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Loss of the Eukaryotic Initiation Factor 3f in Melanoma

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Abstract

Aberrant regulation of the translation initiation is known to contribute to tumorigenesis. eIF3 plays an important role in translation initiation. eIF3f is the p47 subunit of the eIF3 complex whose function in cancer is not clear. Initial studies from our group indicated that eIF3f expression is decreased in melanoma. Overexpression of eIF3f inhibits translation and induces apoptosis in melanoma cells. The eIF3f gene is located at chromosome region 11p15.4. Loss of 11p15.4 is a common event in many tumors including melanoma. In order to investigate the molecular mechanism of the decreased expression of eIF3f in melanoma, we performed loss of heterozygosity (LOH) analysis in 24 melanoma specimens using three microsatellite markers encompassing the eIF3f gene. We showed that the prevalence of LOH ranged from 75% to 92% in melanoma. We also performed eIF3f gene copy number analysis using quantitative real-time PCR to further confirm the specific allelic loss of the eIF3f gene in melanoma. We demonstrated a statistically significant decrease of the eIF3f gene copy number in melanoma compared with normal tissues with a tumor/normal ratio of 0.52. To further elucidate the somatic genetic alterations, we carried out mutation analysis covering the entire coding region and 5'UTR of the eIF3f gene in melanoma tissues and cell lines. Despite some polymorphisms, we did not find any mutations. Furthermore, immunohistochemistry analysis demonstrated that eIF3f protein expression is decreased in melanoma compared to benign nevi. These data provide new insight into the understanding of the molecular pathogenesis of eIF3f during melanoma tumorigenesis.

Keywords

eukaryotic initiation factor 3f; loss of heterozygosity; apoptosis; melanoma

Introduction

Cancer of the skin is one of the most common types of human cancers. Melanoma accounts for about 4% of skin cancer cases but causes a large majority of skin cancer deaths. The number of new melanomas diagnosed in the United States is increasing (American Cancer Society 2006, National Cancer Institute 2006) [1]. The median survival time for patients with disseminated melanoma is 8.1 mo with only approximately 2% surviving for 5 yr [2]. Treatments for advanced disease are inadequate [3,4]. An improved understanding of

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melanoma genetics is the only means to provide new markers for early diagnosis and to identify potential targets for therapeutic intervention [5].

Altering the expression of genes involved in cell proliferation or apoptosis may cause abnormal cell growth and malignant transformation. Although most studies have focused on the transcriptional regulation of gene expression, recent studies indicate that deregulation of protein synthesis may also contribute to cancer development [6,7]. Translation initiation factor eIF4F activation is an essential component of the malignant phenotype in breast carcinoma [8]. eIF4E controls the translation of various malignancy-associated mRNAs which are involved in polyamine synthesis, cell cycle progression, activation of proto-oncogenes, angiogenesis, autocrine growth stimulation, cell survival, invasion and communication with the extracellular environment. eIF4E-mediated translational modulation of these mRNAs plays an important role in both tumor formation and metastasis [9]. It was also reported that abrogation of the phosphorylation of the translation initiation factor eIF-2 causes malignant transformation of NIH3T3 cells [10]. These observations indicate the importance of translational regulation in oncogenesis.

The rate of synthesis of any given protein is determined by the rate of translation initiation [11]. The eukaryotic initiation factor 3 (eIF3) is a multiprotein complex containing at least 13 nonidentical subunits, that plays a central role in translation initiation. The function of eIF3 is not only linked to prevent premature association of the 40S with the 60S ribosomal subunits but also is linked to prevent the premature association of the 40S ribosome to the mRNA by binding to eIF4F complex generating a preinitiation complex called 48S. When the expression or level of eIF3 is altered, this may influence the synthesis of some growth-regulating proteins [6]. Several lines of evidence suggest that alterations in eIF3 subunits can contribute to tumorigenesis. Studies from our group have demonstrated high frequency loss of heterozygosity (LOH) and eIF3f gene allele loss in pancreatic cancer [12]. Recent studies by Zhang et al. [6] indicated that overexpression of five individual subunits of eIF3 promotes malignant transformation of NIH3T3 cells. Aberrant mRNA and protein levels of several eIF3 subunits have been detected in several different solid tumors and cancer cell lines. Different isoforms of eIF3a are overexpressed in human breast, cervical, lung, and gastric cancer, as well as mouse melanoma and HeLa cells [13,14]. eIF3a mRNA is increased in proliferate tissues such as bone marrow, thymus, and developing fetal tissues. eIF3b is overexpressed in human breast cancer [13]. eIF3c is also found over-expressed in testicular seminomas [15]. High-level amplification of eIF3h has been found in prostate tumors [16]. In addition, overexpression of a truncated eIF3e protein has been reported to induce malignant transformation of NIH3T3 cells [17]. Insertion of the mouse mammary tumor virus genome into the eIF3e gene induces neoplasia in mice, suggesting a tumor suppressor role for eIF3e [18,19].

The eIF3f gene, mapped to human chromosome band region 11p15.4, is one of the 13 nonidentical subunits of the eIF3 complex. The function of eIF3f is not clear. We can demonstrate that eIF3f mRNA expression is significantly decreased in many human tumors including melanoma using a cancer profiling array and real-time RT-PCR analysis [20]. Previous studies from our group identified eIF3f subunit as a protein associated with the caspase-processed isoform of the cyclin dependent kinase 11 (CDK11^{p46}) which appears to be a down stream effector in apoptotic signaling using a yeast two-hybrid screening strategy [21]. This interaction occurs in vitro and in vivo. eIF3f can be phosphorylated by CDK11^{p46} during apoptosis [21]. We can demonstrate that the enforced expression of eIF3f inhibits translation, cell growth, cell proliferation and induces apoptosis in melanoma cells [20]. Knockdown of eIF3f prevents apoptosis in melanoma cells [20]. Recent studies from Zhang et al. [6] also showed decreased cell growth, cell proliferation, colony formation and increased apoptosis in eIF3f-overexpressing NIH3T3 cells. These findings suggest that eIF3f may be an important negative regulator of cell growth and proliferation. However the molecular

mechanisms by which the expression of eIF3f is decreased in melanoma is not known. In the present study, we address whether there is allelic loss of eIF3f gene in melanoma using LOH and gene copy number analysis. We also investigated the eIF3f gene mutational status in melanoma cell lines and tissues. Decreased eIF3f expression in melanoma was also confirmed at the mRNA and protein level in human tissue specimens.

Materials and Methods

Tumor Samples

Twenty-four specimens of melanoma were obtained from archived fixed human tissue bank of the Department of Pathology at the University of Arizona with IRB approval. Tumor tissues were reviewed by the same pathologist to confirm histopathologic status for a detailed selection of cells to be microdissected. The tissues were all formalin fixed paraffin-embedded. Five frozen melanoma and adjacent normal skin tissues were obtained from Dr. Stanley P.L. Leong at UCSF for mRNA analysis.

Laser Capture Microdissection (LCM)

Melanoma cells were microdissected out of 8 mm sections, stained with HistoGene staining solution, under direct microscopic visualization using the Arcturus PixCell II Laser Capture Microdissection System (Arcturus Molecular Devices Co, Sunnyvale, CA) available at the Arizona Cancer Center. For each case at least two caps were obtained including one cap with normal tissue as control and another with tumor cells. The conditions for the microdissection were: laser power: 50–70 mW, laser pulse: 2000–2700, spot size of >2 mm².

LOH Analysis

After microdissection, the caps were placed in 0.5 mL microfuge tubes containing 50 µL of fresh lysis buffer (Proteinase K in reconstitution buffer from Arcturus Bioscience, Inc., Basel, Switzerland). The tubes were placed upside down and incubated at 65°C overnight. Then genomic DNA was extracted and three microsatellite markers (D11S1318, D11S1338, and D11S1331) located at 11p15.4 were amplified as described previously by our group [12]. The markers were chosen according to the Gene-bridge4 and Stanford G3 and for maximal heterozygosity. The DNA fragments were separated by capillary electrophoresis (ABI 3100, Applied Biosystems, Foster City, CA). To assess LOH, the peak areas of both alleles were measured using Genescan software (Applied Biosystems, version 3.1) and the ratios of the normal and tumor samples were compared. LOH index: $(P_{T2}/P_{T1})/(P_{N2}/P_{N1})$ of <0.6 or >1.7 were considered positive [12,22]. Each LOH positive case was repeated at least twice.

Mutation Analysis of the eIF3f Gene

Genomic DNA from each sample was amplified by PCR using the primer pairs that flanked every exon and promoter region of the eIF3f gene (Table 1). PCR amplifications were performed under standard conditions in a final volume of 20 µL containing a mixture of 2.5 mM each dNTP, 1X reaction buffer (New England BioLabs, Ipswich, MA), 1 µM final concentration of each primers, 1X PCR enhancer solution (Invitrogen, Carlsbad, CA), 2.5U *Taq* DNA polymerase (New England BioLabs) and 100 ng of genomic DNA. PCR conditions consisted of an initial denaturation at 95°C for 5 min, 40 cycles of 95°C for 1 min, annealing for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were purified with GFX Kit (Amersham Biosciences, Pittsburgh, PA) before sequencing. Automated sequencing was performed on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Mutations were verified in both sense and anti-sense directions on independent PCR products.

Quantitative Real-time PCR

Quantitative real-time PCR was performed on a PRISM 5700 sequence detector (Applied Biosystems) using iTaq™ SYBR Green Supermix with ROX (Bio-Rad Laboratories, Inc., Hercules, CA) as described previously by our group [12]. This method is based on the binding of the dye to double stranded DNA. When SYBR Green binds to double stranded DNA, the intensity of the fluorescent emissions increases as previously described [23]. Threshold cycle (Ct) during the exponential phase of amplification was determined by real-time monitoring of fluorescent emission. Primer sets were designed to span intron–exon boundaries in order to eliminate the contamination of pseudogenes and RNA. Relative quantification was calculated using β -actin as an endogenous control [12,24]. The reactions were performed in triplicates and no template control was included. Dissociation curves were calculated to determine the specificity of the amplicon. Relative quantification was calculated as the target gene/ β -actin ratio [12,24,25]. For quantitative real-time RT-PCR, total RNAs were extracted from tissues using Trizol reagent (Invitrogen) and RNeasy Mini Kit (QIAGEN, Valencia, CA). Reverse transcription was performed using Omniscript RT kit (QIAGEN). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control gene. Primers for GAPDH were purchased from PerkinElmer Life Sciences, Boston, MA. Primers for eIF3f gene are: forward 5'-TGACAGTGAAATACGC GTAC-3', and reverse 5'-GTCACCTGAGAGTCCAATCAC-3'.

Immunohistochemistry Analysis

eIF3f antibody was developed by Rockland as described previously [21]. Biotinylated anti-goat antibody raised in horse was purchased from Vector Laboratories (Burlingame, CA). Paraffin-embedded normal skin, nevus or melanoma tissue sectioned at 5 μ m were deparaffinized in xylene, followed by rehydration in graded series of ethanol, ending with water immersion. Antigen retrieval was performed by microwave exposure in sodium citrate buffer (0.01 M, pH 6.1). Endogenous peroxidase blocking was performed with 3% H₂O₂ in methanol and sections were blocked with 1.5% normal horse serum (Vector Laboratories). The sections were then incubated with a goat polyclonal anti-eIF3f antibody (1:200), followed by a biotinylated secondary anti-goat antibody (1:200; Vector Laboratories). Sections were then treated with Vectastain Elite ABC Reagent followed by diaminobenzidine activated with H₂O₂ used according to the manufacturer's instructions (Vector Laboratories). The slides were finally counterstained with hematoxylin and coverslipped.

Statistical Analysis

All data are reported as mean $\pm$ s.d. When appropriate, differences between two groups were compared by the Student's *t*-test. Differences were considered significant at *P* < 0.05.

Results

Allelic Loss of the Chromosome Region Encompassing eIF3f Gene in Human Melanoma Specimens

We studied 24 melanoma tissues. The clinical and pathological characteristics of the primary melanoma and derived metastasis are described in Table 2. We captured melanoma and adjacent normal skin samples using LCM. An example of microdissected tissues is shown in Figure 1. In order to explore allelic loss of eIF3f gene on chromosome 11p15.4, three microsatellite markers were chosen in close proximity to the eIF3f gene. These markers were chosen according to the Genebridge4 and Stanford G3 for maximal heterozygosity. The results of LOH for each microsatellite marker are shown in Table 3. A representative genotyping profile of D11S1338 is shown in Figure 2. All cases were informative and LOH positive for at least one microsatellite marker. We found that the highest prevalence LOH was 92%

observed on D11S1338 microsatellite marker whereas the lowest was 75% on D11S1331 (Table 4). Out of 24 samples studied on D11S1318 marker, 19 were informative, and out of the informative cases, 15 samples (79%) were positive. Out of 24 samples studied on D11S1338 marker, 13 were informative, and out of the informative cases, 12 samples (92%) were positive. Whereas out of 24 samples studied on D11S1331 marker, 20 were informative, and out of the informative cases, 15 samples (75%) were positive. The average fractional allele loss (FAL) was 0.81, calculated as the total number of chromosomal arms having allelic loss divided by the total number of informative chromosomal arms [12,22]. These results indicate the chromosome region between D11S1318 and D11S1338 microsatellite markers encompassing eIF3f gene could be the region of allelic loss on chromosome band region 11p15.4 in human melanoma.

Decreased eIF3f Gene Copy Number in Melanoma Tissues

To further investigate whether eIF3f gene is specifically lost in melanoma, eIF3f gene copy number was studied in 18 melanoma samples and 10 normal skin tissues from different patient using quantitative real-time PCR analysis [25,26]. The relative gene copy number of eIF3f gene was normalized against β -actin gene copy number. The mean of the eIF3f relative gene copy in the melanoma samples was 0.52, whereas the mean of the eIF3f relative gene copy in the normal tissues was 1.0 with a tumor/normal ratio of 0.52 (Figure 3). There was a statistically significant decrease of eIF3f relative gene copy number in melanoma compared to normal tissues ($P = 0.037$). Taken together with the LOH results, we are able to confirm the allelic loss of eIF3f gene in human melanoma.

eIF3f Gene Mutation Analysis

To identify eIF3f gene mutations in melanoma, we sequenced all 8 exons and the 5'UTR of eIF3f gene in fibroblast, normal melanocytes, 8 melanoma cell lines, normal lung, normal skin from different patient, and 20 human melanoma specimens. The results of our study are shown in Table 5. There were some alterations, mainly in exon 1 and 5'UTR of eIF3f gene. Specifically, we found a C to T transition at nt 348 in exon 1 in UACC1227 and UACC647 melanoma cell lines as well as several melanoma tissue specimens (163, I-0163, 5060, 7361, and 7928), but not in normal cells or tissues. However, this alteration does not cause amino acid change. We also found a G to C transition at nt 1 in 5'UTR in UACC827 and A375 melanoma cells. However, we did not find this transition in human melanoma tissue specimens. A deletion of T at position -224 in the 5'UTR of eIF3f gene was found in 2 melanoma cell lines and 4 melanoma tissues. However, this deletion also exists in normal tissues (Table 5). A C to T polymorphism at nt 122 occurs in both normal melanocytes and a melanoma cell line. Thus, our results suggest that mutation may not be the mechanism of decreased eIF3f expression in melanoma.

Decreased eIF3f mRNA in Melanoma Tissues

We have shown that eIF3f expression is decreased in melanoma using a cDNA cancer profiling array and quantitative real-time RT-PCR [20]. To further confirm that eIF3f mRNA is decreased in human melanoma tissue compared to normal adjacent skin tissue from the same patient, quantitative real-time RT-PCR was performed in five normal adjacent skin and five melanoma tissue specimens from the same patient. It is clear that eIF3f expression is decreased in the majority of melanomas compared to normal skin samples from the same patient (Figure 4). These results further support our previous observations that the expression of eIF3f is decreased in melanoma tissues.

Decreased eIF3f Protein Level in Melanoma Tissues

To further investigate whether eIF3f protein expression is also decreased in melanoma, 9 melanoma, 9 benign nevus, and 10 normal skin tissues from different cases were used for the immunohistochemistry analysis of eIF3f protein expression using eIF3f-specific antibody. The mean score of the eIF3f protein expression was significantly decreased in melanoma tissues compared to both benign nevi and normal skin tissues ($P < 0.00001$; Table 6 and Figure 5). There is no significant difference of eIF3f protein expression between normal skin and benign nevi tissues (Table 6). In addition, eIF3f appears to localize exclusively to the cytoplasm. Therefore, we were able to confirm that eIF3f protein was significantly decreased in human melanoma tissues.

Discussion

It has been previously demonstrated that the LOH of alleles on chromosome 11p15 is a common event in numerous types of tumors, including melanoma [27]. A number of publications suggest the presence of tumor suppressor gene/s on chromosome 11. It has been reported that LOH occurs on 11p in malignant melanoma [28]. The higher frequency of LOH observed in this region in malignant tissues as compared to benign ones suggests that 11p15 chromosome region is the target of genetic alterations. However, which specific gene is responsible for the tumorigenesis is not clear.

We have demonstrated that eIF3f expression is significantly decreased at the mRNA level in many human tumors including melanoma [20]. However, the molecular mechanism of the decreased expression is unknown. The eIF3f gene is located at chromosome region 11p15.4. We can demonstrate that loss of eIF3f gene allele is associated with the decreased expression of eIF3f in pancreatic cancer [12]. Here, we demonstrated that loss of eIF3f gene allele is also associated with the decreased expression of eIF3f in melanoma. Similarly, LOH of eIF3e (INT6) gene has been reported in breast carcinomas [29]. We did not find any mutations in the coding region and the promoter of eIF3f gene, except several polymorphisms at nt 1, 122, 348 and a deletion of T at position -224. It is suggested that polymorphism in the promoter can regulate gene transcription [30]. Polymorphism at nt 1 may affect eIF3f gene splicing. A search for the consensus sequences of transcription factors in the putative eIF3f gene promoter region revealed a possible binding site for REB1 at nt -224 to -217. Therefore, it is possible that the deletion at nt -224 in the promoter region of eIF3f gene may have functional significance. Further studies are needed to clarify this issue.

In the current study, we were able to demonstrate both at the protein and mRNA levels that eIF3f expression is decreased in melanoma tissues. Expression of another eIF3 subunit that was shown to be decreased in human tumors is eIF3e (INT6) [19]. Enforced expression of eIF3f inhibits translation, cell growth, cell proliferation and induces apoptosis in melanoma and pancreatic cancer cells [20]. Knockdown of eIF3f prevents apoptosis in melanoma cells [20]. Recent studies from Zhang et al. [6] also showed decreased cell growth, cell proliferation, colony formation and increased apoptosis in eIF3f-overexpressing NIH3T3 cells. These findings suggest that eIF3f may be an important negative regulator of cell growth and proliferation. The mechanisms by which decreased eIF3f expression contributes to melanoma development are not clear. One possible explanation is that decreased eIF3f may lead to the stabilization of rRNA and ribosomes, which in turn leads to increased translation of oncogenic proteins and malignant transformation [20].

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Abbreviations

eIF3f	eukaryotic initiation factor 3 subunit f
LOH	loss of heterozygosity

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Figure 1. Representative examples of LCM in melanoma and normal tissues captured on the caps and the remaining tissues. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 2.

A representative example of the genotyping profile at the D11S1338 locus. The electropherograms show the disappearance of one allele peak area in melanoma tissue.

Figure 3.

Decreased eIF3f gene copy number in melanoma tissue specimens. Laser captured microdissected melanoma tissues were collected and genomic DNAs were isolated. Relative eIF3f gene copy number was analyzed by quantitative real-time PCR. The numbers are expressed as a ratio of eIF3f gene copy number over β -actin gene. We analyzed 10 normal samples and 18 melanoma tissues in triplicate.

Figure 4. eIF3f mRNA expression is decreased in melanoma tissues. Total RNA was isolated from melanoma or normal adjacent skin tissues from the same patient. Real-time RT-PCR was performed as described in Material and methods. eIF3f expression was normalized against GAPDH.

Figure 5.

eIF3f protein expression is decreased in melanoma. Immunohistochemistry (IHC) was performed on normal skin, benign nevus and melanoma using eIF3f antibody. Representative IHC analysis of eIF3f protein in normal skin, benign nevus and melanoma tissues was shown. Magnification: 400×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1

Primers Used in Mutation Analysis of the eIF3f Gene

Location	Primer sequences
Promoter	Forward 5'-CCCGAAGTGCTGAGATTAC-3' Reverse 5'-GAGGAGCACTTACTGGTACC-3'
Exon 1	Forward 5'-CTAGCTCACGTGGTGATATTC-3' Reverse 5'-GAAAGGACGGTAGGACAC-3'
Exon 2	Forward 5'-CATTGACATTGAAGAGATCATC-3' Reverse 5'-CTACAGGAGCTCTAGTTATGAGTG-3'
Exon 3	Forward 5'-GGCCTCTTCTGTTTATTGG-3' Reverse 5'-GGAAGAGTATAAGATCCAGTCC-3'
Exon 4	Forward 5'-ATGCCACTGTTGTGTTTG-3' Reverse 5'-CAGCAAGTTACACATGGAAG-3'
Exon 5	Forward 5'-TTCTATCAGAACCTTGAGAGC-3' Reverse 5'-ACAAACCTTAGATCAATAGTCC-3'
Exon 6	Forward 5'-AGAGTTAGTAGGACGTTAAGACC-3' Reverse 5'-ATCAGCACATGCCTCAGT-3'
Exon 7	Forward 5'-GGACATAGTTCTCTATCCTGG-3' Reverse 5'-CTGTCTCTATCCTACTCCAGG-3'
Exon 8	Forward 5'-CCCAGTGTCTACCATAGAGC-3' Reverse 5'-ACACATAACCAACTTCTCTACC-3'

Table 2

Clinical and Pathological Characteristics of the Human Melanoma Specimens

Melanoma cases	Local invasion	Metastasis
1	Yes	Yes
2	Yes	Yes
3	Yes	No
4	Yes	Yes
5	Yes	No
6	Yes	No
7	Yes	No
8	Yes	Yes
9	Yes	Yes
10	Yes	Yes
11	Yes	No
12	Yes	Yes
13	Yes	Yes
14	Yes	No
15	Yes	No
16	Yes	No
17	Yes	No
18	Yes	Yes
19	Yes	Yes
20	Yes	No
21	Yes	Yes
22	Yes	No
23	Yes	No
24	Yes	Yes

Table 3
Detailed LOH Analysis of Three Different Microsatellite Markers Encompassing eIF3f Gene Locus at 11p15.4 in Melanoma

		Marker																							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
D11S1338	+	+	+	+	NI	+	+	NI	NI	+	NI	+	NI	+	+	+	NI	+	-	NI	NI	NI	NI		
LOH index	86	6	2.01	188		4.68	1.73			1.9		40		40	0.08	2.64		4	0.7						
D11S1318	+	+	-	+	+	+	+	NI	-	NI	+	-	+	+	+	+	+	NI	+	+	-	NI	NI		
LOH index	16.36	0.34	0.8	0.06	0.27	0.05	0.13	0.25	1		0.3	1.2	0.04	3.8	3.5	0.11	0.08		39	0.11	1.7				
D11S1331	+	+	-	+	+	-	-	+	+	+	+	-	NI	NI	NI	+	+	NI	-	+	+	+	+		
LOH index	3.05	1.8	1	0.35	0.06	1.27	1	0.52	13	0.46	0.44	0.12	1			2.8	1		0.86	3	0.12	7.6	0.25		

+, LOH positive; -, LOH negative; NI, noninformative.

Table 4

Informative Cases and Frequency of Positive LOH Cases for Each Microsatellite Marker for eIF3f Gene

Marker	Total number of cases	Informative cases (% total)	Positive lesions (out of informative cases)	
			Number	Percentage
D11S1318	24	19 (79%)	15	79
D11S1338	24	13 (54%)	12	92
D11S1331	24	20 (83%)	15	75

Table 5

Mutation Analysis of eIF3f Gene in Melanoma Cell Lines and Melanoma Tissue Specimens

Cell lines or tissues	Nucleotide changes	Amino acid changes
Normal fibroblast	C -476 T, G 66 T	No
Normal melanocyte	C 122 T	P 43 L
UACC1227	C 348 T	No
UACC827	Del -224 T, G 1 C	No
UACC502	C 122 T	P 43 L
UACC612	No	No
UACC647	C 348 T	No
UACC2565	No	No
UACC903	No	No
A375	Del -224 T, G 1 C	No
Normal lung	Del -224 T	No
Normal skin	Del -224 T	No
163	C 348 T	No
I-0163	C 348 T	No
1666	No	No
MM0011	No	No
1020	Del -224 T	No
14951	No	No
A1	No	No
A6	No	No
A7	No	No
A9	No	No
A11	No	No
A12	No	No
A13	No	No
AIM	No	No
5060	C 348 T	No
7361	C 348 T	No
7928	C 348 T	No
1017	Del -224 T	No
1013	Del -224 T	No
1019	Del -224 T	No

Table 6

Mean Score of eIF3f Protein Expression in Normal Skin, Benign Nevi and Melanoma Tissue Specimens

Tissue type	# of cases	Score	P-value
Normal skin	10	2.64±0.24	
Benign nevi	9	2.73±0.22	0.39*
Melanoma	9	0.87±0.49	<0.00001**

* Compared to normal skin.

** Compared to both normal skin and benign nevi.