, it is interesting that a germline mutation of CDK4 gene located on 12q13 was found in a few melanoma-prone families [16]. The mutated gene product, CDK4(Arg24Cys), is defective in binding to p16, but hold the ability to bind cyclin D. Cytotoxic T cells (CTLs) recognizing the mutated CDK4 were detected in a few melanoma patients. Taken together, disruptions of CDKN2A/CDK4 pathway surely play a major role in melanoma development.

Multiple atypical nevi (dysplastic nevi, Clark’s nevi) are frequently observed in the members of melanoma-prone families. However, relationship between CDKN2A and atypical nevi has not been yet determined. In a 9p-linked melanoma family reported by Puig et al. [17], only two out of 10 individuals with atypical nevi showed the mutation of CDKN2A. They suggested that a gene responsible for development of atypical nevus was different from CDKN2A. In familial melanoma kindreds reported by Hussussian et al., 92% (33/36) of melanoma patients had CDKN2A mutations but only 30% (10/33) of atypical nevi patients had the mutations, and they also suggested that CDKN2A mutations were not responsible for the development of atypical nevi in the families [18]. In contrast, according to Newton Bishop et al., familial melanoma patients with atypical nevi were three times more likely to carry mutated CDKN2A than those who did not have atypical nevi [19].
Alterations of 9p or CDKN2A were investigated also in sporadic melanocytic nevi without history of familial melanoma. Healy et al. reported that only one out of 32 melanocytic nevi showed LOH at 9p [15]. Histopathologically, the nevus with LOH of 9p showed disorderly intraepidermal proliferation of somewhat atypical melanocytes. Thus the nevus could be considered as early melanoma in situ. Lee et al. detected point mutations of CDKN2A in four out of 12 atypical nevi [20]. By immunostaining, we found that expression of CDKN2A was detected in 61% (17/28) of melanocytic nevi and in 16% (3/19) of melanomas [21]. These data imply that alterations of CDKN2A are not essential in the development of melanocytic nevi including atypical nevi. If melanocytic nevi are the initial step to melanoma development, disruptions of CDKN2A do not seem to be involved at least in this first step. Another interpretation of the normal expression of CDKN2A in melanocytic nevi may be that melanocytic nevi are not the first step to melanoma. As the author reported before [22,23], acquired melanocytic nevus is probably a benign neoplasm of epidermal melanocytes, and vast majority of cutaneous melanomas seem to arise de novo, that is, not in association with acquired melanocytic nevi including atypical nevi (Clark’s nevi). To clarify this point, however, definition and diagnostic specificity of atypical nevi are essential, particularly regarding its relation to melanoma in situ [24].

Interestingly, the locus encoding CDKN2A gives rise to two distinct transcripts from different promoters. Each transcript has a specific 5’ exon, E1α or E1β, which is spliced into common exons E2 and E3. The E1α-containing transcript encodes p16 and the E1β-containing transcript encodes p19ARF [25]. p19ARF has the ability to arrest cell proliferation outside the CDK/ RB pathways, exerting its effects through the binding and blockade of a factor MDM2. MDM2 binds p53 and target it for degradation via the ubiquitin pathway [3]. Up-regulation of p19ARF blocks the MDM2-induced turnover of p53. There is a report that 2 of 5 melanoma cell lines exhibited deletions confined to p19ARF exon E1β and not the 3 exons of CDKN2A [26]. However, other studies using uncultured melanomas found no mutations within E1β. Significance of alterations of p19ARF in melanoma development must wait for further studies. p15, a CDKN2A homologue, also resides on 9p21, ~20–30 kbp centromerically to CDKN2A. There is little evidence of p15 involvement in melanoma development.

2.1.2. Genes on chromosome 1

Chromosome 1p36 was the first gene locus reported to have linkage to familial melanoma in 1989 [27], though the following studies by other groups could not confirm it. According to Goldstein et al. [28], 1p36 seemed to be in linkage with the phenotype of melanoma along with dysplastic nevus in melanoma-prone families, whereas 9p showed much higher linkage if only melanoma phenotype was considered. Recently, Ariza et al. reported that expression of cdc2, located on 1p36 and encoding PITSLE protein kinases, was abnormal in melanoma cells that were resistant to Fas-mediated apoptosis [29].

2.1.3. PTEN/MMAC1

LOH of chromosome 10q is also frequently (30–50%) observed in primary melanoma with rather thin tumor thickness [15,30]. PTEN/MMAC1, located at 10q23.3, is a gene responsible for Cowden’s disease, which develops various types of tumors including intestinal polyposis, breast cancers, and other malignancies [31]. Multiple trichilemmomas are also observed in Cowden’s disease. PTEN/MMAC1 was deleted or mutated in more than 40% of melanoma cell lines and the mutations were demonstrated even in uncultured melanoma specimens [32]. Disruption of this gene is also frequently detected in glioma. It is noteworthy that glial cells and melanocytes are common in origin from the neural crest. PTEN/MMAC1 encodes a 55 kD protein which has tyrosine phosphatase activity and is considered as a tumor suppressor gene. It seems that also this gene is not involved in the early developmental stages of melanoma but in the later progression stages.
2.1.4. AIM1

LOH of chromosome 6q is frequently detected in thick primary melanoma as well as in metastatic melanoma [15,30]. Microcell fusion study introducing normal chromosome 6 into a highly tumorigenic human melanoma cell line defective in this chromosome converted its phenotype dramatically; the derivative melanoma cells restored contact inhibition and lost the ability to form tumors in nude mice [33]. Later, AIM1, a gene located at 6q21, was identified. It belongs to a novel non-lens member of the bg-crystallin superfamily, and parts of the AIM1 protein sequence have weak similarity with actin-binding proteins [34]. Thus, AIM1 could exert its effects through interactions with cytoskeleton. Defect of this gene in melanoma may be involved in the development of the tumorigenic vertical growth phase (VGP) and metastases.

2.1.5. ras and p53

Rates of mutation of ras genes in human melanoma are substantially different among reports. Albino et al. reported that the rate was low, 5–6%, in uncultured melanoma cells [35]. According to van't Veer et al., N-ras mutation was detected in 20% of primary melanomas; most of the lesions with ras mutation were from sun-exposed sites, suggesting that ultraviolet irradiation caused the mutation [36]. Ball et al. recently reported that mutation of ras genes (N-ras, H-ras) was not detected in the lesions of melanoma in situ but found in 38% of invasive melanomas [37]. Shellman et al. transfected mutant N-ras or mutant H-ras genes into WM35 cells, a human melanoma cell line of low invasive potential derived from the radial growth phase (RGP) melanoma [38]. The transfectant gained characters of VGP melanoma: anchorage-independent proliferation, increased motility, invasiveness in in vitro assay, and tumor formation in nude mice. Moreover, they found that the transfectants abrogated growth inhibitory effect by TGF-β. TGF-β inhibits growth of normal melanocytes as well as melanoma cells derived from the early phase such as parental WM35 cells. These data suggest that activated ras plays a crucial role in melanoma progression from RGP to VGP. There is a report suggesting that ras activation is associated with the nodular type of melanoma [39], also supporting the involvement of this gene in rather later advanced stages.

In contrast, p53, a representative tumor suppressor gene commonly deleted in various types of malignancies, is rarely deleted in malignant melanoma [40]. Reasons for this low rate in melanoma are not clear, but it could be explained by the overlapping tumor suppressor functions of CDKN2A and p53.

2.2. Adhesion molecules in melanoma cells

2.2.1. Mel-CAM/MUC18 and αβ3 integrin

Herlyn’s group identified Mel-CAM/MUC18 as an adhesion receptor in melanocytes [41,42]. Its expression is up-regulated in melanoma cells and may play a major role not only in melanoma–melanoma cell interactions but also in melanoma–endothelial interactions. Mel-CAM/MUC18 appears to act in concert with αβ3 integrin, a vitronectin receptor, in promoting metastasis. Herlyn’s group also found that β3 subunit of αβ3 was considered as a notable marker of melanoma progression [43]. According to them, αβ3 is a specific marker that distinguishes RGP from VGP. Overexpression of αβ3 in melanoma cell lines derived from RGP by gene transfer changed the properties of the cells to those of VGP [44]. An immunohistochemical study by Van Belle et al. using monoclonal antibodies to β3 revealed that expression of β3 was not observed in epidermal melanocytes and was absent or low in most melanocytic nevi, except for Spitz’s nevi, which showed strong β3 expression [43]. The junctional components of a few atypical nevi showed focal reactivity for β3. Expression of β3 was absent or low even in melanoma in situ and RGP melanoma. In contrast, increased expression of β3 was detected in most VGP compartments of primary melanomas and metastatic melanomas.

2.2.2. Significance of cadherin profile in melanoma development

The cadherin molecules function as homophilic calcium-dependent cell–cell adhesion and are in-
volved in cell recognition, motility, and tissue integrity. In normal human skin, E-cadherin is expressed on the surface of all kinds of epidermal cells; keratinocytes, melanocytes and Langerhans cells. In contrast, P-cadherin is expressed only on the surface of keratinocytes in the basal layer. In the skin, N-cadherin is expressed by fibroblasts and endothelial cells but not by keratinocytes or melanocytes [5].

Herlyn’s group recently found the importance of changes of cadherin expression in melanoma development. Normal melanocytes, if cultured in vitro, showed a phenotype similar to melanoma cells, such as increased expression of α,β3, and Mel-CAM: MUC18. However, when the melanocytes were co-cultured with epidermal keratinocytes, expression of these markers was lost [5]. Moreover, cultured keratinocytes, when maintained in low calcium-containing medium, controlled proliferation of melanocyte through direct cell–cell contact. E-cadherin-mediated adhesion between the two types of cells was critical for this regulation. In contrast, melanoma cells were refractile to the growth inhibition by keratinocytes, probably due to loss of the contact-mediated regulatory controls.

Immunohistochemically, E-cadherin was positive in four out of 14 melanomas, P-cadherin in eight out of 12, and N-cadherin in 12 of 16 [45]. Flow cytometry revealed that only one of 16 melanoma cell lines was positive for E-cadherin, none was positive for P-cadherin, and all but one were positive for N-cadherin. The expression of N-cadherin by melanoma cells allowed them to form gap junctions with fibroblasts and endothelial cells [46]. Transduction of E-cadherin cDNA into E-cadherin-negative melanoma cells restored the cell adhesion to keratinocytes, rendering them susceptibility to keratinocyte-mediated control [47]. This forced expression of E-cadherin in melanoma cells reconstructed the gap junction with keratinocytes. In an in vitro skin model, the ectopic E-cadherin expression inhibited invasion of melanoma cells into dermis by down-regulating invasion-related adhesion receptors, Mel-CAM/ MUC18 and β, integrin subunit [47]. These findings suggest that escape of epidermal melanocytes from E-cadherin-mediated regulatory control by keratinocytes is an important event relevant to the development of melanoma.

β-catenin links the cytoplasmic domain of E-cadherin to the actin-based cytoskeleton and is required for proper E-cadherin expression and function. It was reported that β-catenin gene was mutated in ~5–25% in melanoma [48,49]. Rubinfield et al. detected abnormally high amounts of β-catenin in 7 of 26 melanoma cell lines. This abnormality was caused by β-catenin gene mutations, unusual β-catenin mRNA splicing, or inactivation of APC (adenomatous polyposis coli tumor suppressor gene), which resulted in stabilization of β-catenin protein [50]. It is interesting that a CTL clone recognizing mutated β-catenin was established from a melanoma patient [50]. According to our recent study, expression rates of β-catenin were correlated with melanoma progression; 96% in melanocytic nevi, 94% in RGP of melanoma, 65% in VGP, and 38% in metastatic melanoma [51]. In addition, the expression patterns were different between melanocytic nevi and melanomas; nuclear expression was lower in melanomas compared to nevi. Roles of β-catenin in melanoma progression must be clarified in the future studies.

2.3. Factors involved in invasion and metastasis of melanoma

The most important nature of malignant melanoma is its extremely high potential to develop metastasis. Thus, investigation of mechanisms involved in metastasis is essential to control this neoplasm. Among various aspects and factors relevant to metastasis, the following two topics are discussed here: changes of matrix metalloproteinase activity and characteristics of vasculature in malignant melanoma. These two factors are crucial in local invasion and hematogeneous metastases of melanoma.

2.3.1. Matrix metalloproteinase in melanoma progression

Degradation of basement membranes and extracellular matrix (ECM) is an essential step in cancer invasion and metastasis. Matrix metalloproteinase (MMP) and their tissue inhibitors
(TIMP) play key roles in this step. MMPs are zinc-dependent endopeptidases responsible for the degradation of ECM components. To date, 19 human MMPs have been cloned and characterized [51]. MMPs are derived not only from tumor cells but also from the surrounding stromal cells. TIMPs modulate activity of MMPs by binding to their catalytic domain. To date, 4 members of TIMPs have been characterized. It must be noted that TIMPs not always inhibit MMP activity. High concentrations of TIMP-2 inhibit MMP-2 activation, but low concentrations of TIMP-2 promote processing of MMP-2 to its proteolytically active form [53].

Regarding malignant melanoma, increased expression of MMP-1, MMP-2, and MMP-9 was shown to correlate with an invasive phenotype [54–56]. In particular, active MMP-2 was detected in highly invasive melanoma cell lines and was absent in non- or poorly invasive cell lines [57]. In a xenograft model, increased expression of MMP-2 was correlated with the metastatic capacity of melanoma cells. There is a report that recombinant TIMP-2 inhibited lung metastases of murine B16-F10 melanoma cells [58]. Recently identified membrane type (MT)1-MMP is also important in the activation of MMP-2 [59]. MMP-9 was detected in cell lines derived from advanced primary melanomas and was absent from cell lines established from early primary lesions [56]. Human melanoma cell lines constitutively expressing MMP-9 produced enhanced lung metastases in nude mice. TIMP-1 seems to inhibit MMP-9 production from melanoma cells, and overexpression of TIMP-1 reduced tumor growth and metastasis of B16 murine melanoma [52].

Hofmann et al. investigated expression of MMP-2, MT1-MMP and TIMP-2 in 60 fresh human melanocytic lesions and found that both MMP-2 and MT1-MMP were strongly expressed in melanoma [60]. The MMP-2 and MT1-MMP positive cells were often localized in subepidermal nests of primary melanoma and at the tumor-stroma interface of primary and metastatic lesions. Benign nevi did not express MMP-2, but TIMP-1 expression was detected not only in melanoma but also in nevi. Cellular localization of active MMP is essential in tumor cell invasion. According to Brooks et al. [61], \(\alpha_\beta_3\) integrin can bind and position active MMP-2 on the cell surface of melanoma. Hofmann et al. showed that MT1-MMP and \(\alpha_\beta_3\) were colocalized on the cell membrane of melanoma [62], suggesting a role of MT1-MMP in the activation of MMP-2 bound to \(\alpha_\beta_3\). They also reported that neither MMP-2 nor \(\alpha_\beta_3\) integrin was detected in melanoma in situ, though increased expression was detected in invasive primary melanoma and metastatic melanoma [63]. Yu et al. recently reported that CD44 promoted invasion of melanoma cells by anchoring active MMP-9 on the cell surface [64]. Our study demonstrated that expression of moesin, a member of the ERM family binding to CD44, was downregulated in melanomas of advanced stages [65]. We also found that increased synthesis of hyaluronate, a ligand to CD44, enhanced motility of melanoma cells in vitro [66]. Taken together, it appears that not only MMPs and \(\alpha_\beta_3\) integrin but also moesin, CD44, and hyaluronate play major roles in the movement and invasion of melanoma cells into the surrounding tissues.

2.3.2. Characteristics of vascularity in malignant melanoma

Malignant melanoma is characterized by high risk of developing hematogeneous metastases even in the early stages. Thus, investigation of vascularity and fragility of blood vessels in this neoplasm have been a major concern. Several authors reported that rich vascularity in primary melanomas was associated with poor prognosis. Using antibodies against factor VIII-related antigen and CD34, Graham et al. compared mean vessel numbers between melanoma pairs matched for prognostic factors [67], and found that, in thin (\(<0.76\) mm) primary lesions, high vascularization was associated with a poorer prognosis. In contrast, Ilmonen et al. reported that, by counting CD31 positive vessels in 84 primary lesions of malignant melanoma, high vascularization in primary lesions suggested a better prognosis [68], though the vascularity was not an independent prognostic factor in multivariate analysis. Significance of vascular density in the prognosis of melanoma patients remains to be determined.
In normal skin, \(\alpha\) smooth muscle actin (\(\alpha\SMA\)) is expressed in vascular smooth muscle cells, pericytes, myoepithelial cells and some other elements. According to Taniguchi's group, expression of \(\alpha\SMA\) was reduced in the blood vessels of malignant melanoma. They also found that melanoma cells released a factor inhibiting the expression of \(\alpha\SMA\) [69]. We recently found reduced expression of calponin-h1 (CNh1) and caldesmon (CD) in the blood vessel walls in melanoma tissues. CNh1 and CD are actin- and calmodulin-binding proteins involved in the structure and function of smooth muscle. Notably, lower expression of CD was closely related to poorer prognosis of melanoma patients (Koganehira et al., manuscript submitted). These data suggest that blood vessel walls in the lesions of melanoma are fragile, and this fragility may contribute to the development of hematogeneous metastasis.

Maniotis et al. have proposed the concept of vasculogenic mimicry in malignant melanoma [70]. Patterned networks of interconnected loops of extracellular matrix are frequently found in metastatic lesions of aggressive melanomas. These patterned channels often contain red blood cells, though they are not lined by real endothelial cells but by melanoma cells. Based on these findings, they have proposed the concept that aggressive melanoma cells generate the patterned channels that facilitate tumor perfusion independent of true angiogenesis. This concept could well explain not only markedly increased blood flow in melanoma lesions as detected by the power Doppler method but also brain metastasis not infrequently manifesting symptoms of cerebral haemorrhage.

### 3. Distinctiveness of acral lentiginous melanoma

Ultraviolet irradiation is supposed to be the most important causative factor of cutaneous melanoma. Among the four subtypes of Clark’s classification, acral lentiginous melanoma (ALM) is unique because it exclusively involves acral skin, such as sole or nail apparatus, which is rarely exposed to sunlight. Therefore, carcinogenesis process of ALM is surely different form that of other subtypes, superficial spreading melanoma (SSM) or lentigo maligna melanoma (LMM), in which sun-exposure may be a major cause. SSM is the main subtype in white populations, accounting for \(>70\%\) of total melanomas. In contrast, In Japanese, \(~50\%\) of cutaneous melanoma are of ALM [71]. The percentage is much higher in black persons. It is noteworthy that the real overall incidence of ALM is probably similar among all races [72]. In white populations, too many melanomas arise on non-acral sites, lowering the relative rate of ALM. If there were no effect of sunlight on the skin, ALM would become the most prevalent subtype in all races. When we consider that the surface size of the soles accounts for \(~3\%\) of the entire body surface, the rates of ALM in non-white populations are extraordinarily high. Therefore, the author believes that analysis of mechanisms involved in the development of ALM has great impact on the investigation of melanocyte transformation.

Bastian et al. recently reported interesting data regarding chromosomal alterations in ALM [73]. They analyzed chromosomal aberrations of 15 ALMs and 15 SSMs using comparative genomic hybridization (CGH). All ALMs had at least one gene amplification, significantly more than SSMs, in which only 2 of 15 (13\%) had one amplification. In ALM, at least 15 different genomic regions were amplified, involving small portions of chromosomal arms, some of which included known oncogenes implicated in melanoma. The most frequent amplified regions in ALM occurred at 11q13 (47\%), 22q11-13 (40\%), and 5p15 (20\%). Fluorescence in situ hybridization (FISH) analysis revealed that the gene amplifications were observed not only in tumor cells of invasive portions of melanoma but also in those of non-invasive macular portions. Moreover, amplifications of 11q13 were also detected in three out of five additional cases of ALM in situ, indicating the alterations are already present in the early developmental phase of ALM. Another noteworthy aspect of ALM is the unique dermoscopic pattern, the parallel ridge pattern, observed in early ALM [74]. This pattern indicates that atypical melanocytes of early phase ALM proliferate preferentially in the epidermal rete ridges situated
under the surface skin ridges. These cytogenetic and dermoscopic data suggest that ALM is a distinct type of cutaneous melanoma, probably reflecting unique pathogenesis of this type of melanoma different from other subtypes.

What is the major carcinogenic factor of ALM? We can not identify it at present, however, subsite distribution of ALM could provide us with some hints about this question. In Japanese, ALM affects soles of the feet much more frequently than palms of hands [71]. In contrast, nail-apparatus melanoma affects fingernails more frequently than toenails. Soles and finger-nails are the sites frequently and easily exposed to mechanical trauma. Thus, trauma may be a possible causative factor in the development of ALM. Another factor that could be relevant to ALM development is physiological paucity of melanin granules in acral skin. Soles, palms, and nail-apparatus are mostly not pigmented even in blacks. Melanin granules may prevent skin damages not only from ultraviolet light but also from free radicals locally released from various kinds of inflammation. Thus, DNA of melanocytes in acral skin devoid of melanin granules could be easily damaged from free radicals induced by trauma. This damage could be causally related to the transformation of melanocytes. The hypothesis described here is challenged by the fact that squamous cell carcinoma is rarely observed in acral skin, and thus it should be critically evaluated in further studies.

4. Brief summary and perspective on melanoma management

Management of malignant melanoma has substantially changed in recent years such as narrow surgical margins of thin primary lesions and sentinel node biopsy instead of elective lymph node dissection. A new staging system has been proposed in order to adjust to these changes [75]. Temozolomide, a more active derivative of dacarbazine (DTIC), will be soon in hand. However, thus far, we have virtually no effective treatment for patients with advanced melanoma [76]. Here the latest topics about melanoma management are briefly reviewed.

4.1. Biochemotherapy

In recent years, two regimens for the treatment of advanced melanoma have attracted our attention. One is the Dartmouth regimen, a combination of cisplatin (CDDP), DTIC and carmustine (BCNU) along with tamoxifen, an anti-estrogenic agent. The other is sequential biochemotherapy, in which chemotherapy including CDDP is immediately followed by administration of interleukin-2 (IL-2) and interferon-\(\alpha\) (IFN-\(\alpha\)). According to McClay’s summing up of eight phase II trials of the Dartmouth regimen, overall response rate was 44% including 14% of complete response [77]. Recent prospective randomized studies, however, failed to confirm the effect of this regimen [78].

A variety of regimens of sequential biochemotherapy have been tried, all exhibiting very high overall response rates mostly over 40%. Complete response rates were also high, ranging from 10 to 30%. More importantly, 5–10% of long-survivors were reported in these trials [79]. It is interesting that responders in these trials often developed vitiligo. Ongoing prospective randomized studies of biochemotherapy will finally determine its effectiveness. Biochemotherapy may work not only cytocidally but also immunologically, as suggested by the development of vitiligo in responding patients. It was reported that CTLs recognizing melanoma antigens were demonstrated in patients receiving biochemotherapy [80]. Increased serum IL-12 levels were also reported in patients responding to biochemotherapy [81]. In our study using B16 murine melanoma, growth of melanoma nodules in mice was significantly inhibited by sequential biochemotherapy composed of CDDP, IL-2, and IFN-\(\beta\) [82]. We found synergistic effects among the three agents. The effects was partly explained by the production of IFN-\(\gamma\). These findings suggest the possibility that biochemotherapy activates the Th1 arm of immunological reaction in melanoma patients. Clinical significance of biochemotherapy and precise mechanisms of its effects remain to be clarified.
4.2. Immunotherapy and gene therapy

Malignant melanoma is the leading neoplasm that provide us with information about tumor immunology in human. CTLs recognizing a variety of melanoma antigens have been successfully cloned, and antigen peptides presented on HLA-class I molecules have been identified [83]. Rosenberg et al. treated patients with advanced melanoma using a peptide of g209-2M, a synthetic gp100 peptides (209–217) in which a methionine replaced the threonine at position 2 to increase binding to HLA-A2, and obtained response rate of 42% (13/31 patients) [84].

Another exciting approach to immunotherapy of melanoma is utilization of dendritic cells. In the study by Nestle et al. [85], dendritic cells obtained from peripheral blood of melanoma patients were propagated ex vivo and pulsed with cocktails of melanoma antigen peptides or autologous melanoma tissue lysates. Then the dendritic cells were directly injected into lymph nodes of melanoma patients. In this trial, 31% (5/16 patients) showed clinical response including two patients with complete response. In the trial by Thurner et al. [86], dendritic cells propagated ex vivo and pulsed with a MAGE-3 peptide were subcutaneously administered to patients with advanced melanoma. Regression of individual metastases including liver metastases was observed in six out of 11 patients and MAGE-3-specific CD8+ lymphocytes were induced in eight patients.

Malignant melanoma is one of the main targets of gene therapy, however, no definitely effective gene therapy has been established yet. Our group is now investigating the effect of transduction of IFN-β gene into melanoma cells using multilayered cationic liposomes as a vector. Human melanoma cells transfected with IFN-β gene (0.6μg DNA) by this method produced substantial amount of IFN-β (up to 67U/ml) in vitro and proliferation of melanoma cells was significantly inhibited. Human melanoma nodules transplanted to nude mice disappeared completely after six times local injection of the liposomes containing IFN-β gene (3 μg DNA/injection) [87]. In contrast, injection of IFN-β protein (5 × 10^4 U/injection) inhibited only slightly the growth of melanoma nodules. The reason for the better effect of IFN-β gene compared to IFN-β protein is not clear, however, data reported by Hanson et al. may be interesting regarding this point [88]. They showed endogeneous expression of IFN gene is related to increased sensitivity to IFN. IFN gene is located at chromosome 9p21, which is often deleted in melanoma cells as described before. Thus, transduction and expression of IFN-β gene by our gene therapy may recover sensitivity of melanoma cells to IFN-β. Because of various effects of IFN-β such as direct growth inhibition, stimulation of antigen expression, and suppression of angiogenesis, IFN-β gene therapy is worthy to be evaluated clinically.

4.3. Molecular classification of melanoma by gene expression profile

Malignant melanoma shows highly complex biologic behavior. Even if the tumor thickness is the same, some melanomas soon metastasize to distant organs but others do not. Much more reliable estimation of prognosis of each melanoma patient is necessary to determine indication of adjuvant therapy. A new horizon has been now opened for predicting biologic properties of individual melanoma. This attractive concept is based on mathematical analyses of gene expression patterns in each melanoma using the DNA microarray technology. According to Bittner et al. [89], the detailed gene expression profiling makes it possible to classify each melanoma into subgroups distinctive in prognosis [88]. Using such an sophisticated method, in the near future, we will be able to predict not only biologic behavior of each melanoma but also sensitivity to chemotherapy and/or immunotherapy, allowing us to give the tailor-made therapy for each melanoma patient.

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