ORIGINAL ARTICLE

CYTOGENOMICS OF MURINE MELANOMA CELL LINES C57/B1 AND B16-F0

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Abstract: In melanoma, one of the most aggressive human tumors, early diagnosis is still the best strategy to increase survival rates. C57/B1 and B16-F0 are murine cell lines frequently applied in basic and applied melanoma research. Thus, it is striking, that cytogenomic features of these two cell lines are not known yet. In the present study, molecular cytogenetics and array-comparative genomic hybridization were done in C57/B1 and B16-F0 cells and the resulting imbalances and breakpoints were translated into the human genome. Both cell lines derived from each other and had an isochromosome 12 and a balanced translocation of chromosomes 3 and 13 in common. Interestingly, both cell lines presented aberrations which were also observed in human skin but not in human eye or uveal melanoma.

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INTRODUCTION

One of the most aggressive human cancer types with adverse prognosis are skin cancers, with melanomas of skin and eye being most frequently observed subtypes.[1] Most deleterious forms of melanoma accompany the metastatic stage, while at early stages the small tumors can be treated by simple surgical removal.[2-4] Most important underlying reason for skin cancers is excessive exposure to ultraviolet (specifically UVB) light.[5] This can cause inflammation, DNA mutations and damage, and go together with chromothripsis-related events and/or cell death. It is a truism that during initiation, promotion and progression of skin cancer, changes in DNA and chromosomes are involved.[6] Thus, genetic studies are necessary to better understand melanoma biology, which also need to be combined with tests of novel melanoma therapeutics.[5]

Genes recently brought to the focus of melanoma research were those involved in pigmentation, DNA repair, immune response, metabolism and/or are vitamin D receptor polymorphisms.[1, 7] Also specifically associated with hereditary melanomas are the so-called high- (*CDKN2A*, *CDK4*, *TERT*, *POT1*), moderate- (*MC1R*, *MITF*) and low-penetrance genes (*KIT*, *SOX10*, *MDM2*), known to be able to act as tumor suppressor genes or oncogenes. Still, molecular genetic diagnostics was not routinely considered in skin cancer treatment, as no significant influence on overall survival rates could be achieved by inclusion of genetic data in the past.[3, 7, 8] Nowadays, BRAF and MEK inhibitors, which are based on genetic data regarding mutations in the *BRAF* gene, are well-established, standard and successful treatments for metastatic melanoma.[9]

In melanoma, as in other cancer research, there is a need for in vivo and in vitro model systems. This can include model animals, and, among them, murine model systems are still the most preferred ones.[10] A special form of murine model systems are cell lines. Most interestingly, murine tumor cell lines like B16-F0 and C57/B1 have been used in dozens of studies as

melanoma models.[11] C57/B1 and B16-F0 derive from a naturally occurring melanoma in the syngeneic C57BL/6 (H-2b) mouse strain – the gender of the mouse is not reported.[12] These C57/B1 and B16-F0 cells were taken in cell culture in 1954.[13] Still, these two cell lines have been applied without any detailed genetic characterization to date. Here, we performed the first cytogenomic characterization of C57/B1 and B16-F0 based on molecular cytogenetic and array-comparative genomic hybridization (aCGH) approaches.

MATERIAL AND METHODS

Cell lines

The cell lines C57/B1 and B16-F0 were obtained from the European Collection of Authenticated Cell Cultures (ECACC 92101204 and ECACC 85011438; Salisbury, UK). They are indicated there as 'not further characterized salivary tumor lines' to be grown adherently (medium: DMEM/10% fetal calf serum + antibiotics). Cells were prepared cytogenetically[14] and whole genomic DNA was extracted as described elsewhere.[15] Cell line-derived chromosomes were subjected to molecular cytogenetics, and extracted DNA to aCGH analyses.

Molecular cytogenetics

Fluorescence in situ hybridization (FISH) was performed using whole chromosome paints ("SkyPaintTM DNA Kit M-10 for Mouse Chromosomes", Applied Spectral Imaging, Edingen-Neckarhausen, Germany) in multicolor-FISH (mFISH), and murine chromosome-specific multicolor banding (mcb) probe mixes for FISH-banding[16], as previously described.[17] For each probe set, at least 30 metaphases were documented and analyzed using Zeiss Axioplan microscopy, equipped with standard black and white CCD-camera and ISIS software (MetaSystems, Altlussheim, Germany). aCGH was done according to standard procedures using "SurePrint G3 Mouse CGH Microarray, 4x180K" (Agilent Technologies, Waldbronn, Germany).[15]

Data analysis

Karyotypes, breakpoints and imbalances observed in murine cell lines C57/B1 and B16-F0 were determined according to aCGH and mcb data and aligned to human homologous regions using Ensembl Browser.[17] The obtained data was compared to genetic changes known from human malignant melanoma according to Höglund et al. (2004)[1] and Di Lorenzo et al. (2016).[7]

RESULTS

Molecular cytogenetic results

C57/B1

C57/B1 is a rather stable diploid cell line. Despite that, this cell line showed five clones. Clone 1 (20%), which could be the ancestor clone, showed the following karyotype: 40,X,t(3;13)(F3;A3),dic(5;15)(A1;A1),+6, +6,idic(12)(A1;A1). Clone 2, with 25%, had the karyotype 39,X,idem,idic(16)(A1;A1). The karyotype of clone 3 (30%) was 40,X,idem,idic(18)(A1;A1) and is shown in Figure 1A. In clone 4 (20%), a complex derivative chromosome 13 replaced the der(13)t(3;13)(F3;A3) – see Figure 1B; karyotype 40,X,der(3)t(3;13)(F3;A3),dic(5;15)(A1;A1),+6,+6,idic (12)(A1;A1),der(13)t(3;13)(3::13::13::3)(3qter→3F3::1 3A3→13A1::CEN::13A1→13A3::3F3→3qter). Finally there was a clone 5 with 40,X,idem,idic(10)(A1;A1) in 5% of the cells.

B16-F0

B16-F0 is a tetraploid cell line with 71-77 chromosomes per metaphase. According to that, the karyotype of the clone 1 (75%) was 71~77,X,der(X)t(X;15)(D;B3), der(1)t(1;16)(F;A1),+del(1)(BE),der(3)t(3;13)(F3A3),d er(3)t(3;13)(F3A3),inv(4)(A1C1),del(5)(DF),inv(6)(B2 G1),+6,+6,+6,idic(7)(A1;A1),der(8)t(8;9)(A4;F3),+der (8)t(8;12)(C2;C1),+del(11)(A2B1),idic(12)(A1;A1),der (13)t(3;13)(F3;A3),-14,del(15)(A1A3),-15,-16,dic(17; 19)t(17;19)(19qter→19A::CEN::17A3→17B1::17C→ 17D::19B→19qter).
In clone 2, a der(4)(pter→D2::A1→C5:) replaced one normal chromosome 4 - except for that, clone 2 was as clone 1 (Figure 2).

aCGH results

aCGH-analysis of imbalanced rearrangements confirmed most of the FISH-detected aberrations (Figure 3). For more information about aCGH results and for the two cell lines, see Suppl. Table. Interestingly, clone 5 of C57/B1 seemed to be present in higher percentages in non-dividing cells than in dividing ones: in aCGH a trisomy 10, caused by idic(10)(A1;A1) and seen only in 5% of the metaphases, was clearly visible. The imbalance patterns of the murine melanoma cell lines C57/B1 and B16-F0 revealed by aCGH were translated to the human genome as depicted in Figure 4. By comparing the corresponding homologous regions for the cell lines in the human karyotype with the imbalances in malignant melanomas[1], a resemblance was detected with skin melanoma (Table 1). Moreover, the loss of chromosome 3, and gain of 8q[18] typical for uveal melanoma were absent in the two studied cell lines.
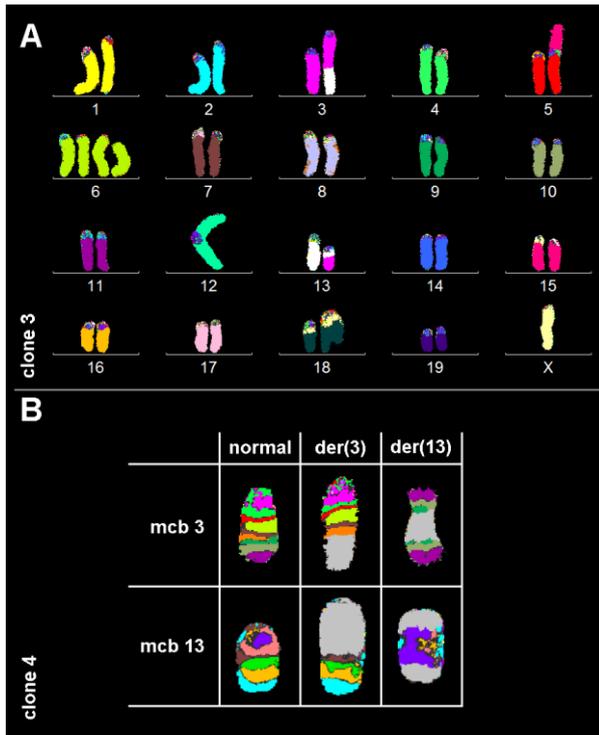
Figure 1. Molecular cytogenetic results in cell line C57/B1
A) mFISH result using all murine whole chromosome painting probes in one experiment is depicted revealing a near-diploid karyotype. The karyotype formula for clone 3 is 40,X,t(3;13)(F3;A3),dic(5;15)(A1;A1),+6,+6,idic(12)(A1;A1),idic(18)(A1;A1).
B) For C57/B1 clone 4 pseudocolor banding for murine chromosomes 3 and 13 is shown. One normal chromosome 3 and 13 together with a der(3)t(3;13)(F3;A3) and a der(13)t(3;13)(3::13::13::3)(3qter→3F3::13A3→13A1::CEN::13A1→13A3::3F3→3qter) are presented after multicolor banding.
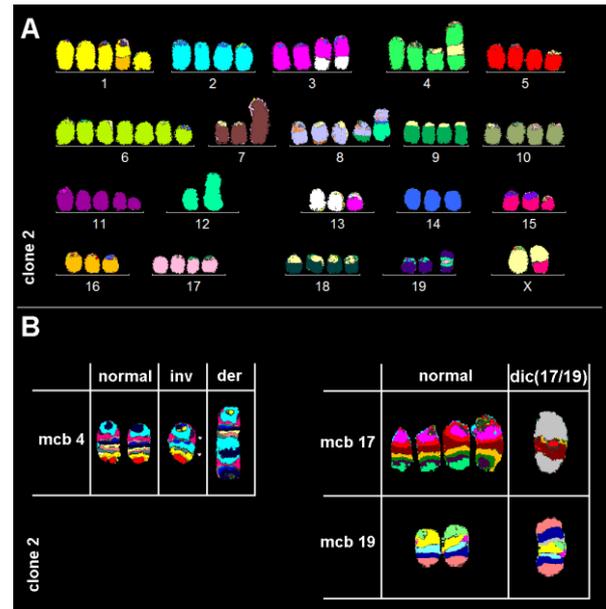
Figure 2. Molecular cytogenetic results in cell line B16-F0
A) mFISH results using all murine whole chromosome painting probes in one experiment revealed a near-tertaploid karyotype. Here shown is clone 2.
B) Results for murine multicolor banding (mcb) using a chromosome 4-, 17- and 19-specific probe are shown. mcb4 revealed a inv(4)(A1C1) and a der(4)(pter→D2::A1→C5:). The other 2 mcb probes identified four normal chromosomes 17, two normal chromosomes 19 and a dic(17;19)t(17;19)(19qter→19A::CEN::17A3→17B1::17C→17D::19B→19qter).
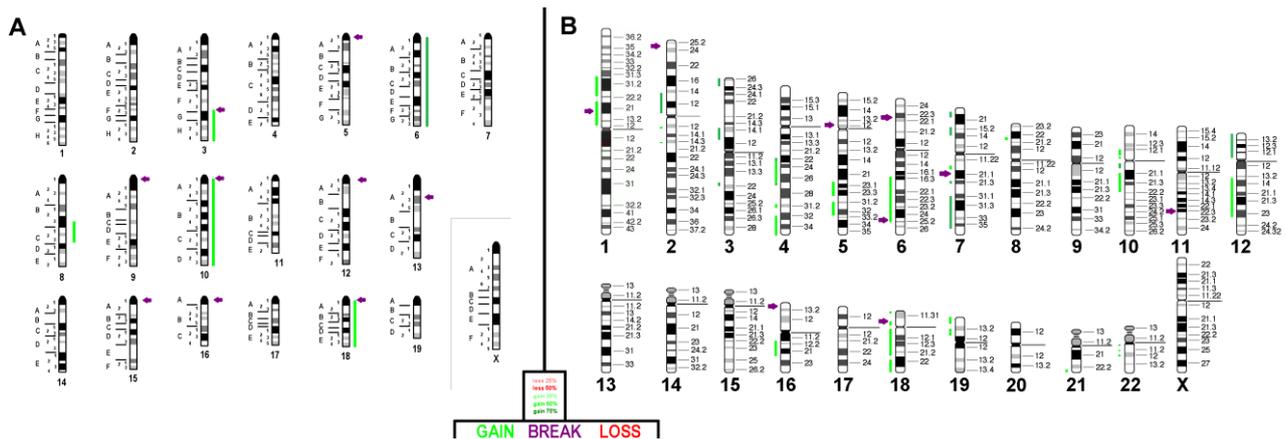
Figure 3. A) aCGH results for murine melanoma cell line C57/B1. The copy number alterations with respect to the diploid karyotype are given as color code depicted in the figure with shades of red (losses) and green (gains), purple arrows indicate breakpoints. Breakpoints are indicated according to mcb results; **B) Projection of the aCGH results for the cell line onto the human genome showing imbalances as gains and losses of specific chromosomal regions with respect to the original near-tetraploid chromosome set.**
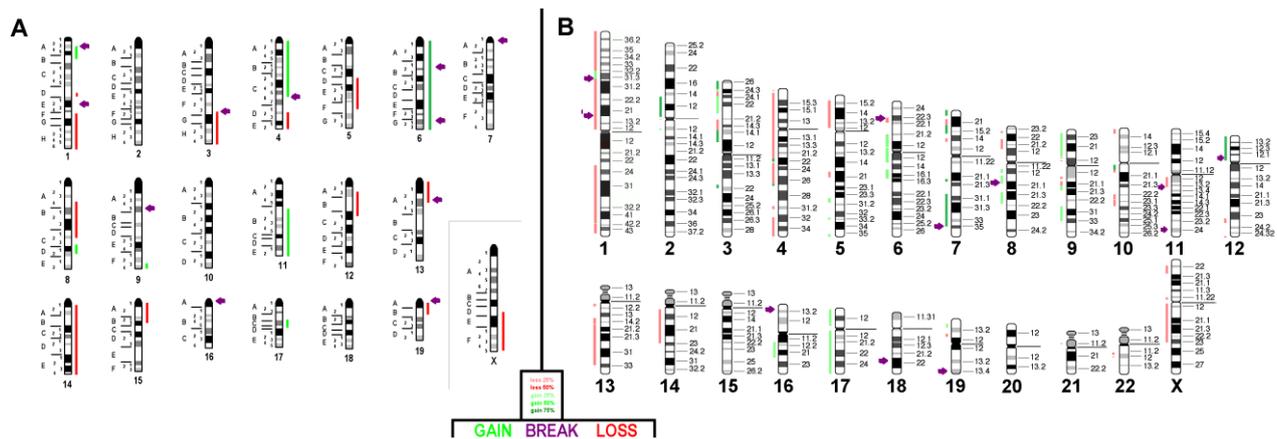
Figure 4. aCGH results for murine melanoma cell line B16-F0 (A) and its projection onto the human genome (B).
For more details see legend of Figure 3.

In Table 2, locations of known low-, moderate- and high-penetrance genes associated with hereditary malignant melanomas were aligned with the imbalances found in C57/B1 and B16-F0. Four imbalances, each, for the nine selected genes were found per cell line (Table 2).

Table 1. Most frequent aberrations of malignant melanoma (MM) derived from skin or eye based on Höglund et al. (2004).

	MM skin [%]	MM eye [%]	C57/B1	B16-F0
-1p10-p36	28	29	+	-
+2	11	11	(+)	(+)
+3	**18**	10	(+)	(+)
-4	**33**	16	-	+
+4q27-q35	9	10	+	-
-5	**32**	16	-	(+)
+7	**36**	26	(+)	(+)
-8p10-p23	25	29	-	(+)
+8q10-q24	25	**38**	-	+
+9q22-q34	15	8	-	+
-12q13-q24	**27**	14	-	(+)
+12q15-q24	5	10	+	-
-14	**35**	18	-	(+)
+17q10-q25	12	9	-	+
+18	**26**	13	+	-
+19	14	8	(+)	-
+22	11	13	(+)	-
-X	23	22	-	(+)
OVERALL + and (+)	-	-	9	12

Legend: x = complete overlap, (x) = partial overlap, - = no overlap.

DISCUSSION

Melanomas constitute aggressive cancers and are mainly caused by UV exposure. Still, melanoma pathogenesis as well as progression are poorly understood, and effective treatment based on genetic data (mutations in *BRAF* gene) has only been developed in recent years.[9] Here we studied the two murine melanoma cell lines derived from each other, C57/B1 and B16-F0, for the first time. These studies were urgently needed, as e.g. B16-F0 was used in some studies as a model for cells with 'poorly invasive' features[19] and in others as a model for studying the metastatic spread of melanoma.[20]

Here it could be shown that the studied cell lines known to derive from each other have cytogenetic features in common, like an isochromosome 12 and a balanced translocation of chromosomes 3 and 13. However, C57/B1 is near-diploid and has much less chromosomal aberrations and imbalances than B16-F0. Interestingly, C57/B1 mainly had gains relative to its diploid karyotype, while B16-F0, presenting a near-tetraploid constitution, has both – gains and losses of copy numbers. Overall, both cell lines are, according to Table 1[1], genetically most similar to human skin melanoma and have very little similarity to eye melanoma.

A comparison of genomic regions known to comprise low-, moderate- and high-penetrance genes associated with hereditary malignant melanomas (Table 2) revealed gains of copy numbers for *MITF* and *POT1* in C57/B1 and B16-F0; both genes are moderate- to high-penetrance genes. However, *MDM2* (low-penetrance gene) and *CDK4* (high-penetrance gene) were amplified only in C57/B1, while *KIT* (low-penetrance gene) and *CDKN2A* (high-penetrance gene) showed loss or gain of copy numbers in B16-F0 (Table 2). Accordingly, no clear conclusions could be drawn from this data alone. Sequencing or expression profiling of the cell lines may shed more light on possible meaningful correlations for

Table 2. Selected high-, moderate and low-penetrance genes playing a role in MM are listed.

	gene	location in human (GRCH37/hg19)	C57/B	B16-F0
low-penetrance genes	*KIT*	chr4:55524095-55606881	no change	loss
	MDM2	chr12:69201971-69239320	gain	no change
	SOX10	chr22:38368319-38380539	no change	no change
moderate-penetrance genes	*MITF*	chr3:69788586-70017488	gain	gain
	MC1R	chr16:89984287-89987385	no change	no change
high-penetrance genes	*TERT*	chr5:1,253,287-1,295,162	no change	no change
	POT1	chr7:124,462,440-124,570,037	gain	gain
	CDKN2A	chr9:21967751-21994490	no change	gain
	CDK4	chr12:58141510-58146230	gain	no change

Legend: It is listed if the corresponding region is altered (gain, loss or no change) in the two cell lines.

these genes. Interestingly, in 2008 Gobeil et al.[21] used a genome wide RNAi screen for B16-F0, without knowing its genetic constitution, to identify the tumor suppressor gene *GAS1*. This was most likely successful, as *GAS1*, located in humans on chromosome 9q21.33, is not affected by copy number variations in B16-F0, acc. to our data (Suppl. Table 1). Another study using B16-F0 worth mentioning is that of Yun et al. (2019)[22]: they selected this cell line for studies of the *MITF* gene - obviously without knowing that this gene is amplified in B16-F0. Thus, a new interpretation of their results may be necessary.

In conclusion, here we performed the first detailed cytogenomic study in the murine malignant melanoma cell lines C57/B1 and B16-F0 and can conclude that they can be used as models for human metastatic melanoma derived from skin.

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REFERENCES

1. Höglund M, Gisselsson D, Hansen GB, White VA, Säll T, Mitelman F, Horsman D. Dissecting karyotypic patterns in malignant melanomas: temporal clustering of losses and gains in melanoma karyotypic evolution. Int J Cancer. 2004;108(1):57-65.
2. Aya-Bonilla C, Gray ES, Manikandan J, Freeman JB, Zaenker P, Reid AL, Khattak MA, Frank MH, Millward M, Ziman M. Immunomagnetic-enriched subpopulations of melanoma circulating tumour cells (CTCs) exhibit distinct transcriptome profiles. Cancers (Basel). 2019;11(2):157.
3. Roesch A. Tumor heterogeneity and plasticity as elusive drivers for resistance to MAPK pathway inhibition in melanoma. Oncogene. 2015;34(23):2951-2957.
4. Nida N, Irtaza A, Javed A, Yousaf MH, Mahmood MT. Melanoma lesion detection and segmentation using deep region based convolutional neural network and fuzzy C-means clustering. Int J Med Inform. 2019;124:37-48.
5. Karimkhani C, Green AC, Nijsten T, Weinstock MA, Dellavalle RP, Naghavi M, Fitzmaurice C. The global burden of melanoma: results from the Global Burden of Disease Study 2015. Br J Dermatol. 2017;177(1):134-140.
6. Yang Y, Wu R, Sargsyan D, Yin R, Kuo HC, Yang I, Wang L, Cheng D, Wang C, Li S, Hudlikar R, Lu Y, Kong AN. UVB drives different stages of epigenome alterations during progression of skin cancer. Cancer Lett. 2019;449:20-30.
7. Di Lorenzo S, Fanale D, Corradino B, Caló V, Rinaldi G, Bazan V, Giordano A, Cordova A, Russo A. Absence of germline CDKN2A mutation in Sicilian patients with familial malignant melanoma: Could it be a population-specific genetic signature? Cancer Biol Ther. 2016;17(1):83-90.
8. Rossi M, Pellegrini C, Cardelli L, Ciciarelli V, Di Nardo L, Fargnoli MC. Familial melanoma: Diagnostic and management implications. Dermatol Pract Concept. 2019;9(1):10-16.
9. Lin PS, Semrad TJ. Molecular testing for the treatment of advanced colorectal cancer: An overview. Methods Mol Biol. 2018;1765:281-297.
10. Horak V, Palanova A, Cizkova J, Miltrova V, Vodicka P, Kupcova Skalnikova H. Melanoma-Bearing Libechov Minipig (MeLiM): The unique swine model of hereditary metastatic melanoma. Genes (Basel). 2019;10(11):915.
11. Pubmed. https://pubmed.ncbi.nlm.nih.gov; accessed on 02. September 2020
12. Danciu C, Oprean C, Coricovac DE, Andreea C, Cimpean A, Radeke H, Soica C, Dehelean C. Behaviour of four different B16 murine melanoma cell sublines: C57BL/6J skin. Int J Exp Pathol. 2015;96(2):73-80.
13. Teicher B. Tumor models in cancer research. 2002, Humana Press, New Jersey, USA
14. Rhode H, Liehr T, Kosyakova N, Rincic M, et al. Molecular cytogenetic characterization of two murine colorectal cancer cell lines. OBM Genetics 2018;2(1):037.
15. Kubicova E, Trifonov V, Borovecki F, Liehr T, Rincic M, Kosyakova N, Hussein SS. First molecular cytogenetic characterization of murine malignant mesothelioma cell line AE17 and in silico translation to the human genome. Curr Bioinform 2017;12(1):11-18.
16. Liehr T, Starke H, Heller A, Kosyakova N, Mrasek K, Gross M, Karst C, Steinhaeuser U, Hunstig F, Fickelscher

I, Kuechler A, Trifonov V, Romanenko SA, Weise A. Multicolor fluorescence in situ hybridization (FISH) applied to FISH-banding. Cytogenet Genome Res. 2006;114(3-4):240-244.

17. Leibiger C, Kosyakova N, Mkrtchyan H, Glei M, Trifonov V, Liehr T. First molecular cytogenetic high resolution characterization of the NIH 3T3 cell line by murine multicolor banding. J Histochem Cytochem. 2013;61(4):306-312.

18. Doherty RE, Alfawaz M, Francis J, Lijka-Jones B, Sisley K. Noncutaneous Melanoma, Chapter 2: Genetics of Uveal Melanoma. Brisbane (AU): Codon Publications, 2018.

19. Silva P, Mendoza P, Rivas S, Díaz J, Moraga C, Quest AF, Torres VA. Hypoxia promotes Rab5 activation, leading to tumor cell migration, invasion and metastasis. Oncotarget. 2016;7(20):29548-29562.

20. Denoyer D, Greguric I, Roselt P, Neels OC, Aide N, Taylor SR, Katsifis A, Dorow DS, Hicks RJ. High-contrast PET of melanoma using (18)F-MEL050, a selective probe for melanin with predominantly renal clearance. J Nucl Med. 2010;51(3):441-447.

21. Gobeil S, Zhu X, Doillon CJ, Green MR. A genome-wide shRNA screen identifies GAS1 as a novel melanoma metastasis suppressor gene. Genes Dev. 2008;22(21):2932-2940.

22. Yun CY, Hong SD, Lee YH, Lee J, Jung DE, Kim GH, Kim SH, Jung JK, Kim KH, Lee H, Hong JT, Han SB, Kim Y. Nuclear entry of CRTC1 as druggable target of acquired pigmentary disorder. Theranostics. 2019;9(3):646-660.