Research Article

Combination PPARγ and RXR Agonist Treatment in Melanoma Cells: Functional Importance of S100A2

Joshua P. Klopper,1,2 Vibha Sharma,1 Reid Bissonnette,3 and Bryan R. Haugen1,2

1 Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado Denver, Aurora, CO 80045, USA
2 University of Colorado Cancer Center, University of Colorado Denver, Aurora, CO 80045, USA
3 Department of Molecular Oncology, Ligand Pharmaceuticals, San Diego, CA 92121, USA

Correspondence should be addressed to Joshua P. Klopper, joshua.klopper@ucdenver.edu

Received 11 May 2009; Accepted 28 July 2009

Academic Editor: Dipak Panigrahy

Copyright © 2010 Joshua P. Klopper et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nuclear hormone receptors, including RXR and PPARγ, represent novel therapeutic targets in melanoma. We have previously shown that the DRO subline of the amelanotic melanoma A375 responds to rexinoid and thiazolidinedione (TZD) treatment in vitro and in vivo. We performed microarray analysis of A375(DRO) after TZD and combination rexinoid/TZD treatment in which the calcium binding protein S100A2 had increased expression after rexinoid or TZD treatment and a synergistic increase to combination treatment. Increased S100A2 expression is dependent on an intact PPARγ receptor, but it is not sufficient to mediate the antiproliferative effects of rexinoid/TZD treatment. Over expression of S100A2 enhanced the effect of rexinoid and TZD treatment while inhibition of S100A2 expression attenuated the response to rexinoid/TZD treatment, suggesting that S100A2 is necessary for optimal response to RXR and PPARγ activation by respective ligands. In summary, we have identified potential downstream mediators of rexinoid and TZD treatment in a poorly differentiated melanoma and found that alterations in S100A2 expression affect RXR and PPARγ signaling in A375(DRO) cells. These studies provide insight into potential mechanisms of tumor response or resistance to these novel therapies.

1. Introduction

Melanoma represents a significant public health problem with a rising incidence over the last 3 decades [1]. More than 7700 patients will die of this disease annually, almost all with metastases [2]. The median survival in patients with metastatic disease is 7–9 months [3]. While some prognostic factors correlate with a more favorable prognosis, such as lack of visceral metastases, younger age, and treatment with biochemotherapy, the 5–10-year survival rates still remain less than 20% [4]. Thus, a search for novel therapies is warranted in this aggressive disease given the suboptimal choices available.

We have reported the efficacy of rexinoid, thiazolidinedione, and combination therapy in the melanoma cell line A375(DRO) (DRO was originally thought to be an anaplastic thyroid cancer cell line) [5–7]. Additionally, we have shown that RXR and PPARγ receptors are necessary for optimal response to rexinoid or TZD therapies, as knock down of either receptor attenuates the antiproliferative response to its own ligand alone or the ligand of its heterodimer partner [8].

In this report, we explore potential downstream mediators of the rexinoid and TZD treatment effect in the A375(DRO) melanoma cancer cell line using comparative gene expression microarray analysis. We have identified the calcium binding protein S100A2 as a potential mediator of rexinoid and TZD signaling in melanoma. S100A2 is one of 24 members of S100 proteins that regulate cellular processes including neoplasia and has significantly increased gene expression in A375(DRO) with rexinoid and TZD, while a synergistic effect is seen with combination therapy [9].

2. Materials and Methods

2.1. Cell Line and Chemicals. A375(DRO) was provided by Dr. G.J. Juillard (University of California at Los Angeles, Los
Angeles, CA). DRO was previously thought to be derived from an anaplastic thyroid cancer. We have shown that it is genetically identical to the melanoma cell line A375 and is therefore designated as a subline of A375, A375(DRO) [7, 8]. A375(DRO) was grown in RPMI 1640 (Invitrogen Corporation) supplemented with 2% fetal bovine serum (Hyclone) and 0.5% penicillin/streptomycin. LGD1069 was provided by Ligand Pharmaceuticals (San Diego, CA), and Rosiglitazone (ROS1) was provided by GlaxoSmithKline.

2.2. Microarray Analysis. Four million A375(DRO) cells were plated in triplicate into 100-mm plates and incubated overnight. The next day, the medium was changed, and medium with volume equivalent vehicle (DMSO) or 1 μmol/L of LGD1069, ROS1, or the combination (500 nM of each) was added in the set of cells to incubate for 24 hours. RNA was extracted from treated cells using the QIAGEN RNaseasy Mini Kit and was quantified by standard spectrophotometry. RNA integrity was verified by gel electrophoresis using an Agilent 2100 Bioanalyzer. Total RNA (5 μg) was converted to ds-cDNA using the Superscript Choice System. In vitro transcription of cRNA was done and the transcript hybridization to the cDNA oligonucleotide array. We used the Affymetrix GeneChip Human Genome U133A platform, and all gene chip processing and analyses occurred in the UCHSC Affymetrix microarray core facility. Each condition was run in triplicate from three independent experiments. Data analysis, including background adjustment and normalization, was done using Affymetrix GeneSpring software.

2.3. Quantitative Reverse Transcription-PCR (qRT-PCR). Total RNA was isolated from A375(DRO) in single samples under the same conditions used for the microarray experiment using the RNeasy Mini Kit (Quiagen, Valencia, CA) as per the manufacturer’s protocol. The mRNA for S100A2 was measured by real-time Quantitative RT-PCR using ABI PRISM 7700. The sequences of forward and reverse primers as designed by Primer Express (PE ABI) were 5'-TTCTGGTGTCTGTCTGCCC-3' and 5'-AGCG-GCCTGCTCCAGAAC-3'. The TaqMan fluorogenic probe used was 6FAM-TGG-TCTGCCACAGATCCATGATGTC-TAMRA.

Amplification reactions, thermal cycling conditions, and generation of a standard curve have been described previously [6].

2.4. S100A2 Overexpression. Human S100A2 in pcDNA3 vector was the generous gift from Professor C. Heizmann (University of Zurich). A375(DRO) cells were stably transfected with S100A2 in pcDNA3 vector and empty vector using lipofectamine method in 6-well cell culture plates (4 μg/well) as previously described [10]. Thereafter stable clones were selected and continuously cultured in 150 μg/mL G418 (Gibco/BRL).

2.5. shRNA. We used a lentiviral mediated shRNA system from Sigma (St. Louis, MO) and followed the manufacturer’s protocol. Lentiviral particles contain shRNA toward S100A2 or PPARγ or RXRγ-specific sequences as well as a scrambled (SCR) sequence that consists of 5 nucleotides that do not match any known gene transcript in both the murine and human genome. The infected cells are selected by a puromycin resistance and then assessed for correct insertion/RNA inhibition by qRT-PCR or western blot for S100A2, PPARγ, or RXRγ. The concentration of puromycin used to select for DNA construct incorporation cells was 0.4 μg/mL.

2.6. Western Blot Analysis. Whole cell protein extracts were obtained from A375(DRO) under conditions of volume equivalent vehicle, LGD1069/ROS1 combination treatment, and with overexpressed S100A2 or shRNA directed at S100A2. The protein content of lysates was measured using a commercial protein assay kit (DC from Bio-Rad). Diluted samples containing equal amounts of protein (60 μg) were mixed with 2x Laemmli sample buffer (Bio-Rad Laboratories). Proteins were separated on a 10% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 1x TBST (20 mmol/L Tris-HCl (pH 7.6), 8.5% NaCl, and 0.1% Tween 20) containing 5% nonfat dry milk at room temperature for 2 hours and incubated in the appropriate primary antibody in 1x TBST containing 5% nonfat dry milk at 4°C overnight. S100A2 protein antibodies (Sigma S6797), RXRα (sc 553 and D-20), RXRβ (sc 831 and C-20), and RXRγ (sc Y-20 and JC-555) receptor antibodies were used at a concentration of 1 : 1000, and PPARγ (sc 7196 and H-100) rabbit polyclonal antibody was used at 1 : 500. After washing, membranes were incubated for 1 hour at room temperature with antirabbit IgG conjugated to horseradish peroxidase at a 1 : 5000 dilution for RXRs and 1 : 1000 for PPARγ (GE Healthcare UK). β-Actin was probed for loading control. The enhanced chemiluminescence detection reagent from Amershams Biosciences was used for immunodetection.

2.7. Cell Growth and Proliferation. A375(DRO) cells at baseline, with S100A2 overexpressed, with infected SCR shRNA, and with shS100A2 cells were grown to approximately 80% confluence in 100 mm tissue culture plates. Cells were then harvested using Trypsin-EDTA (Invitrogen Corporation, Carlsbad, CA) and counted using a hemocytometer. Cells were then transferred to a 96-well plate at a concentration of 500 cells/200 μL of media. Each row of eight wells received the same cell type and subsequently the same drug. After
cells were plated, media with the appropriate concentration of ligand or equivalent volume of vehicle was added to each well. Cells were treated with volume equivalent vehicle, 1 μM LGD1069, 1 μM rosiglitazone, or the 1 μM combination (500 nM of each). Fresh media with vehicle or ligand was added every 72 hours. At the completion of 6 days, cell proliferation was assessed following the manufacturers instructions using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Following a two-hour incubation at 37 °C, each plate was analyzed by a MRX Micro plate Reader (Dynatech Laboratories, Chantilly, VA) using Revelation software.

2.8. Statistics. Cell growth between control and treatment conditions quantified using the group mean ± SE and significance was compared between control and treatment conditions with a Student’s t-test between conditions (SISA online statistical tool).

3. Results

3.1. Microarray Analysis of LGD1069/ROSI-Treated A375 (DRO) Cells. The antiproliferative effects of rexinoid and TZD treatment on A375(DRO) occur at or beyond six days of treatment [5]. However, we chose to analyze gene expression changes in A375(DRO) at 24 hours since RXR and PPAR are nuclear hormone receptors, and we would predict direct gene expression effects of these liganded transcription factors to occur early. The results of the LGD1069 1 μM treatment arm have been previously published [6]. Microarray analysis revealed that the combination rexinoid/TZD treatment resulted in 212 genes with increased expression and 1050 genes with decreased expression (one-way ANOVA P < .05). These genes broadly fell into the categories of cell growth, nucleic acid binding, and cell signal transduction. The 20 genes with the largest change in expression after treatment are listed in Tables 1, 2, 3, and 4 (excluding affymetrix specific cDNA sequences without related searchable genes). The complete data set is in the supplementary materials (see Supplementary Material available online at doi:10.1155/2010/729876). Four genes were upregulated by rexinoid/TZD combination therapy greater than 20-fold: TIE1 (121.5-fold), S100A2 (69.1-fold), ILB-1 (40.1-fold), and ANGPTL4 (32.2-fold) (Table 5). Of these genes, S100A2 was increased by both the rexinoid (3.4-fold) and TZD (4.9-fold) but also demonstrated a synergistic stimulation (69.1-fold) with the combination treatment. In addition, 171 genes had increased expression and 1006 genes had decreased expression by at least 2-fold with ROSI alone (Tables 1, 2, 3, and 4—one-way ANOVA P < .05). Based upon the significant increase of S100A2 mRNA levels with each ligand alone and the synergistic increase with combination therapy, we performed additional experiments with S100A2 to define its role in mediating the effects of combination rexinoid/TZD treatment in melanoma cells. We have previously published confirmation of ANGPTL4 regulation by rexinoids [6]. The effects of TZD and rexinoids on TIE-1 and ILB-1 mRNA and protein expression have not yet been confirmed by other methods.

Table 1: Microarray Analysis of A375(DRO) cells after TZD/rexinoid combination treatment—2-fold up regulated genes. A375(DRO) cells were treated in vitro with 1 μmol/L of PIO or combination LGD1069/PIO for 24 hours compared with volume-equivalent vehicle in triplicate with a cutoff of a 2-fold change (P < .5, one-way ANOVA). Gene symbols were derived from the Affymetrix web site.

| Gene Symbol | fold change | GenBank ID |
|-------------|-------------|------------|
| TIE1        | 121.5       | NM_005424  |
| S100A2      | 69.1        | NM_005978  |
| IL1B        | 40.1        | NM_000576  |
| ANGPTL4     | 32.2        | NM_01039667|
| AREG        | 18.2        | NM_001657  |
| RPL37A      | 12.6        | NM_000998  |
| CORO2B      | 10.9        | NM_006091  |
| RPL27A      | 8.7         | NM_000990  |
| INHBA       | 7.6         | NM_002192  |
| ST3GAL1     | 5.4         | NM_003033  |
| TXN         | 5.0         | NM_003329  |
| ITGA3       | 5.0         | NM_002204  |
| FGFR2       | 5.0         | NM_000141  |
| GEM         | 4.9         | NM_005261  |
| COL5A2      | 4.9         | NM_000939  |
| RPL38       | 4.8         | NM_000999  |
| PMEPA1      | 4.7         | NM_020182  |
| MCL1        | 4.5         | NM_021960  |
| RPL38       | 4.2         | NM_000999  |
| SMARCA2     | 3.8         | NM_003070  |

3.2. An Intact RXR and PPAR Receptor Is Required for Optimal S100A2 Expression. S100A2 levels were measured after treatment of A375(DRO) with 1 μM combination of LGD1069 and ROSI (500 nM each). To determine the relative contribution of PPAR and RXR, we compared control cells stably infected with scrambled shRNA with sublines stably infected with shRNA against PPAR and RXR which greatly reduced the levels of each receptor [8]. Figure 1 shows that the rexinoid/TZD-induced expression of S100A2 was attenuated by lack of either receptor. PPAR appears to have the greatest effect on this response.

3.3. S100A2 Overexpression Enhances the Antiproliferative Response to LGD1069 and ROSI Treatment. S100A2 protein was overexpressed in A375(DRO) cells, and the empty vector (EV) was used as a control. Figure 2 shows that the levels of S100A2 protein in the overexpressing subline (S100A2) are similar to levels seen after treating A375(DRO) cells with rexinoid/TZD combination. After plating equivalent numbers of control (A375(DRO) + EV) and S100A2 overexpressing cells, we observed no difference in growth rate at 3 and 6 days (Figure 3(a)). However, with 6 days of 1 μM LGD1069, 1 μM ROSI, or 1 μM combination therapy the S100A2 overexpressing cells had a significant decrease in proliferation compared to the EV cells relative to vehicle treatment (64% versus 46% for LGD1069, 86% versus 72%
Table 2: Microarray Analysis of A375(DRO) cells after TZD/rexinoid combination treatment—2-fold down regulated genes.

| Gene Symbol | fold change | GenBank ID   |
|-------------|-------------|--------------|
| ABAT        | −17.5       | NM_000663    |
| TSFM        | −10.4       | NM_005726    |
| XRCC4       | −9.0        | NM_003401    |
| NUDT1       | −8.9        | NM_002452    |
| ZIC1        | −8.3        | NM_003412    |
| BARD1       | −8.2        | NM_000465    |
| CDC25A      | −8.2        | NM_001789    |
| MAP9        | −8.1        | NM_001039580 |
| QPCT        | −7.9        | NM_012413    |
| C12orf52    | −7.8        | NM_032848    |
| PTCD2       | −7.3        | NM_024754    |
| TLE1        | −6.8        | NM_005077    |
| MYO6        | −6.4        | NM_004999    |
| WDR59       | −6.0        | NM_030581    |
| RARS        | −5.6        | NM_002887    |
| BIN1        | −5.2        | NM_004305    |
| WBP1        | −5.2        | NM_012477    |
| CSGALNACT1  | −5.1        | NM_018371    |
| CAMTA1      | −5.1        | NM_015215    |
| TRIM23      | −5.1        | NM_001656    |

Table 3: Microarray Analysis of A375(DRO) cells after TZD/rexinoid combination treatment—2-fold up regulated genes.

| Gene Symbol | fold change | GenBank ID   |
|-------------|-------------|--------------|
| ORC4L       | −15.9       | NM_002552    |
| TAF12       | −8.9        | NM_005644    |
| ZNF443      | −8.1        | NM_005815    |
| HIGD1A      | −8.0        | NM_032775    |
| CYB5R2      | −7.9        | NM_001039580 |
| DAZAP2      | −7.5        | NM_005726    |
| STK32B      | −7.1        | NM_024754    |
| CAMTA1      | −6.6        | NM_015215    |
| HCG4        | −6.4        | NR_002139    |
| BSC1L2 // HNRNPUL2 | −6.3 | NM_001079559 |
| SL3C9A4     | −6.0        | NM_017414    |
| DZIP3       | −6.0        | NM_014648    |
| MSRB2       | −6.0        | NM_018009    |
| KLHL22      | −5.8        | NM_032848    |
| MPP5        | −5.7        | NM_024899    |
| RNF41       | −5.6        | NM_005785    |
| VRK3        | −5.5        | NM_030581    |
| ABAT        | −5.4        | NM_000663    |
| BRCA2       | −5.3        | NM_000059    |
| RARS        | −5.3        | NM_002887    |

Table 4: Microarray Analysis of A375(DRO) cells after TZD/rexinoid combination treatment—2-fold down regulated with ROSI alone.

| Gene Symbol | fold change | GenBank ID   |
|-------------|-------------|--------------|
| LGD1069/ROSI| 121.5       | NM_001039580 |
| ROSI        | 13.1        | NM_000578    |
| LGD1069*    | <2          | 3.4          | <2          | 6.5 |

Table 5: Four genes with the highest mRNA stimulation after combination TZD/rexinoid treatment.

| Gene Symbol | fold change | GenBank ID   |
|-------------|-------------|--------------|
| LGD1069/ROSI| 121.5       | NM_001039580 |
| ROSI        | 13.1        | NM_000578    |
| LGD1069*    | <2          | 3.4          | <2          | 6.5 |

3.4. Knock Down of S100A2 Attenuates the Growth Inhibition of A375(DRO) Cells by Rexinoid, TZD, and Combination Treatment. Knock down of S100A2 with specific shRNA did not affect growth of A375(DRO) cells (data not shown). S100A2 shRNA stably expressed in A375(DRO) cells resulted in a decrease in S100A2 mRNA and protein expression after treatment with LGD1069/ROSI (Figure 4). Stable expression of scrambled shRNA (SCR) had no effect (Figure 3(b)). Knock down of S100A2 with specific shRNA did significantly blunt growth suppression by treatment with rexinoid, TZD, or combination (P ≤ .008 for all conditions; Figure 3(b)).
Figure 1: S100A2 mRNA stimulation by TZD/rexinoid treatment is dependent on intact PPARγ and RXRγ. One microgram of total RNA was used for the S100A2 quantitative reverse transcription-PCR analysis (ABIPRISM7700; Perkin-Elmer), and absolute values were derived from a standard curve using a known amount of sense strand RNA (ag, attograms of sense strand RNA). Isoform RNA was normalized to total input RNA (18s rRNA measured from 1 ng of total RNA). A375(DRO) cells were infected with either shPPARγ or shRXRγ lentiviral particles and then treated with LGD1069/ROSI 1 μM for 24 hours. S100A2 mRNA levels were compared to levels from A375(DRO) cells infected with the shSCR control under the same treatment conditions.

Figure 2: Protein expression of S100A2 in A375(DRO) cells. 60 μg of nuclear protein extract from A375(DRO) before (DMSO—lane 1) and after combination treatment (lane 2) or transfected with empty vector (EV—lane 3) or S100A2 in pcDNA3 vector with no treatment (lane 4) was size-separated on a 10% SDS-PAGE gel and transferred to nitrocellulose. The blot was blocked with 10% nonfat milk and incubated with S100A2 receptor antibodies (sc Y-20). β-Actin was measured as a loading control.

are very low in these cells [11]. Analysis of shS100A2 and RXRγ sequences revealed no sequence homology.

4. Discussion

In this report, we have examined global gene expression in a poorly differentiated cancer model, the amelanotic melanoma cell line A375(DRO), after treatment with PPARγ and RXR ligands. S100A2 was shown to be a potentially important target based on increased levels with rexinoid or TZD treatment and synergistically increased levels with combination therapy. Furthermore, S100A2 appears to be required for the maximal antiproliferative effects of rexinoids and TZD in these melanoma cells.

The S100 proteins have a broad range of intracellular functions including the regulation of protein phosphorylation and enzyme activity, calcium homeostasis, regulation of cytoskeletal proteins, and transcriptional factors [12]. S100 proteins appear to regulate tumorigenesis. For example, S100A2 proteins enhance p53 transcriptional activity whereas S100A4 increases p53 apoptosis in models of adenocarcinoma, osteosarcoma, and oral carcinoma [12–14]. Thus, a relative imbalance of S100 proteins may promote or inhibit neoplastic transformation or progression. S100A2 seems to have a variable pattern of expression with some evidence pointing to higher expression in normal tissues and
In melanoma, S100 proteins may play a critical role in regulating the transformation of nevi to melanoma. S100A4 levels are lower in metastatic melanoma compared with primary tumors, while S100A7, S100A8, and S100A9 levels appear to be higher in malignant melanoma compared with normal melanocytes [17]. In a study of 105 patients with stage IV melanoma, elevated serum levels of S100B were associated with a significantly shorter survival [18].

S100A2 expression is higher in premalignant nevi than in cells from primary melanoma tumors or metastases suggesting that loss of S100A2 may be important for neoplastic transformation [19]. In an in vitro model of uveal melanoma, S100A2 gene expression was significantly up-regulated by the methyltransferase inhibitor decitabine, which was correlated with cell death [20]. We have demonstrated that S100A2 levels are low in the A375(DRO) melanoma cell line, and these levels are increased by treatment with PPARγ and RXR agonists, which is associated with a significant reduction in growth and increase in apoptosis [8].
Though other S100 proteins have been shown to increase with retinoid therapy in models of cancer including teratocarcinoma, breast cancer, and gastric carcinoma [21–23], this is the first report of increased S100A2 expression by either rexinoids or TZDs. We have previously demonstrated that, in A375(DRO), the combination of LGD1069 and ROSI synergistically decreases in vitro cell proliferation and in vivo tumor growth [5, 8]. Our data indicates that S100A2 is necessary to mediate the antiproliferative effects of rexinoid and TZD treatment but is not sufficient to mediate this effect. However, with overexpression of S100A2, we observe an enhanced effect of rexinoid and TZD treatment on the melanoma cells.

This observed relative resistance to rexinoid and TZD treatment with shS100A2 was found in conjunction with decreased RXRγ protein levels (though the shS100A2 sequence does not overlap with RXRγ). We have previously shown that decreasing RXRγ by shRXRγ in A375(DRO) decreases response to rexinoid, TZD, and the combination [8]. Modulators of retinoid receptors have been described in melanoma and include HSP 90 and Cyclophilin B [24], but we were unable to find any direct link between S100A2 expression and RXRγ regulation. It appears as if the presence of S100A2 is important for optimal RXRγ expression, but this is most likely not a direct interaction as measurable and at least partially functional RXRγ (as evidenced by response to LGD1069) was seen after shS100A2 infection. Further studies will be needed to elucidate the exact interaction of S100A2 and retinoid receptors.

In summary, we have performed a microarray analysis of a poorly differentiated melanoma after rexinoid and TZD treatment. S100A2 gene expression is significantly increased by both rexinoid and rexinoid treatment alone and is synergistically increased by combination therapy. S100A2 is necessary for the maximal antiproliferative effect of rexinoid and TZD in this model, but it is not sufficient to mediate this effect.

Acknowledgments

The first author is supported by the American Cancer Society MRSG-06-193-01-TBE, Endocrine Fellows Foundation Grant, American Cancer Society, Institutional Research Grant/University of Colorado Cancer Center Fellows Grant. The fourth author is supported by NIH CA100560. This research was made possible by the support of the University of Colorado Cancer Center (UCCC) Microarray Core.

References

[1] C. Garbe and T. K. Eigentler, “Diagnosis and treatment of cutaneous melanoma: state of the art 2006,” Melanoma Research, vol. 17, no. 2, pp. 117–127, 2007.
[2] G. D. Shah and P. B. Chapman, “Adjuvant therapy of melanoma,” Cancer Journal, vol. 13, no. 3, pp. 217–222, 2007.
[3] P. Lorigan, T. Eisen, and A. Hauschild, “Systemic therapy for metastatic malignant melanoma—from deeply disappointing to bright future?” Experimental Dermatology, vol. 17, no. 5, pp. 383–394, 2008.
[4] A. Y. Bedikian, M. M. Johnson, C. L. Warneke, et al., “Prognostic factors that determine the long-term survival of patients with unresectable metastatic melanoma,” Cancer Investigation, vol. 26, no. 6, pp. 624–633, 2008.
[5] J. P. Klopper, W. R. Hays, V. Sharma, M. A. Baumbusch, J. M. Hershman, and B. R. Haugen, “Retinoid X receptor-γ and peroxisome proliferator-activated receptor-γ expression predicts thyroid carcinoma cell response to retinoid and thiazolidinedione treatment,” Molecular Cancer Therapeutics, vol. 3, no. 8, pp. 1011–1120, 2004.
[6] J. P. Klopper, A. Berenz, W. R. Hays, et al., “In vivo and microarray analysis of rexinoid-responsive anaplastic thyroid carcinoma,” Clinical Cancer Research, vol. 14, no. 2, pp. 589–596, 2008.
[7] R. E. Schwegge, J. P. Klopper, C. Korch, et al., “Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification,” Journal of Clinical Endocrinology and Metabolism, vol. 93, no. 11, pp. 4331–4341, 2008.
[8] J. P. Klopper, V. Sharma, A. Berenz, et al., “Retinoid and thiazolidinedione therapies in melanoma: an analysis of differential response based on nuclear hormone receptor expression,” Molecular Cancer, vol. 8, article 16, 2009.
[9] D. Nonaka, L. Chiriboga, and B. P. Rubin, “Differential expression of S100 protein subtypes in malignant melanoma, and benign and malignant peripheral nerve sheath tumors,” Journal of Cutaneous Pathology, vol. 35, no. 11, pp. 1014–1019, 2008.
[10] S. Foser, I. Redwanz, M. Ebeling, C. W. Heizmann, and U. Certa, “Interferon-α and interferon-α co-induce growth inhibition of human tumor cells,” Cellular and Molecular Life Sciences, vol. 63, no. 19-20, pp. 2387–2396, 2006.
[11] B. R. Haugen, L. L. Larson, U. Pugazhenthhi, et al., “Retinoic acid and retinoid X receptors are differentially expressed in thyroid cancer and thyroid carcinoma cell lines and predict response to treatment with retinoids,” Journal of Clinical Endocrinology and Metabolism, vol. 89, no. 1, pp. 272–280, 2004.
[12] A. Muelleri, B. W. Schäfer, S. Ferrari, et al., “The Calcium-binding protein S100A2 interacts with p53 and modulates its transcriptional activity,” Journal of Biological Chemistry, vol. 280, no. 32, pp. 29186–29193, 2005.
[13] I. Salama, P. S. Malone, F. Mihaimeed, and J. L. Jones, “A review of the S100 proteins in cancer,” European Journal of Surgical Oncology, vol. 34, no. 4, pp. 357–364, 2008.
[14] M. Grigorian, S. Andresen, E. Tulchinsky, et al., “Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein: functional consequences of their interaction,” Journal of Biological Chemistry, vol. 276, no. 25, pp. 22699–22708, 2001.
[15] H. Wang, Z. Zhang, R. Li, et al., “Overexpression of S100A2 protein as a prognostic marker for patients with stage I non small cell lung cancer,” International Journal of Cancer, vol. 116, no. 2, pp. 285–290, 2005.
[16] B. Bartling, G. Rebhein, W. D. Schmitt, H.-S. Hofmann, R.-E. Silber, and A. Simm, “S100A2-S100P expression profile and diagnosis of non-small cell lung carcinoma: Impairment by advanced tumour stages and neoadjuvant chemotherapy,” European Journal of Cancer, vol. 43, no. 13, pp. 1935–1943, 2007.
[17] A. Riker, S. A. Enkemann, O. Fodstad, S. Liu, S. Ren, and C. Morris, “The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor
progression and metastasis," *BMC Medical Genomics*, vol. 1, article 13, 2008.

[18] F. Egberts, A. Pollex, J.-H. Egberts, K. C. Kaehler, M. Weichenthal, and A. Hauschild, "Long-term survival analysis in metastatic melanoma: serum S100B is an independent prognostic marker and superior to LDH," *Onkologie*, vol. 31, no. 7, pp. 380–384, 2008.

[19] G. M. Maelandsmo, V. A. Florenes, T. Mellingsaetr, E. Hovig, R. S. Kerbel, and Ø. Fodstad, "Differential expression patterns of S100A2, S100A4 and S100A6 during progression of human malignant melanoma," *International Journal of Cancer*, vol. 74, no. 4, pp. 464–469, 1997.

[20] J. A. Gollob and C. J. Sciambi, "Decitabine up-regulates S100A2 expression and synergizes with IFN-gamma to kill uveal melanoma cells," *Clinical Cancer Research*, vol. 13, pp. 5219–5225, 2007.

[21] R.-Y. Shyu, S.-L. Huang, and S.-Y. Jiang, "Retinoic acid increases expression of the calcium-binding protein S100P in human gastric cancer cells," *Journal of Biomedical Science*, vol. 10, no. 3, pp. 313–319, 2003.

[22] Y. Tsutsui, T. Nogami, M. Sano, A. Kashiwai, and K. Kato, "Induction of S-100b (ββ) protein in human teratocarcinoma cells," *Cell Differentiation*, vol. 21, no. 2, pp. 137–145, 1987.

[23] H.-T. Kim, G. Kong, D. DeNardo, et al., "Identification of biomarkers modulated by the retinoid LGD1069 (Bexarotene) in human breast cells using oligonucleotide arrays," *Cancer Research*, vol. 66, no. 24, pp. 12009–12018, 2006.

[24] B. Richards, J. Karpilow, C. Dunn, et al., "Genetic selection for modulators of a retinoic-acid-responsive reporter in human cells," *Genetics*, vol. 163, no. 3, pp. 1047–1060, 2003.