RAGE and S100 protein transcription levels are highly variable in human melanoma tumors and cells

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Abstract. The Receptor for Advanced Glycation Endproducts (RAGE) has been suggested to play an important role in melanoma. Animal studies with anti-RAGE antibodies have shown that RAGE blockade leads to reduced melanoma tumor growth and metastasis formation. RAGE is a multiligand receptor and among its ligands are the Ca-binding S100 proteins. Certain S100 proteins are differentially expressed in melanoma. For example, S100B is currently used as a reliable prognostic biomarker in patients with malignant melanoma. We have surveyed 40 human melanoma tumor samples for the transcription of RAGE and five of its known S100 protein ligands. Compared to normal skin tissue, we found highly significant ($p < 0.0001$) over-expression of S100B and under-expression of S100A2, whereas no significant difference in transcription of S100A6 and S100A10 was observed. RAGE showed slightly increased transcription in stage IV. Between individual tumor samples tremendous differences in transcription of the S100 proteins were observed, whereas RAGE expression showed relatively little variance. We also analyzed three well-characterized melanoma cell lines for S100 and RAGE expression. The S100 protein transcription profile showed clear differences between cultured melanoma cells and melanoma tumor tissue. Detailed profiling of S100 and RAGE transcription in melanoma tumors in combination with imunohisto-chemical and clinical data may lead to improved molecular diagnostic of melanoma and subsequently may facilitate improved treatment in the future.

Key words: RAGE — sRAGE — S100 protein — Melanoma

Introduction

Metastatic melanoma is characterized by poor (<20%) five-year survival rates. Many melanomas respond poorly to chemotherapy and aggressively form metastases. A better understanding of melanoma tumor biology may allow personalized chemotherapeutic treatment strategies. One of the most reliable biomarkers for melanoma is S100B. It is used as a marker for overall tumor load as well as for tumor response and survival prognosis during the therapeutic process: high serum levels of S100B predict poor treatment outcome (Egberts et al. 2008; Hamberg et al. 2003; Hauschild et al. 1999; Heizmann 2004; Schultz et al. 1998; Bolander et al. 2008; Oberholzer et al. 2008; Andres et al. 2008).

S100B is a member of the S100 protein family, which encompasses 21 members of sequence and structure related proteins (Marenholz et al. 2006). S100 proteins are small EF-hand calcium binding proteins that possess various intra- and extra-cellular functions, including cell proliferation, migration, muscle contraction, dynamics of the cytoskeleton and metabolic regulation (Donato 2003; Heizmann 2002). Members of the S100 protein family have been demonstrated to play important roles in tumor progression and tumorigenesis in several cancers, including melanoma, through their interaction with a number of intracellular target proteins (Emberley et al. 2004; Harpio and Einarsson 2004; Van Ginkel et al. 1998; Donato 2003; Zimmer et al. 2003). Extracellular S100 proteins have been described as signals of tissue damage and to function as alarmins (Bianchi 2007;

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Foell et al. 2007). Recently, extracellular S100 proteins have been implicated with cancer through their interaction with RAGE (Donato 2003; Heizmann et al. 2007). We are focusing in this study on five S100 proteins (S100B, S100A2, S100A4, S100A6 and S100A10) that have been associated in cancer progression and development.

S100B is highly expressed in melanocytes and in the brain. Intracellular S100B interacts with the p53 tumor suppressor (Baudier et al. 1992) and is an active target for anti-cancer drug development(Markowitz et al. 2005).

S100A2 was first described as a tumor-suppressor in mammary carcinoma cells (Lee et al. 1992) but recent studies have found that it is over-expressed in ovarian and gastric cancers, suggesting a more complex role for S100A2 in cancer (El-Rifai et al. 2002; Hough et al. 2001). In cultured epithelial cells, S100A2 has been shown to inhibit cell motility, probably through modification of actin polymerization/depolymerization mechanisms (Nagy et al. 2001).

The gene of S100A4 was first identified in metastatic tumor cell lines and is thought to play an important role in tumor progression and metastasis (Ambartsumian et al. 2005; Ambartsumian et al. 2001; Ebralidze et al. 1989). The role of S100A4 in melanoma tumor progression is not yet clear (Maelandsmo et al. 1997; Nonaka et al. 2008).

S100A6 is mainly expressed in fibroblasts and epithelial tissue but can also be found in neurons and smooth muscle cells (Kuznicki et al. 1989; Kuznicki et al. 1989). S100A6 expression is up-regulated in several cancers such as acute myeloid leukemia (Calabretta et al. 1985), pancreatic cancer (Vimalachandran et al. 2005), neuroblastomas and human melanomas (Boni et al. 1997; Shrestha et al. 1998; Weterman et al. 1992; Weterman et al. 1993)(Nonaka et al. 2008).

S100A10 was first identified in complex with annexin 2 (Gerke and Weber 1984; Glenney and Tack 1985) and is insensitive to calcium due to crucial mutations in its calcium binding domains resulting in a locked active conformation (Rety et al. 1999). S100A10 is thought to play an important role in the trafficking of plasma membrane proteins (Rescher and Gerke 2008). S100A10 has been shown to play a role in invasiveness of colorectal cancer cells through the interaction with plasminogen (Zhang et al. 2004). High expression of S100A10 has also been shown in anaplastic large cell lymphoma (Rust et al. 2005) and in renal cell carcinoma (Domoto et al. 2007). Down-regulation of S100A10, both at the transcript and at the protein levels has also been observed in three melanoma cell lines in culture suggesting a putative role of S100A10 in melanoma progression (Petersson et al. 2009).

S100 proteins have specific and common target proteins: the tumor suppressor p53 protein appears to be a common intracellular target for S100 proteins. p53 is a transcription factor that directs cell cycle arrest and apoptosis under stress conditions, probably through its tetramerization (Natan et al. 2009). S100B, S100A2, S100A4, S100A6 and S100A11 have been shown to interact with p53 but in distinct ways that could result in different effects of p53 dependent cell-cycle regulation (Baudier et al. 1992; Rustandi et al. 2000; Rustandi et al. 1998; Mueller et al. 2005; Fernandez-Fernandez et al. 2008; Fernandez-Fernandez et al. 2005).

The Receptor for Advanced Glycation Endproducts, RAGE, has been suggested as a common extracellular receptor for S100 proteins (Donato 2007; Heizmann et al. 2007; Leclerc et al. 2008; Schmidt et al. 2000). RAGE itself is important for tumor development, progression and metastasis in several cancers including melanoma (Fuentes et al. 2007; Heizmann et al. 2007; Logsdon et al. 2007; Turovskaya et al. 2008; Sparvero et al. 2009; Abe et al. 2004). RAGE is a cell surface receptor of the large immunoglobulin like receptor family and can be activated by structurally unrelated ligands that include members of the S100 protein family, the advanced glycation endproducts (AGE), amphoterin, and amyloid forming peptides or proteins. In cells, RAGE is present in multiple splice- and proteolytically truncated isoforms, the most important being soluble RAGE (sRAGE) that plays the role of decoy to antagonize ligand induced RAGE signaling (Hudson et al. 2008; Hudson et al. 2005; Ramasamy et al. 2009).

Melanomas express RAGE and S100 proteins and the stimulation of RAGE by S100 proteins may be a critical factor for tumor development, progression and metastasis. The splice isoforms of RAGE might also play an important role in melanoma. In order to explore this hypothesis we have undertaken a quantitative analysis of mRNA transcript levels of S100 proteins (S100B, S100A2, S100A4, S100A6 and S100A10) and of RAGE (full-length and sRAGE) of tissue samples of advanced melanoma (stage III and IV). We have also analyzed S100 and RAGE transcription levels in three cultured melanoma cell lines to complement the data obtained from the melanoma tumor samples.

Materials and methods

Quantitative PCR

A human melanoma tissue cDNA array (TissueScan MERT501) was purchased from OriGene (Rockville, MD) and consisted of normalized cDNA (against β-actin) from three non-tumor skin samples, 21 stage III malignant melanoma tumor samples and 19 stage IV malignant melanoma tumor samples. The age and gender distribution of tumor samples was as followed: stage III, 11 females and 10 males ranging from age 34 to 81 (median 56); stage IV, 5 females and 14 males ranging from age 42 to 81 (median 56). The average tumor content was 82 % (Supplemental Table 1). The cDNA of the non-tumor and tumor samples

Supplemental Table 1. Description of the melanoma tumor tissue cDNA array: Metastatic melanomas can be classified as stages III to IV depending of the spreading of the tumor cells. In stage III, the melanoma has spread to 1 to 3 lymph nodes near the primary tumors. In IIIA, the melanoma is not ulcerated and has not spread to other parts of the body. In stages IIIB and C, the melanoma has not spread to other parts of the body, it may or may not be ulcerated and may have spread to nearby small areas of skin or lymphatic channels. Stage IV melanomas have spread to other part of the body or to distant lymph nodes.

Sample	Gender	Age	Tissue	Diagnosis	Stage	% Tumor	% Necrosis	% Other tissue
1	Male	36	Skin	Fracture of bone	0	0	0	100
2	Not Sp	69	Skin	Skin Carcinoma, Merkel cell	0	0	0	100
3	Male	51	Skin	Obesity	0	0	0	100
4	Female	42	Lymph node	Malig. melanoma, metastatic	III	80	15	5
5	Female	59	Lymph node	Malig. melanoma, metastatic	III	95	5	0
6	Female	66	Lymph node	Malig. melanoma, metastatic	III	80	0	20
7	Male	56	Lymph node	Malig. melanoma, metastatic	III	85	5	10
8	Male	46	Lymph node	Malig. melanoma, metastatic	III	96	2	2
9	Female	66	Lymph node	Malig. melanoma, metastatic	III	80	20	0
10	Female	34	Lymph node	Malig. melanoma, metastatic	III	90	0	10
11	Female	56	Lymph node	Malig. melanoma, metastatic	III	80	20	0
12	Male	59	Lymph node	Malig. melanoma, metastatic	III	90	5	5
13	Female	49	Lymph node	Malig. melanoma, metastatic	III	95	5	0
14	Female	67	Lymph node	Malig. melanoma, metastatic	IIIA	80	0	20
15	Male	61	Lymph node	Malig. melanoma, metastatic	IIIB	90	10	0
16	Male	43	Lymph node	Malig. melanoma, metastatic	IIIB	85	7	8
17	Male	42	Lymph node	Malig. melanoma, metastatic	IIIB	90	0	10
18	Female	49	Lymph node	Malig. melanoma, metastatic	IIIB	90	0	10
19	Female	72	Lymph node	Malig. melanoma, metastatic	IIIB	75	0	25
20	Male	50	Groin	Malig. melanoma, metastatic	IIIB	60	0	40
21	Male	56	Lymph node	Malig. melanoma, recurrent	IIIC	95	0	5
22	Male	81	Lymph node	Malig. melanoma, metastatic	IIIC	90	5	5
23	Male	66	Lymph node	Malig. melanoma, metastatic	IIIC	70	5	25
24	Female	52	Lymph node	Malig. melanoma, metastatic	IIIC	55	0	45
25	Male	66	Lymph node	Malig. melanoma, metastatic	IV	75	5	20
26	Female	42	Lung	Malig. melanoma, metastatic	IV	75	20	5
27	Female	45	Lymph node	Malig. melanoma, metastatic	IV	80	15	5
28	Female	74	Lung	Malig. melanoma, metastatic	IV	98	0	2
29	Male	54	Lymph node	Malig. melanoma, metastatic	IV	70	20	10
30	Male	48	Mesentery	Malig. melanoma, metastatic	IV	95	0	5
31	Male	60	Jejunum	Malig. melanoma, metastatic	IV	80	0	20
32	Male	58	Lung	Malig. melanoma, metastatic	IV	80	0	20
33	Male	45	Lung	Malig. melanoma, metastatic	IV	90	5	5
34	Male	75	Small intest.	Malig. melanoma, metastatic	IV	80	15	5
35	Male	56	Liver	Malig. melanoma, metastatic	IV	75	1	24
36	Male	46	Jejunum	Malig. melanoma, metastatic	IV	90	10	0
37	Male	56	Lung	Malig. melanoma, metastatic	IV	90	10	0
38	Female	66	Omentum	Malig. melanoma, metastatic	IV	90	0	10
39	Male	56	Small intest.	Malig. melanoma, metastatic	IV	90	5	5
40	Male	81	Lymph node	Malig. melanoma, metastatic	IV	70	0	30
41	Male	56	Lung	Malig. melanoma, metastatic	IV	60	40	0
42	Male	73	Lung	Malig. melanoma, metastatic	IV	65	30	5
43	Female	42	Skin	Malig. melanoma, metastatic	IV	90	5	5

of the plate were provided already normalized against β-actin. Description of the tumor samples can be found in the Supplemental Table 1. PCR primers were designed with the help of PrimerBank (Wang and Seed 2003; Spandidos et al. 2008). Primers used are summarized in Table 1. Brilliant II SYBR Green QPCR master mix was from Stratagene (La Jolla CA). Real-time PCR measurements were performed on a Stratagene Mx3005p instrument, in duplicate for the tissue sample array and in triplicate for the cell samples. At the end of each RT-PCR run, the melting curves were obtained. In addition, the correct size of the amplicons was controlled by agarose gel electrophoresis.

Cell lines and tissue culture

Melanoma cell lines WM-115 (passage P80), WM-266-4 (P42) and SK-MEL2 (P40) were generously provided by Dr. G.B. Fields (University of Texas Health Science Center, San Antonio, Texas). Cells were maintained in opti-MeM (Invitrogen, Carlsbad, CA) supplemented with 4% FBS 100 U/ml penicillin, 100 μg/ml streptomycin. Cells at 70-80% confluence were used for mRNA preparation using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. The mRNA was immediately reverse transcribed into cDNA using a reverse transcription kit (Promega, Madison, WI). The cDNA was used in quantitative PCR using a standard protocol. The cDNAs were normalized to β-actin.

Statistical analysis

Statistical analysis was performed on the Ct values using the Student t test for 2 groups of unpaired data with unequal variance.

Results

RAGE transcription levels in human melanoma.

We have determined the transcription levels of RAGE in a panel of 40 human melanoma tumor tissue samples. 33 out of 43 tumor samples contained 75% or more tumor tissue according to the manufacturer's documentation (Origene). The tumor samples provided were classified as tumor stage III, IIIA-C, tumor stage IV, or lesion stage IV. Three samples of normal skin tissue stage 0 were also provided and originated from non-melanoma patients (Supplemental Table 1). We measured the transcript levels of full length RAGE (Fig. 1A) and sRAGE (Fig. 1B) using the isoform specific primer pairs (Table 1). Transcript levels were normalized relative to healthy skin and data for individual tumor samples are shown in Figure 1. Immediately notable are large differences between tumor samples. Some tumors showed close to ten-fold over-expression of RAGE whereas other samples showed over five-fold under-expression relative to normal skin. We averaged and compared RAGE transcription in stage III and stage IV tumors and analyzed them for significant differences. We found statistically significant differences ($p < 0.05$) between stage III and stage IV melanoma tumors, with stage IV tumors over-expressing RAGE relative to stage III (Fig. 1A).

The analysis of the transcription levels of the sRAGE isoform (Fig. 1B) also showed great differences between samples, with up to 22 fold difference between the lowest and the highest transcription levels in either stage III or stage IV melanoma tumors. 90% of stage III and stage IV tumors were significantly ($p < 0.05$) under-expressed compared to non-melanoma tissue sample. The over-expression of full-length RAGE between stage III and stage IV tumors and the under-expression of sRAGE in both stage III and stage IV tumors support the hypothesis of a protective role of sRAGE against the detrimental effects of RAGE ligands in normal conditions. In pathological conditions, such as melanoma, reduced sRAGE expression could enhance the amount of free ligand available to full-length RAGE,up-regulated, resulting in increased RAGE dependent signaling.

S100 transcription levels in melanoma tumors.

We next analyzed the transcription levels of four S100 genes in our tumor samples. Tremendous differences in transcription levels for S100B of over 2,000 fold were observed between tumor samples (Fig. 1C). On average transcription levels for S100B exceeded normal skin by 100 fold in stage III and 200 fold in stage IV tumors. We found a strong statistical correlation between S100B expression and tumor stage ($p < 0.0001$). Higher stage tumors showed higher S100B transcript

Table 1. Primer pairs used in real-time PCR experiments

Primer	5'-3' sequence	Amplicon size
RAGE fwd RAGE rev	TGTGTGGCCACCCATTCCAG GCCCTCCAGTACTACTCTCG	309
sRAGE fwd sRAGE rev	AGCCCTCTCCTCAAATCCACT CTTTATCAAACCCCTCACCTGC	304
S100B fwd S100B rev	CCGAACTGAAGGAGCTCATC AGAACTCGTGGCAGGCAGTA	155
S100A2 fwd S100A2 rev	CACTACCTTCCACAAGTACT GAAGTCATTGCACATGAC	229
S100A6 fwd S100A6 rev	GTGGCCATCTTCCACAAGTA TTCACCTCCTGGTCCTTGTT	285
S100A10 fwd S100A10 rev	AAAGACCCTCTGGCTGTGG AATCCTTCTATGGGGGAAGC	247
β-actin fwd β-actin rev	CATGTACGTTGCTATCCAGGC CTCCTTAATGTCACGCACGAT	250

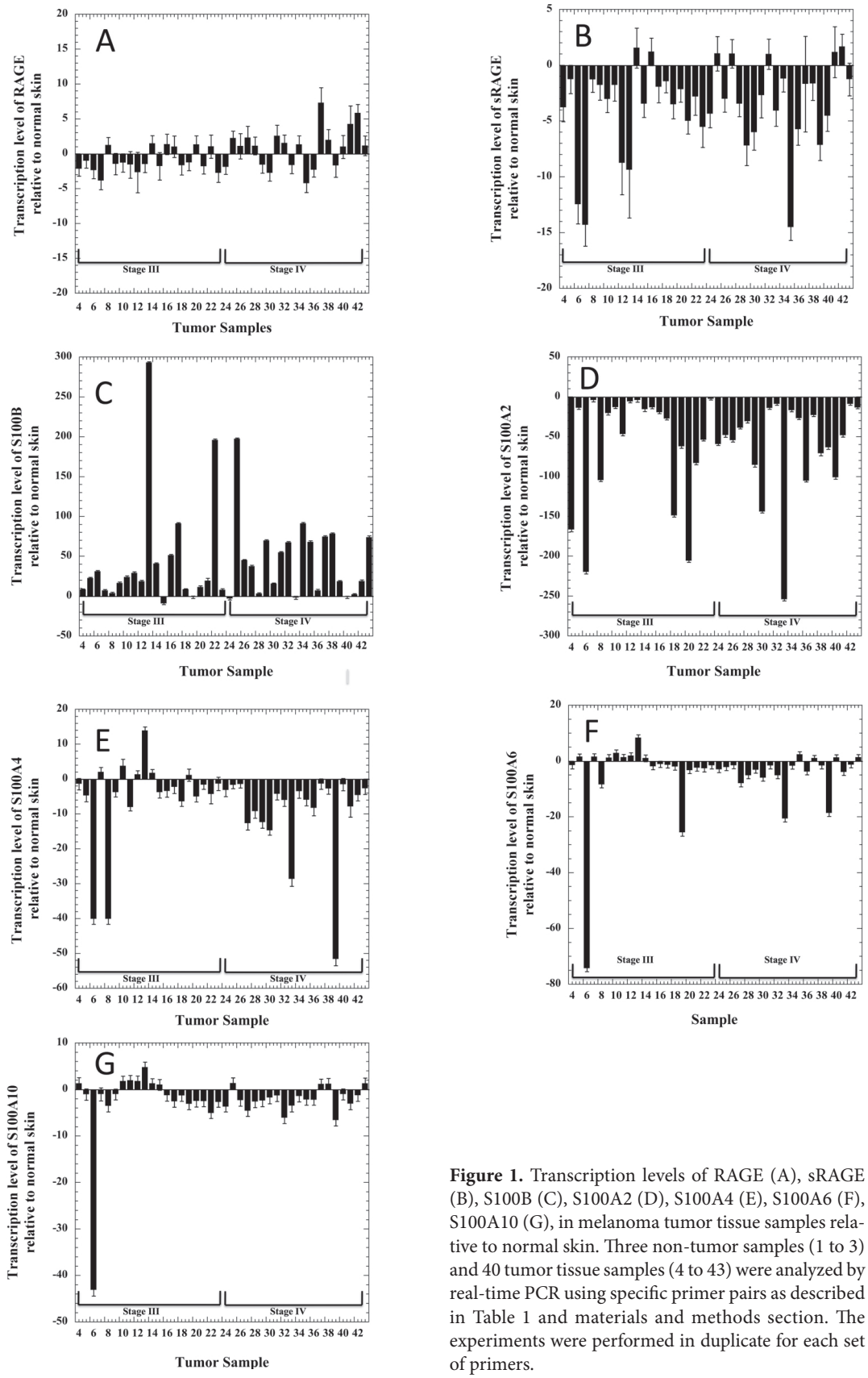

Figure 1. Transcription levels of RAGE (A), sRAGE (B), S100B (C), S100A2 (D), S100A4 (E), S100A6 (F), S100A10 (G), in melanoma tumor tissue samples relative to normal skin. Three non-tumor samples (1 to 3) and 40 tumor tissue samples (4 to 43) were analyzed by real-time PCR using specific primer pairs as described in Table 1 and materials and methods section. The experiments were performed in duplicate for each set of primers.

levels. Interestingly, five out of 40 melanoma tumor samples presented under-expression of S100B of 3.2 and 9.5 fold compared to non tumor samples, in agreement with previous studies showing the absence of S100B over-expression in 4% of all melanoma tumors (Aisner et al. 2005).

We also measured the transcription levels of the tumor suppressor S100A2, which we found to be generally in lower abundance in tumor samples (Fig. 1D). S100A2 was statistically significantly down-regulated ($p < 0.0001$) in both stage III and stage IV tumors when compared to normal skin. S100A2 was down-regulated on average 40- to 50-fold but showed tremendous differences between individual tumors. We did not detect up-regulation of S100A2 transcripts in any of the tumor samples (Fig. 1D).

S100A4 was found in higher abundance than S100B in normal skin ($Ct_{S100A4} = 20.8$; $Ct_{S100B} = 27.8$) and its transcript levels were significantly decreased ($p < 0.05$) in stage IV tumors (Fig. 1E). We also observed a significant ($p < 0.05$) under-expression of S100A4 transcripts in 76% of stage III tumors. Again, large differences in transcripts levels (>500 fold) were observed between melanoma tumors (Fig. 1E).

S100A6 did not show any significant difference in transcription levels between either stage III or stage IV tumors and normal skin even so individual tumors show great differences in relative expression: S100A6 transcription levels varied by more than a factor of 600 fold among stage III tumors and by 50 fold among stage IV tumors (Fig. 1F). A detailed analysis showed that 43% and 38% stage III tumors showed significant ($p < 0.05$) over-expression and under-expression, respectively, of S100A6 transcripts relative to non-tumor samples. Similarly 58% stage IV melanoma tumors showed significant ($p < 0.05$) under-expression of S100A6 transcripts compared to reference skin. Similar to S100A4 (Ct = 20.8), S100A6 (Ct = 22.2) was highly expressed in normal skin. Our results support an earlier study where 33% of melanomas, characterized by dermato-pathologists were found to express S100A6 at the protein level (Ribe and McNutt 2003).

S100A10 transcription levels in stage III and stage IV tumor samples did not show significant differences when compared to reference skin (Fig. 1G). Here again large variations between samples were observed with more than 200 fold differences between the lowest and the highest transcription levels in melanoma tumors. Detailed analysis showed that 58% stage IV melanoma tumors showed significant ($p < 0.05$) under-expression of S100A10.

The analysis of tumor samples for transcription of S100 proteins and RAGE revealed that S100B was generally strongly over-expressed and that the transcription level increased with the tumor stage. S100A2 on the other side was strongly under-expressed compared to normal skin. In average, the transcription ratio between S100A2 and S100B was approximately 6:1 in normal skin but reached 1:600 in stage III and 1:1500 in stage IV melanomas. Also, the ratio between full length RAGE and sRAGE changed between normal skin and melanoma tumor tissues from 6:1 to 12,1 for stage III and to 38,1 for stage IV melanomas.

RAGE and S100 transcription in melanoma cell lines

Although our panel of tumor tissue samples contained a high percentile of tumor tissue (mean = 82%), they also contained non-tumor tissue that could influence the evaluation of the transcription levels of the S100 proteins and RAGE. Furthermore "tumor tissue" is intrinsically heterogeneous due to non-melanoma cells that invade and constitute to tumor mass. We therefore decided to evaluate the transcription levels of S100B, S100A2, S100A4, S100A6, S100A10, RAGE and sRAGE in three well characterized melanoma cell lines: WM-115, WM-266 and SK-Mel2. These cell lines are free of non-melanocyte derived cell types. WM-115 and WM-266 originate from the same patient, WM-115 derives from the primary tumor whereas WM-266 comes from a secondary tumor. The transcription levels of RAGE and S100 proteins in these cell lines were normalized to the transcription level of β-actin in order to be comparable to the results from tissue culture arrays. As in the case of tumor tissue, we observed large variations in the transcription levels of RAGE and the S100 proteins between the three cell lines (Fig. 2).

As a general trend gene transcription levels for RAGE and the S100s relative to β-actin were always highest in the primary tumor cell line WM-115. RAGE was transcribed at lower levels than the S100s, by about two to three orders of magnitudes and varied by a factor of approximately 2.5 between the primary (WM-115) and the metastatic cell line WM-266 (Fig. 2A). sRAGE was found in ten times lower amount than full-length RAGE in all three cell lines. Interestingly, the ratio between RAGE and sRAGE was generally constant between the three melanoma cell lines although it varied significantly between the normal skin and melanoma stage III ($p < 0.0001$) or stage IV ($p < 0.0001$) tumor samples (Fig. 2A).

S100B was most highly expressed and exceeded RAGE transcription levels by more than three orders of magnitude (Fig. 2B). Differences between cell lines did not exceed four-fold. S100A2 transcripts were found at low levels and in the same order of magnitude as RAGE transcripts (Fig. 2C). Eight fold variation in transcription levels were observed between the three cell lines.

The S100B:S100A2 ratios were approximately 625:1, 2800: 1 and 560:1 for WM-115, WM-226 and Sk-Mel2, respectively.

S100A4 was strongly transcribed and showed greater differences between cell lines than the other genes. An almost 20-fold difference between WM-115 and WM-266 and a six-fold difference between WM-266 and SK-Mel2 were detected (Fig. 2D).

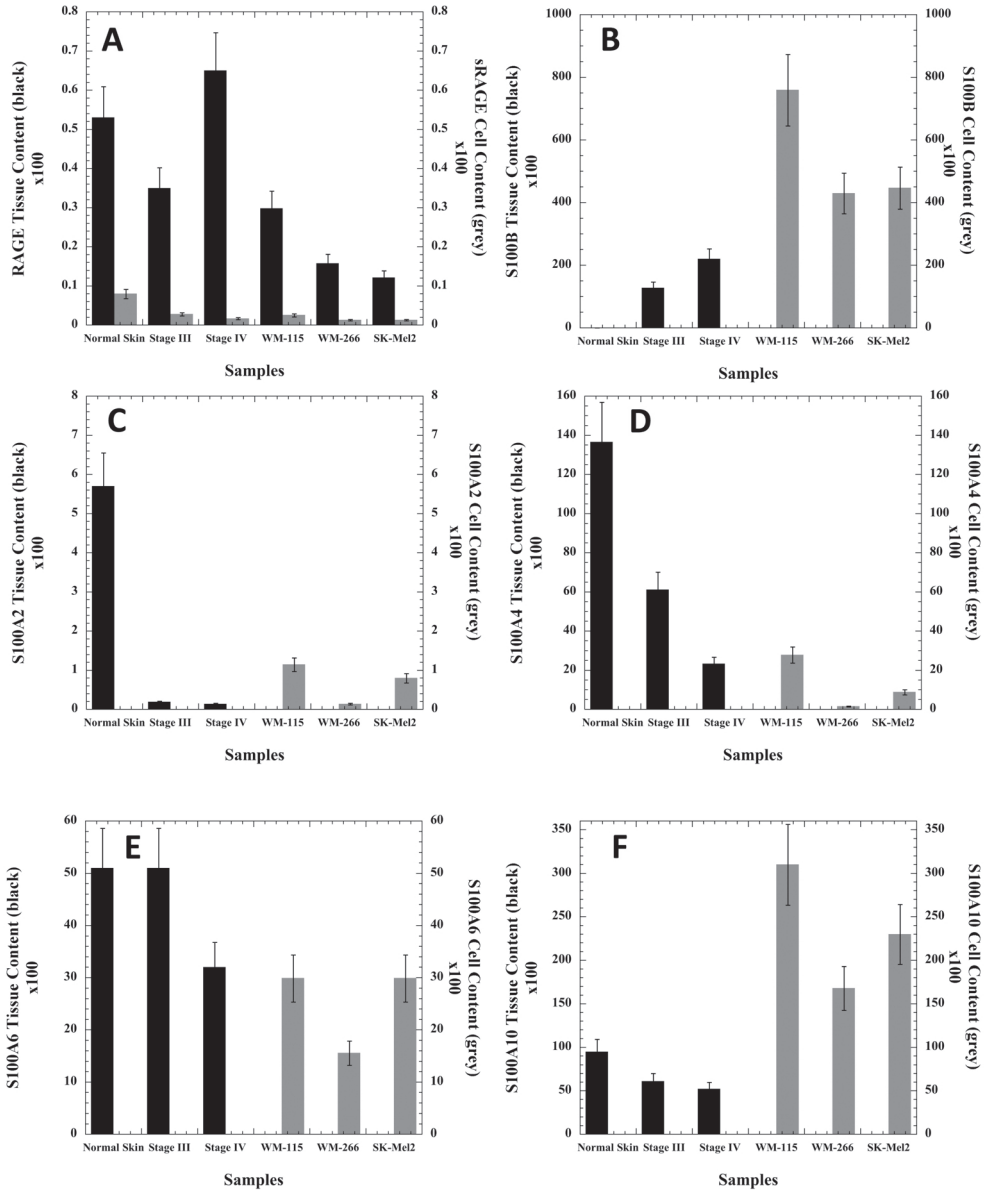

Figure 2. Transcription levels of RAGE and sRAGE (A), S100B (B), S100A2 (C), S100A4 (D), S100A6 (E), S100A10 (F), in melanoma cell lines WM-115, WM-266, sk-Mel2. The expression levels of the transcripts were normalized against β-actin. The experiments were performed in triplicate. The values of the transcript levels were also normalized by an additional factor of 100 for better representation.

S100A6 differed from the emerging pattern between the cell lines in that WM-115 and SK-Mel2 had approximately equal transcription levels (Fig. 2E).

S100A10 transcription level in the three cell lines was the closest to the one of S100B and was similar in the three cell lines (Fig. 2F).

The increase in the S100B:S100A2 and RAGE:sRAGE ratios observed by the comparison of normal skin with melanoma tumor tissues were also observed for cultured melanoma cells.

Discussion

RAGE has been suggested to be involved in melanoma progression and development (Abe et al. 2004). Animal studies using anti-RAGE antibodies have demonstrated that RAGE blockade can reduce melanoma tumor growth and metastasis formation. Among the RAGE ligands are several S100 proteins including S100B, S100A2 and S100A6 that have also been suggested to be potential biomarkers in melanoma: S100B is currently used by clinicians as prognostic marker

and high serum levels of S100B correlate with poor survival rates (Bolander et al. 2008; Oberholzer et al. 2008; Andres et al. 2008). The exact role of S100B in melanoma progression is still unknown. S100A2, generally described as tumor suppressor, was found to be strongly expressed in benign nevi and absent in metastatic melanoma, suggesting that loss of S100A2 expression could play a role in melanoma (Maelandsmo et al. 1997). A recent report also showed that over-expression of S100A2 could synergize with interferon α resulting in growth inhibition of cutaneous melanoma (Foser et al. 2006). In uveal melanoma, up-regulation of S100A2 was observed following treatment with decibatine and resulted in cell death (Gollob and Sciambi 2007). S100A6 gene expression was also found in melanocytic lesions (Weterman et al. 1993) and was shown to correlate with melanoma survival rate, a higher S100A6 expression rate being less favorable (Maelandsmo et al. 1997).

In our present study, we used a panel of cDNA prepared from 40 melanoma tissue samples and from three normal skin samples. The objective of our study was to evaluate differences in transcription levels between individual tumors and between tumor stages relative to normal skin.

Our study detected RAGE, sRAGE, S100B, S100A2, S100A4, S100A6 and S100A10 transcripts in melanoma tumor cDNA. Averaging stage III and stage IV tumor samples and comparing them to normal skin we found 100 to 200 fold increased transcript levels for S100B and 50 fold lower transcript levels for S100A2. These differences were statistically highly significant ($p < 0.0001$) and in the case of S100B differences between stage III and stage IV were also significant ($p < 0.001$). Differences between normal skin and melanoma for RAGE, S100A6 and S100A10 were not statistically significant on average. However, differences were significant for RAGE and S100A4 between tumor stage III and IV, for sRAGE between stage III tumors and normal skin and for S100A4 between tumors stage IV in comparison to normal skin. However, although no significant difference in sRAGE transcription levels was observed when comparing all stage IV tumors with normal skin, a significant decrease in expression level ($p < 0.05$) was observed for 89% of these tumors. Moreover, although no significant difference could be observe, on average, in the transcript levels of S100A6 and S100A10 between normal skin and melanoma tumors, the finding that 58% of stage IV tumors showed significant ($p < 0.05$) decrease in transcript levels of S100A10 and that 43% of stage III tumors showed a significant ($p < 0.05$) increase in transcript levels of S100A6 supports previous studies performed on both cell lines and tumor tissue samples (Petersson et al. 2009; Ribe and McNutt 2003).

More important than the average values are the differences in transcription levels observed between individual tumor samples: 500 fold for S100B, S100A4, S100A6, 200 fold for S100A10, 100 fold for S100A2. These variations between individual tumors are too large to be the result of tumor tissue content in the biopsied sample. We suggest instead that these differences are intrinsic properties of the tumor itself and that melanoma tumors develop a molecular signature, which can differ between tumors.

Molecular signatures in tumors are an emerging concept. Cluster classification of melanoma tumors according to the expression of several S100 proteins could be useful at several levels: it could help to understand the role of these S100 proteins in the development and progression of the tumor and also help in the diagnosis and prognosis of the cancer. S100B is already an established biomarker in melanoma. S100A2 shows promising properties as well. The presence of large variations in RAGE transcription among melanoma tumors may also indicate a possible role of RAGE in a subset population of melanoma tumors.

Tumor profiling based on gene transcription levels does not necessarily mirror protein levels in the tumor. However, transcript levels can be determined with very high specificity for a given gene and at very low cost in a very fast manner. PCR based diagnostic methods are significantly faster and more economical when compared to traditional immuno-histochemical methods. This is an important consideration when it comes to diagnostic and preventive screening efforts offered to an expanded spectrum of patients. The integration of the genetic profiling of RAGE and S100 transcription in combination with immuno-histological analysis of tumors could result in earlier diagnosis and treatment of melanoma in the future (Kashani-Sabet et al. 2009; Viros et al. 2008). For example, suspicious skin moles are routinely removed by family doctors and send in for immuno-histopathological characterization. A routine PCR based ratio determination of S100B:S100A2 and RAGE:sRAGE may provide an additional and quantitative measure for potential malignancy.

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