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What Is New in Melanoma Genetics and Treatment?

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Genetics · Melanoma · Therapy

Abstract

New therapies for advanced melanoma have led to major advances, which, for the first time, showed improved survival for patients with this very challenging neoplasm. These new treatments are based on gene-targeted therapies or stimulation of immune responses. However, these treatments are not without challenges in terms of resistance and toxicity. Physicians should be aware of these side effects as prompt treatment may save lives. Melanoma genetics is also unravelling new genetic risk factors involving telomere genes as well as new gene pathways at the somatic level which may soon become therapeutic targets. It is also shedding new light onto the pathology of this tumour with links to neural diseases and longevity.

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What Is New in the Genetics of Cutaneous Melanoma?

Whole-genome sequencing of tumour DNA, expression arrays and whole-genome methylation have been bringing up a large amount of data over the last few years

as seen with other cancers. It is clear that melanoma pathology involves many gene pathways, and trying to identify driver mutations is not an easy task.

Susceptibility Genes*High-Risk Genes*

Melanoma high-risk genes are defined as genes that when mutated in an individual confer a high risk of developing melanoma and are usually associated with multiple melanoma cases within the family. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) was the first gene associated with melanoma susceptibility.

CDKN2A (p16) has been found to be relevant for around 25% of melanoma families but the prevalence of these mutations in the sporadic melanoma is very low so genetic testing is reserved for families at high risk [1]. Specific inclusion criteria for genetic testing for p16 or *CDK4* (another gene linked to p16) is the presence of multiple melanomas within a family, presence of pancreatic cancer in a melanoma family or presence of multiple primary melanomas even affecting one individual only. Other family cancer syndromes may be found in the context of

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melanoma susceptibility, and these families may need screening for breast, ovary or colon cancer as appropriate.

More recently, less common germline mutations in genes such as *MITF*, *BAP1* and p14 *ARF* have been linked to melanoma and have explained the clustering of kidney, breast and brain tumours within some melanoma families [2]. *BAP1* is located in the chromosomal region 3p21 and encodes a protein that acts as a tumour suppressor through transcription regulation via chromatin remodeling and the ubiquitin-proteasome system. The frequency of *CDKN2A* wild-type melanoma-prone families with mutations in *BAP1* is not well established, but beyond cutaneous melanoma, families bearing *BAP1* mutations seem to suffer also more frequently from other cancers such as uveal melanoma, mesothelioma, kidney, other cutaneous tumours and other cancers [3]. It is therefore important to document a family history of all cancers and not just skin cancer when screening patients at high risk of melanoma.

Genome-wide analyses using a case-control design have reported many more common low-penetrance single-nucleotide polymorphisms associated with increased melanoma risk [4]. Many of these genes can be grouped into pigmentation or naevus genes but many others are not really linked to specific skin phenotypes. Some of these genes are involved in telomere biology and neural diseases for example. Although pigmentation may affect the ability to tan, there are other factors associated with fair skin that may also affect melanoma risk such as body weight and immune responses via neuro-endocrine factors through the very complex melanocortin pathways [5, 6].

A high number of naevi, the strongest phenotypic risk factor for melanoma, has been found to be associated with longer telomeres in circulating white cells [7].

Telomeres consist of tandem nucleotide repeats (TTAGGG) and are located at the ends of chromosomes, which guard against loss of genetic material during cellular replication. Due to an inherent end replication problem, chromosomes are exposed to a potential loss of genetic material, with telomeres acting as a buffer against loss of chromatin. Repeated cell cycles eventually lead to a critically shortened telomere length, which signals cellular senescence and triggers apoptosis. This arrest in proliferation is thought to protect against malignant transformation, and a failure to do so results in catastrophic genomic instability and carcinogenesis [8].

Telomere length in white cells is predictive of biological ageing and is seen as 'biological age clock'. Telomeres shorten both with age and following environmental ex-

posures associated with cancer risk, such as increased caloric intake, smoking, low bone mineral density and ultraviolet irradiation [9–11].

Several publications have now replicated the association with longer telomeres in melanoma cohorts: both telomere length and single-nucleotide polymorphisms predicting telomere length are important for predicting naevus number and melanoma risk. Horn et al. [12] identified a germline mutation in the promoter of the telomerase reverse transcriptase (*TERT*) gene in a melanoma-prone family with many melanoma cases using multipoint linkage analyses and target-enriched high-throughput sequencing. *TERT* is located in 5p15 and encodes the catalytic subunit of the telomerase, which is the ribonucleoprotein complex that maintains telomere length. However, other genes also maintain telomere length [13]. Another gene which controls telomerase is *POT1*, which plays an important role in telomere maintenance by preventing the inappropriate processing of the exposed chromosome ends [14]. *POT1* is located within the 7q31 chromosomal region and encodes a protein of the telomeric shelterin complex. Two other telomere genes have been described in melanoma-prone families involved in the shelterin complex, *ACD* and *TERF2IP* [15]. Overall, germline mutations in genes involved in telomere maintenance (*TERT*, *POT1*, *ACD* and *TERF2IP*) may explain around 1% of familial melanoma cases, showing the relevance of telomere maintenance in melanoma susceptibility [16]. However, methylation of telomere genes and other mechanisms affecting telomere maintenance other than mutations need to be investigated.

This suggests that individuals at risk of melanoma with a large number of naevi may have reduced senescence and potentially increased longevity of melanocytes. So in clinical practice, this may translate as having a large number of naevi in patients aged over 50 years, and this should be regarded as a significant risk factor for melanoma.

Low- to Moderate-Risk Genes

Melanocortin 1 receptor (*MC1R*) is considered a moderate-risk gene, and its role in melanoma susceptibility has been widely studied [17]. *MC1R*, located on 16q24, is one of the master regulator genes of human pigmentation and encodes the α -melanocyte-stimulating hormone receptor 1. Variants in *MC1R* have different functional effects, either at the level of α -melanocyte-stimulating hormone binding or cAMP signalling, resulting in changes in the ratio between eumelanin (brown pigment) and pheomelanin (red-yellow pigment, potentially mutagen-

ic). *MC1R* variants are associated with skin and hair pigmentation and, independently of their phenotypic effect, *MC1R* variants are associated with an increased risk of developing melanoma [18].

Somatic Melanoma Genetics

At the somatic level in the tumour, the mitogen-activated protein kinase (MAPK) pathway is highly relevant in melanoma. Approximately 50% of melanoma tumours have activating mutations in the *BRAF* gene [19]. *RAS* mutations are also informative and prevalent in 20% of melanomas which are more likely to be fast-growing and nodular types of melanomas [20, 21].

Mutations of *c-KIT* are preferentially observed in acral lentiginous melanoma and mucosal tumours [22]. Overall, 10–22% of patients with mucosal melanomas have tumours containing *KIT* mutations or amplifications [23]. Thus, sensitivity to *KIT* inhibition exists in a subset of melanoma tumours but these are not common [24].

In the clinical setting, *BRAF* mutations are routinely screened in melanoma tumours in stage III and above but when *BRAF* mutations are not detected, the tumour may need to be screened for *NRAS*, *KIT* and *GNAQ* mutations if appropriate [25].

Strategies to target melanomas that are *BRAF*/*NRAS*/*KIT* wild type (triple wild type) have proven even more elusive. Mutations in the *PTEN* gene have been detected in a variety of human cancers including breast, thyroid, glioblastomas, endometrial, prostate and melanoma [26]. Initial studies demonstrated a mutation rate in *PTEN* of 30–40% in melanoma cell lines and 10% in primary melanomas [27]. *PTEN* is also involved in mediating growth arrest and other cellular functions of the MAPK pathway [28]. Germline mutations in this gene also predispose carriers to develop Cowden's disease, a heritable cancer risk syndrome which is extremely rare [29].

The identification of PI3K/AKT signalling as a core pathway for melanoma development and therapeutic escape suggested the possibility of cotargeting MAPK and PI3K/AKT signalling in *BRAF*-mutant melanoma. Many melanoma mutations are in fact potentially actionable with drug targets, and drug development in these gene pathways is moving fast [30].

Genome-wide sequencing of melanoma tumours is generating interesting data. Melanoma is one of the most mutated cancers in terms of somatic changes as well as lung and bowel cancer, and the presence of many somatic changes in these tumours may be because of the increased interactions with the environment in the respective organs [18].

What Is New in the Treatment of Cutaneous Melanoma?

Sentinel Node Biopsies and Lymphadenectomies

Melanoma patients with intermediate and thick tumours are offered a sentinel node biopsy to identify lymph node spread as this procedure has prognostic value stratifying patients in different risk categories [31–33]. Other parameters such as mitoses and ulceration are also helpful in thinner melanomas [34]. After a positive sentinel lymph node has been detected, the current guidelines recommend a complete lymph node dissection of all involved metastatic basins but in cases with very small deposits in the lymph node, the patient may be given the choice of avoiding a lymphadenectomy [35]. The number of positive lymph nodes and its ratio represent the two most important prognostic factors in stage III melanoma patients [36, 37]. We are still awaiting the results of the Multicenter Selective Lymphadenectomy Trial II (MSLT-II) in which patients with a positive sentinel node are randomized to completion lymphadenectomy or observation with ultrasound. Despite the risk of a false-negative sentinel lymph node [38], its role in predicting survival is still high enough to justify the procedure. Patients need to be well informed about the prognostic value of this procedure but access to adjuvant treatment for stage III melanoma may also justify the use of this procedure in the future.

Metastatic Melanoma

Advances in the understanding of signalling pathways, melanoma oncogenes and immune system regulation have recently resulted in a new generation of therapeutics that have significantly improved response rates and survival.

Signalling Pathway-Directed Therapies

Approximately 50% of melanoma tumours have an activating mutation in the *BRAF* oncogene, which results in the constitutive activation of the MAPK signalling pathway [39]. Potent selective antagonists of mutant *BRAF* (vemurafenib and dabrafenib) are regarded as standard of care for metastatic *BRAF*-mutant melanoma [40, 41]. Of at least as much clinical importance, however, is the fact that approximately 90% of patients whose cancers carry the *BRAF* mutation have some tumour shrinkage with targeted inhibitors and that these responses occur very rapidly. This rapid response rate can provide palliative relief for patients with significant tumour-related symptoms but usually last 6–9 months when other therapies may need to be considered [42–46].

Activated BRAF phosphorylates MEK, the next downstream target in the MAPK pathway. Although *MEK* is rarely mutated in untreated melanoma, it is a conduit for signalling, and inhibition of this target results in significant benefit in comparison to chemotherapy. Trametinib and cobimetinib are selective MEK inhibitors, and both have been shown to improve median progression-free and overall survival in comparison to chemotherapy in BRAF-mutant melanoma [46–48]. Dual inhibition of both BRAF and MEK pathways in *BRAF*-mutant disease is now proven to be significantly more potent than single-agent inhibition.

The side effects of these gene-targeted therapies with BRAF inhibitors (vemurafenib) include a variety of different effects with the majority occurring on the skin [49, 50]. The most common adverse events recorded in the BRIM-3 registration trial included arthralgia, fatigue, nausea, rashes, photosensitivity and cutaneous squamous cell carcinoma or kerato-acanthoma [51]. The dose was modified or interrupted due to adverse events in 38% of patients treated with vemurafenib and permanently discontinued in only 7% of the patients treated.

Clinical trials of dabrafenib, another BRAF inhibitor, began in 2009 with final results published in 2012. The most common adverse effects included cutaneous squamous cell carcinoma (11%), fatigue (8%) and pyrexia (6%). Thirteen (7%) patients required a reduction in the dose. Squamous cell carcinomas and kerato-acanthoma appeared less frequently than in patients treated with dabrafenib than with vemurafenib, although a direct comparison has never been investigated [41, 52, 53].

To reduce these cutaneous side effects, it is recommended to use soap substitutes, emollients, keratolytics containing salicylic acid or urea, topical or oral antibiotics (in case of acne type eruptions on the face/trunk) and sunscreens applied every day even on non-sunny days as photosensitivity can be severe especially for vemurafenib.

The issue with RAF kinase inhibitors is acquired resistance caused by reactivation of the MAPK pathway. This results in a median progression-free survival of 6–8 months. BRAF inhibitors may also lead to new melanoma primary development induced by a paradoxical activation of the MAPK pathway in cells without *BRAF* mutations. This led to the introduction of a MEK inhibitor, trametinib, combined with a BRAF inhibitor, dabrafenib, for combination therapy. A significant improvement in the response rate (76 vs. 54%, $p = 0.03$) and progression-free survival was observed in the combination arm, compared to dabrafenib monotherapy [54] with fewer adverse events for squamous cell carcinoma (7 vs. 19%, respec-

tively), although the incidence of pyrexia (71 vs. 26%) was increased by the introduction of a MEK inhibitor.

These patients under targeted gene therapies are also more likely to develop multiple keratotic lesions that may progress to kerato-acanthomas and squamous cell carcinomas, and may need a referral to the local dermatologist via the 2-week wait if the lesions are growing quickly. During treatment, patients can notice changes in size, shape and colour of naevi, and as new primary melanoma can occur, it is important to have dermatological input in oncology clinics treating melanoma [55]. This also highlights the need of a dermoscopy and digital monitoring to assess changing naevi in these patients and the early diagnosis of BRAF-inhibitor-related skin cancer (i.e. squamous cell carcinoma, etc.).

Immunotherapy

Melanoma is considered one of the most immunogenic tumours, and the role of the immune system in driving prognosis is well known even in localized tumours [56–58].

Improved understanding of the control mechanisms regulating T-cell activation has led to a new generation of immunotherapies for many cancers and especially melanoma. The first checkpoint inhibitor in routine clinical use is ipilimumab, a humanized monoclonal antibody against the CTLA-4 protein, which stimulates immune responses against the tumour [59, 60]. Ipilimumab benefits only approximately 15% of patients, and unfortunately there is currently no available biomarker that identifies which patients will derive benefit. Although it does improve median overall survival, the real attraction of the agent is the possibility of a long-term durable remission up to 5 years.

Recent attention has focused on an alternative checkpoint, PD-1, and its ligand PDL-1 [61]. The blockade of the PD-1/PDL-1 interaction is able to overcome immune resistance [62–64]: nivolumab and pembrolizumab have now been shown to be superior to chemotherapy and to ipilimumab alone in patients with advanced melanoma. They are licensed for use in the USA, and, at the time of writing, European licenses were obtained for pembrolizumab [65–67]. Thirty to forty percent of patients experience significant shrinkage of their tumours with these new agents, which is significantly higher than for ipilimumab.

The side effect profile of these drugs is severer than that of BRAF and MEK inhibitors with toxicity to the bowel, lung and liver with potential severe life-threatening colitis, pneumonitis and hepatitis. Although patients

are instructed to contact their oncology team urgently in case of lung or gastrointestinal symptoms under immune therapies, general practitioner and emergency physicians may be faced with these emergencies, and swift actions contacting the oncology team with rapid use of high-dose steroids can be life saving. Pituitary failure is also a severe side effect of immunotherapy and needs to be recognized early. Other side effects that are less threatening are: fatigue, skin rashes (except extensive vasculitic rash which may signal a severe auto-immune reaction), vitiligo and neuropathy.

Conclusions

In primary care, it is important to identify patients most at risk of melanoma with a full skin examination looking for suspicious naevi as well as documenting whether the patient has a high number of naevi [68]. Documenting the family history of all types of cancers is also very important as melanoma can be found in cancer-prone families, and cancer screening may be recommended. Documenting past sun exposure is unfortunate-

ly not identifying patients most at risk of melanoma. It is likely that in the next 5 years, panels of melanoma susceptibility genes will be tested in high-risk patients to help devise follow-up strategies for dermatologists but also to give advice about other cancer screening, such as for bowel, breast or kidney cancer, which may be more prevalent in high-risk families. Melanoma treatment is changing at great pace with improved survival that had not been seen previously for this very challenging tumour. Many clinicians and cancer nurses may be faced with drug toxicity with these new drugs and need to be aware of the side effects for prompt treatment and/or referral. Although oncology units prescribing these drugs will have all the procedures in place to support melanoma patients, general practitioners and emergency physicians need to be vigilant for early signs of toxicity especially for the lung and gut as the prognosis is much improved if identified early and treated promptly.

Disclosure Statement

The authors have no conflict of interest to disclose.

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