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Molecular Testing in Melanoma

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Abstract

Melanoma is the deadliest form of skin cancer and is increasing in incidence. Recent treatment advances have been made, but there remains a need for continued development of effective therapy options, as treatment rarely leads to cure. Many melanomas contain somatic mutations involved tumor pathogenesis. Accurate identification of these mutations is necessary in order to stratify patients for the purpose of treatment and potential for clinical trials, given the absence or presence of a specific mutation. There are a number of techniques available that will identify genetic mutations and genomic aberrations present within melanoma tumor samples which are reviewed here. The type of mutation and sample number will drive selection of a given mutation detection strategy. The strengths and weaknesses, along with limitations, of the various methods will also be discussed. The discovery of somatic mutations integral in melanoma will increase our understanding of tumor pathogenesis and should facilitate identification of mutations relevant to clinical treatment decisions, advancing progress towards personalized medicine.

Keywords

Melanoma; Molecular testing; Somatic mutation testing; Single nucleotide extension; Direct sequencing; Copy Number; Next generation sequencing; BRAF; NRAS; KIT

Introduction

Melanoma incidence is increasing and anticipated to continue to rise. In the United States, the estimated new cases and deaths related to melanoma for 2011, were 70,230 and 8,790, respectively ¹. Until recently, few effective treatment options were available for patients with advanced stage melanoma. However, this past year saw the FDA approval of the antagonist CTLA-4 antibody, Ipilimumab, and the targeted mutant BRAF inhibitor, Vemurafenib ^{2–4}. Although a small percentage of cures have been observed with both IL-2 and anti-CTLA-4 antibody therapy ^{3,5,6} and long term responses can be observed with BRAF inhibition ^{2,7}, the majority of patients eventually relapse after treatment at varying time periods. Thus, despite these advances, there remains a need for continued development of effective treatment options. Additionally, there are limited options available for adjuvant therapy for Stage III melanoma.

Through genetic and molecular studies, a number of somatic mutations have been identified which play key roles in melanoma pathogenesis, either in the early stages of tumor development, during metastasis, or both. Molecular and genetic understanding of the underlying mechanisms driving melanoma tumor cells has led to the development of targeted therapies in the treatment of this disease (reviewed in ^{8–10}). The identification of

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mutant *BRAF* as a factor in melanoma pathogenesis was integral in the development of targeted BRAF inhibition, which has led to improved overall survival for patients with advanced melanoma^{2,11,12}. Continued discovery of genetic mutations in melanoma will allow for the development of specific therapies directed towards individual mutations and for the development of personalized medicine. In this review, we discuss the various techniques available for the identification of somatic genetic mutations and genomic aberrations in melanoma.

Testing for Somatic Genetic Mutations – Genes and Technology

Genetic mutations and genomic aberrations have been implicated in the pathogenesis in a number of cancers, such as *EGFR* in lung cancer, *KRAS* in colon cancer, and *BRAF* in melanoma^{2,13–16}. In melanoma, a number of specific genetic and genomic aberrations have been identified to be important in tumorigenesis. Most of the identified driving changes are point mutations; however others are genomic aberrations, such as loss of heterozygosity at specific genetic loci or amplification, and epigenetic silencing can be observed. Somatic genetic and genomic changes in genes such as *NRAS*, *BRAF*, *KIT*, *PTEN*, *GNAQ/GNA11*, and *MAP2K1/2 (MEK1/2)* are known to be crucial for melanoma pathogenesis. Understanding of these genetic underpinnings of melanoma has enabled the development of targeted therapy directed against KIT, MEK 1/2, and mutant BRAF led to a number of mutation driven clinical trials, and most recently, the FDA approval of vemurafenib^{2,17–19}. Other genes have both inherited mutations in familial melanoma and somatic changes in sporadic melanoma, such as *MITF*, *CDKN2A* and *CDK4*. The roles of some of these mutations have been well defined in melanoma, while others continue to be under study. Identification of the array of mutations in patients with melanoma will be useful in determining a genetic profile of the tumor with potential implications for treatment decisions.

Integral to the development of therapies directed against genetic mutations in any cancer, and particularly melanoma, is the reliable identification of these genetic aberrations in tumor samples, both fresh and formalin-fixed paraffin embedded. Moreover, the presence or absence of specific mutations may determine the use of a particular therapy and inclusion and exclusion in a clinical trial specific for patients carrying a known mutation in their melanoma. A number of different types of techniques are available to test for specific mutations; for this review, we focus on somatic mutation testing as it is currently used for therapeutic selection in melanoma. Evaluating somatic mutations can be broken down into a number of types of testing, including single nucleotide extension techniques, direct sequencing techniques, copy number analysis, and high density sequencing. The type of mutation, as well as the number of samples, being analyzed will most likely determine the techniques used for mutation detection.

Testing for genetic and genomic changes in melanoma

Direct sequencing of DNA from tumor samples can identify all point mutations in a given stretch of DNA. PyrosequencingTM (Qiagen, Inc., Alameda CA) provides short sequencing of DNA between 300 – 500 nucleotides²⁰. Thus, if several mutations are clustered in a small region, it provides advantage over single nucleotide extension. Mutations can be detected when the mutant DNA comprises 5% of the total DNA sample, which has been demonstrated to occur even in DNA samples from FFPE tumors²¹. Many molecular pathology laboratories use this technique to evaluate regions with multiple different mutations clustered over several adjacent nucleotides, such as the region surrounding BRAF V600. Traditional Sanger sequencing continues to be used to evaluate genes with mutations throughout an exon. Although it is less sensitive than Pyrosequencing or the single

nucleotide extension techniques described below at 25% mutation rate, it remains the assay of choice for genes such as *KIT*.

Single nucleotide extension assays are useful for identifying a known point mutation. These assays evaluate a specific base of interest, and will not identify mutations outside the interrogated base. Additionally depending on the design of the assay, if only one type of base change is anticipated (e.g. A to C), they may miss any other base changes (e.g. A to G). Two commonly used nucleotide extension techniques that are used include iPlex™ (Sequenom, Inc, San Diego CA) ^{22,23} and SNaPshot™ (Applied Biosystems, Inc, Foster City CA) ²⁴. These platforms use primers of certain lengths for amplification, designed around the mutated single base of interest, and a second primer for the assay is used, which terminates at the base prior to the base of interest. Single base extension reactions are then carried out and depending on the design potentially evaluate all four bases. The designed primers contain common tags, either 10-mers in the iPlex platform or poly(dT) in the SNaPshot platform, to allow for PCR amplification and multiplexing ²⁵. The different nucleotides are then detected by matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF) analysis for iPlex ²⁶ and analysis of fluorescent labeled nucleotides for SNaPshot ²⁵. These techniques have a level of sensitivity higher than direct sequencing, with mutation accounting for 10% and 5% of total DNA for iPlex ²⁷ and SNaPshot ²⁵, respectively. They also are successful genotyping DNA extracted from formalin-fixed paraffin embedded tumors, with lower quality DNA. These techniques are designed for multiplexing and the interrogation of a number of different point mutations (or single nucleotide polymorphisms) simultaneously. They cannot be used efficiently for interrogating multiple base changes in a short stretch of nucleotides unless several multiplexes are designed, which usually include other genes, and the overlapping regions are distributed among the multiplexes. Single nucleotide extension techniques are commonly used in clinical molecular pathology for diagnosis. They are best suited for genes that contain mutation hotspots, such as *NRAS*, *BRAF*, and *GNAQ/GNA11*.

Large genomic changes can be identified using Fluorescence in Situ Hybridization (FISH). FISH allows for the detection of larger amplifications, deletions, and rearrangements in tumors spanning approximately 100,000 base pairs. This technique is widely used in hematologic malignancies to provide detailed cytogenetic information with direct bearing on risk stratification, leading to specific treatment decisions. FISH also is used in solid malignancies as well ^{28,29}, mostly recently with clinical identification of *ALK* rearrangement in lung cancers for determining treatment with the *ALK* inhibitor crizotinib ³⁰ and genomic analysis of malignant mesothelioma ³¹. Investigators have attempted to use FISH as a way to aid in the diagnosis of melanoma in ambiguous samples. Senetta et al. have evaluated the use of three specific probes, targeted towards *RREB1*, *MYB*, and *CCND1* genes, as well as centromere control probe in the differentiation of benign nevi and malignant melanoma ³². Specific probe patterns were predictive of benign lesions vs. malignant melanoma in the validation samples, while results are still unclear in the ambiguous samples. Hossain et al. examined the use of chromosome specific FISH probes in order to distinguish benign lesions vs. malignant melanoma ³³. Chromosomal abnormalities were identified in 94% melanoma samples, 6% of compound nevi, and 0% of normal skin with most frequent abnormality being gain of chromosome 11, followed by gains in chromosomes 7, 20, and 6 ³³. While FISH is not reliable as an individual diagnostic tool for diagnosis of malignant melanoma, it may be used as a supplementary test when samples are ambiguous and the diagnosis is not straightforward.

DNA copy number alterations have been shown to be involved in the pathogenesis of a number of human cancers ³⁴ and may play a role in prediction of progression or clinical outcome of several tumor types ³⁵. Copy number profiling in melanoma can be performed in

order to analyze multiple genomic aberrations in melanoma tumor samples and evaluate distinct genomic changes, including high copy gains and losses of chromosomes or parts of chromosomes, as well as minute gains or losses. Previous studies have evaluated melanoma cell lines looking for genomic alterations involved in the pathogenesis of melanoma; amplifications have been identified in a number of genes including *BRAF*, *NRAS*, *MITF*, *CCND1*, *MDM2*, *CCNE1*, and *NOTCH2*, and homozygous deletions were identified in *CDKN2A* and *PTEN*^{36–38}. Copy number analysis also allows for the investigation and identification of novel genes and pathways involved in melanoma. This technique is useful in the clinical setting when samples are ambiguous and difficult to diagnosis, providing additional information, like expression profiles, which may aid in the final diagnosis. Genome wide copy number analysis technologies have evolved over the years. Initially, probe sequences were derived from bacterial artificial chromosomes using array comparative genomic hybridization (aCGH), but now have progressed to single nucleotide polymorphism (SNP)-based arrays and oligonucleotide aCGH. SNP arrays have oligonucleotide probes of about 25 nucleotides in length, whereas oligonucleotide platforms used for aCGH have 60-mer probes. Greshock et al. compared a number of different profiling platforms and determined that the longer oligonucleotide probes were better at detecting low-amplitude genomic alterations in melanoma tumor cells and SNP arrays superior for detecting copy number alteration in non-coding elements³⁹. This technique allows for the assessment of genomic changes throughout the tumor sample and allows for high throughput of samples. Using this technique, a genomic signature can be obtained for each melanoma tumor, which can inform therapeutic choice, along with the genetic changes in the tumor.

Whereas copy number profiling provides a broad look at the genetic signature of tumor samples, targeted copy number analysis allows you to evaluate one gene or a few genes of interest, and can evaluate a number of regions across a specific gene of interest. One technique used to perform targeted analysis is multiplex probe ligation amplification (MLPA; MRC-Holland, Amsterdam, Netherlands). MLPA identifies genomic amplifications and deletions^{40,41}. This technique can be multiplexed and has an advantage over aCGH in that it uses less tumor DNA starting material. In MLPA, probes annealed adjacent to each other and ligated. Copy number of test probes is determined by normalization to control probes. For specific gene analyses, the probes are targeted to the exons. This technique has been used to detect genomic rearrangements in genes associated with Mendelian syndromes, contiguous gene deletion syndromes and evaluate somatic copy number changes, among others^{42–45}.

Massively Parallel Sequencing

Massively parallel sequencing techniques are now available, and far surpass traditional sequencing, as they enable the sequencing of exomes and entire genomes of tumor samples. These techniques allow for the simultaneous sequencing of a large number of genes and determination of mutations, genetic alterations, and copy number changes. Ross and Cronin review the different types of next-generation technologies used in research laboratories currently in detail⁴⁶. Next generation sequencing utilizes libraries constructed from genomic DNA or exons and flanking DNA and run on a flow cell surface, with short DNA fragments subjected to adapter ligation, capture of the DNA fragments, amplification, and repetitive cycles of sequencing and sequence detection. These techniques are continually improved to increase multiplexing within each flow cell. Next generation sequencing generates a large amount of sequence data and information that must be processed and then analyzed. Analysis of these data is a constantly evolving field.

Whole genome sequencing has been performed to evaluate malignant melanoma genomes. In the initial study, analysis was performed on a cell line derived from a metastasis of a

malignant melanoma and compared to a matched lymphoblastoid cell line⁴⁷. They identified 33,345 somatic mutations, 680 small deletions, 303 small insertions, and 51 somatic rearrangements. Mutational changes were noted that were consistent with ultraviolet exposure, predominantly C>T/G>A transitions. They identified mutations known to be important in melanoma, including *BRAF*V600E, a large genomic deletion of *PTEN*, and two base pair deletion of *CDKN2A*. Novel mutations were also identified including *SPDEF*, which encodes for a member of the ETS transcription factor family⁴⁸; *MMP28*, which is a matrix metalloproteinase gene and was reported to be mutated in melanoma⁴⁹; and *UVRAG*, which has been shown to be involved in autophagy and is a proposed tumor suppressor gene^{50,51}. It is anticipated that additional sequencing of melanoma genomes will provide new information on novel mutations involved in melanoma pathogenesis.

Recently, whole exome sequencing of melanoma samples have implicated other genes involved in melanoma pathogenesis. An analysis of 14 metastatic melanoma tumors revealed a number of genes that contained somatic mutations at an elevated frequency, beyond those previously identified⁵². Specifically, Wei et al. identified a recurrent mutation in *TRRAP* at position p.S722F, found to be mutated in ~4% (6/167) of the samples, with subsequent functional analysis suggestive of *TRRAP* functioning as an oncogene. *GRIN2A* was also found to be mutated in 33% (17/52) of melanoma samples. Similar to what had been previously observed, *BRAF* was found to be mutated in ~50% of tumor samples. *NRAS* mutations were not identified in this set sequencing, but are known to be present in approximately 15%–20% of melanomas^{53–55}. Recently, additional exome sequencing of melanoma identified recurrent somatic mutations in *MAP2K1* and *MAP2K2*⁵⁶. Seven melanoma cell lines, generated from separate metastases, were analyzed in order to identify additional somatic mutations important in melanoma pathogenesis⁵⁶. The seven samples analyzed contained either *BRAF* or *NRAS* mutations; mutations in homologous codons in *MAP2K1* and *MAP2K2* were identified in two melanoma samples. Further evaluation revealed mutations in either *MAP2K1* or *MAP2K2* in 8% of melanoma samples (10/127 samples)⁵⁶. Additional genes, *FAT4*, *DSC1*, and *LRPIB*, were also identified as mutated, but their significance in melanoma is unknown at this time. Genetic information generated identified known mutated genes involved in melanoma pathogenesis and has led to the identification of additional candidate genes which may be involved in melanoma.

The introduction of massively parallel sequencing techniques, both exome and whole genome, will provide us with a vast amount of new data regarding the genetics and genomics of malignant melanoma. This information can be assembled to molecularly profile melanomas and assist to stratify patients. The molecular characterization can be utilized to predict how patients will respond to particular therapies and may help predict effective treatment selection and resistance to therapies. Moreover, the identification of novel mutations may provide for new targets for therapeutic intervention. Massively parallel sequencing techniques and analysis of the data are still in the process of being refined. Currently, these assays are higher in cost than traditional sequencing, however, in the long term, these methods will dramatically reduce cost per sample, allowing many more samples to be sequenced. The \$1000 genome was recently announced, but it is not clear how long it will take to put massively parallel sequencing into place for every cancer. Furthermore, source DNA for massively parallel sequencing is currently restricted to fresh – frozen tumor samples, although it is being developed for use in FFPE. The most costly component will remain the analysis of the large volume of generated data. The techniques currently used to screen for mutations (ie. single base extension, pyrosequencing) are optimized to process a big number of samples in a relatively quick period of time and at a reasonable cost per samples. It will be crucial to have the infrastructure to process and analyze all the data generated from next-generation sequencing, including the interpretation of results and how this information translates to clinical practice. This new technique holds great promise and

will lead us closer to personalized medicine and treatment for cancer, particularly melanoma.

It remains to be seen what impact next generation sequencing will have on clinical application. This technique has provided investigators with increasing information regarding melanoma samples and the identification of a number of novel candidate genes involved in melanoma pathogenesis. These discoveries will hopefully translate into future therapies. While next generation sequencing provides a vast array of information about a melanoma tumor sample, known mutations with available therapeutic intervention also can be identified by other, well established methods. Thus, the use of whole genome and whole exome sequencing may not be the ideal first choice for tumor analysis based on costs and available therapeutics. It is reasonable to assess the mutation status of *BRAF*, *NRAS*, and *KIT*, respectively, in cutaneous melanoma samples before pursuing alternative mutation interrogation. Next generation sequencing may be applicable in situations where known mutations are not identified and the identification of additional genetic mutations is needed. Moreover, it might be a good technique to use once a patient has developed resistance to current therapy, thereby providing information relating to the development of resistance and other mutations to target with therapy. One could also postulate that with continued next generation sequencing on melanoma samples and increased coverage of the genome it is possible that all mutations would be identified and that no new information would be discovered by next generation sequencing. It is also quite possible that given the heterogeneous nature of melanoma samples, and in cancer in general, next generation sequencing will identify mutations specific to each patient's melanoma and therefore directly affect therapeutic interventions with small molecules. Further investigation with these techniques and data interpretation will help elucidate the optimal testing that should occur with tumor samples.

Genetic mutations and genomic aberrations in melanoma

NRAS

Activation of the MAP kinase pathway has been identified as a key player in a number of cancers and is identified to be important in melanoma; a number of activating mutations in the MAP kinase pathway have been found^{57,58}. Activating mutations in *NRAS* have been identified in approximately 15% – 20% of melanoma tumors^{53–55}. The most common *NRAS* mutations are in exon 2 at codon 61, specifically Q61L (leucine substitution for glutamine)⁵⁹, although other amino acid changes also are observed (ie, Q61R, Q61H)⁶⁰. *NRAS* mutations also are observed in exon 1 at codons 12 and 13⁵⁷. The Q61 mutations result in a constitutively active form of the protein leading to uncontrolled cellular proliferation⁵⁹. A recent meta-analysis was performed looking at the presence of *NRAS* mutations in melanoma tumor samples and found it to be associated with nodular melanomas and melanomas at sites of chronic sun-damaged skin⁶¹. Moreover, *NRAS* mutations were associated with thicker tumor samples, increased mitotic rate, and demonstrated a worse clinical outcome compared to patients with *BRAF*V600E mutations or without *NRAS* or *BRAF* mutations⁶².

BRAF

BRAF is a downstream molecule in the MAP kinase pathway and has been demonstrated to be integral in the pathogenesis of melanoma. *BRAF* has been found to be mutated in 40% to 60% of melanoma samples, with the most common *BRAF* mutation at codon 600, resulting in the substitution of glutamic acid for valine (V600E mutation)^{63–65}. -This mutation results in constitutive kinase activity of *BRAF* and subsequent downstream signaling through the MAP kinase pathway⁶⁶. Additional *BRAF* V600 mutations have been observed in

melanoma tumor samples, as well as melanoma cell lines ^{65,67,68}. Occasionally, mutations have been identified in *BRAF* in the loop domain (exon 11) ⁶⁵. Interestingly, *BRAF* has also been shown to be mutated in a significant percentage of benign nevi ⁶⁹. Out of 77 benign nevi that were evaluated, 63 (82%) contained *BRAF* mutations, suggesting they are an early event in the progression to melanoma ⁶⁹. While *BRAF* and *NRAS* mutations are the most common mutations in melanoma and both result in activation of the MAPK signaling pathway, these two mutations are generally mutually exclusive.

Vemurafenib, a targeted therapy directed against V600-mutant *BRAF*, was recently approved by the FDA to treat advanced stage melanoma with a known V600E mutation ², providing a specific example of how identifying a specific somatic mutation in melanoma tumors has led to direct targeted therapy that has also demonstrated a significant clinical benefit. As this therapy is directed against this specific mutation, molecular testing of tumor samples is required in order to determine the presence or absence of this mutation. This mutation can be assessed by a number of different methods that have been discussed above. Recently, a commercially available test identifying the *BRAF* V600E mutation was approved by the FDA – the cobas® 4800 *BRAF* V600 Mutation Test. The cobas® test is a real-time PCR assay which uses TaqMan probes that are labeled with different fluorescent tags which bind to wild-type and V600E mutant *BRAF* sequences, and are detected when they match to the correct sequence, and a second set of primers for DNA amplification. As with all techniques used to detect somatic mutations, it is limited by the amount of mutant sequence in the initial sample as well as DNA integrity. This test is also limited in its detection of non-V600E *BRAF* mutations (package insert). Additional limitations of the test exist. It demonstrates a 66% cross-sensitivity for *BRAF* V600K mutations. Moreover, based on the nature of mutant detection by the cobas® test, this test will not detect two base pair mutations that result in the V600E mutation. Given this information, it is imperative that accurate mutation testing is performed when trying to determine clinical treatment options for patients with melanoma.

KIT

KIT is a receptor tyrosine kinase that is situated on the cell surface and has been identified to play a role normal melanocyte development ⁷⁰, as well as in the pathogenesis of acral, lentiginous, and mucosal melanomas ⁷¹. *KIT* is involved in the activation of a number of intracellular signaling pathways, including the MAPK pathway and PI3K pathway ^{70,72}. Analysis of 102 primary melanomas from mucosa, acral skin, chronic sun-damaged skin and skin without chronic sun-damage by aCGH demonstrated somatic mutations and/or increased copy number of *KIT* in close to 30% of each melanoma tumor type (except those arising from areas of skin without chronic sun-damage) and this corresponded with increased *KIT* expression ⁷¹. Imatinib, which inhibits the tyrosine kinase activity of c-KIT and PDGFR, has been shown to be effective in the treatment of other tumors, including gastrointestinal stromal tumors, where *KIT* has been amplified or mutated ^{73–75}, so it is not unreasonable to expect a response in melanoma. Indeed, in limited studies, individual responses have been seen with imatinib ^{76–80} and dasatinib (another tyrosine kinase inhibitor, similar to imatinib) ⁸¹ in melanoma. There is no one predominant *KIT* mutation and mutations are found in exons 9, 11, 13, and 17. Because of this, molecular testing for mutations must evaluate multiple regions of the gene. Emerging data, however, indicates that not all mutations may result in functional dependence upon c-KIT, or at least correspond with sensitivity to KIT inhibitors ¹⁷.

PTEN

PTEN is a tumor suppressor gene found on chromosome 10q23 and functions as a lipid phosphatase, regulating the PI3K pathway. The PI3K/AKT pathway is well characterized,

and is implicated in melanoma pathogenesis^{82–85}. *PTEN* is involved in controlling cell cycle progression, cell growth and cell proliferation, through its regulation and inhibition of AKT, a downstream component of the PI3K signaling pathway. *PTEN* mutations have been identified in a number of tumors in addition to melanoma, such as breast and prostate cancer⁸⁶, and when involved mutated in the germline, is associated with autosomal dominant inherited *PTEN* hamartomatous syndrome (formerly known as Cowden disease and Bannayan-Riley-Ruvalcaba⁸⁷). *PTEN* mutations and loss have been observed to principally co-exist with *BRAF* mutations^{55,87,88}. Studies continue to investigate the association of *PTEN* and melanoma and in conjunction with other known mutations in melanoma pathogenesis. In melanoma, *PTEN* mutations include missense mutations, deletions, and insertions^{82,89,90,95}, as well as LOH and epigenetic silencing^{82,89,90} making interrogation for mutations and genomic rearrangements in *PTEN* necessary⁸⁹.

GNAQ/GNA11

Uveal melanomas are the most common primary tumors of the eye in adults, arising from melanocytes in the choroid, ciliary body, or iris⁹¹. While *BRAF* and *NRAS* mutations have been shown to play an integral role in cutaneous melanomas, they are not involved in the pathogenesis of uveal melanoma^{92,93}. Mutations affecting the pathogenesis of uveal melanomas have been identified in *GNAQ* and *GNA11*. *GNAQ* and *GNA11* encode for alpha subunits of G-protein-coupled receptors. Mutations at amino acid 209 in both genes result in a constitutively active G-protein and subsequent cell signaling. Van Raamsdonk et al. demonstrated that 83% of uveal melanomas contained activating somatic mutations in *GNAQ* or *GNA11*, which were mutually exclusive⁹⁴. The most frequent observed mutation was in exon 5 (Q209) in both genes and was observed in blue nevi, primary uveal melanoma, and uveal melanoma metastases^{92,94}. Additional mutations in *GNAQ* and *GNA11* were also identified in blue nevi and uveal melanoma, but not to the extent as the Q209 mutation. *GNAQ* Q209 transfection into melanocytes resulted in increased anchorage independent growth and when injected into mice, increased tumorigenicity was observed⁸⁷. The *GNA11* Q209 mutation increased oncogenic potential when injected into mice and cells transduced with this variant demonstrated increased MAP kinase activation, evident through increased ERK phosphorylation⁹⁴. These and other observations are highly suggestive that *GNAQ* and *GNA11* play a role in the pathogenesis of uveal melanoma. These mutations are potential targets for which specific therapy can be directed. Given the limited, site-specific mutations that occur in these genes, it is possible to identify mutations through sequencing of individual exons of interest or by single base pair extension of specific mutations.

Conclusion

A number of genetic mutations have been identified to play a role in both familial and spontaneous melanoma. Molecular testing is evolving as technological advances improve. These techniques have made it possible to determine more precise and specific information from melanoma tumors, and it is anticipated that as technology progresses, so will our understanding of melanoma pathogenesis. These new developments have contributed to the expansion of therapies to target melanoma tumors, as conventional chemotherapy treatments had not been effective, and should facilitate identification of mutations relevant to clinical treatment decisions. The information on somatic mutations will help to advance the development of clinical trials, looking for therapies that will provide durable responses. These advances will allow clinicians to determine the optimal treatment strategy based on the genetic and genomic profile of a patient's tumor, thereby furthering personalized medicine.

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Table 1

Mutation detection methods with description. Brief description of mutation detection methods discussed in the review available to test melanoma samples. Important features and key similarities and differences between techniques are featured.

Mutation Detection Method	Description
Direct Sequencing	<ul style="list-style-type: none"> Sanger sequencing, addition and detection of terminal dideoxynucleotide Only detects mutations in specific DNA sequence with read length approximately 500–700 nucleotides
Pyrosequencing	<ul style="list-style-type: none"> Enzymatic assay detecting pyrophosphate after nucleotide incorporation into growing DNA chain Only detects mutations in specific DNA sequence with read length between 300–500 nucleotides
iPlex™ (Sequenom)	<ul style="list-style-type: none"> Single nucleotide extension assay Multiple mutations can be detected due to multiplexing Difficult to detect multiple base changes in specific sequence Difficult to detect two base pair mutations
SNaPshot™	<ul style="list-style-type: none"> Single nucleotide extension assay Multiple mutations in different DNA sequences can be detected due to multiplexing Difficult to detect multiple base changes in specific sequence Difficult to detect two base pair mutations
cobas® 4800 BRAF V600	<ul style="list-style-type: none"> Single nucleotide extension assay -Specific for single base mutation resulting in BRAF V600E mutation in melanoma samples Does not reliably detect non-BRAF V600E mutations and does not detect two base pair mutations resulting in BRAF V600E mutation
Fluorescence In Situ Hybridization (FISH)	<ul style="list-style-type: none"> Detects large genomic changes such as amplifications, deletions, and rearrangements at the chromosome level
SNP arrays	<ul style="list-style-type: none"> Genome wide detection of multiple genetic and genomic alterations including amplifications, deletions, copy number variation Oligonucleotide probes of approximately 25 nucleotides in length
aCGH	<ul style="list-style-type: none"> Genome wide detection of multiple genetic and genomic alterations including amplifications, deletions, copy number variation Oligonucleotide probes of approximately 60 nucleotides in length
Multiplex probe ligation amplification (MLPA)	<ul style="list-style-type: none"> Targeted copy number analysis of specific genes and detection of amplifications and deletions Can be multiplexed Uses less DNA starting material than aCGH
Whole genome sequencing	<ul style="list-style-type: none"> Simultaneous sequencing of DNA from a sample with detection of mutations, genetic alterations, and copy number changes DNA library constructed from genomic DNA from tumor samples requires DNA from fresh-frozen tumors

Mutation Detection Method	Description
Whole exome sequencing	<ul style="list-style-type: none">• Simultaneous sequencing of DNA from a sample with detection of mutations, genetic alterations, and copy number changes• DNA library constructed from exons and flanking DNA from tumor samples• requires DNA from fresh-frozen tumors