

Genetic Predisposition to Malignant Melanoma

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Abstract

Predisposition to malignant melanoma may be inherited via germ-line mutations that affect proteins of the cell cycling mechanism, increasing the chance of developing the disease on exposure to environmental agents. Germ-line mutations occurring in the CDKN2A loci mapping to chromosomal region 9p21, or the CDK4 loci mapping to 12q14, alter the function of p16. This ultimately results in uncontrolled proliferation of cells. These mutations are most often missense mutations, but insertions and deletions have also been reported. There are phenotypic differences in melanoma between those with mutations in these genes and those without. No phenotypic differences have been reported between those with CDKN2A mutations and those with CDK4 mutations. Germ-line testing of malignant melanoma could be a possibility if more conclusive genetic information can be found and the cost of the test can be reduced.

Introduction

Melanoma is a neoplasia of the melanocytes, primarily of the skin, but occasionally affecting other organs such as the digestive tract, meninges and lymph nodes. Melanoma accounts for only four percent of skin cancer cases, but is responsible for most skin cancer related death due to its highly malignant nature. The incidence of melanoma in Australia has been on the increase and currently sits at three times the incidence rate in the United States. The most recent figures (1998) indicate an age standardised incidence of 46.4 per 100,000 people in Australia, accounting for 10.1 percent of all new cancers (Australian Institute of Health and Welfare 2002).

Five to 12 percent of malignant melanoma develop in individuals with more than one first-degree relative exhibiting the disease. Familial malignant melanoma is postulated to be an autosomal dominant condition. The main gene associated with this disease is CDKN2A, which maps to chromosomal region 9p21. Mutations in the CDK4 gene, mapping to 12q14, have been found in three melanoma prone families. Histological differences between sporadic cases of melanoma and familial melanoma have been found, but there are no differences in the melanomas associated with the different gene loci (Goldstein & Tucker 2001).

Mutations in the CDKN2A and CDK4 genes result in the encoding of abnormal p16 and cdk4 proteins respectively. The abnormal proteins are no longer able to serve their particular functions in cell cycle arrest. The majority of mutations within these genes are missense mutations. Germ-line testing for the mutations is available. However, such tests are limited in usefulness due to the large proportion of melanomas that may arise by mutations that have not been identified.

Symptoms

The first indications of cutaneous malignant melanoma are irregular or abnormal changes to pigmented lesions on the skin. Normal benign pigmented melanocytic lesions (nevi) are small in size, being less than 6 mm in diameter. They have a smooth edge with a regular surface and regular pigmentation. Therefore any nevi appearing larger than normal with an irregular edge, an abnormal surface and pigmentation should be regarded as an indication of possible malignant change. An excision biopsy should be performed to examine this possibility (Stevens & Lowe 2000). Other indications of malignant melanoma include ulceration at the site of the lesion, an irregular notched margin, itching, bleeding, oozing, nodularity and scab formation (McCance & Heuther 2002, pp. 1460-1). It should also be noted that some types of melanoma appear flat while others develop into a raised nodular lesion (Stevens & Lowe 2000). The ABCD rule has been developed as a guide to identify possible melanomas, representing: Asymmetry, Border irregularity, Colour variation, and a Diameter larger than 6 mm (McCance & Heuther 2002).

The awareness of risk factors associated with malignant melanoma allows those at risk to take preventative measures and monitor any irregular changes to melanocytic lesions on their skin. Risk factors include genetic predisposition, exposure to ultraviolet light, steroid hormone use, fair hair and light skin with a tendency to burn, and freckles (McCance & Heuther 2002). Studies have shown that in those with melanoma predisposing mutations, the risk of melanoma is increased with a pale complexion and solar injury to the skin. As sun exposure has been shown to increase the risk of melanoma beyond that accounted for by germ-line mutations alone (Greene 1998), people with a susceptibility to melanoma can reduce their risk by limiting sun exposure.

Chromosomal Regions Involved In Melanoma Predisposition

9p21

Chromosomal region 9p21 (Figure 1) is frequently subjected to rearrangements and deletions in cutaneous melanoma and its linkage to melanoma inheritance has been demonstrated in many studies (Platz et al. 1997). The melanoma gene mapped to 9p21 is the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene. Germ-line mutations in CDKN2A have been identified in about 20 percent of melanoma prone families (Greene 1998).



Figure 1. Chromosome 9
The 9p21 region is shown by an arrow
(Adapted from NCBI 2004a).



Figure 2. Chromosome 12
The 12q14 region is shown by an arrow
(Adapted from NCBI 2004b).

12q14

Germ-line mutations in the cyclin-dependant kinase 4 (CDK4) gene, located in chromosomal region 12q14 (Figure 2), have been described in three melanoma families. This gene therefore accounts for only a fraction of all hereditary melanoma (Greene 1998).

Inheritance

The melanoma predisposition genes, CDKN2A and CDK4, are inherited in an autosomal dominant pattern (Goldstein & Tucker 2001). Segregation analysis, a statistical method that uses the phenotypes of individuals in families to determine the most likely mode of inheritance (Nussbaum et al. 2001), has determined that an autosomal dominant model best fits the pattern of inheritance in cutaneous malignant melanoma families (Greene 1998). This was shown in a recent study where half of the offspring of affected parents manifested a melanoma, dysplastic nevi or pancreatic carcinoma phenotype (Rulyak et al. 2003).

Normal and Mutated Gene Products

CDKN2A gene products – p16 and p14^{ARF}

The CDKN2A gene codes for the protein, p16, also known as p16^{INK4a} or MTS1. This is a low molecular weight protein involved in regulation of the cell cycle (Rulyak et al. 2003). p16 controls the cell cycle by forming a complex with cyclin dependent kinase 4 or 6 (cdk4/6). Formation of this complex inhibits the phosphorylation of retinoblastoma protein (pRb) resulting in the arresting of cell proliferation and growth (Figure 3).

When cdk4/6 is not bound by the p16 inhibitor, it complexes with cyclin D proteins. This allows phosphorylation of pRb to proceed unimpeded. Phosphorylation of multiple serine or threonine residues in pRb allows the release of transcription factors that induce the expression of S phase genes and increase the rate of transition through G1 to the S phase of the cell cycle. A mutation in CDKN2A giving an abnormal p16 gene product therefore ultimately results in uncontrolled proliferation of cells (Walker et al. 1998). p16 can be thus considered as a tumour suppressor protein and mutations in the CDKN2A tumour suppressor gene can result in unregulated cell growth and neoplastic progression (Rulyak et al. 2003). A tumour suppressor gene is a normal gene involved in the regulation of cell proliferation. Such genes block tumour development. Loss of function in both alleles of a tumour suppressor gene results in uncontrolled cell division, abnormal cell growth and defective apoptosis (Nussbaum et al. 2001).

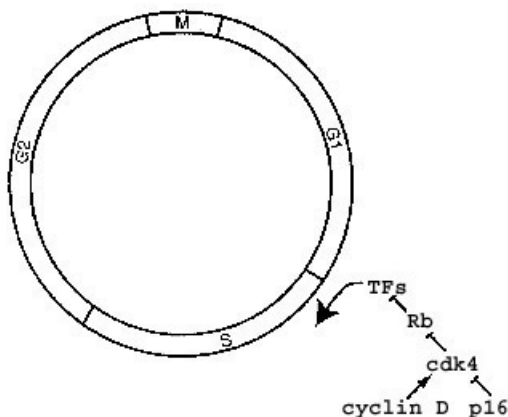


Figure 3. The role of p16 in the cell cycle (Adapted from Vogelstein & Kinzler 1998). p16 controls cell division by acting on the proteins cyclin-dependant kinase 4 (cdk4) and retinoblastoma protein (Rb), and releases transcription factors (TFs). Arrows indicate a positive effect while a flat-ended arrow indicates an inhibitory effect.

The splicing of different 5' exons in alternate reading frames (ARFs) of the CDKN2A melanoma predisposition gene results in the encoding of another, structurally distinct, tumour suppressor protein. The protein is called p14^{ARF} and is a tumour suppressor gene that mediates G1 and G2 cell cycle arrest (Hewitt et al. 2002). p16 is comprised of exons 1, 2 and 3 while p14^{ARF} comprises exons 1, 2 and 3 (Goldstein & Tucker 2001).

CDK4 gene product – cdk4

The product of the CDK4 gene, cyclin-dependant kinase 4 (cdk4), is a key component in the regulation of the cell cycle. As outlined above, the completion of the G1 phase of the cell cycle and entry into the S phase is tightly regulated by cyclin D proteins and their associated cdk4 and 6, p16 and pRb. Amplification of the CDK4 gene has been found in some forms of cancer and could result in levels of cdk4 that are too high to be inhibited by p16. This would lead to a loss of the pRb-mediated control in cell proliferation. Another explanation for the pathological basis of mutations in this gene is the finding that they can result in a disruption of the domain of cdk4 that binds to p16 (Guldberg et al. 1997). This results in an activation of cdk4, due to p16 no longer being able to bind and play its inhibitory role. CDK4 therefore functions as a dominant oncogene. An oncogene is a mutated gene whose altered function results in abnormal cell proliferation (Nussbaum et al. 2001). The uninhibited cdk4 is free to complex with cyclin D proteins. This allows the phosphorylation of pRb to occur unchecked, ultimately resulting in uncontrolled proliferation of cells (Greene 1998).

Mutations

Germ line mutations in the CDKN2A gene on chromosome 9p21 have been detected in about 20 percent of all families demonstrating a predisposition to melanoma. Mutations in the CDK4 gene on chromosome 12q14 have only been detected in three melanoma-prone families (Greene 1998). At this stage, the genetic defects in other families are largely unknown (Rulyak et al. 2003).

CDKN2A Mutations

Germ-line mutations in the CDKN2A gene have been found in melanoma prone families from North America, Europe and Australasia. They have been found in approximately 20 percent of melanoma prone families with three or more members exhibiting the disease. The frequency of mutations increases with the number of family members affected. For example, the frequency of detectable mutations is less than five percent in those families with two members exhibiting melanoma, compared with over 50 percent in those families with over six members with melanoma. Also, approximately 10 percent of patients with multiple melanomas carry germ-line mutations although they do not have a family history of the disease (Goldstein & Tucker 2001). This shows that not necessarily all germ-line mutations that predispose a person to melanoma are inherited.

Studies have shown that most CDKN2A mutations have originated from a common ancestor (Goldstein & Tucker 2001). Analysis of markers flanking the CDKN2A locus has suggested common haplotypes for families with recurrent mutations in this region, providing evidence for common founders of CDKN2A mutations (Goldstein et al. 2000). CDKN2A mutations are therefore the main known genetic predisposition for malignant melanoma.

There have been numerous mutations reported in the CDKN2A gene. The majority are missense mutations involving the alteration of the coding strand of DNA due to the specification of a different amino acid. This occurs because a single nucleotide substitution in the DNA alters the code of a triplet of bases (Nussbaum et al. 2001). Missense mutations are scattered throughout the CDKN2A coding region (Goldstein & Tucker 2001).



113insArg

In a Swedish study it was found that nine of the ten families with mutations in CDKN2A had an arginine insertion at codon 113 (113insArg) (Borg et al. 2000). This had already been described in other Swedish melanoma studies and could therefore be a founder mutation of potential importance in Scandinavian countries. The 113insArg mutation affects the fourth ankyrin repeat of the p16 protein. Mutations in the ankyrin consensus sequence have been shown to affect the binding of p16 to cdk4 (Borg et al. 2000).

Ile49Ser

The Ile49Ser mutation leads to an isoleucine to serine substitution at codon 49 in exon 1. This mutation has been shown to segregate with the disease in Australian melanoma kindreds. The p16 product resulting from this genetic mutation has demonstrated decreased binding to cdk4, suggesting that it may predispose to melanoma (Lal et al. 2000).

Met53Ile

The Met53Ile mutation is a methionine to isoleucine substitution at codon 53 in exon 2. Like with the Ile49Ser mutation, the p16 gene product also shows impaired binding to cdk4 protein (Lal et al. 2000). It is a commonly found mutation amongst families with malignant melanoma and is believed to occur due to a C to G point transversion (Pollock et al. 1998) meaning a pyrimidine is substituted by a purine (Nussbaum et al. 2001), in exon 2.

Duplication of a 24bp Repeat

One melanoma kindred carries a duplication of a 24bp repeat. This mutation is present in the 5' region of CDKN2A. The duplication is believed to have occurred from an unequal crossing over between the 24bp repeats which are normally present in the wildtype sequence, or through polymerase slippage which can occur during replication (Pollock et al. 1998).

Gly101Trp

The Gly101Trp mutation has been described in more than 20 families worldwide. It has been established that the Gly101 residue may not be involved in cdk4 binding, but instead plays a structural role (Auroy et al. 2001).

CDK4 Mutations

Mutations in the CDK4 melanoma susceptibility gene have only been found in three families world wide (Greene 1998). Such mutations therefore account for a very small proportion of cases of familial malignant melanoma. Like families with mutations in CDKN2A, those with CDK4 mutations also share a common haplotype, indicating they are founder mutations (Goldstein et al. 2000).

Arg24Cys

Two of the three families with mutations in CDK4 have a germ-line Arg24Cys mutation which results in the production of a mutated cdk4 protein that will not bind to p16 (Goldstein et al. 2000). It does this by disrupting the highly conserved p16 binding domain of the cdk4 protein. This cdk4 variant acts as a dominant oncogene unresponsive to p16 inhibition (Platz 1998).

Correlations Between Genotype and Phenotype

Melanomas resulting from CDKN2A and CDK4 mutations have similar characteristics including age at diagnosis, number of melanoma tumours and number of nevi (Goldstein & Tucker 2001). Histological differences have been found between cutaneous melanomas associated with germ-line mutations and melanomas not associated with such mutations. Mutation associated melanomas are less invasive, with less inflammation and regression but more histological ulceration. They are also more often located on the head and neck and have a significantly

younger age of presentation. In comparison, there are no significant differences found in tumour thickness (Masback et al. 2002). Also, higher nevus counts and densities have been found in mutation carriers than in those people without the mutations (Greene 1998).

Germ-Line Testing For Melanoma Susceptibility

Germ line testing for melanoma predisposition is now possible, as the major genetic factors for malignant melanoma have been identified. As malignant melanoma results from environmental factors as well as genetic predisposition, screening would allow individuals at a greater genetic risk to modify their behaviour to minimise their exposure to environmental risks. For example, an individual who is found to have an increased risk of melanoma can limit their sun exposure and be more vigilant in checking for abnormal growths on their skin (Vogelstein & Kinzler 1998).

The genes examined in germ-line testing are relatively small in size. This reduces technical difficulties, resulting in a cost effective test that would allow screening of populations with an increased susceptibility to melanoma. However, there are other considerations that reduce the effectiveness of germ-line screening. The prevalence of mutations in known melanoma predisposition genes is so low that a positive test would be a rare outcome in population screening. This would be of minor value compared to the large cost of establishing the test. Also, a negative result cannot exclude the possibility of genetic predisposition to melanoma (Vogelstein & Kinzler 1998) due to the probable existence of mutations in melanoma predisposition genes that have not yet been identified.

Conclusion

Malignant melanoma is inherited in approximately 10 percent of all melanoma cases. It is inherited by germ-line mutations passed on by autosomal dominant inheritance. The most common mutations occur in the CDKN2A gene. Mutations in this gene have been found in approximately 20 percent of familial melanomas. The p16 encoded can no longer bind to cdk4/6, resulting in the lack of inhibition of pRb, which is responsible for the normal functioning of the cell cycle. CDK4 is another gene involved in malignant melanoma inheritance. Mutations in CDK4 have been found in three families worldwide. They result in the encoding of cdk4 protein that can no longer bind to p16 leading to uncontrolled cell proliferation.

Most mutations found in the CDKN2A gene are missense mutations. These mutations result in the substitution of an amino acid altering the function of the protein CDKN2A encodes. Two examples are Ile49Ser and Met53Ile. The most common mutation in CDK4 associated with melanoma is also a missense mutation, Arg24Cys.

There are phenotypic differences between melanoma associated with CDKN2A germ-line mutations and melanoma without germ-line mutations. These are histological differences such as reduced invasion, less inflammation and regression. Such phenotypic differences have not been found between melanomas associated with CDKN2A mutations opposed to those with CDK4 mutations; for example, the thickness of the tumours is the same.

There are techniques available for the screening of CDKN2A and CDK4 germ-line mutations. However, due to the fact that these mutations only occur in 20 percent of melanoma cases, such a test is not viable. Further research may lead to the discovery of more mutations associated with malignant melanoma, allowing any increases of melanoma predisposition to be detected.



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