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Gene expression signature associated with *BRAF* mutations in human primary cutaneous melanomas

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ABSTRACT

With the aim to correlate *BRAF* mutation status with gene expression in human primary cutaneous melanomas, and thus to get more insight on the consequences of *BRAF* mutation on cell biology, we analyzed all expression data obtained in melanomas from which DNA was extracted from the same tissue slides that were used for the expression study.
A cohort of 69 frozen primary melanoma whose oligonucleotide micro-array expression data were available, were genotyped for *BRAF* and *NRAS* genes. The expression data from these melanomas were re-analyzed according to *BRAF* mutational status.
A set of 250 probes representing 209 genes that were significantly (raw $P \leq 0.001$) associated with BRAF mutation status was identified and 17 of these were previously shown to be implicated in cutaneous melanoma progression or pigmentation pathway-associated genes driven by the microphthalmia transcription factor (MITF). The list of 34 top probes contained no more than 1% of false discoveries with a probability of 0.95. Among the genes that differentiated most strongly between *BRAF* mutated and non-mutated melanomas, there were those involved in melanoma immune response such as *MAGE-D2*, *CD63*, and *HSP70*.
These findings support the immunogenicity of $BRAF^{V600E}$, eliciting patients T-cell responses in various *in vitro* assays. The genes whose expression is associated with *BRAF* mutations are not simply restricted to the MAPK/ERK signaling but also converge to

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enhanced immune responsiveness, cell motility and melanosomes processing involved in the adaptative UV response.

1. Introduction

Cutaneous melanoma (CM) is a complex multifactorial disease in which genetic and environmental factors play an important role. In this respect, the discovery of frequent *BRAF* mutations in CM represents a major breakthrough in the genetics of CM. While we and others have reported that *BRAF* is not a melanoma susceptibility gene (Jackson et al., 2005; Lang et al., 2003; Laud et al., 2003), somatic mutation of *BRAF* appears to be an early event during melanoma development and is maintained during its progression (Omholt et al., 2003; Pollock et al., 2003). The most common *BRAF* mutation corresponds to a T > A transversion at position 1799, resulting in the substitution of Valine by Glutamate at position 600 of the protein (Michaloglou et al., 2007). The V600E mutation renders BRAF constitutively active and increases its kinase activity (Davies et al., 2002). Activated BRAF proteins phosphorylates and activates MEK1 and 2 (MAPKKs), which in turn phosphorylate and activate ERK1 and 2 (MAPKs). These phosphorylate several cytoplasmic and nuclear targets, including transcription factors such as Ets-1, c-Jun and c-Myc (Michaloglou et al., 2007). Ectopic expression of $BRAF^{V600E}$ is sufficient to promote nevus formation in transgenic zebrafish (Patton et al., 2005), but progression to malignancy requires the acquisition of additional genetic events (Michaloglou et al., 2005). Using cell lines and animal models, it has even been demonstrated that $BRAF^{V600E}$ alone can induce cellular senescence (Michaloglou et al., 2005). The effect of *BRAF* mutations on global cell biology and gene expressions is still largely unknown, and several attempts have been made to identify a *BRAF* signature by microarray gene expression profiling. However, as accessibility to frozen human primary cutaneous melanomas is extremely restricted, these studies were performed on cell lines (Bloethner et al., 2006; Johansson et al., 2007; Pavey et al., 2004).

In the framework of the EORTC Melanoma group, we have previously performed a multi-expression study of 83 frozen primary melanomas of the skin. We investigated the relationship between gene expression profiles and clinical outcome, and we applied class comparison and class prediction analyses to identify genes whose expression was associated with clinical outcome (Winnepenninckx et al., 2006). This allowed us to identify 254 genes whose expression was associated with 4-year distant metastasis-free survival (4 yr DMFS).

With the aim to correlate *BRAF* mutation status with gene expression in human primary cutaneous melanomas, and thus to get more insight on the consequences of *BRAF* mutation on cell biology, we analyzed all expression data obtained in 69 melanomas from which DNA was extracted from the same tissue slides that were used for the expression study. We identified 250 probes representing 209 genes whose expression significantly (raw $P \leq 10^{-3}$) differed between melanomas with and without *BRAF* mutation. In addition to genes previously reported as being implicated in melanoma progression, several upregulated genes in *BRAF* mutated melanomas encode proteins that are involved in the host's immune response.

2. Materials and methods

2.1. Genotyping of tumor samples

The initial patient population and samples preparation have been described elsewhere (Winnepenninckx et al., 2006). The same samples were used for both DNA and RNA recovery to prevent sampling bias effect. RNA was extracted using RNeasy Micro Kit protocol (Qiagen, ref 74004) and DNA was extracted using DNeasiy Kit (Qiagen, ref 69582) and proved successful in 69 of the 83 samples included in the expression study. In 14 samples, there was no left over tissue nor DNA available. These 69 samples represent the present study population. The coding exons and intron-exon junctions of the *BRAF* and *NRAS* genes were screened for mutations by direct sequencing of exons 11 and 15 for *BRAF*, and exons 2 and 3 for *NRAS* (Laud et al., 2003).

2.2. Statistical analysis

Statistical analyses were performed with the BRB-ArrayTools software, version 3.3. To identify sequences whose expression was associated with *BRAF* mutation we analyzed the data obtained after hybridization on Agilent whole genome 44K oligonucleotide microarray for the 69 patients with documented mutation status. Patients were separated into two groups, one group with *BRAF* mutation, and one group without. The default gene selection procedure from BRB ArrayTools was used as previously (Winnepenninckx et al., 2006): the sequences that were differentially expressed at a statistical significance level of $P = 0.001$. We applied a multivariate permutation based on 1000 random permutations to control the false discoveries rate (Korn et al., 2007). Distant metastasis-free survival was defined as the time interval between the diagnosis of the primary cutaneous melanoma and a metastasis located beyond the first regional lymph node or death from melanoma. Patients alive without distant metastasis or with a death not related to cancer were censored at the date of last follow-up or at the date of death, respectively. Patients alive at the date of last follow-up were censored at that date. We used Fisher's exact tests, Wilcoxon tests, and chi-squared tests to assess differences between the clinicopathologic variables of the two groups of patients. All statistical tests were two-sided.

2.3. Data availability

The microarray data analyzed in this paper are publicly available in the Array Express data repository at the European Bioinformatics Institute (http://www.ebi.ac.uk/arrayexpress/) under accession number E-TABM-1 (IGR_MELANOMA_STUDY).

3. Results

3.1. BRAF mutation status has no impact on DMFS or overall survival

Thirty-two out of 69 (46%) primary melanomas harbored a *BRAF* mutation. Most of the mutations (30/32) consisted in the classical Val600 to Glu substitution. Two samples carried T599I and D594N mutations respectively. Among the 37 samples that did not display a *BRAF* mutation, 6 harbored a Q61R activating mutation in *NRAS*. *BRAF* and *NRAS* mutations were mutually exclusive. The clinical and pathological variables of patients are shown in Table 1. No significant differences were observed between patients with and without *BRAF* mutations. Particularly, there was no association between BRAF mutation status and distant-metastasis-free survival ($P = 0.59$, logrank test).

3.2. BRAF mutation-associated gene expression signature in primary melanomas

The gene expression data were then used to identify genes discriminating the 32 melanomas with *BRAF* mutations from the 37 wild type (WT) melanomas. At a significance level of raw $P \leq 0.001$, 250 probes corresponding to 209 genes were differentially expressed between both groups (see supplementary Table). Applying a controlling procedure of the false discovery rate, we found that the list of 34 top probes contained no more than 1% of false discoveries with a probability of 0.95. These 34 probes are listed in Table 2.

3.3. CD63 and melanoma-driven immune response factors are overexpressed in BRAF mutated melanomas

Among the genes that differentiated most strongly between *BRAF* mutated and non-mutated melanomas, there were genes involved in melanoma immune response such as *CD63*, *MAGE-D2*, *S100A*, and *HSP70*. In the list of 209 discriminant genes, 17 were previously shown to be implicated in cutaneous melanoma progression, such as osteopontin, *SERPINE2*, cathepsin B, and insulin-like growth factor binding protein 2, or in the pigmentation pathway driven by the microphthalmia transcription factor (*MITF*), such as *S100B* and melanoma inhibitory activity-1 (*MIA-1*).

Table 1 – Comparison of patient characteristics between the wild type and the mutated *BRAF* melanoma groups

Variable	WT BRAF group ($n = 37$)	Mutated BRAF group ($n = 32$)	P value
Median age, years (range)*	64 (1–93)	61 (22–92)	0.50
Median thickness (Breslow), mm (range)*	3.0 (0.5–18.6)	3.76 (1.0–14.6)	0.45
AJCC stage at diagnosis, No.**,[a]			
Stage I	13	7	0.42
Stage II	18	17	
Stage III or IV	6	8	
Sex, No.***			0.46
Male	15	10	
Female	22	20	
Site, No.***			0.99
Axial	16	13	
Extremities	21	19	
Melanoma type, No.***			0.25
SMM[a]	25	16	
Others	7	10	
Ulceration***			0.99
Absent	23	20	
Present	14	12	
Regression***			0.99
Absent	30	26	
Present	6	6	
Mitotic rate, No.***			0.99
<6 mitoses per mm^2	16	13	
≥ 6 mitoses per mm^2	21	19	
Predominant cell-type***			0.76
Spindle	7	5	
Others	30	27	
TILs**			0.26
Absent	16	13	
Non-brisk	9	13	
Brisk	12	6	
Neovascularization or angiotropism, No.***			0.99
Absent	34	29	
Present	3	3	
Elastolysis***			0.71
Absent to moderate	28	27	
Severe	5	3	

a AJCC = American Joint Committee on Cancer; SSM = superficial spreading melanoma; TILs = host response by tumor infiltrating lymphocytes; non-brisk = focal or discontinuous; brisk = continuous. *, Wilcoxon test; **, chi-squared test; ***, Fisher's exact test. All statistical tests were two-sided.

4. Discussion

The present study is the extension of a previous gene expression profiling in primary melanomas carried out to better characterize the effect of *BRAF* mutations on global gene expression and therefore to hint key events associated with disease progression in patients whose melanoma harbors *BRAF* mutation. From 69 samples, characterized for both expression profile and *BRAF* and *NRAS* mutation status, 46% and 8.7% were mutated in *BRAF* and *NRAS* respectively. These figures are in agreement with the reported mutation rate in primary melanomas, and the fact that both mutations were exclusive one to another is also in line with published data (Davies et al., 2002; Smalley and Herlyn, 2004). No difference in survival was observed between patients with and without *BRAF* mutations. In addition, the *BRAF*-associated signature was clearly distinct from the prognosis signature associated with distant metastasis disclosed previously (Winnepenninckx et al., 2006). Although this issue has been a matter of

Table 2 – List of the 34 top probes whose expression differs significantly between wild type and mutated *BRAF* melanomas (FDR < 0.01 with $P = 0.95$)

Rank	Raw_p value	Accession code	Gene symbol	Chromosome band	Description
1	3,09E-08	X61382	SYPL	7q22.3	Synaptophysin-like protein
2	3,53E-08	NM_001780	CD63	12q13.2	CD63 antigen (melanoma 1 antigen)
4	1,06E-07	NM_004956	ETV1	7p21.2	Ets variant gene 1
5	5,37E-07	NM_024297	MGC2941	17p13.1	Hypothetical protein MGC2941
6	7,53E-07	U79286	HRMT1L1	21q22.3	HMT1 hnRNP methyltransferase-like 1 (S. *cerevisiae*)
7	7,83E-07	NM_000181	GUSB	7q11.21	Glucuronidase, beta
8	1,04E-06	NM_001383	DPH2L1	17p13.3	DPH2-like 1 (S. *cerevisiae*)
9	1,09E-06	NM_005979	S100A13	–	S100 calcium binding protein A13
10	1,48E-06	NM_001897	CSPG4 (=MCSP)	15q24.2	Chondroitin sulfate proteoglycan 4 (melanoma-associated)
11	1,51E-06	NM_017828	FLJ20452	15q24.2	COMM domain containing 4
13	1,56E-06	NM_005720	ARPC1B	7q22.1	Actin related protein 2/3 complex, subunit 1B, 41kDa
14	1,57E-06	AJ004914	Tmp21-II	8q24,3	Tmp21-II, transcribed pseudogene
16	1,66E-06	NM_001344	DAD1	14q11.2	Defender against cell death 1
17	1,75E-06	NM_002337	LRPAP1	4p16.3	Low density lipoprotein receptor-related protein associated protein 1
19	3,24E-06	NM_014599	MAGED2	Xp11.21	melanoma antigen, family D, 2
20	3,29E-06	BC038098	BC038098		Homo sapiens, Similar to general transcription factor II, i, clone IMAGE:6021750, mRNA
21	3,84E-06	X97299	Ptg-2	13q14.11	Transforming growth factor beta 1 induced transcript 4
23	4,06E-06	BC037430	BC037430		Homo sapiens cDNA clone IMAGE:5531727, partial cds
24	4,16E-06	BC028093	BC028093	7q33	Myotrophin
25	4,79E-06	D38500	PMS2L4	–	Postmeiotic segregation increased 2-like 4
26	5,57E-06	NM_004546	NDUFB2	7q34	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8 kDa
27	8,40E-06	NM_006533	MIA	19q13.2	Melanoma inhibitory activity
28	9,58E-06	NM_032476	MRPS6	21q22.11	Mitochondrial ribosomal protein S6
29	1,02E-05	NM_006216	SERPINE2	2q36.1	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
30	1,14E-05	NM_015602	LAP1B	1q25.2	Lamina-associated polypeptide 1B
31	1,16E-05	NM_002155	HSPA6	1q23.3	Heat shock 70 kDa protein 6 (HSP70B')
12	1,42E-05	BC000837	FLJ20452	15q24.2	COMM domain containing 4
32	1,43E-05	NM_152495	FLJ38993	1q42.12	Hypothetical protein FLJ38993
33	1,50E-05	NM_014026	HSPC015	11q24.2	mRNA decapping enzyme
34	1,60E-05	Y16893	HUS1	7p12.3	HUS1 checkpoint homolog (S. *pombe*)

All of these probes are overexpressed in the *BRAF* mutated melanomas.

debate, our data therefore strongly support the notion that *BRAF* mutations are not directly correlated with the clinical outcome of CM but most likely contribute to tumor initiation (Omholt et al., 2003; Smalley and Herlyn, 2004; Chang et al., 2004; Dong et al., 2003; Kumar et al., 2003; Shinozaki et al., 2004). The *NRAS* mutated samples do not display the *BRAF* signature as already observed in melanoma cell lines (Bloethner et al., 2006; Pavey et al., 2004), and in primary thyroid papillary carcinoma (Giordano et al., 2005). This suggests that although both mutations activate the MAPK/ERK pathway, their biological outcome might be distinct. It has recently been shown that in *NRAS* mutated melanoma cell lines, the MEK/ERK pathway is activated through a switching signaling from *BRAF* to *RAF1* resulting in distinct intracellular signaling responses as compared to *BRAF* mutated cell lines (Dumaz et al., 2006). These observations suggest the existence of a *BRAF* specific downstream signaling distinct from the MEK/ERK pathway.

According to its gene expression status, the $BRAF^{D594N}$ sample clustered among the V600E mutated samples suggesting an active role for this mutation in the development of melanoma. The D594N mutations occur much more rarely than the V600E ones and are mostly found in colorectal cancers (Kim et al., 2007; Kumar et al., 2004). Their occurrence in primary melanoma raises the question of their mode of action and contribution to tumor progression.

Different *BRAF* signatures were previously reported in studies that used melanoma cell lines (Bloethner et al., 2006; Pavey et al., 2004; Hoek et al., 2006). Gene expression profiling data from cell lines however do not always reflect those obtained from primary tumor samples, possibly because of the particular conditions imposed to culture these cell lines. Interestingly however, 5 genes of the *BRAF* signature described here (*MAGED2, SERPINE2, DUSP6, PRMT2/HRMT1L1*, and *PTPRF*) were also present in the *BRAF* signatures reported in melanoma cell lines (Bloethner et al., 2006; Pavey et al., 2004; Hoek et al., 2006). Among these genes, *MAGE D2* and *SERPINE 2* expressions were the most strongly correlated with BRAF mutation status in our patients series. *MAGE-D2* encodes one of the cancer testis genes. Unlike the testis- and tumor-specific expression of many MAGE genes, *MAGE-D2* mRNA is also expressed in healthy human tissues and in most cell types examined. It does not encode any of the known *MAGE* antigenic peptides and cannot therefore be considered a classic cancer testis antigen (Kidd et al., 2006; Papageorgio et al., 2007). The function of *MAGE*-D2 protein is unknown, but recent data support the view that MAGED2 is a p53-dissector and can sequestrates wild type p53 to negatively regulate its

activity (Papageorgio et al., 2007). It has been demonstrated that the *p53* and *BRAF* pathways can interact genetically to produce melanoma (Patton et al., 2005). Therefore *MAGED2* overexpression in *BRAF* mutated melanomas may represent another way for the melanoma cell to inactivate p53. This may also explain why wild type p53 frequently accumulates in the nuclei in melanoma cells. The serine protease inhibitor nexin 2 (*SERPINE2*) is a member of the serpin superfamily of serine protease inhibitors which can rapidly inhibit thrombin, urokinase, and plasmin. It is an important modulator of tumor cell/host interactions in solid tumors and may contribute to invasion of experimentally induced pancreatic carcinoma (Buchholz et al., 2003). It is therefore likely that $BRAF^{V600E}$ can promote tumor cells invasion through *SERPINE2* overexpression in the presence of other events. The *MIA* (melanoma inhibitory activity) gene expression is significantly increased in tumor samples harboring the *BRAF* mutation. MIA has been postulated to competitively mask integrin binding sites by specifically associating with the extracellular matrix proteins fibronectin and laminin, leading to decreased adhesiveness of the connective tissue to melanoma cells, and thus enhanced migratory ability of melanoma cells (Rothhammer and Bosserhoff, 2006; Cao et al., 2007).

BRAF mutation correlates with overexpression of *CD63* (melanoma 1 antigen) and *HSP70* whose proteins are involved in cancer immunoediting. The correlation between V600E mutation and *CD63* overexpression is extremely strong in the current study. CD63 belongs to the tetraspanins family (Gesierich et al., 2005; Iida et al., 2005), which is increasingly studied in the context of cancer biology. In particular, members of the tetraspanins family are able to increase cell motility and particle binding (Le Naour et al., 2006; Latysheva et al., 2006; Lazo, 2007). Tetraspanins do not function as binding receptors by themselves but promote outer segment particle processing through functional interaction with alpha v beta integrins. It is noteworthy that this role of tetraspanins in particle processing has a specific relevance in the melanosomes-producing cells that are melanocytes. Finally, Heat shock protein (Hsp) 70B' is a human Hsp70 chaperone that is strictly inducible, having little or no basal expression levels in most cells (Le Naour et al., 2006; Latysheva et al., 2006; Lazo, 2007). As other HSP70's, it can increase the immunogenicity of melanoma cells and induce *in vivo* CTL and NK responses (Elsner et al., 2007). HSP70-inducers are under clinical investigation but preclinical data show that HSP70 induction leads to chemosensitization and increased melanoma apoptosis. Whether the strong correlation between BRAF mutation and *HSP70B*' expression results from a direct effect of $BRAF^{V600E}$ on HSP70B' expression or whether this is an indirect consequence of different responses to stress in mutated melanomas as compared to melanomas with no mutation needs further investigation. As shown in Table 1, there is no indication of an increased TILs response in the BRAF mutant tumors, but this needs to be specifically studied in a larger population.

In conclusion, our analysis of a specific expression signature associated with *BRAF* mutations in primary melanoma samples provides new data to better understand the role of the constitutive activation of the BRAF/MEK/ERK pathway and its possible contribution toward disease progression. The genes whose expression is associated with *BRAF* mutations are not simply restricted to the MAPK/ERK signaling but also converge to enhanced immune responsiveness, cell motility and tetraspanin-related vesiculization involved in the adaptative UV response.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found at doi:10.1016/j.molonc.2008.01.002.

REFERENCES

Bloethner, S., Hemminki, K., Thirumaran, R.K., Chen, B., Mueller-Berghaus, J., Ugurel, S., Schadendorf, D., Kumar, R., 2006. Differences in global gene expression in melanoma cell lines with and without homozygous deletion of the CDKN2A locus genes. Melanoma Res. 16, 297–307.

Buchholz, M., Biebl, A., Neesse, A., Wagner, M., Iwamura, T., Leder, G., Adler, G., Gress, T.M., 2003. SERPINE2 (protease nexin I) promotes extracellular matrix production and local invasion of pancreatic tumors in vivo. Cancer Res. 63, 4945–4951.

Cao, M.G., Auge, J.M., Molina, R., Marti, R., Carrera, C., Castel, T., Vilella, R., Conill, C., Sanchez, M., Malvehy, J., Puig, S., 2007. Melanoma inhibiting activity protein (MIA), beta-2 microglobulin and lactate dehydrogenase (LDH) in metastatic melanoma. Anticancer Res. 27, 595–599.

Chang, D.Z., Panageas, K.S., Osman, I., Polsky, D., Busam, K., Chapman, P.B., 2004. Clinical significance of BRAF mutations in metastatic melanoma. J. Transl. Med. 2, 46.

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B.A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G.J., Bigner, D.D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J.W., Leung, S.Y., Yuen, S.T., Weber, B.L., Seigler, H.F., Darrow, T.L., Paterson, H., Marais, R., Marshall, C.J., Wooster, R., Stratton, M.R., Futreal, P.A., 2002. Mutations of the BRAF gene in human cancer. Nature 417, 949–954.

Dong, J., Phelps, R.G., Qiao, R., Yao, S., Benard, O., Ronai, Z., Aaronson, S.A., 2003. BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma. Cancer Res. 63, 3883–3885.

Dumaz, N., Hayward, R., Martin, J., Ogilvie, L., Hedley, D., Curtin, J.A., Bastian, B.C., Springer, C., Marais, R., 2006. In melanoma, RAS mutations are accompanied by switching signaling from BRAF to CRAF and disrupted cyclic AMP signaling. Cancer Res. 66, 9483–9491.

Elsner, L., Muppala, V., Gehrmann, M., Lozano, J., Malzahn, D., Bickeboller, H., Brunner, E., Zientkowska, M., Herrmann, T., Walter, L., Alves, F., Multhoff, G., Dressel, R., 2007. The heat shock protein HSP70 promotes mouse NK cell activity against tumors that express inducible NKG2D ligands. J. Immunol. 179, 5523–5533.

Gesierich, S., Paret, C., Hildebrand, D., Weitz, J., Zgraggen, K., Schmitz-Winnenthal, F.H., Horejsi, V., Yoshie, O., Herlyn, D., Ashman, L.K., Zoller, M., 2005. Colocalization of the tetraspanins, CO-029 and CD151, with integrins in human pancreatic adenocarcinoma: impact on cell motility. Clin. Cancer Res. 11, 2840–2852.

Giordano, T.J., Kuick, R., Thomas, D.G., Misek, D.E., Vinco, M., Sanders, D., Zhu, Z., Ciampi, R., Roh, M., Shedden, K., Gauger, P., Doherty, G., Thompson, N.W., Hanash, S., Koenig, R.J., Nikiforov, Y.E., 2005. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene 24, 6646–6656.

Hoek, K.S., Schlegel, N.C., Brafford, P., Sucker, A., Ugurel, S., Kumar, R., Weber, B.L., Nathanson, K.L., Phillips, D.J., Herlyn, M., Schadendorf, D., Dummer, R., 2006. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. Pigment Cell Res. 19, 290–302.

Iida, J., Skubitz, A.P., McCarthy, J.B., Skubitz, K.M., 2005. Protein kinase activity is associated with CD63 in melanoma cells. J. Transl. Med. 3, 42.

Jackson, S., Harland, M., Turner, F., Taylor, C., Chambers, P.A., Randerson-Moor, J., Swerdlow, A.J., dos Santos Silva, I., Beswick, S., Bishop, D.T., Newton Bishop, J.A., 2005. No evidence for BRAF as a melanoma/nevus susceptibility gene. Cancer Epidemiol. Biomarkers Prev. 14, 913–918.

Johansson, P., Pavey, S., Hayward, N., 2007. Confirmation of a BRAF mutation-associated gene expression signature in melanoma. Pigment Cell Res. 20, 216–221.

Kidd, M., Modlin, I.M., Mane, S.M., Camp, R.L., Eick, G., Latich, I., 2006. The role of genetic markers—NAP1L1, MAGE-D2, and MTA1—in defining small-intestinal carcinoid neoplasia. Ann. Surg. Oncol. 13, 253–262.

Kim, I.J., Kang, H.C., Jang, S.G., Ahn, S.A., Yoon, H.J., Park, J.G., 2007. Development and applications of a BRAF oligonucleotide microarray. J. Mol. Diagn. 9, 55–63.

Korn, E.L., Li, M.C., McShane, L.M., Simon, R., 2007. An investigation of two multivariate permutation methods for controlling the false discovery proportion. Stat. Med. 26, 4428–4440.

Kumar, R., Angelini, S., Czene, K., Sauroja, I., Hahka-Kemppinen, M., Pyrhonen, S., Hemminki, K., 2003. BRAF mutations in metastatic melanoma: a possible association with clinical outcome. Clin. Cancer Res. 9, 3362–3368.

Kumar, R., Angelini, S., Snellman, E., Hemminki, K., 2004. BRAF mutations are common somatic events in melanocytic nevi. J. Invest. Dermatol. 122, 342–348.

Lang, J., Boxer, M., MacKie, R., 2003. Absence of exon 15 BRAF germline mutations in familial melanoma. Hum. Mutat. 21, 327–330.

Latysheva, N., Muratov, G., Rajesh, S., Padgett, M., Hotchin, N.A., Overduin, M., Berditchevski, F., 2006. Syntenin-1 is a new component of tetraspanin-enriched microdomains: mechanisms and consequences of the interaction of syntenin-1 with CD63. Mol. Cell. Biol. 26, 7707–7718.

Laud, K., Kannengiesser, C., Avril, M.F., Chompret, A., Stoppa-Lyonnet, D., Desjardins, L., Eychene, A., Demenais, F., Lenoir, G.M., Bressac-de Paillerets, B., 2003. BRAF as a melanoma susceptibility candidate gene? Cancer Res. 63, 3061–3065.

Lazo, P.A., 2007. Functional implications of tetraspanin proteins in cancer biology. Cancer Sci. 98, 1666–1677.

Le Naour, F., Andre, M., Greco, C., Billard, M., Sordat, B., Emile, J.F., Lanza, F., Boucheix, C., Rubinstein, E., 2006. Profiling of the tetraspanin web of human colon cancer cells. Mol. Cell. Proteomics. 5, 845–857.

Michaloglou, C., Vredeveld, L.C., Soengas, M.S., Denoyelle, C., Kuilman, T., van der Horst, C.M., Majoor, D.M., Shay, J.W., Mooi, W.J., Peeper, D.S., 2005. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 436, 720–724.

Michaloglou, C., Vredeveld, L.C., Mooi, W.J., Peeper, D.S., 2007. BRAF(E600) in benign and malignant human tumours. Oncogene. 2007 [Epub ahead of print].

Omholt, K., Platz, A., Kanter, L., Ringborg, U., Hansson, J., 2003. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin. Cancer Res. 9, 6483–6488.

Papageorgio, C., Brachmann, R., Zeng, J., Culverhouse, R., Zhang, W., McLeod, H., 2007. MAGED2: a novel p53-dissociator. Int. J. Oncol. 31, 1205–1211.

Pollock, P.M., Harper, U.L., Hansen, K.S., Yudt, L.M., Stark, M., Robbins, C.M., Moses, T.Y., Hostetter, G., Wagner, U., Kakareka, J., Salem, G., Pohida, T., Heenan, P., Duray, P., Kallioniemi, O., Hayward, N.K., Trent, J.M., Meltzer, P.S., 2003. High frequency of BRAF mutations in nevi. Nat. Genet. 33, 19–20.

Patton, E.E., Widlund, H.R., Kutok, J.L., Kopani, K.R., Amatruda, J.F., Murphey, R.D., Berghmans, S., Mayhall, E.A., Traver, D., Fletcher, C.D., Aster, J.C., Granter, S.R., Look, A.T., Lee, C., Fisher, D.E., Zon, L.I., 2005. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr. Biol. 15, 249–254.

Pavey, S., Johansson, P., Packer, L., Taylor, J., Stark, M., Pollock, P.M., Walker, G.J., Boyle, G.M., Harper, U., Cozzi, S.J., Hansen, K., Yudt, L., Schmidt, C., Hersey, P., Ellem, K.A., O'Rourke, M.G., Parsons, P.G., Meltzer, P., Ringner, M., Hayward, N.K., 2004. Microarray expression profiling in melanoma reveals a BRAF mutation signature. Oncogene 23, 4060–4067.

Rothhammer, T., Bosserhoff, A.K., 2006. Influence of melanoma inhibitory activity on transforming growth factor-beta signaling in malignant melanoma. Melanoma Res. 16, 309–316.

Shinozaki, M., Fujimoto, A., Morton, D.L., Hoon, D.S., 2004. Incidence of BRAF oncogene mutation and clinical relevance for primary cutaneous melanomas. Clin. Cancer Res. 10, 1753–1757.

Smalley, K.S., Herlyn, M., 2004. Loitering with intent: new evidence for the role of BRAF mutations in the proliferation of melanocytic lesions. J. Invest. Dermatol. 123, xvi–xvii.

Winnepenninckx, V., Lazar, V., Michiels, S., Dessen, P., Stas, M., Alonso, S.R., Avril, M.F., Ortiz Romero, P.L., Robert, T., Balacescu, O., Eggermont, A.M., Lenoir, G., Sarasin, A., Tursz, T., van den Oord, J.J., Spatz, A., 2006. Gene expression profiling of primary cutaneous melanoma and clinical outcome. J. Natl. Cancer Inst. 98, 472–482.