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(54) **MITF AS A MARKER FOR PREDISPOSITION TO CANCER**

(75) Inventors: **Robert Ballotti**, Nice (FR); **Corine Bertolotto**, Nice (FR); **Brigitte Bressac De Paillerets**, Sceaux (FR); **Mahaut De Lichy**, Paris (FR); **Fabienne Lesueur**, Lyon (FR)

(73) Assignees: **INSTITUT GUSTAVE ROUSSY**, VILLEJUIF CEDEX (FR); **UNIVERSITE SOPHIA NICE ANTIPOLIS**, NICE CEDEX 02 (FR); **INSERM (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE)**, PARIS CEDEX 13 (FR)

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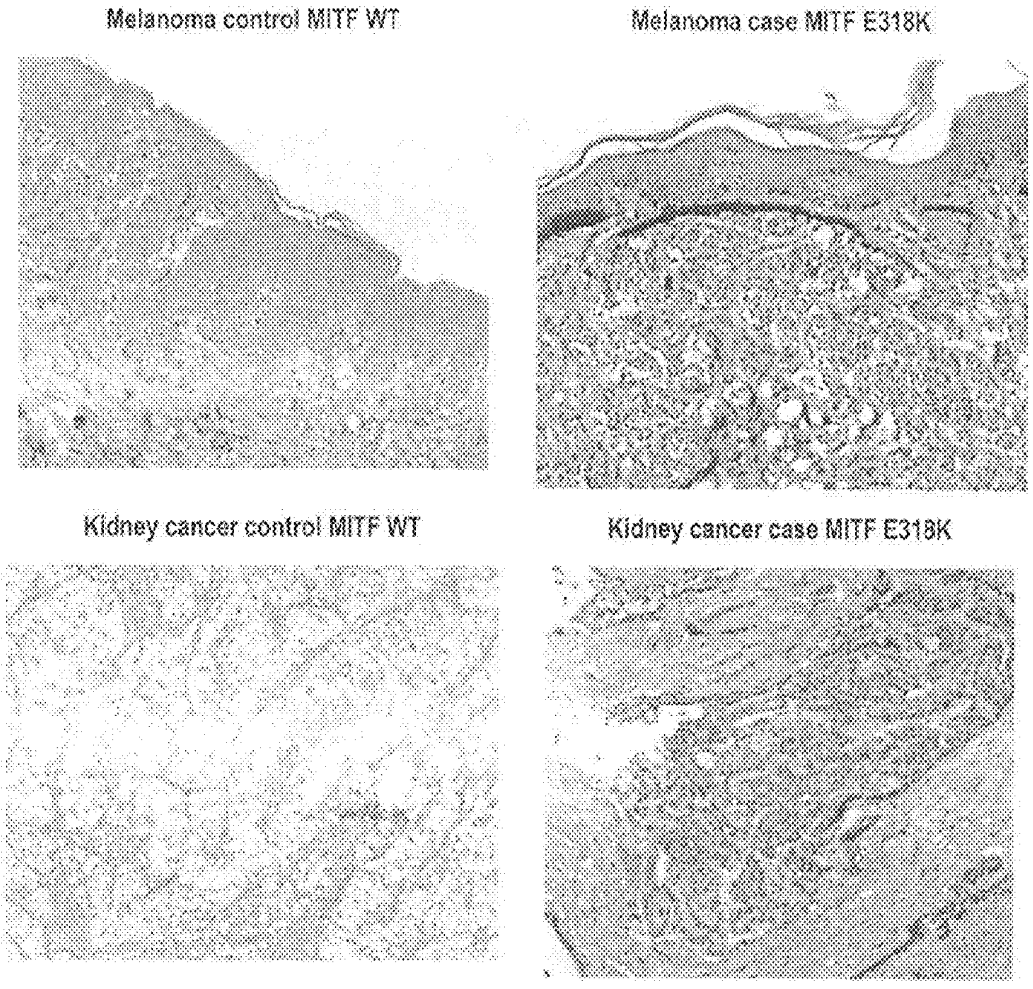
(57) **ABSTRACT**

The present invention relates to a mutant of MITF which is useful as a marker for predisposition to the development of cancer and to uses thereof in diagnosis and preventive treatments, sumoylation being reduced or absent in this mutant.



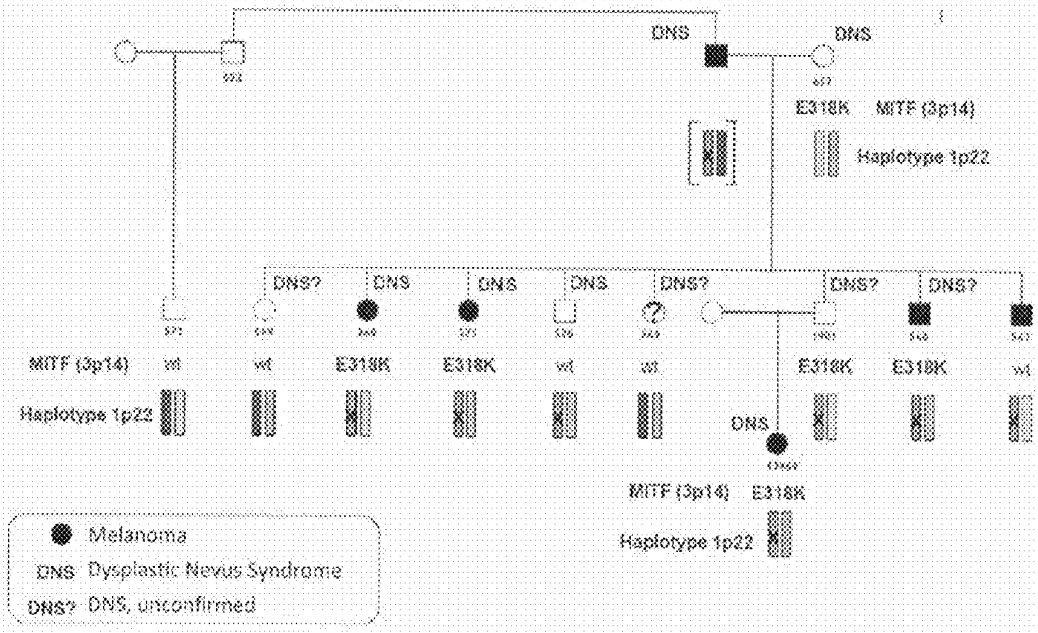


FIGURE 1a

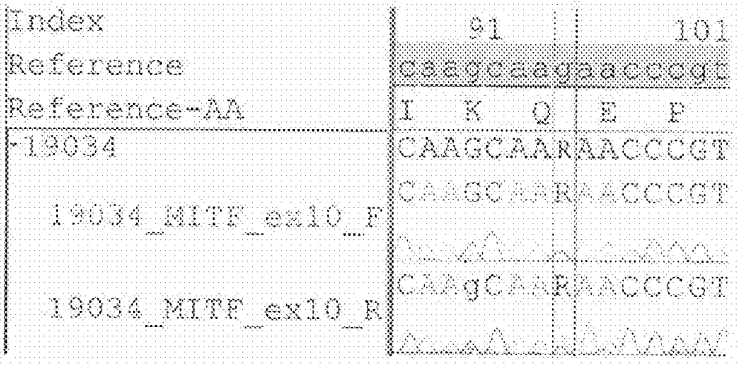


FIGURE 1b

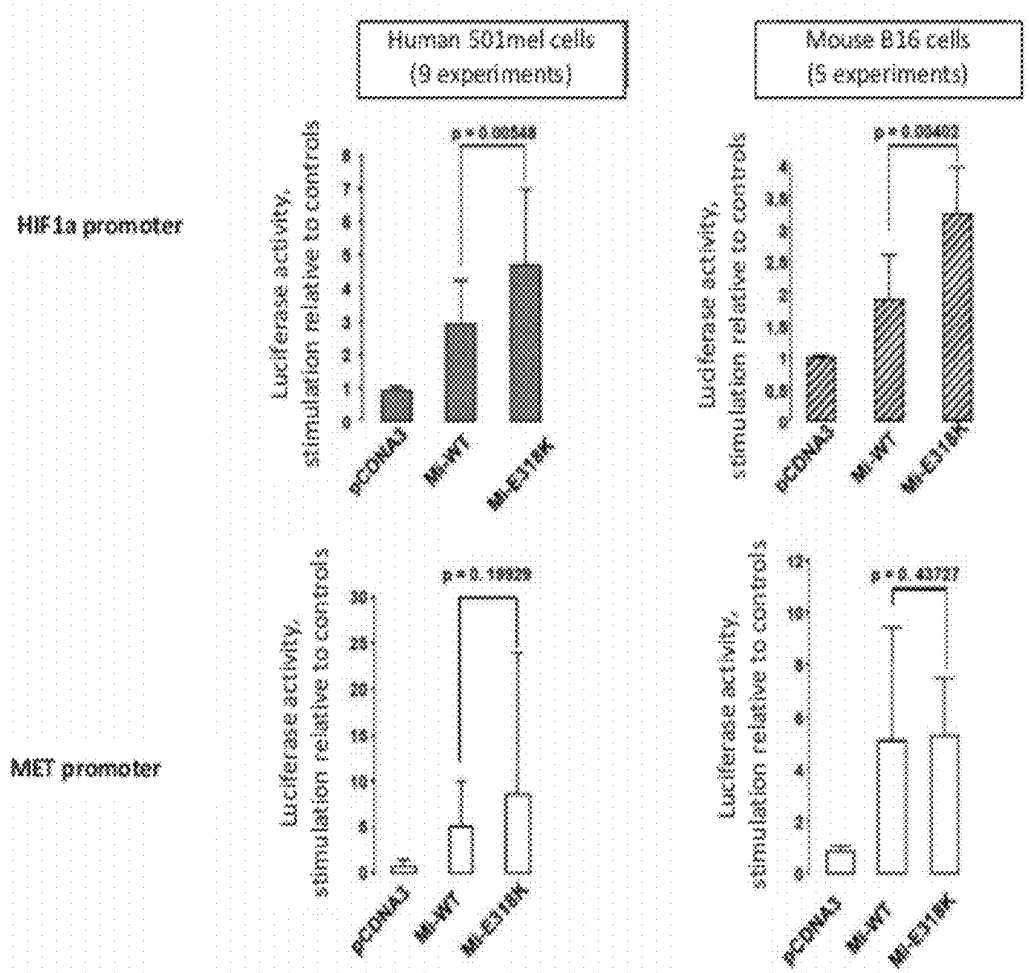
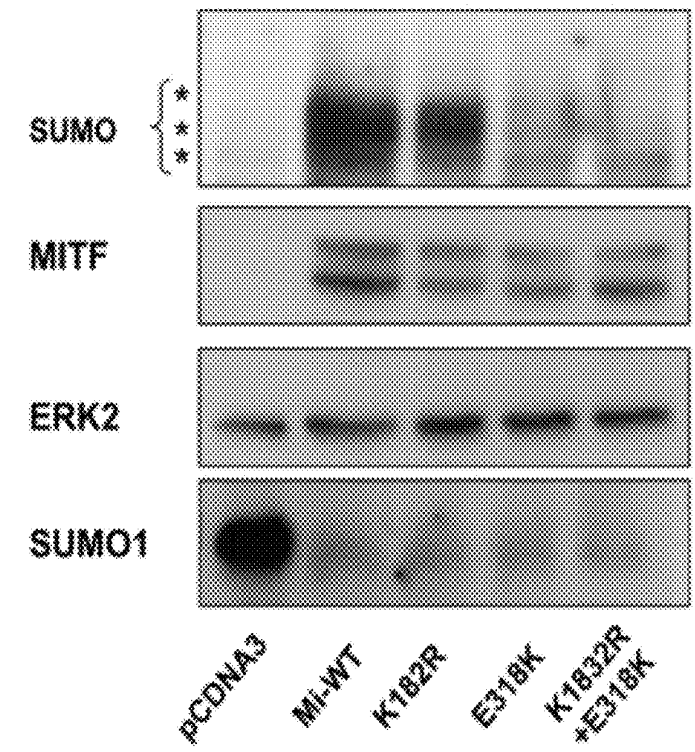

Human 501mel cells
(9 experiments)
Mouse B16 cells
(5 experiments)
HIF1a promoter
MET promoter
Luciferase activity,
stimulation relative to controls
pCDNA3
MI-WT
MI-E318K

FIGURE 2


SUMO
MITF
ERK2
SUMO1
pCDNA3
MI-WT
K182R
E318K
K182R +E318K

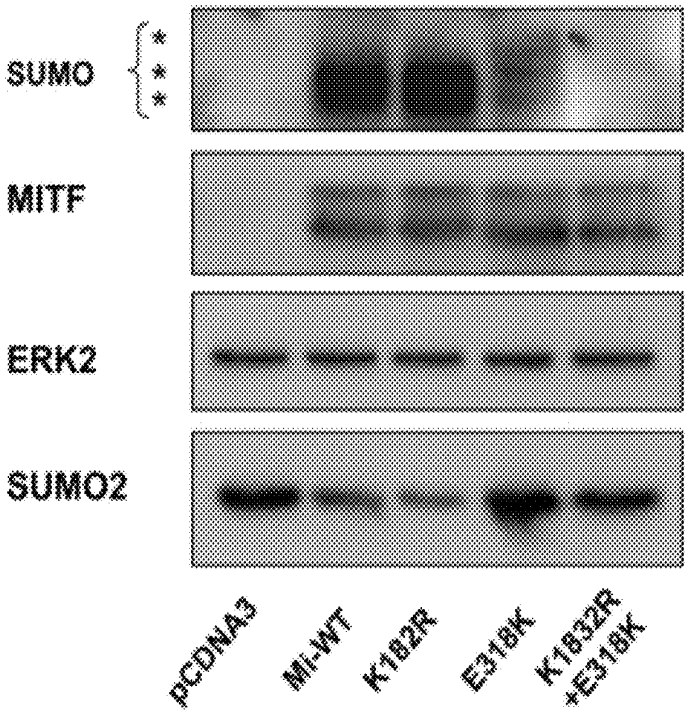

SUMO
MITF
ERK2
SUMO2
pCDNA3
MI-WT
K182R
E318K
K182R +E318K

Figure 3

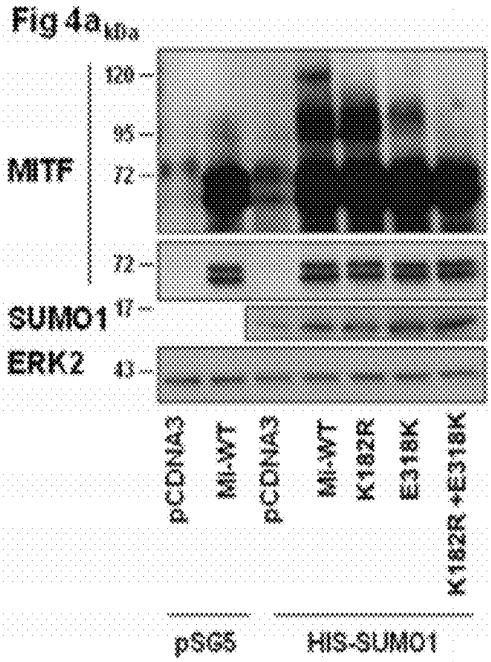


Fig 4a
kDa
120
95
72
MITF
72
SUMO1
17
ERK2
43
pCDNA3
Mi-WT
pCDNA3
Mi-WT
K182R
E318K
K182R +E318K
pSG5
HIS-SUMO1

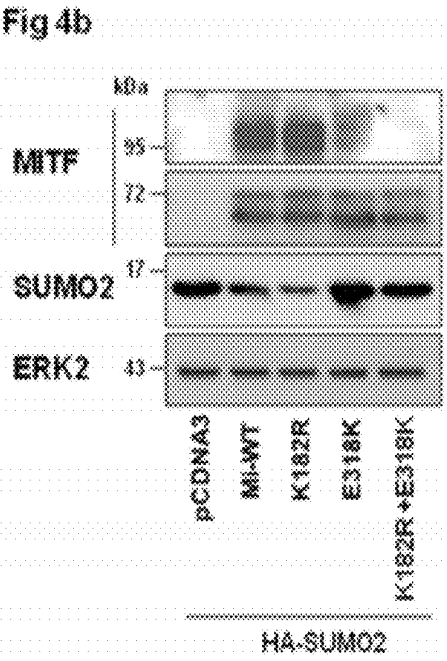


Fig 4b
kDa
95
72
MITF
SUMO2
17
ERK2
43
pCDNA3
Mi-WT
K182R
E318K
K182R +E318K
HA-SUMO2

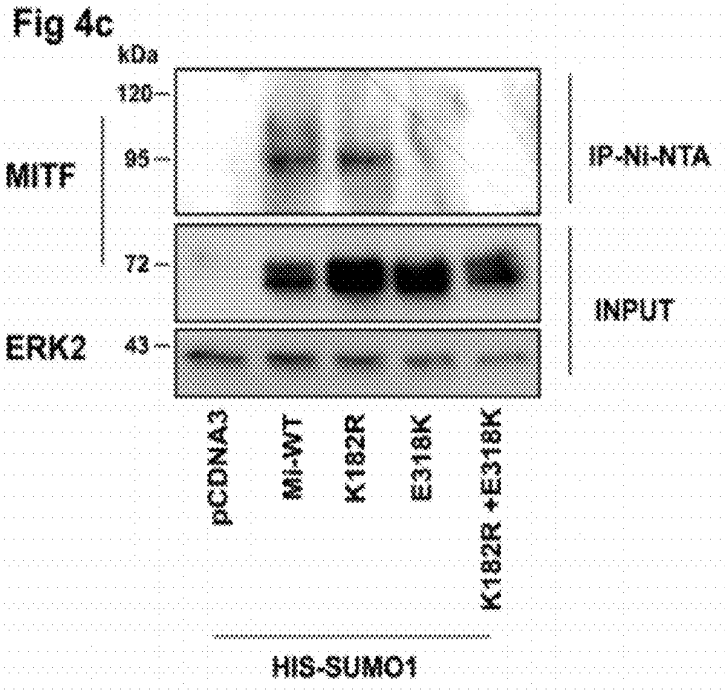


Fig 4c
kDa
120
95
MITF
72
ERK2
43
IP-Ni-NTA
INPUT
pCDNA3
Mi-WT
K182R
E318K
K182R +E318K
HIS-SUMO1

Fig 5a

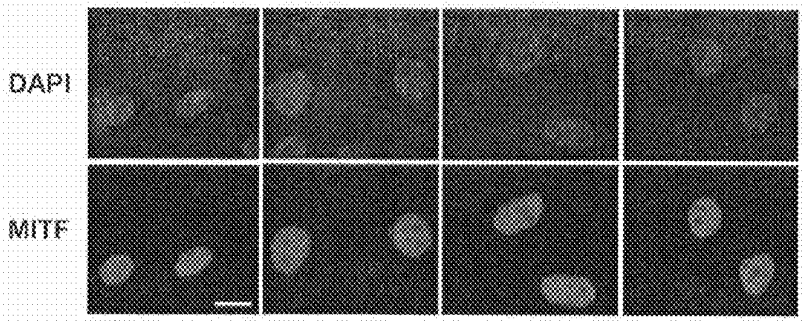

DAPI
MITF
Mi-WT
K182R
E318K
K182R
+E318K

Fig 5b

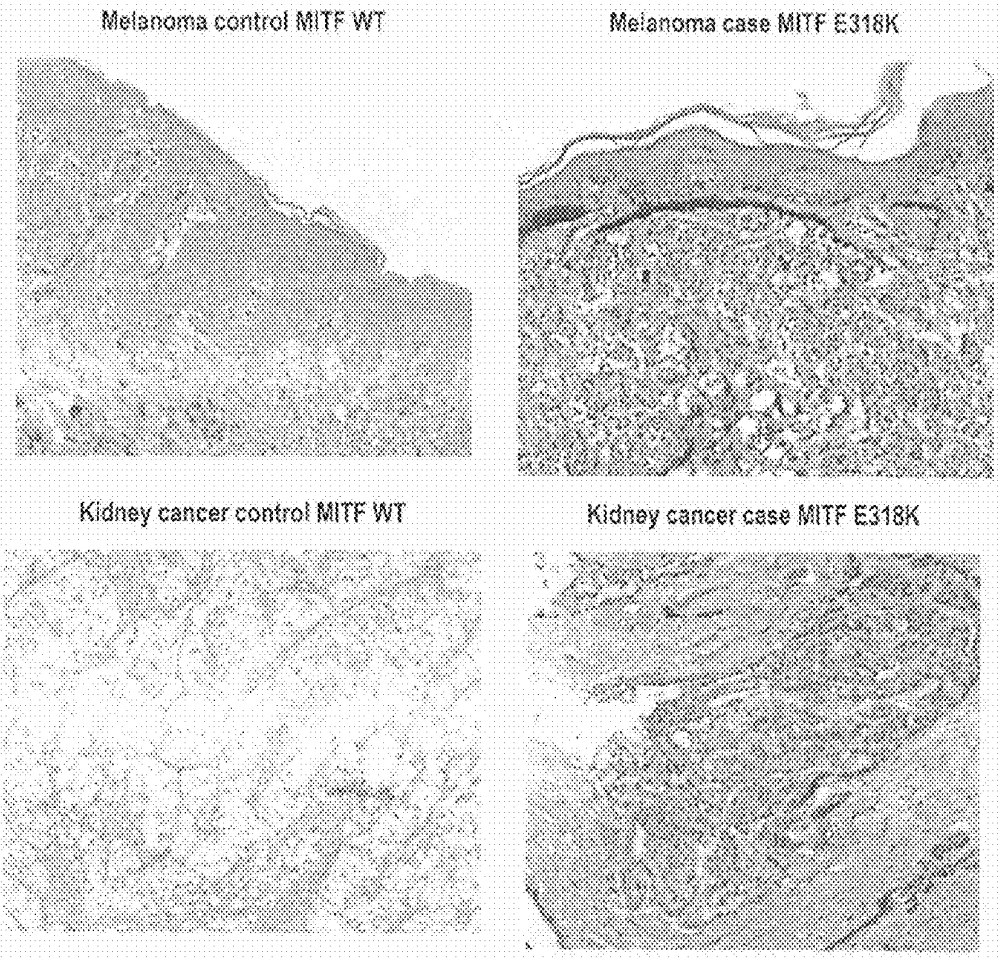
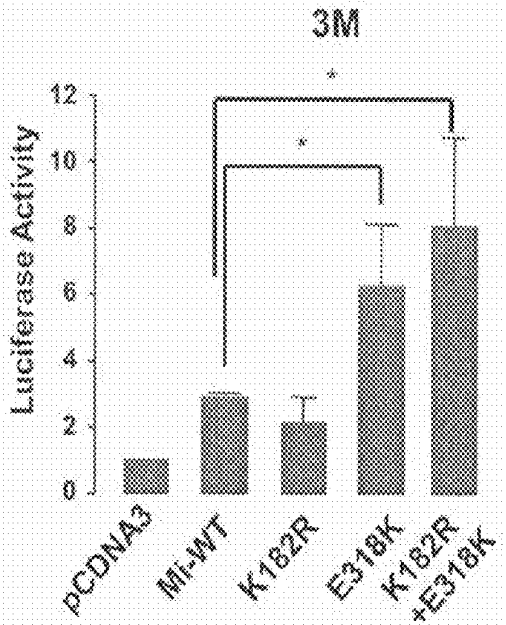

Melanoma control MITF WT
Melanoma case MITF E318K
Kidney cancer control MITF WT
Kidney cancer case MITF E318K

Fig 5c



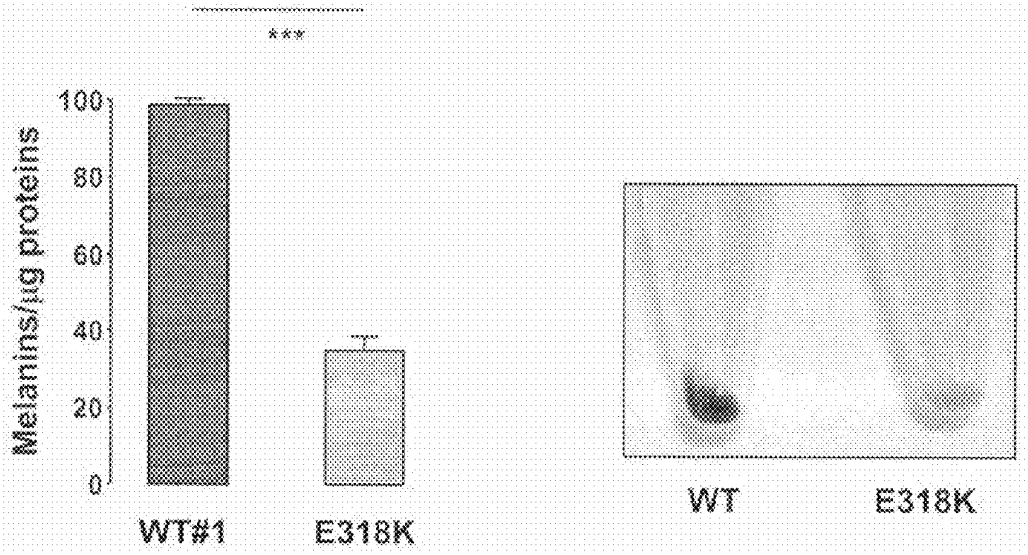


Figure 6

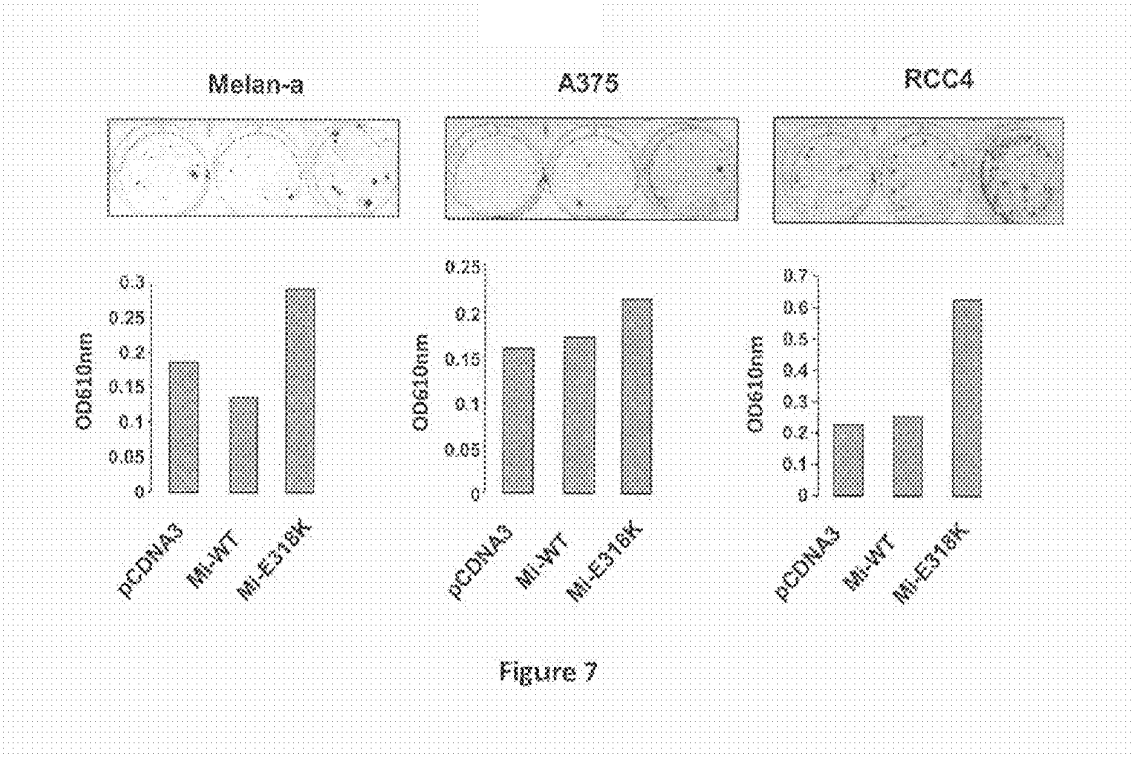

Melan-a
A375
RCC4
OD610nm
0.3
0.25
0.2
0.15
0.1
0.05
0
0.25
0.2
0.15
0.1
0.05
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pCDNA3
MI-WT
MI-E316K

Figure 7

MITF AS A MARKER FOR PREDISPOSITION TO CANCER

[0001] The present application relates to the field of medicine, and more particularly to that of determining a predisposition to the development of cancer.

[0002] In approximately 5% of cancers, constitutional mutations that activate oncogenes or inactivate tumor suppressor genes (known as major genes or strong effect genes) confer a high risk (>50% lifetime risk) of developing cancer to the individuals who carry them, and are responsible for familial forms of cancer, cancers with an early age of onset, or multiple primary cancers. The expression of the disease may vary under the influence of other genetic factors (known as modifiers, or weak effect genes) or environmental factors. Identifying individuals at risk allows them to benefit from prevention and surveillance aimed at early detection. In some cases, the constitutional germline mutation can guide the anticancer therapy, as in the case of anti-PARP treatments in patients with BRCA1 and BRCA2 germline mutations (Hennessy B. T. J., JCO, 2010, 28, 3570-3576).

[0003] Melanoma is a malignant tumor of melanocytes. It is one of the rarest forms of skin cancer but accounts for the majority of skin cancer deaths. Despite many years of intensive research, the only effective treatment is surgical resection of the primary tumor before it reaches a thickness of more than 1 mm. According to a WHO report, there are approximately 48,000 melanoma deaths per year. Some studies have speculated that patients with cutaneous melanoma may have an increased risk of developing breast cancer, lymphoma or kidney cancer.

[0004] In melanoma, two strong effect genes have been identified to date: CDKN2A coding for the $p16^{INK4A}$ and $p14^{ARF}$ proteins, and CDK4. The major environmental factor is UV exposure. Known weak effect genes are mainly those which encode proteins involved in skin pigmentation, MC1R being the most widely studied to date. In 50% of families with three melanoma cases, no susceptibility gene has been identified.

[0005] In this context, the inventors have studied the MITF gene, a major regulatory gene of melanocytes (1) and an oncogene (2,3), as a candidate gene predisposing to melanoma. MITF is a transcription factor from the bHLH-LZ family which plays a major role in melanocyte survival and growth. MITF is involved in the regulation of melanogenesis. The role of MITF is unusual in that it both induces and represses cell proliferation. Indeed, this factor is necessary for terminal melanocyte differentiation and/or pigmentation, on the one hand, and for malignant behavior by inducing cell proliferation, on the other hand. Constitutional "loss of function" mutations of the MITF gene are associated with autosomal dominant diseases such as Waardenburg syndrome and Tietz syndrome, characterized by hearing loss and pigmentation anomalies of the skin, hair and/or iris.

[0006] The MITE gene comprises 9 exons. Six MITF isoforms have been identified. In humans they are generally referred to as isoforms 1 to 6, while isoform 4 is more commonly known as isoform M. In the mouse, the letter nomenclature is used instead. These isoforms are transcribed by specific promoters. In addition, they can be distinguished by their N-terminal region and all contain exons 2 to 9, whereas exon 1 is specific of each isoform (1). Isoform 4, more commonly known as MITF-M, differs from the other isoforms by an insertion of six amino acids. This isoform has been detected only in melanocytes or in vivo transformed cells (nevus, melanoma, etc.) or in vitro cell lines. The other isoforms are expressed in many tissues and cell lines, sometimes also with tissue specificities.

[0007] WO 00/47765 teaches that alternative splicing of the MITE gene produces mitf+ and mitf− transcripts coding for proteins differing by the insertion of six additional amino acids in mitf+. mitf+ and mitf− are predominantly expressed in healthy and tumor cells, respectively. This patent application therefore discloses a semi-quantitative method for evaluating, predicting or monitoring the risk and the treatment of melanoma. WO 05/116249 also describes a quantitative method based on these MITF splicing variants.

[0008] In the present invention, the inventors have identified a recurrent germline mutation in the MITF gene, herein named E318K (based on the nomenclature of isoform 4), which is useful as a marker for predisposition to cancer.

[0009] The MITE gene (microphthalmia-associated transcription factor) is well known to one of skill in the art and can be characterized by its references in data bases such as UniGene (Hs.166017), HomoloGene (4892) and GeneID (4286). It is also called MI, WS2A or bHLHe32.

[0010] Said E318K mutation maps to exon 9 of the MITF gene, in particular to position 952 of isoform M according to HGVS nomenclature. It corresponds to a substitution of a nucleotide G by a nucleotide A (c.952G>A) in the coding sequence and results in the substitution of a glutamic acid by a lysine (p.Glu318Lys). Exon 9 is common to all MITF splicing variants. In the MITF-M isoform, this residue is in position 952 of the transcript and results in the mutation of the amino acid in position 318. The term "E318K mutation" designates the mutation, regardless of its position in the MITF isoforms. In particular, the position of the mutated nucleotide and the amino acid in the different splicing variants is shown below.

Isoform	Other name	Transcript ref.	Protein ref.	Mutation	SEQ ID No
ISOFORM 1	MITF-A	NM_198159	NP_937802	E419K	35
ISOFORM 2	MITF-H	NM_198177	NP_937820	E403K	36
ISOFORM 3	MITF-C	NM_006722	NP_006713	E418K	37
ISOFORM 4	MITF-M	NM_000248	NP_000239	E318K	38
ISOFORM 5		NM_198158	NP_937801	E312K	39
ISOFORM 6		NM_198178	NP_937821	E394K	40

[0011] The MITF E318K mutant is more potent than wild-type MITF at activating transcription of the HIF1A gene, known to play a major role in renal carcinogenesis (secondarily activated with "loss of function" mutations of genes

predisposing to kidney cancer, such as VHL, FH, SDHB). Furthermore, it has been shown that this mutation reduces sumoylation of MITF, thereby perhaps influencing the stability of the protein or the amount of proteins coded by the target genes. In fact, amino acid E318 is part of one of the two sumoylation sites in the MITF protein. As the MITE gene encodes a transcription factor, the MITF E318K mutant protein may continuously activate some of its target genes. It is also possible that the mutation changes the localization of the MITF protein or its nuclear-cytoplasmic ratio.

[0012] The inventors have discovered that the MITF E318K mutant is more frequent in patients with cutaneous malignant melanoma and concomitant kidney cancer. The mutation is also thought to be more frequent in individuals who developed cutaneous malignant melanoma and kidney cancer or in families with a history of melanoma and kidney cancer in relatives, or cutaneous melanoma and another cancer, in particular a polycythemia or a lymphoma. This mutant is present at a very low frequency (2/2846 subjects, i.e., a frequency of heterozygotes of 0.0007 in a first cohort, and 11/1824 subjects, i.e., a frequency of heterozygotes of 0.003 in a second cohort) in healthy control subjects. The inventors have shown that the non-sumoylated form of MITF leads to less differentiated melanocytes and more highly proliferative cells.

[0013] Moreover, it is likely that this mutant is also more frequent in subjects having developed one or more tumors originating in the neural crest such as a neuroendocrine cancer, a sarcoma, a neuroblastoma or a nervous system tumor (NST), or other types of cancers according to the preliminary results of the inventors, such as a lymphoma, a lung cancer, a kidney cancer, a breast cancer, a pancreatic cancer, a pediatric tumor, a hematopoietic malignancy, a gastrointestinal cancer, a polycythemia or a combination of these types of cancer. The sumoylation sites classically comprise the W-K-X-E consensus motif in which W is a hydrophobic amino acid and X is any amino acid. MITF possesses two sumoylation sites: the first site has the sequence IKRE (with the K in positions 289, 273, 288, 182, 182 and 126, respectively, in isoforms 1, 2, 3, 4, 5 and 6), and the second site has the sequence IKQE (with the K in positions 417, 401, 416, 316, 310 and 254, respectively, in isoforms 1, 2, 3, 4, 5 and 6). The K182 and K316 mutations increase the transcription of a target gene, melastatin/TRPM, but do not affect DNA binding, localization or stability of the protein (Miller A J et al., JBC, 2005, 280: 146-155).

[0014] The teaching with regard to the MITF E318K mutant of the present invention may be generalized to any MITF mutation reducing or abolishing the sumoylation of the MITF protein at one of the sumoylation sites or at both sites.

[0015] Therefore, the present invention relates to a method for determining whether a subject has a predisposition or a susceptibility to develop a cancer selected from the group consisting of: a cutaneous malignant melanoma, a kidney cancer, a thyroid cancer, a sarcoma, a neuroblastoma, a central nervous system tumor (CNST), a lymphoma, a lung cancer, a polycythemia, and combinations thereof, comprising determining in a biological sample from the subject the presence of an MITF mutation (microphthalmia-associated transcription factor) reducing or abolishing the sumoylation of MITF, the presence of said mutation indicating that the subject has a predisposition or a susceptibility to develop such cancer. In a particular embodiment, notably when the MITF mutation is E318K (that is to say, E318K in isoform 4 or substitution of the corresponding Glu residue in the other MITF isoforms by a Lys residue), the cancer is a cutaneous malignant melanoma or a combination of a cutaneous malignant melanoma and another cancer, particularly a cancer selected from the group consisting of a neuroendocrine cancer, a sarcoma, a neuroblastoma or a nervous system tumor (NST), a lymphoma, a lung cancer, a kidney cancer, a breast cancer, a pancreatic cancer, a pediatric tumor, a hematopoietic malignancy, a gastrointestinal cancer, a polycythemia and combinations thereof. The cancer may also be selected from among a kidney cancer, a thyroid cancer, a sarcoma, a neuroblastoma, a central nervous system tumor (CNST), a lymphoma, a lung cancer, a polycythemia, and combinations thereof. In a particular embodiment, the combination is that of a cutaneous malignant melanoma and a kidney cancer.

[0016] Preferably, the mutation is a substitution of a lysine residue and/or of a glutamic acid residue of one of the MITF sumoylation sites or of both sites. For instance, the mutation is the substitution of a residue selected from the following table by any of the other 19 amino acids.

Isoform	Transcript ref.	Protein ref.	1st sumoylation site		2nd sumoylation site	
ISOFORM 1	NM_198159	NP_937802	K289	E291	K417	E419
ISOFORM 2	NM_198177	NP_937820	K273	E275	K401	E403
ISOFORM 3	NM_006722	NP_006713	K288	E290	K416	E418
ISOFORM 4	NM_000248	NP_000239	K182	E184	K316	E318
ISOFORM 5	NM_198158	NP_937801	K182	E184	K310	E312
ISOFORM 6	NM_198178	NP_937821	K126	E128	K254	E256

[0017] More specifically, a lysine residue may be substituted by any of the other 19 amino acids; and/or, a glutamic acid residue may be substituted by any of the other 19 amino acids.

[0018] Preferably, the method comprises detecting a substitution of the "K316" residue (that is to say, K316 in isoform 4 or the corresponding Lys residue in the other MITF isoforms) and/or of the "E318" residue (that is to say, E318 in isoform 4 or the corresponding Glu residue in the other MITF isoforms) by any of the other 19 amino acids.

[0019] In a preferred embodiment, the method comprises detecting a substitution of the "E318" residue (that is to say, E318 in isoform 4 or the corresponding Glu residue in the other MITF isoforms) by any of the other 19 amino acids. In an even more preferred embodiment, the method comprises detecting the "E318K" mutation (that is to say, E318K in isoform 4 or the substitution of the corresponding Glu residue by a Lys residue in the other MITF isoforms).

[0020] The mutation may be detected at the protein or nucleic level. The methods for identifying the mutation such as defined earlier in the MITF gene or transcripts thereof (mRNA) are well known to one of skill in the art and include in particular and not by way of limitation, sequencing, selective hybridization and/or selective amplification. At the nucleic level, detection may be carried out on a sample of genomic DNA, mRNA or cDNA.

[0021] In particular, sequencing of MITF may be complete or partial. In fact, the method may comprise solely the sequencing of the region comprising the residue suspected to be mutated and even the sequencing of only this particular residue.

[0022] Selective hybridization is understood to mean that the genomic DNA, RNA or cDNA is placed in the presence of a probe specific of the mutant MITF and optionally a probe specific of MITF not harboring said mutation or wild-type MITF. The probes may be in suspension or immobilized on a substrate. Typically, the probes will be labeled for easier detection. In particular, the probes are single-stranded nucleic acid molecules of 8 to 1000 nucleotides, preferably 10 to 800 or 15 to 50 nucleotides.

[0023] The nucleic acid may be amplified before detection of the mutation. For instance, a primer pair specific of the regions flanking the position of the mutation to be detected (that is to say, upstream or downstream) will be constructed. Typically, the primers are single-stranded nucleic acid molecules of 5 to 60 nucleotides, preferably 8 to 25 nucleotides. Perfect complementarity is preferred because it ensures high specificity. However, some mismatches may be tolerated. Once the MITF gene or the exon containing the mutation, or else one of its transcripts, has been amplified, the amplicon is used for detecting the presence of the mutation by sequencing or specific hybridization or by any other suitable method known to one of skill in the art. The mutation may also be detected by melting curve analysis (see WO2007/035806 for example).

[0024] The presence of the mutation may also be detected by selective amplification of the mutant. For instance, a primer pair is prepared, one of the primers specifically hybridizing with the sequence carrying the mutation to be detected. Said primer will be able to initiate amplification or to hybridize with its target only if the sequence carries the mutated nucleotide. As a result, the presence of an amplicon would indicate that MITF harbors the tested mutation, whereas the absence of said amplicon would indicate that MITF does not harbor this mutation.

[0025] It shall be understood that these methods may be readily adapted by one of skill in the art to detect simultaneously or in parallel several mutations of the sumoylation site(s). Thus, the methods would allow the detection of one or more mutations coding for the substitution of residues selected from the group consisting of K182, E184, K316 and E318 in MITF isoform 4 or the corresponding residues in the other MITF isoforms.

[0026] When the mutation is detected at the protein level, the method makes use of an antibody able to discriminate between MITF harboring the mutation to be detected and MITF not harboring said mutation. In particular, the biological sample is contacted with an antibody directed against MITF harboring the mutation to be detected and the presence of an immune complex is detected. Different methods allow the detection of said immune complexes such as ELISA, radioimmunoassay (RIA) and immunoenzymatic assay (IEMA). "Antibody" also refers to any antibody fragments and derivatives conserving the ability to specifically bind to the MITF mutant to be detected as compared to MITF not harboring this mutation. Here again, these methods may be easily adapted by one of skill in the art for the simultaneous or in parallel detection of several mutations of one or more sumoylation site(s). Thus, the methods would allow the detection of one or more substitutions of residues selected from the group consisting of K182, E184, K316 and E318 in MITF isoform 4 or the corresponding residues in the other MITF isoforms, for example with the aid of a combination of antibodies specific for each MITF mutant harboring one or several of these substitutions.

[0027] Alternatively, the mutation reducing or abolishing MITF sumoylation may be detected at the protein level in an indirect manner. For instance, the presence of the mutation may be detected by measuring the sumoylation of MITF, a reduction of sumoylation relative to the wild-type MITF protein indicating the presence of the mutation. The examples describe how said sumoylation is measured. Likewise, the mutation reducing sumoylation, the mutant MITF protein is stabilized and therefore detectable in tissue slices by immunohistochemistry or immunofluorescence. Detection of the mutation or of the presence/absence of sumoylation may also be carried out by mass spectrometry (WO/2005/003390).

[0028] In a particularly interesting embodiment of the present invention, sumoylation of MITF is measured indirectly by determining the cellular localization of MITF detected by immunohistochemistry or immunofluoresence. In fact, in a surprising and highly original manner, the reduction of sumoylation of the MITF protein causes a modification of the cellular localization of the MITF protein visible by immunohistochemistry in tumor cells. The wild-type protein is located only or mainly in the nucleus whereas the protein harboring the mutation reducing sumoylation of the protein, in particular the E318K mutation, is located in both the nucleus and the cytoplasm. Thus, the present invention relates to a method wherein the mutation reducing sumoylation of MITF is detected by determining the cellular localization of MITF by immunohistochemistry, a nuclear localization indicating the wild-type MITF protein and a cytoplasmic localization indicating the MITF protein harboring a mutation reducing sumoylation of MITF, in particular the MITF E318K mutant. The mutation may also allow detection of the MITF protein by immunohistochemistry or immunofluoresence in tissues where the wild-type protein is undetectable by these same methods.

[0029] The mutation may be detected in any MITF isoform. In a particular embodiment, the mutation is detected in isoform 4.

[0030] In the spirit of the invention, the term "subject" refers to a mammal, preferably a human.

[0031] In the spirit of the invention, the term "biological sample" refers to a sample of healthy or tumor tissue, for example a biopsy and in particular a biopsy of the skin, kidney, thyroid, lung, or a biological fluid, for example a sample of blood, cerebrospinal fluid, urine or lymph. Preferably, the biological sample is a blood sample. The methods of the present invention may comprise a preliminary step of collecting the biological sample.

[0032] It shall be understood that the methods of the present invention also encompass, in addition to the detection of the mutation reducing or abolishing MITF sumoylation, the detection of other markers for predisposition to cancer.

[0033] The present invention also relates to the use of the means for detecting the mutation reducing or abolishing sumoylation of MITF for preparing a diagnostic kit for determining whether a subject has a predisposition or a susceptibility to develop a cancer selected from the following list: a cutaneous malignant melanoma, a neuroendocrine cancer, a sarcoma, a neuroblastoma or a nervous system tumor (NST), a lymphoma, a lung cancer, a kidney cancer, a breast cancer, a pancreatic cancer, a pediatric tumor, a hematopoietic malignancy, a gastrointestinal cancer, a polycythemia, and combinations thereof, the presence of said mutation indicating that the subject has a predisposition or a susceptibility to develop such cancer. In a particular embodiment, the cancer is

selected from the group consisting of a cutaneous malignant melanoma, a kidney cancer, a thyroid cancer, a sarcoma, a neuroblastoma, a central nervous system tumor (CNST), a lymphoma, a lung cancer, a polycythemia and combinations thereof In a preferred manner, the cancer is selected from among a cutaneous malignant melanoma, a kidney cancer and a combination thereof The methods of detection may comprise or consist in a probe specific of MITF harboring the mutation to be detected, a primer pair allowing amplification of a nucleotide segment comprising the mutation to be detected, a pair of primers one of which specifically hybridizes with the sequence carrying the mutation to be detected (thereby allowing selective amplification of the MITF mutant to be detected), an antibody directed against the MITF mutant to be detected, means by which to detect and measure the sumoylation of MITF, negative controls for detecting MITF not carrying the mutation to be detected, or combinations thereof In a particular embodiment, notably when the MITF mutation is E318K (that is to say, E318K in isoform 4 or the substitution of the corresponding Glu residue in the other MITF iso forms by a Lys residue), the cancer is a cutaneous malignant melanoma or a combination of a cutaneous malignant melanoma and another cancer, in particular a cancer selected from the group consisting of a neuroendocrine cancer, a sarcoma, a neuroblastoma or nervous system tumor (NST), a lymphoma, a lung cancer, a kidney cancer, a breast cancer, a pancreatic cancer, a pediatric tumor, a hematopoietic malignancy, a gastrointestinal cancer, a polycythemia, and combinations thereof In a particular embodiment, the cancer is selected from the group consisting of a cutaneous malignant melanoma, a kidney cancer, a thyroid cancer, a sarcoma, a neuroblastoma, a central nervous system tumor (CNST), a lymphoma, a lung cancer, a polycythemia and combinations thereof In a preferred manner, the cancer is selected from among a cutaneous malignant melanoma, a kidney cancer, and a combination thereof.

[0034] The interest of detecting a predisposition or a susceptibility to cancer is that the subject can benefit from clinical monitoring or surveillance allowing the detection of a cancer at an early stage and therefore increasing the chances of cure. Furthermore, detection of the mutation may make it possible to guide the therapeutic algorithm of the patient and/or enhance the efficacy of the treatments. Moreover, the subject so identified can also benefit from a preventive treatment. Said treatment is intended to prevent or delay the development of the cancer.

[0035] Thus, the present invention also relates to a method for selecting patients who may benefit from a preventive treatment or a medical surveillance comprising determining the patient's susceptibility to cancer by the method according to the present invention and selecting subjects presenting the mutation reducing or abolishing the sumoylation of MITF.

[0036] The preventive treatment in question may comprise administering polyphenolic compounds. In fact, it has been shown in particular that polyphenolic compounds from fermented rice hulls reduce the level of the MITF protein (5). Thus, said treatment might counteract the reduction of sumoylation of the MITF protein and its functional effects on its target genes, MITF being a transcription factor. Moreover, polyphenols from black tea have a chemopreventive action which might occur by cell cycle arrest and by a pro-apoptotic mechanism (6). Therefore, the present invention relates to polyphenolic compounds for a use in the preventive treatment of cancer in subjects carrying an MITF mutation reducing or abolishing the sumoylation of MITF and to the use of polyphenolic compounds for preparing a medicament intended for the preventive treatment of cancer (chemoprevention) in subjects carrying an MITF mutation reducing or abolishing the sumoylation of MITF. The invention further relates to a method of treatment comprising administering an effective therapeutic dose of polyphenolic compounds to subjects carrying an MITF mutation reducing or abolishing the sumoylation of MITF, thereby preventing or delaying the development of a cancer. Preferably, the polyphenolic compounds are polyphenolic compounds from fermented rice hulls or polyphenols from black tea. Patent application WO 05/099721 also describes many other polyphenolic compounds useful for the prevention of cancer, in particular through their antioxidant effect.

[0037] The invention will become clearer in the following examples which are given for purposes of illustration and not by way of limitation.

BRIEF DESCRIPTION OF DRAWINGS

[0038] FIG. **1**: (FIG. **1***a*) Pedigree of the first family with multiple melanoma cases in which the MITF E318K variant was identified; (FIG. **1***b*) electrophoregram of the germline mutation (blood) in a person with melanoma and kidney cancer. (FIG. **1***b*).

[0039] FIG. **2**: Transcriptional activation of the HIF1A but not the MET promoter in the presence of the MITF protein harboring the E318K mutation compared with wild-type MITF protein.

[0040] FIG. **3**: Reduction of sumoylation of the MITF protein harboring the E318K mutation.

[0041] FIG. **4**: The E318K mutation reduces sumoylation of the MITF protein. FIG. **4***a*) HEK293 cells were cotransfected with a plasmid coding for myc-tagged wild-type or mutant MITF (K182R; E318K; K182R/E318K) and pSG5His-SUMO1 or pSG5 empty vector. Cells were lysed in boiling buffer and tested by western blot for MITF and ERK2 to control for sufficient loading of each lane. FIG. **4***b*) HEK293 cells were cotransfected with a plasmid coding for myc-tagged wild-type or mutant MITF (K182R; E318K; K182R/E318K) and pSG5His-SUMO2 or pSG5 empty vector. Cells were tested by western blot for MITF and ERK2. FIG. **4***c*) HEK293 cells were cotransfected with a plasmid coding for myc-tagged wild-type or mutant MITF (K182R; E318K; K182R/E318K) and pSG5His-SUMO1 or pSg5 empty vector. Cell lysates were purified on Ni-NTA columns and analyzed by western blot for MITF (upper panel). The lower panel shows the western blot of cell lysates before purification to control MITF expression and sufficient loading of each lane.

[0042] FIG. **5**: The E318K mutation might affect the cellular localization of MITF and alter its transcriptional activity. FIG. **5***a*) Immunofluorescence analysis of HEK293 cells transfected with a plasmid coding for myc-tagged wild-type or mutant MITF (K182R; E318K; K182R/E318K E318K) and stained with an anti-myc antibody, then stained secondarily with Alexafluor 594-labeled anti-mouse antibody. Cell nuclei were counterstained with DAPI. The bar represents 10 μm. FIG. **5***b*) Immunohistochemical staining of melanoma and renal tumor tissue with anti-MITF antibody (×200). Melanoma harboring wild-type MITF show nuclear staining while melanoma harboring the MITF E318K mutation show nuclear and cytoplasmic staining Renal cancer tissue harboring wild-type MITF does not stain whereas renal cancer tis-

sue harboring the MITF E318K mutation shows nuclear and cytoplasmic staining FIG. **5***c*) HEL293 cells were transiently transfected with a synthetic 3× M-box luciferase reporter plasmid and with the mutant or wild-type MITF pCDNA3 construct or empty pCDNA3 vector. Luciferase activity was normalized to β-galactosidase activity and the results were expressed as fold-stimulation over basal luciferase activity for unstimulated cells.

[0043] FIG. **6**: Mutant melanocytes show lighter pigmentation. Cell pellets and melanin determination in the melanocytes of two healthy donors compared with melanocytes isolated from a skin biopsy of patients harboring the E318K mutation (***) show a significant difference ($p<0.001$). The two donors and the mutant carriers were Caucasian.

[0044] FIG. **7**: The E318K mutation confers a growth advantage. Melanocytes from melan-a immortalized mice (left), the human metastatic melanoma A375 cell line (center) and human renal carcinoma RCC4 cells (right) were transfected with an empty vector, or a vector coding for wild-type MITF (Mi-WT) or mutant MITF (Mi-E318K). Photographs at 2 weeks (upper) and absorbance after crystal violet staining/destaining of the clones are shown.

EXAMPLES

Example 1

[0045] The inventors studied the MITF gene, which is considered an oncogene and therefore a candidate gene. They first studied whether there were mutations in the MITE gene (3p14) at the caspase cleavage site (anti-apoptotic effect) in melanoma families (4), by sequencing all the isoforms. Their preliminary results revealed the presence of a germline variant of MITF-M c.952G>A, p.Glu318Lys (E318K) in a Spanish Basque family (TRY) with multiple cases of melanoma (with 2 kidney cancers, 3 central nervous system tumors, 1 lung cancer and 1 stomach cancer on the maternal side from whence the E318K mutation originated), with paternal transmission of a 1p22 haplotype. The variant was absent in 180 French/Caucasian and 96 Spanish Basque controls (FIGS. **1***a* and *b*).

[0046] In B16 mouse melanoma cells, the inventors showed that the MITF variant c.952G>A, p.Glu318Lys (E318K) was more active than the wild-type form at inducing transcription of the HIF1a gene but not of the MET gene (FIG. **2**). The inventors therefore hypothesized that HIF is the transforming factor of renal cells in the absence of VHL, in hypoxic conditions; VHL (loss of function mutations of a tumor suppressor gene) and MET (activating mutations of an oncogene) are two genes predisposing to kidney cancer; HI1aF activated by the MITF E318K mutant might therefore have the same effect as absence of VHL. The inventors therefore showed that the MITF E318K mutation was present in 4/55 patients and absent in 276 controls ($p=0.0007$) of the "melanoma and renal cancer (sporadic cases)" subgroup of the MELARISK cohort. The inventors then investigated the frequency of the E318K mutant in the different biological sample collections of IGR (Institut Gustave Roussy, Villejuif, France). Other carriers of this mutation were identified: a female who developed juvenile TFE3 translocation-associated kidney cancer whose mother had breast cancer followed by melanoma; a female who developed 3 melanomas and a lymphoma; an index case of a family with multiple cases of melanoma; an index case who developed several melanomas; two males who developed nodular melanomas; a female who developed melanoma, one of whose uncles had a CNST; two males who developed a renal papillary carcinoma; a male with polycythemia. In fact, VHL is one of the three genes predisposing to polycythemia.

TABLE 1

Results of MITF E318K mutation screening

Melanoma and renal cancer	Sample No.	MITF wild-type	MITF E318K	p-value
Sporadic	55	51	4	0.0007
Familial	50	49	1	0.1534
Sporadic and familial	105	100	5	0.0015
Papillary renal cancer	24	22	2	0.0062
Melanoma and CNST, familial			1	0.1687
Polycythemia	14	13	1	0.0483
Lymphoma Melanomas	16	15	1	0.0548
Multiple melanomas Familial	107	106	1	0.2794
Multiple melanomas Sporadic	34	33	1	0.1097
Nodular melanomas	90	88	2	0.0600
Multiple cutaneous melanomas Familial	49	48	1	0.1508

Control populations were blood and CEPH donors (N=276).

Example 2

[0047] Identification of MITF Germline Mutations in Patients with Melanoma and Renal Cell Carcinoma

[0048] To confirm the identification of the MITF variant resulting from a missense substitution p.E318K (c.952G>A in MITF isoform M, NM_000248) associated with coexisting melanoma and renal cancer, the inventors sequenced the entire coding sequence of the gene, the intron-exon boundaries and the 8 alternative promoters in 62 patients with coexisting melanoma and renal cell carcinoma. This substitution was observed in 5 of the 62 patients. The frequency of this variant is significantly higher than in the control population of 1824 subjects (4% vs 0.3%, $p=9.7\times10^{-5}$). Thus, p.E318K carriers have a 14-fold higher risk of developing both melanoma and renal cell carcinoma (Odds Ratio=14.46 [95% confidence interval: 3.79-46.82]) (Table 2). To confirm that this variant affects the susceptibility to melanoma alone, the inventors genotyped 704 patients with melanoma (who were negative for the CDKN2A and CDK4 mutations predisposing to melanoma) including 422 independent cases with a family history of melanoma, 242 sporadic cases with multiple primary melanomas and 40 sporadic nodular melanomas (Table 2). The latter cases were tested because 4 of the 5 patients with both melanoma and renal cell carcinoma and carrying the p.E318K mutation had at least one nodular melanoma (the rarest histologic type of melanoma). The frequency of p.E318K was significantly higher in all patients with melanoma alone as compared to controls (1.3% vs 0.3%, $p=4.5\times10^{-5}$) and p.E318K carriers had a more than fourfold

increased risk of developing melanoma (Odds Ratio=4.57 [95% confidence interval: 2.05-10.68]). This increased risk appears to be due mainly to the sporadic multiple primary melanoma patients (Odds Ratio=7.10 [95% confidence interval: 2.67-18.62]) whereas the effect of p.E318K was not significant for melanoma patients from a melanoma family (Odds Ratio=2.78 [95% confidence interval: 0.9-7.90]) or for patients with nodular melanoma (Odds Ratio=8.67 [95% confidence interval: 0.91-41.70] (Table 3)). However, the test of homogeneity of the frequency of the p.E318K allele across these three groups was only marginally significant (p=0.06). Biological material was available for additional affected family members in three of the seven melanoma families in which the proband carried the variant. In each of these families, p.E318K cosegregated with melanoma. Alternatively, to examine the effect of p.E318K on susceptibility to renal cancer, the inventors genotyped this variant in 187 patients with renal cell carcinoma. The frequency of p.E318K was also higher in patients with renal cancer than in controls (1.3% vs 0.3%, p=0.01) and the increase in the risk of renal cancer associated with p.E318K was similar to that seen for melanoma alone (Odds Ratio=4.53 [95% confidence interval: 1.22-14.30]) (Table 2). There was no significant demonstration of heterogeneity of the p.E318K allele frequency across the three groups of patients (melanoma +renal cell carcinoma, melanoma alone, renal cell carcinoma alone; p=0.08). Combining all the patient groups increased the degree of significance of the difference in p.E318K allele frequency between cases and controls (1.5% vs 0.3%, $p=2.5\times10^{-7}$). On the whole, carriers of the p.E318K mutation had a more than fivefold increased risk of developing melanoma, renal cell carcinoma or both (Odds Ratio=5.17 [95% confidence interval: 2.49-11.52]).

[0049] To determine whether p.E318K predisposes to the co-occurrence of melanoma and another cancer other than renal cancer, the investigators genotyped 172 patients with melanoma and another primary tumor but none carried the p.E318K mutation (Table 2). Since association of p.E318K with another primary tumor is a rare event, the inventors plan to study larger series.

TABLE 2

Frequency of the p.E318K germline mutation in patients with cancer

Type of tumor	Number of non-carriers	Number of carriers*	Total	Minority allele frequency	FET p-value	OR [95% CI]
Controls	1813	11	1824	0.003	—	Ref
CM or/and RCC	924	29	953	0.015	2.5×10^{-7}	5.17 [2.49-11.52]
Both	57	5[a]	62	0.040	9.7×10^{-5}	14.46 [3.79-46.82]
CM alone[b]	685	19[c]	704	0.013	4.5×10^{-5}	4.57 [2.05-10.68]
RCC alone[d]	182	5[e]	187	0.013	0.012	4.53 [1.22-14.30]
Melanoma and another cancer[f]	172	0	172	0	0.61	—

OR = Odds Ratio;

95% CI = 95% confidence interval;

p-value = critical probability of the test;

CM = cutaneous melanoma;

RCC = renal cell carcinoma.

*All carriers are heterozygotes for the p.E318K variant.

[a]The 5 patients developed clear cell renal cell carcinoma (ccRCC), 4 of the 5 patients developed at least one nodular melanoma, and the 5th patient developed a superficial spreading melanoma (SSM).

[b]Familial melanomas (in particular with at least 2 confirmed melanoma cases in the family), 422 cases; sporadic cases with multiple primary melanomas (MPM), 242 cases; sporadic nodular melanoma, 40 cases.

[c]Out of 19 carriers, 7 are familial cases, 9 are sporadic MPM cases and 2 are sporadic nodular melanoma cases.

[d]Clear cell renal cell carcinoma (ccRCC), 54 sporadic cases; papillary renal cell carcinoma (PRC), 55 cases (22 cases with type I, 30 cases with type II, and 3 cases with unknown histologic subtype); mixed renal cell carcinoma phenotypes (in particular, papillary and clear cell), 2 cases; pediatric renal cell carcinoma, 5 cases; renal cell carcinoma with unknown histologic subtype, 71 cases.

[e]Out of 5 carriers, one is a clear cell renal cell carcinoma (ccRCC), 2 are type II papillary renal cell carcinomas, 1 is a juvenile carcinoma with a somatic translocation t(X; 17)(p11; q25) and 1 is a type I papillary renal cell carcinoma.

[f]Breast cancer, 97 cases; brain cancer, 27 cases; non-medullary thyroid cancer, 28 cases; colon cancer, 10 cases; other cancers (testicular, uterine, ovarian, prostate, sarcoma and endometrial), 10 cases.

TABLE 3

Frequency of the MITF p.E318K germline mutation in patients with melanoma alone

Type of tumor	Number of non-carriers	Number of carriers*	Total	Minority allele frequency	FET p-value	OR [95% CI]
Controls	1813	11	1824	0.003	—	Ref
CM alone	685	19	704	0.013	4.5×10^{-5}	4.57 [2.05-10.68]

TABLE 3-continued

Frequency of the MITF p.E318K germline mutation in patients with melanoma alone

Type of tumor	Number of non-carriers	Number of carriers*	Total	Minority allele frequency	FET p-value	OR [95% CI]
Familial	415	7	422	0.008	0.06	2.78 [0.91-7.90]
Multiple primary melanoma (MPM)	232	10	242	0.021	4.1×10^{-5}	7.10 [2.67-18.62]
Nodular melanoma	38	2	40	0.025	0.029	8.67 [0.90-41.70]

Test of homogeneity of allele frequency among the 3 categories of melanoma alone (familial, MPM, nodular): p = 0.065 (exact test).
Test of homogeneity comparing familial versus MPM: p = 0.072 (exact test)

Example 3

[0050] Functional Effects of the MITF p.E318K Mutation

[0051] This E318K mutation is located at one of the two sumoylation sites of the protein (WKXE motif, 2 sites K182 and K316). The inventors have demonstrated a reduction in sumoylation of MITF in the presence of the E318K mutation (FIG. **3**).

[0052] More specifically, the inventors have produced the E318K variant by site directed mutagenesis. In addition, they have also prepared the K182R variant and a K182R:E318K double mutant.

[0053] After coexpression of His-SUMO-1 with wild-type MITF, western blots on total extracts using an anti-MITF antibody revealed the presence of a 120 kD band and a doublet of approximately 90 kD, suggesting that MITF undergoes sumoylation which increases its molecular weight (FIG. **4**A). While addition of exogenous SUMO protein increased the overall level of MITF sumoylation, western blots revealed that MITF is also sumoylated in basal conditions showing the 90 kD doublet, thereby excluding a non-specific effect of SUMO overexpression. The K182R mutation led to complete disappearance of the higher molecular weight form of MITF but had practically no effect on the 90 kD band. When codon 318 was mutated to lysine, the inventors observed a considerable reduction in the level of all the high molecular weight bands of MITF. Lastly, no high molecular weight forms of MITF were observed when the double mutant was used. Similar results were seen with coexpression of HA-SUMO-2 (FIG. **4**B), demonstrating that wild-type MITF was modified by SUMO-1 or SUMO-2 and that the E318K mutation affected both SUMO-1 and SUMO-2 modifications. To confirm SUMO-1 binding to MITF, the His-SUMO-1 plasmid was transfected alone or with the MITF constructs. Then, proteins containing His-SUMO-1 were purified on a Ni-TFA column. In cells transfected with wild-type MITF, the western blot with anti-MITF antibody revealed sumoylated forms of MITF migrating at approximately 90 and 120 kD (FIG. **4**C). The K182R mutation mainly affected the 120 kD sumoylated form of MITF whereas no sumoylated form was found with E318K or the double mutant. Together, these results show that codon 316 is a major SUMO acceptor site in MITF and that the E318K mutation dramatically reduces sumoylation of MITF.

Example 4

[0054] The E318K Mutation Might Change the Localization of MITF and Alters its Transcriptional Activity

[0055] Sumoylation orchestrates many cellular processes, partly by controlling nuclear-cytoplasmic signal transduction and transcription. The inventors investigated whether the E318K mutant with reduced sumoylation could modify the cellular localization of MITF (FIG. **5**A). Immunofluorescence staining with anti-MITF antibody showed that the E318K mutant but also the K182R and K182R:E318K mutants were detected in the nuclei of melanoma cells, which is consistent with the nuclear localization of wild-type MITF. However, immunohistochemistry experiments on melanoma and kidney cancer cells revealed that E318K mutants showed both nuclear and cytoplasmic staining (FIG. **5**B).

Type of staining	Nuclear	Nuclear + cytoplasmic	None
Control melanoma	8/9	1/9	0/9
Melanoma with E318K mutation	0/8	8/8	0/8
Control kidney cancer	0/6	0/6	6/6
Kidney cancer with E318K mutation	0/6	2/6	4/6

[0056] The inventors also explored the possible effect of the E318K substitution on the transcriptional activity of MITF by comparing the activity of the wild-type versus E318K mutant on a synthetic reporter containing 3 copies M box linked to an SV40 minimal promoter. The E318K mutant had 2-3 times more transcriptional activity than the wild-type (FIG. **5**C). The double mutant was even more active than the E318K mutant while the single mutant K182R had an activity similar to wild-type. These data indicate that sumoylation reduces the transcriptional activity of MITF. The inventors then checked the effect of the E318K mutation on physiological promoters, focusing on MET and HIF1A, two MITF target genes involved in melanocyte and renal carcinogenesis. Wild-type MITF and the E318K mutant had similar transcriptional activity on the MET promoter (FIG. **2**) whereas the E318K mutant had higher transcriptional activity than wild-type on the HIF1A promoter. Therefore, the p.E318K mutation may exert its oncogenic effect through transcriptional up-regulation of HIF1A.

Example 5

[0057] The E318K Mutation Confers a Less Differentiated and More Highly Proliferative Cell Phenotype

[0058] Production of pigment is one of the features of melanocyte differentiation, characterized by slower growth, in contrast to weakly pigmented cells which correlate closely with less differentiated, proliferative phenotypes. In addition, hypoxia and HIF1A sustain the survival, proliferation and transformation of melanocytes and the progression of melanoma. Accordingly, the inventors found that melanocytes isolated from skin biopsies of a patient with the germline mutation were less highly pigmented than melanocytes from two healthy mutation-negative donors, all three of which were Caucasian (FIG. **6**). MITF transcription is modified by two receptor signaling pathways, including the melanocortin-1 receptor (MC1R). Lastly, compared with wild-type MITF, expression of E318K stimulated the growth of immortalized melanocytes (FIG. **7**A, Melan-a), melanoma cells (FIG. **7**B, A375) and VHL-deficient kidney cancer cells (FIG. **7**C, RCC4). Taken together, the data suggest that the MITF E318K mutant confers a constitutive growth advantage.

[0059] Materials and Methods

[0060] Melarisk is a unique registry of melanoma-prone families (MELARISK) initiated in 1985 by Institut Gustave Roussy (Prof. Avril) and INSERM (Florence Demenais, U946), with participation of dermatologists (in particular, since 2005, Cochin University Hospital Center, Prof. Avril and Hospices Civils de Lyon, Prof. Thomas) and oncogeneticists. The biological materials are stored in the IGF cancer susceptibility Biobank (blood, frozen lymphocytes, lymphoblastic cell lines established by Genethon, DNA). Family, demographic, clinical and risk factor data for melanoma have been collected for several years now and are stored in a MySQL data base in INSERM unit U946.

[0061] Direct Sequencing of MITF

[0062] Primers used to sequence MITF are shown in the following table. The amplification protocol consisted of 35 cycles with 30-sec temperature steps at 94° C., 60° C. and 72° C.

[0063] PCR products were sequenced with the "Big Dye Terminator", version 3.0 (Applied Biosystems, Foster City, Calif.) on an ABI Prism© 3730 Genetic Analyzer (Applied Biosystems, Foster City, Calif.).

TABLE

Primer sequences for the MITF gene

Amplified fragment	Sense Sequence 5' > 3'	Antisense Sequence 5' > 3'	Size of product (bp)
Exon 1a_part1	SEQ ID NO 1	SEQ ID NO 2	377
Exon 1a_part2	SEQ ID NO 3	SEQ ID NO 4	394
Exon 1b	SEQ ID NO 5	SEQ ID NO 6	298
Exon 1e	SEQ ID NO 7	SEQ ID NO 8	232
Exon 2/Exon 1c	SEQ ID NO 9	SEQ ID NO 10	396
Exon 1 (M)	SEQ ID NO 11	SEQ ID NO 12	389
Exon 2	SEQ ID NO 13	SEQ ID NO 14	426
Exon 3	SEQ ID NO 15	SEQ ID NO 16	271
Exon 4	SEQ ID NO 17	SEQ ID NO 18	257
Exon 5	SEQ ID NO 19	SEQ ID NO 20	447
Exon 6a/6b	SEQ ID NO 21	SEQ ID NO 22	280
Exon 7	SEQ ID NO 23	SEQ ID NO 24	320
Exon 8	SEQ ID NO 25	SEQ ID NO 26	563
Exon 9	SEQ ID NO 27	SEQ ID NO 28	544

[0064] Genotyping of the MITF E318K Mutation by PCR with a MGB Taqman Primer

[0065] PCR reactions were carried out with 10 ng of genomic DNA in the presence of 0.2 μmol/L of MGB Taqman primers, either (5'-VIC-ATC AAG CAA GAA CCC G-3'—SEQ ID No 29) which perfectly matches the wild-type MITF sequence or with (5'-6-FAM-CAA GCA AAA ACC CG-3'—SEQ ID No 30) which perfectly matches the MITF sequence coding the E318K mutation. Final concentrations of the other reagents were as follows: 1× Universal Master Mix (Applied), 0.4 μmol/L of sense primer (5'-TGCTCTCCA-GATTTGGTGAATCG-3'—SEQ ID No 31), 0.4 μmol/L of antisense primer (5'-GGTCTTGGCTGCAGTTCTCAA-3'—SEQ ID No 32). The size of the PCR amplicon was 67 bp. PCR cycling was carried out on a ABI™ 2720 thermocycler as follows: 95° C. for 15 min; 30 cycles at 95° C. for 15 sec and 60° C. for 1 min. Allelic discrimination was performed by a final fluorescence measurement on an ABI™ 7900HT Fast Real Time PCR system and analyzed with ABI™ SDS v2.3 software. Either wild-type or E318K DNA samples were included as controls in each genotyping experiment. Genotyped mutant samples were checked by direct sequencing using the protocol and primers described above for exon 9 of MITF.

[0066] Plasmids

[0067] The MITF M-form construct pCDNA3-Mi has been described previously (7). Mutations of MITF at K182R and/or E318K were generated using the QuickChange method (Stratagene) using the following sense primers with their reverse complements: Mi-K182R 5'-cttcccaacataagaagg-gagctcacagc-3' (SEQ ID No 33); MI-E318K 5'-ggatcatcaag-caaaaaccagttcttgag-3' (SEQ ID No 34). The presence of the mutations was confirmed by sequencing.

[0068] His-SUMO1 and His-HA-SUMO2 were kindly provided by M. A. Dejean and are described in the following publication (8).

[0069] Cotransfections and Immunoblots

[0070] HEK293 cells grown in 6-well dishes (10^4 cells/well) were transfected with the indicated plasmids (2 μg of total DNA/well) using FuGENE 6™ (Roche Applied Science). 48 hours later, cells were rinsed in PBS followed by lysis at 95° C. in 1× loading buffer (41.6 mM Tris, pH 6.8, 1.5% SDS, 6.7% glycerol) and boiling for an additional 5 minutes.

[0071] Proteins were resolved by electrophoresis in 10% SDS-polyacrylamide gels and transferred to PVDF membranes. Proteins were detected using ECL (Amersham) and anti-MITF (Abcam), anti-HA tag (Abcam), anti-SUMO1 (Santa Cruz Biotech) or anti-ERK2 (Santa Cruz Biotech) antibodies.

[0072] Reporter Assays

[0073] Human 501mel and mouse B16 cells were plated into 24-well plates (25×10^3 cells/well) and, the following days, the cells were transiently transfected with 0.3 μg of reporter plasmid (pHIF1α and pMet), 0.05 μg of pCDNA2 MITF or empty pCDNA2 vector, 2 μl of lipofectamine reagent (Invitrogen) and 0.05 μg of pCMVβGal to control variability of transfection efficiency. Cells were lysed and assayed for luciferase and β-galactosidase activity 48 hours later. Transfections were performed at least in triplicate.

[0074] Immunofluorescence

[0075] HEK293 cells were plated on glass coverslips (100×10^3 cells) in 6-well dishes and transfected with 3 μg of pCDNA3 MITF or empty pCDNA3 vector, using 10 μl of

lipofectamine. 48 hours later, cells were fixed for 10 min with 4% paraformaldehyde in PBS, washed in PBS, and permeabilized for 2 min with 0.1% Triton X-100, 1% bovine serum albumin (BSA). Next, samples were washed once in PBS and treated with 50 mM NH_4Cl for 2 min, then washed three times in PBS and stained for 1 hour with anti-MITF antibody (Abcam) in 1% BSA/PBS. Samples were then washed three times with PBS for 5 min and stained secondarily for 1 hour with Alexa-488 conjugated goat anti-mouse antibody (Molcular Probes) in 1% BSA. Cells were washed once in PBS, counterstained with 4,6-diamino-2-phenylindole (DAPI), washed 3 times in PBS and mounted using Fluromount-G (Southern Biotech, Birmingham Ala.). Cells were examined under a Zeiss Axiophot microscope with epifluorescence illumination.

[0076] Determination of Melanin Content

[0077] Approximately 6×10^6 melanocytes were pelleted by centrifugation at 1000 g for 5 min and washed twice in phosphate buffer. A fraction of the pellet was dissolved in 0.5% NaOH for 1 hour at 80° C. and optical density was measured at 405 nm. The other fraction was used to determine protein content by the BCA™ method (Pierce). Melanin content was corrected for protein concentration and expressed as a percentage of control cells (100%).

[0078] Test of Colony Formation

[0079] Human melanoma A375 cells and human kidney cancer RCC4 cells (80,000 per well) were transfected with a total of 3 μg DNA per well (wild-type MITF or E318K) and 10% pBABE-puro using Fugene (Roche). Puromycin (1 μg/ml) was added to the medium 48 hours after transfection. Fourteen days later the cells were fixed, stained with 0.4% crystal violet and plates were photographed. Cells were also destained with 10% acetic acid in PBS and cell counts were determined by measuring absorbance at 610 nm.

[0080] Immunohistochemistry

[0081] After dewaxing the coverslips and unmasking the antigens in hot Antigen Unmasking solution (Vector Laboratories), sections were permeabilized in 0.3% Triton/PBS for 15 min, then rinsed rapidly in PBS. After blocking endogenous peroxidases, the sections were saturated in PBS/1% BSA/5% goat serum for 30 min, then incubated overnight at 4° C. with the first anti-MITF antibody clone C5 (1:10 or 1:100 dilution) in PBS/1% BSA. After rinsing in PBS, sections were incubated with the second biotinylated antibody in PBS/1% BSA for 1 hour at room temperature. Sections were then rinsed in PBS and incubated in HRP Avidin/Biotin solution (ABC Elite kit, Vector Laboratories). After rinsing in PBS, the sections were revealed in the presence of a peroxidase substrate (VIP kit, Vector Laboratories). Finally, slides were mounted using Mountex (Cell Path).

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[0089] 8. Bischof O, et al. (2006) *Mol. Cell.* 22, 783-94.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 40

<210> SEQ ID NO 1
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<213> ORGANISM: artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 1

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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 2

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<210> SEQ ID NO 3
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<220> FEATURE:
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 13

tttgtgcctg aaggaagagc 20

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
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caaaggctgg taaatgtggc 20

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<223> OTHER INFORMATION: primer

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<220> FEATURE:
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<212> TYPE: DNA
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tacacggctt gggtggtg 18

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gcttaaaagt cctctgtgct ctg 23

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<400> SEQUENCE: 28

caagaaaacc ccttcaggta ag 22

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atcaagcaag aacccg 16

<210> SEQ ID NO 30
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caagcaaaaa cccg 14

<210> SEQ ID NO 31
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<212> TYPE: DNA
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<223> OTHER INFORMATION: primer

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tgctctccag atttggtgaa tcg 23

<210> SEQ ID NO 32
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<400> SEQUENCE: 32

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<210> SEQ ID NO 33
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 33

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<210> SEQ ID NO 34
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<212> TYPE: DNA
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ggatcatcaa gcaaaaacca gttcttgag 29

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<212> TYPE: PRT
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<223> OTHER INFORMATION: Xaa = any amino acid
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<223> OTHER INFORMATION: Xaa = any amino acid
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<223> OTHER INFORMATION: Xaa = any amino acid
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<222> LOCATION: (419)..(419)
<223> OTHER INFORMATION: Xaa = any amino acid

<400> SEQUENCE: 35

Met Gln Ser Glu Ser Gly Ile Val Pro Asp Phe Glu Val Gly Glu Glu
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Phe His Glu Glu Pro Lys Thr Tyr Tyr Glu Leu Lys Ser Gln Pro Leu
20 25 30

Lys Ser Ser Ser Ser Ala Glu His Pro Gly Ala Ser Lys Pro Pro Ile
35 40 45

Ser Ser Ser Ser Met Thr Ser Arg Ile Leu Leu Arg Gln Gln Leu Met
50 55 60

Arg Glu Gln Met Gln Glu Gln Glu Arg Arg Glu Gln Gln Gln Lys Leu
65 70 75 80

Gln Ala Ala Gln Phe Met Gln Gln Arg Val Pro Val Ser Gln Thr Pro
85 90 95

Ala Ile Asn Val Ser Val Pro Thr Thr Leu Pro Ser Ala Thr Gln Val
100 105 110

Pro Met Glu Val Leu Lys Val Gln Thr His Leu Glu Asn Pro Thr Lys
115 120 125

Tyr His Ile Gln Gln Ala Gln Arg Gln Gln Val Lys Gln Tyr Leu Ser
130 135 140

Thr Thr Leu Ala Asn Lys His Ala Asn Gln Val Leu Ser Leu Pro Cys
145 150 155 160

Pro Asn Gln Pro Gly Asp His Val Met Pro Pro Val Pro Gly Ser Ser
165 170 175

Ala Pro Asn Ser Pro Met Ala Met Leu Thr Leu Asn Ser Asn Cys Glu
180 185 190

Lys Glu Gly Phe Tyr Lys Phe Glu Glu Gln Asn Arg Ala Glu Ser Glu
195 200 205

Cys Pro Gly Met Asn Thr His Ser Arg Ala Ser Cys Met Gln Met Asp
210 215 220

Asp Val Ile Asp Asp Ile Ile Ser Leu Glu Ser Ser Tyr Asn Glu Glu
225 230 235 240

Ile Leu Gly Leu Met Asp Pro Ala Leu Gln Met Ala Asn Thr Leu Pro
245 250 255

Val Ser Gly Asn Leu Ile Asp Leu Tyr Gly Asn Gln Gly Leu Pro Pro
260 265 270

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Pro Gly Leu Thr Ile Ser Asn Ser Cys Pro Ala Asn Leu Pro Asn Ile
        275                 280                 285

Xaa Arg Xaa Leu Thr Glu Ser Glu Ala Arg Ala Leu Ala Lys Glu Arg
    290                 295                 300

Gln Lys Lys Asp Asn His Asn Leu Ile Glu Arg Arg Arg Arg Phe Asn
305                 310                 315                 320

Ile Asn Asp Arg Ile Lys Glu Leu Gly Thr Leu Ile Pro Lys Ser Asn
                325                 330                 335

Asp Pro Asp Met Arg Trp Asn Lys Gly Thr Ile Leu Lys Ala Ser Val
            340                 345                 350

Asp Tyr Ile Arg Lys Leu Gln Arg Glu Gln Gln Arg Ala Lys Glu Leu
        355                 360                 365

Glu Asn Arg Gln Lys Lys Leu Glu His Ala Asn Arg His Leu Leu Leu
    370                 375                 380

Arg Ile Gln Glu Leu Glu Met Gln Ala Arg Ala His Gly Leu Ser Leu
385                 390                 395                 400

Ile Pro Ser Thr Gly Leu Cys Ser Pro Asp Leu Val Asn Arg Ile Ile
                405                 410                 415

Xaa Gln Xaa Pro Val Leu Glu Asn Cys Ser Gln Asp Leu Leu Gln His
            420                 425                 430

His Ala Asp Leu Thr Cys Thr Thr Thr Leu Asp Leu Thr Asp Gly Thr
        435                 440                 445

Ile Thr Phe Asn Asn Asn Leu Gly Thr Gly Thr Glu Ala Asn Gln Ala
    450                 455                 460

Tyr Ser Val Pro Thr Lys Met Gly Ser Lys Leu Glu Asp Ile Leu Met
465                 470                 475                 480

Asp Asp Thr Leu Ser Pro Val Gly Val Thr Asp Pro Leu Leu Ser Ser
                485                 490                 495

Val Ser Pro Gly Ala Ser Lys Thr Ser Ser Arg Arg Ser Ser Met Ser
            500                 505                 510

Met Glu Glu Thr Glu His Thr Cys
        515                 520


<210> SEQ ID NO 36
<211> LENGTH: 504
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<213> ORGANISM: Homo sapiens
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<223> OTHER INFORMATION: Xaa = any amino acid
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<223> OTHER INFORMATION: Xaa = any amino acid
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<223> OTHER INFORMATION: Xaa = any amino acid

<400> SEQUENCE: 36

Met Glu Ala Leu Arg Val Gln Met Phe Met Pro Cys Ser Phe Glu Ser
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Leu Tyr Leu Ser Ser Ala Glu His Pro Gly Ala Ser Lys Pro Pro Ile
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           20                  25                  30

Ser Ser Ser Ser Met Thr Ser Arg Ile Leu Leu Arg Gln Gln Leu Met
        35                  40                  45

Arg Glu Gln Met Gln Glu Gln Glu Arg Arg Glu Gln Gln Gln Lys Leu
    50                  55                  60

Gln Ala Ala Gln Phe Met Gln Gln Arg Val Pro Val Ser Gln Thr Pro
65                  70                  75                  80

Ala Ile Asn Val Ser Val Pro Thr Thr Leu Pro Ser Ala Thr Gln Val
                85                  90                  95

Pro Met Glu Val Leu Lys Val Gln Thr His Leu Glu Asn Pro Thr Lys
            100                 105                 110

Tyr His Ile Gln Gln Ala Gln Arg Gln Gln Val Lys Gln Tyr Leu Ser
        115                 120                 125

Thr Thr Leu Ala Asn Lys His Ala Asn Gln Val Leu Ser Leu Pro Cys
    130                 135                 140

Pro Asn Gln Pro Gly Asp His Val Met Pro Pro Val Pro Gly Ser Ser
145                 150                 155                 160

Ala Pro Asn Ser Pro Met Ala Met Leu Thr Leu Asn Ser Asn Cys Glu
                165                 170                 175

Lys Glu Gly Phe Tyr Lys Phe Glu Glu Gln Asn Arg Ala Glu Ser Glu
            180                 185                 190

Cys Pro Gly Met Asn Thr His Ser Arg Ala Ser Cys Met Gln Met Asp
        195                 200                 205

Asp Val Ile Asp Asp Ile Ile Ser Leu Glu Ser Ser Tyr Asn Glu Glu
    210                 215                 220

Ile Leu Gly Leu Met Asp Pro Ala Leu Gln Met Ala Asn Thr Leu Pro
225                 230                 235                 240

Val Ser Gly Asn Leu Ile Asp Leu Tyr Gly Asn Gln Gly Leu Pro Pro
                245                 250                 255

Pro Gly Leu Thr Ile Ser Asn Ser Cys Pro Ala Asn Leu Pro Asn Ile
            260                 265                 270

Xaa Arg Xaa Leu Thr Glu Ser Glu Ala Arg Ala Leu Ala Lys Glu Arg
        275                 280                 285

Gln Lys Lys Asp Asn His Asn Leu Ile Glu Arg Arg Arg Arg Phe Asn
    290                 295                 300

Ile Asn Asp Arg Ile Lys Glu Leu Gly Thr Leu Ile Pro Lys Ser Asn
305                 310                 315                 320

Asp Pro Asp Met Arg Trp Asn Lys Gly Thr Ile Leu Lys Ala Ser Val
                325                 330                 335

Asp Tyr Ile Arg Lys Leu Gln Arg Glu Gln Gln Arg Ala Lys Glu Leu
            340                 345                 350

Glu Asn Arg Gln Lys Lys Leu Glu His Ala Asn Arg His Leu Leu Leu
        355                 360                 365

Arg Ile Gln Glu Leu Glu Met Gln Ala Arg Ala His Gly Leu Ser Leu
    370                 375                 380

Ile Pro Ser Thr Gly Leu Cys Ser Pro Asp Leu Val Asn Arg Ile Ile
385                 390                 395                 400

Xaa Gln Xaa Pro Val Leu Glu Asn Cys Ser Gln Asp Leu Leu Gln His
                405                 410                 415

His Ala Asp Leu Thr Cys Thr Thr Thr Leu Asp Leu Thr Asp Gly Thr
            420                 425                 430
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Ile Thr Phe Asn Asn Asn Leu Gly Thr Gly Thr Glu Ala Asn Gln Ala
        435                 440                 445

Tyr Ser Val Pro Thr Lys Met Gly Ser Lys Leu Glu Asp Ile Leu Met
    450                 455                 460

Asp Asp Thr Leu Ser Pro Val Gly Val Thr Asp Pro Leu Leu Ser Ser
465                 470                 475                 480

Val Ser Pro Gly Ala Ser Lys Thr Ser Ser Arg Arg Ser Ser Met Ser
                485                 490                 495

Met Glu Glu Thr Glu His Thr Cys
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Met Gly His Leu Glu Asn Thr Ser Val Val Phe Pro Arg Ala Ile Phe
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Ser Leu Cys Glu Lys Glu Thr Arg Lys Leu Thr Leu Cys Leu Phe Ser
            20                  25                  30

Arg Ser Ser Ser Ala Glu His Pro Gly Ala Ser Lys Pro Pro Ile Ser
        35                  40                  45

Ser Ser Ser Met Thr Ser Arg Ile Leu Leu Arg Gln Gln Leu Met Arg
    50                  55                  60

Glu Gln Met Gln Glu Gln Glu Arg Arg Glu Gln Gln Gln Lys Leu Gln
65                  70                  75                  80

Ala Ala Gln Phe Met Gln Gln Arg Val Pro Val Ser Gln Thr Pro Ala
                85                  90                  95

Ile Asn Val Ser Val Pro Thr Thr Leu Pro Ser Ala Thr Gln Val Pro
            100                 105                 110

Met Glu Val Leu Lys Val Gln Thr His Leu Glu Asn Pro Thr Lys Tyr
        115                 120                 125

His Ile Gln Gln Ala Gln Arg Gln Gln Val Lys Gln Tyr Leu Ser Thr
    130                 135                 140

Thr Leu Ala Asn Lys His Ala Asn Gln Val Leu Ser Leu Pro Cys Pro
145                 150                 155                 160

Asn Gln Pro Gly Asp His Val Met Pro Pro Val Pro Gly Ser Ser Ala
                165                 170                 175

Pro Asn Ser Pro Met Ala Met Leu Thr Leu Asn Ser Asn Cys Glu Lys
            180                 185                 190
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Glu Gly Phe Tyr Lys Phe Glu Glu Gln Asn Arg Ala Glu Ser Glu Cys
        195                 200                 205

Pro Gly Met Asn Thr His Ser Arg Ala Ser Cys Met Gln Met Asp Asp
    210                 215                 220

Val Ile Asp Asp Ile Ile Ser Leu Glu Ser Ser Tyr Asn Glu Glu Ile
225                 230                 235                 240

Leu Gly Leu Met Asp Pro Ala Leu Gln Met Ala Asn Thr Leu Pro Val
                245                 250                 255

Ser Gly Asn Leu Ile Asp Leu Tyr Gly Asn Gln Gly Leu Pro Pro Pro
            260                 265                 270

Gly Leu Thr Ile Ser Asn Ser Cys Pro Ala Asn Leu Pro Asn Ile Xaa
        275                 280                 285

Arg Xaa Leu Thr Glu Ser Glu Ala Arg Ala Leu Ala Lys Glu Arg Gln
    290                 295                 300

Lys Lys Asp Asn His Asn Leu Ile Glu Arg Arg Arg Arg Phe Asn Ile
305                 310                 315                 320

Asn Asp Arg Ile Lys Glu Leu Gly Thr Leu Ile Pro Lys Ser Asn Asp
                325                 330                 335

Pro Asp Met Arg Trp Asn Lys Gly Thr Ile Leu Lys Ala Ser Val Asp
            340                 345                 350

Tyr Ile Arg Lys Leu Gln Arg Glu Gln Gln Arg Ala Lys Glu Leu Glu
        355                 360                 365

Asn Arg Gln Lys Lys Leu Glu His Ala Asn Arg His Leu Leu Leu Arg
    370                 375                 380

Ile Gln Glu Leu Glu Met Gln Ala Arg Ala His Gly Leu Ser Leu Ile
385                 390                 395                 400

Pro Ser Thr Gly Leu Cys Ser Pro Asp Leu Val Asn Arg Ile Ile Xaa
                405                 410                 415

Gln Xaa Pro Val Leu Glu Asn Cys Ser Gln Asp Leu Leu Gln His His
            420                 425                 430

Ala Asp Leu Thr Cys Thr Thr Thr Leu Asp Leu Thr Asp Gly Thr Ile
        435                 440                 445

Thr Phe Asn Asn Asn Leu Gly Thr Gly Thr Glu Ala Asn Gln Ala Tyr
    450                 455                 460

Ser Val Pro Thr Lys Met Gly Ser Lys Leu Glu Asp Ile Leu Met Asp
465                 470                 475                 480

Asp Thr Leu Ser Pro Val Gly Val Thr Asp Pro Leu Leu Ser Ser Val
                485                 490                 495

Ser Pro Gly Ala Ser Lys Thr Ser Ser Arg Arg Ser Ser Met Ser Met
            500                 505                 510

Glu Glu Thr Glu His Thr Cys
        515


<210> SEQ ID NO 38
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<221> NAME/KEY: SITE
<222> LOCATION: (182)..(182)
<223> OTHER INFORMATION: Xaa = any amio acid
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<223> OTHER INFORMATION: Xaa = any amio acid
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<223> OTHER INFORMATION: Xaa = any amio acid
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<223> OTHER INFORMATION: Xaa = any amio acid

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Met Leu Glu Met Leu Glu Tyr Asn His Tyr Gln Val Gln Thr His Leu
1 5 10 15

Glu Asn Pro Thr Lys Tyr His Ile Gln Gln Ala Gln Arg Gln Gln Val
            20 25 30

Lys Gln Tyr Leu Ser Thr Thr Leu Ala Asn Lys His Ala Asn Gln Val
        35 40 45

Leu Ser Leu Pro Cys Pro Asn Gln Pro Gly Asp His Val Met Pro Pro
    50 55 60

Val Pro Gly Ser Ser Ala Pro Asn Ser Pro Met Ala Met Leu Thr Leu
65 70 75 80

Asn Ser Asn Cys Glu Lys Glu Gly Phe Tyr Lys Phe Glu Glu Gln Asn
                85 90 95

Arg Ala Glu Ser Glu Cys Pro Gly Met Asn Thr His Ser Arg Ala Ser
            100 105 110

Cys Met Gln Met Asp Asp Val Ile Asp Asp Ile Ile Ser Leu Glu Ser
        115 120 125

Ser Tyr Asn Glu Glu Ile Leu Gly Leu Met Asp Pro Ala Leu Gln Met
    130 135 140

Ala Asn Thr Leu Pro Val Ser Gly Asn Leu Ile Asp Leu Tyr Gly Asn
145 150 155 160

Gln Gly Leu Pro Pro Pro Gly Leu Thr Ile Ser Asn Ser Cys Pro Ala
                165 170 175

Asn Leu Pro Asn Ile Xaa Arg Xaa Leu Thr Ala Cys Ile Phe Pro Thr
            180 185 190

Glu Ser Glu Ala Arg Ala Leu Ala Lys Glu Arg Gln Lys Lys Asp Asn
        195 200 205

His Asn Leu Ile Glu Arg Arg Arg Arg Phe Asn Ile Asn Asp Arg Ile
    210 215 220

Lys Glu Leu Gly Thr Leu Ile Pro Lys Ser Asn Asp Pro Asp Met Arg
225 230 235 240

Trp Asn Lys Gly Thr Ile Leu Lys Ala Ser Val Asp Tyr Ile Arg Lys
                245 250 255

Leu Gln Arg Glu Gln Gln Arg Ala Lys Glu Leu Glu Asn Arg Gln Lys
            260 265 270

Lys Leu Glu His Ala Asn Arg His Leu Leu Leu Arg Ile Gln Glu Leu
        275 280 285

Glu Met Gln Ala Arg Ala His Gly Leu Ser Leu Ile Pro Ser Thr Gly
    290 295 300

Leu Cys Ser Pro Asp Leu Val Asn Arg Ile Ile Xaa Gln Xaa Pro Val
305 310 315 320

Leu Glu Asn Cys Ser Gln Asp Leu Leu Gln His His Ala Asp Leu Thr
                325 330 335

Cys Thr Thr Thr Leu Asp Leu Thr Asp Gly Thr Ile Thr Phe Asn Asn
            340 345 350

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Asn Leu Gly Thr Gly Thr Glu Ala Asn Gln Ala Tyr Ser Val Pro Thr
        355                 360                 365

Lys Met Gly Ser Lys Leu Glu Asp Ile Leu Met Asp Asp Thr Leu Ser
    370                 375                 380

Pro Val Gly Val Thr Asp Pro Leu Leu Ser Ser Val Ser Pro Gly Ala
385                 390                 395                 400

Ser Lys Thr Ser Ser Arg Arg Ser Ser Met Ser Met Glu Glu Thr Glu
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His Thr Cys


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<213> ORGANISM: Homo sapiens
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<222> LOCATION: (182)..(182)
<223> OTHER INFORMATION: Xaa = any amino acid
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Met Leu Glu Met Leu Glu Tyr Asn His Tyr Gln Val Gln Thr His Leu
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Glu Asn Pro Thr Lys Tyr His Ile Gln Gln Ala Gln Arg Gln Gln Val
            20                  25                  30

Lys Gln Tyr Leu Ser Thr Thr Leu Ala Asn Lys His Ala Asn Gln Val
        35                  40                  45

Leu Ser Leu Pro Cys Pro Asn Gln Pro Gly Asp His Val Met Pro Pro
    50                  55                  60

Val Pro Gly Ser Ser Ala Pro Asn Ser Pro Met Ala Met Leu Thr Leu
65                  70                  75                  80

Asn Ser Asn Cys Glu Lys Glu Gly Phe Tyr Lys Phe Glu Glu Gln Asn
                85                  90                  95

Arg Ala Glu Ser Glu Cys Pro Gly Met Asn Thr His Ser Arg Ala Ser
            100                 105                 110

Cys Met Gln Met Asp Asp Val Ile Asp Asp Ile Ile Ser Leu Glu Ser
        115                 120                 125

Ser Tyr Asn Glu Glu Ile Leu Gly Leu Met Asp Pro Ala Leu Gln Met
    130                 135                 140

Ala Asn Thr Leu Pro Val Ser Gly Asn Leu Ile Asp Leu Tyr Gly Asn
145                 150                 155                 160

Gln Gly Leu Pro Pro Pro Gly Leu Thr Ile Ser Asn Ser Cys Pro Ala
                165                 170                 175

Asn Leu Pro Asn Ile Xaa Arg Xaa Leu Thr Glu Ser Glu Ala Arg Ala
            180                 185                 190

Leu Ala Lys Glu Arg Gln Lys Lys Asp Asn His Asn Leu Ile Glu Arg
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        195                 200                 205

Arg Arg Arg Phe Asn Ile Asn Asp Arg Ile Lys Glu Leu Gly Thr Leu
    210                 215                 220

Ile Pro Lys Ser Asn Asp Pro Asp Met Arg Trp Asn Lys Gly Thr Ile
225                 230                 235                 240

Leu Lys Ala Ser Val Asp Tyr Ile Arg Lys Leu Gln Arg Glu Gln Gln
                245                 250                 255

Arg Ala Lys Glu Leu Glu Asn Arg Gln Lys Lys Leu Glu His Ala Asn
            260                 265                 270

Arg His Leu Leu Leu Arg Ile Gln Glu Leu Glu Met Gln Ala Arg Ala
        275                 280                 285

His Gly Leu Ser Leu Ile Pro Ser Thr Gly Leu Cys Ser Pro Asp Leu
    290                 295                 300

Val Asn Arg Ile Ile Xaa Gln Xaa Pro Val Leu Glu Asn Cys Ser Gln
305                 310                 315                 320

Asp Leu Leu Gln His His Ala Asp Leu Thr Cys Thr Thr Thr Leu Asp
                325                 330                 335

Leu Thr Asp Gly Thr Ile Thr Phe Asn Asn Asn Leu Gly Thr Gly Thr
            340                 345                 350

Glu Ala Asn Gln Ala Tyr Ser Val Pro Thr Lys Met Gly Ser Lys Leu
        355                 360                 365

Glu Asp Ile Leu Met Asp Asp Thr Leu Ser Pro Val Gly Val Thr Asp
    370                 375                 380

Pro Leu Leu Ser Ser Val Ser Pro Gly Ala Ser Lys Thr Ser Ser Arg
385                 390                 395                 400

Arg Ser Ser Met Ser Met Glu Glu Thr Glu His Thr Cys
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<223> OTHER INFORMATION: Xaa = any amino acid
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Met Leu Glu Met Leu Glu Tyr Asn His Tyr Gln Val Gln Thr His Leu
1               5                   10                  15

Glu Asn Pro Thr Lys Tyr His Ile Gln Gln Ala Gln Arg Gln Gln Gly
            20                  25                  30

Phe Tyr Lys Phe Glu Glu Gln Asn Arg Ala Glu Ser Glu Cys Pro Gly
        35                  40                  45

Met Asn Thr His Ser Arg Ala Ser Cys Met Gln Met Asp Asp Val Ile
    50                  55                  60
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Asp Asp Ile Ile Ser Leu Glu Ser Ser Tyr Asn Glu Glu Ile Leu Gly
65                  70                  75                  80

Leu Met Asp Pro Ala Leu Gln Met Ala Asn Thr Leu Pro Val Ser Gly
                85                  90                  95

Asn Leu Ile Asp Leu Tyr Gly Asn Gln Gly Leu Pro Pro Pro Gly Leu
            100                 105                 110

Thr Ile Ser Asn Ser Cys Pro Ala Asn Leu Pro Asn Ile Xaa Arg Xaa
        115                 120                 125

Leu Thr Glu Ser Glu Ala Arg Ala Leu Ala Lys Glu Arg Gln Lys Lys
    130                 135                 140

Asp Asn His Asn Leu Ile Glu Arg Arg Arg Arg Phe Asn Ile Asn Asp
145                 150                 155                 160

Arg Ile Lys Glu Leu Gly Thr Leu Ile Pro Lys Ser Asn Asp Pro Asp
                165                 170                 175

Met Arg Trp Asn Lys Gly Thr Ile Leu Lys Ala Ser Val Asp Tyr Ile
            180                 185                 190

Arg Lys Leu Gln Arg Glu Gln Gln Arg Ala Lys Glu Leu Glu Asn Arg
        195                 200                 205

Gln Lys Lys Leu Glu His Ala Asn Arg His Leu Leu Leu Arg Ile Gln
    210                 215                 220

Glu Leu Glu Met Gln Ala Arg Ala His Gly Leu Ser Leu Ile Pro Ser
225                 230                 235                 240

Thr Gly Leu Cys Ser Pro Asp Leu Val Asn Arg Ile Ile Xaa Gln Xaa
                245                 250                 255

Pro Val Leu Glu Asn Cys Ser Gln Asp Leu Leu Gln His His Ala Asp
            260                 265                 270

Leu Thr Cys Thr Thr Thr Leu Asp Leu Thr Asp Gly Thr Ile Thr Phe
        275                 280                 285

Asn Asn Asn Leu Gly Thr Gly Thr Glu Ala Asn Gln Ala Tyr Ser Val
    290                 295                 300

Pro Thr Lys Met Gly Ser Lys Leu Glu Asp Ile Leu Met Asp Asp Thr
305                 310                 315                 320

Leu Ser Pro Val Gly Val Thr Asp Pro Leu Leu Ser Ser Val Ser Pro
                325                 330                 335

Gly Ala Ser Lys Thr Ser Ser Arg Arg Ser Ser Met Ser Met Glu Glu
            340                 345                 350

Thr Glu His Thr Cys
        355
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1-13. (canceled)

14. A method for determining whether a subject has a predisposition or a susceptibility to develop a cancer selected from the group consisting of a cutaneous malignant melanoma, a neuroendocrine cancer, a sarcoma, a neuroblastoma, a nervous system tumor (NST), a lymphoma, a lung cancer, a renal cancer, a breast cancer, a pancreatic cancer, a pediatric tumor, a hematopoietic malignancy, a gastrointestinal cancer, a polycythemia, and combinations thereof, comprising determining in a biological sample from the subject the presence of a mutation in microphthalmia-associated transcription factor (MITF), said mutation reducing or abolishing the sumoylation of MITF and the presence of said mutation indicating that the subject has a predisposition or a susceptibility to develop such cancer.

15. The method according to claim **14**, wherein the MITF mutation is a substitution of a lysine residue and/or a glutamic acid residue of one of the MITF sumoylation sites or of both sites.

16. The method according to claim **15**, wherein the mutation is the substitution of a residue selected from the table below by any of the other 19 amino acids.

Isoform	SEQ ID No	Transcript ref.	Protein ref.	1st sumoylation site		2nd sumoylation site	
ISOFORM 1	35	NM_198159	NP_937802	K289	E291	K417	E419
ISOFORM 2	36	NM_198177	NP_937820	K273	E275	K401	E403
ISOFORM 3	37	NM_006722	NP_006713	K288	E290	K416	E418
ISOFORM 4	38	NM_000248	NP_000239	K182	E184	K316	E318
ISOFORM 5	39	NM_198158	NP_937801	K182	E184	K310	E312
SOFORM 6	40	NM_198178	NP_937821	K126	E128	K254	E256

17. The method according to claim **16**, wherein the mutation is a substitution of K316 or E318 of isoform 4 or of the corresponding residue in the other MITF isoforms.

18. The method according to claim **17**, wherein the mutation is a substitution of E318 of isoform 4 or of the corresponding residue in the other MITF isoforms.

19. The method according to claim **18**, wherein E318 is substituted with a lysine.

20. The method according to claim **14**, wherein the mutation is detected at the protein or nucleic level.

21. The method according to claim **14**, wherein the mutation is detected indirectly by measuring sumoylation of MITF, a reduction of sumoylation relative to the wild-type MITF protein indicating the presence of the E318K mutation.

22. The method according to claim **21**, wherein the mutation is detected by determining the cellular localization of MITF by immunohistochemistry, a nuclear localization being indicative of the wild-type MITF protein while a nuclear and cytoplasmic localization being indicative of the MITF E318K mutant protein.

23. The method according to claim **14**, wherein the cancer is selected from among the combination of a cutaneous malignant melanoma and a renal cell carcinoma, polycythemia, and lymphomas, preferably a cutaneous malignant melanoma and a kidney cancer.

24. The method according to claim **14**, said method further comprising the administration of an effective therapeutic dose of polyphenolic compounds to a subject having reduced or abolished sumoylation of MITF.

25. The method according to claim **24**, wherein the polyphenolic compounds are polyphenolic compounds from fermented rice hulls or polyphenols from black tea.

26. A method for selecting patients who may benefit from a preventive treatment of cancer or a medical surveillance for early detection of cancer comprising determining the patient's susceptibility to cancer by the method according to claim **14** and selecting subjects carrying the mutation reducing or abolishing the sumoylation of MITF.

27. The method according to claim **26**, wherein it further comprises administering an effective therapeutic dose of polyphenolic compounds to selected subjects, thereby preventing or delaying the development of a cancer.

28. The method according to claim **27**, wherein the polyphenolic compounds are polyphenolic compounds from fermented rice hulls or polyphenols from black tea.

29. A method for delaying cancer in a subject carrying a mutation that reduces or abolishes the sumoylation of MITF, comprising administering an effective therapeutic dose of polyphenolic compounds to a subject having reduced or abolished sumoylation of MITF, said effective therapeutic dose delaying the development of a cancer.

30. The method according to claim **29**, wherein the polyphenolic compounds are polyphenolic compounds from fermented rice hulls or polyphenols from black tea.

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