Biochemical and Biophysical Research Communications 398 (2010) 146–152

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Targeting glutamine metabolism sensitizes melanoma cells to TRAIL-induced death

J.-Z. Qin, H. Xin, B.J. Nickoloff *

Department of Pathology, Loyola University Medical Center, Maywood, IL 60153, USA

ARTICLE INFO

Article history:
Received 11 June 2010
Available online 19 June 2010

Keywords:
Aminooxyacetate
Glutamine
Melanoma
Mcl-1
Noxa
TRAIL

ABSTRACT

Targeting specific metabolic pathways has emerged for cancer therapeutics. For melanoma, metabolic studies have solely focused on high glucose uptake. By contrast, little is known regarding addiction to glutamine. Using five melanoma lines and two normal cell types, addition of aminooxyacetate (AOA), an inhibitor of glutamate-dependent transaminase regulating glutaminolytic pathway, two lines underwent low levels of apoptosis (>30%), while the other three lines were resistant, as were normal cells to AOA. However, three resistant lines (but not normal cells), became sensitized to undergoing apoptosis when TRAIL was combined with AOA. TRAIL by itself had minimal effects on all cell lines and normal cells, and did not augment AOA-induced killing in the two sensitive melanoma lines. AOA plus TRAIL induced a caspase-dependent apoptotic response. AOA did not influence TRAIL DR4 or DR5 cell surface death receptor levels, but AOA enhanced pro-apoptotic protein levels of Noxa, while reducing pro-survival protein Mcl-1. To verify AOA was targeting glutamine pathway, depletion of glutamine produced similar results, because absence of glutamine sensitized three melanoma lines, but not fibroblasts to killing by TRAIL. Glutamine depletion also led to Noxa induction. These results indicate some lines are addicted to glutamine, and treatment with AOA or glutamine depletion sensitizes melanoma to TRAIL-mediated killing, while sparing normal cells. Future studies are indicated to translate these discoveries to metastatic melanoma as there is currently no treatment available to prolong survival.

1. Introduction

Glutamine metabolism, like glucose metabolism is abnormal in cancer cells; and glutamine usage in cancer cells is very inefficient [1]. Even though glutamine is the most common amino acid, no previous reports focus on glutamine metabolism in melanoma cells from a therapeutic perspective as regards using antagonists combined with death receptor ligands. Metastatic melanoma is a highly aggressive malignancy, and is nearly always fatal, with a median survival of only 6–9 months once distant sites become seeded from skin [2]. While dacarbazine (DTIC) is the only FDA approved chemotherapeutic agent for metastatic melanoma (a tumor response of 5–20%), many other therapeutic approaches have been tried, including multi-agent chemotherapy, radiation therapy, and immunotherapy. One important obstacle in identification of therapeutic approaches in the past has been the relatively poorly understood biochemical pathways that govern proliferation and survival pathways of melanoma cells. Given rapid progress in understanding of the biology of melanoma cells, new opportunities have arisen for investigative skin biologists and clinicians to attack this deadly neoplasm [3].

Because of the notorious difficulty in killing melanoma by targeting a single pathway, it is reasonable to consider using a combination of agents that target different pathways to achieve enhanced beneficial results. Recently, a growing body of literature has shifted from conventional emphasis on cell proliferation and resistance to apoptosis of tumor cells to focus on cancer cell metabolomics [4,5]. In this report, we target glutamine metabolism and use a two agent strategy; first focusing on aminooxyacetate (AOA) interfering with glutamine metabolism, and combining AOA with tumor necrosis factor-related apoptosis inducing-ligand (TRAIL). To further emphasize the glutamine addiction pathway, cells were also studied using medium depleted of glutamine.

As there are two major apoptotic pathways (i.e. so-called extrinsic or direct/death receptor-mediated, and the intrinsic/indirect or mitochondrial-based) responsible for mediating death of tumor cells [6], we sought to determine if enhanced killing of melanoma cells addicted to glutamine could be accomplished by activating the extrinsic death pathway. Activating the extrinsic death pathway using TRAIL in combination with AOA, overcame the apoptotic resistance of several of the melanoma cell lines; while leaving two different normal cell types (e.g. melanocytes and fibroblasts) unscathed. Enhanced killing by AOA plus TRAIL was mediated by caspases because a pan-caspase inhibitor

* Corresponding author. Address: Oncology Institute, Department of Pathology, Cardinal Bernardin Cancer Center, Loyola University Medical Center, Bldg. 112, Room 301, 2160 S. First Ave., Maywood, IL 60153, USA. Fax: +1 708 327 3239.
E-mail address: bnickol@lumc.edu (B.J. Nickoloff).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.06.057

(e.g. ZVAD) reversed the cell death, and also involved damaging the outer mitochondrial membrane with increased permeabilization and induction of Noxa.

Taken together, the current findings indicate that overcoming the notorious resistance of melanoma to conventional therapy is achievable, by combining AOA together with TRAIL, or by using glutamine depletion plus TRAIL [7]. Further studies are warranted as regards altered melanoma metabolomics and new therapeutic agents targeting glutamine metabolism are emerging and are likely to be exploited for consideration in future clinical trials [8].

2. Materials and methods

2.1. Cell cultures and reagents

Five human melanoma cell lines OCM1A, Mum2C, A375, UACC3093, and c8161 cells were grown in either DMEM medium (OCM1A, A375 cells), or RPMI-1640 medium (all other cell lines). Both conventional and glutamine-free RPMI-1640 medium and DMEM medium were purchased from Lonza (Walkersville, MD) and supplemented with 10% FCS (Gemini Bio-Products, Woodland, CA). Normal adult human skin-derived melanocytes were purchased from Lonza, and grown in the medium provided by the company as previously described [9]. AOA and propidium iodide were purchased from Sigma (St. Louis, MO). Both recombinant human TRAIL and Annexin-V–FITC were obtained from Biovision Research Products (Mountain View, CA). Mouse monoclonal abs against TRAIL receptors (DR4, DR5) and fibroblasts were used as previously described [10]. All cells were maintained in a humidified incubator under standard conditions (37 °C, 5% CO_2). To detect and quantify cell death receptor expression, melanoma lines were treated with AOA (1 mM) for 24 h. After harvesting, cells were incubated with specific abs detecting human DR4 or DR5 at 5 μg/ml for 1 h. Cells were washed with PBS and incubated with Alexa-488 labeled goat anti-mouse IgG (Invitrogen, Carlsbad, CA) for 0.5 h followed by FACS analysis.

2.2. Cell treatment and assessment of cell death

Cells were plated into 24-well plates overnight, and on the second day cells were pre-treated with AOA at different concentrations (0.1, 0.5, 1 mM) for 0.5 h followed by adding recombinant human TRAIL (100 ng/ml) for 24 h. Deprivation of glutamine was accomplished by washing cells with PBS, and replacing conventional medium with glutamine-free medium (Lonza) plus 10% dialyzed FCS. TRAIL was added at 1 h after cells were maintained in glutamine-free medium. Cell death was assessed by flow cytometry after staining with Annexin-V–FTIC and 1 μg/ml propidium iodide (Sigma). Both Annexin-V positive, and Annexin-V positive plus PI positive, or only PI positive cells, were considered as dead cells.

2.3. Mitochondrial membrane potential assay

Cells were harvested (either untreated or treated) and incubated with fresh medium containing 5 μg/ml of Rhodamine 123, a fluorescent dye that selectively accumulates in mitochondria of living cells with normal $\Delta\psi_m$ for 0.5 h. Cells were washed with PBS to remove excess probe, and analyzed by FACS. Reduction of Rhodamine 123 fluorescence intensity indicates loss of mitochondrial membrane potential.

2.4. Immunoblot assay

Cells were harvested by scraping and lysed with M-Per Mammalian Protein Extraction Reagent (Thermo Scientific, Rockville, IL) supplemented with protease inhibitor cocktail (Roche Diagnostics GmbH) and phosphatase inhibitor cocktail set II (Calbiochem, Los Angeles, CA), followed by shaking and centrifugation at 4 °C. Supernatants were collected as whole cell extracts and protein concentrations were measured using Bradford reagents (Bio-Rad Laboratories, Hercules, CA). Thirty micrograms of protein were resolved by SDS–PAGE and transferred to PVDF membrane followed by 1 h blocking buffer supplied by Li-COR Biosciences (Lincoln, NE). Blots were probed with primary abs overnight at 4 °C, washed and incubated with fluorescence-labeled secondary abs for 1 h at room temperature in dark. Protein levels on the membrane were visualized with Li-COR Infrared Imaging System. Abs used were obtained as follows: caspase 3, GAPDH, Mcl-1, Bcl-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal ab against Noxa and caspase 8 were from purchased from Calbiochem (San Diego, CA) and Fischer Scientific (Pittsburgh, PA), respectively. Ab against Bid was purchased from Cell Signaling (Beverly, MA).

2.5. RT-PCR

Total RNAs were isolated from cells with TRIzol (Invitrogen). One microgram of total RNA was converted to cDNA using reverse transcription reagents (Applied Biosystems, Foster City, CA). PCR was performed with platinum Taq DNA polymerase (Invitrogen). The following specific primer pairs were used: Noxa: 5-AGA TGC CTG GGA AGA AG-3 (forward), 5-AGT CCC CTC ATG CAA GT-3 (reverse); Mcl-1: 5-CGG TAA TCG GAC TCA A CCTC-3 (forward), 5-CCT CCT TCT CCG TAG CCA A-3 (reverse); 18s RNA: 5-GGC GCC CCC TCG ATG CTC TTA G-3, (forward), 5-GCT CGG GCC TGC TTT GAA CAC TCT-3 (reverse). PCR products were separated by electrophoresis using 1.5% agarose gels and visualized with ethidium bromide.

2.6. Statistical analysis

Statistical analysis was performed using unpaired, two sided Student's *t*-test, and results considered significant when p values <0.05.

3. Results

3.1. Differential AOA-mediated cytotoxicity of melanoma lines, melanocytes and fibroblasts in the absence and presence of TRAIL

The sensitivity of melanoma lines to AOA alone (0.1, 0.5, and 1 mM, 24 h) or TRAIL alone (100 ng/ml), or combination of AOA plus TRAIL, revealed three melanoma lines (A375, c8161, and UACC3093 cells) relatively resistant to killing by AOA alone (Fig. 1A), while two melanoma lines (OCM1A and Mum2C cells) were killed by AOA alone (Fig. 1B). The sensitivity of melanoma lines to TRAIL alone revealed increased killing ranging from approximately 10% to 40% among the AOA resistant cell lines (Fig. 1A); but complete resistance in the AOA sensitive lines (Fig. 1B). Interestingly, when AOA and TRAIL were combined, a concentration-dependent enhanced killing by TRAIL occurred when AOA was present at 0.5 and 1 mM concentrations (Fig. 1A, double asterisks). However, in two cell lines (OCM1A and Mum2C cells), co-presence of AOA and TRAIL did not enhance overall killing (Fig. 1B). To examine potential cytotoxicity for AOA and TRAIL on normal cells, similar experiments were performed using melanocytes and fibroblasts (Fig. 1C). Neither AOA alone (1 mM) or TRAIL alone, or combination of AOA plus TRAIL triggered significant killing of normal cells. Thus, combining AOA plus TRAIL showed promise as we could achieve significant killing (>60% of total population) in three different melanoma cell lines, without harming two different normal cells.

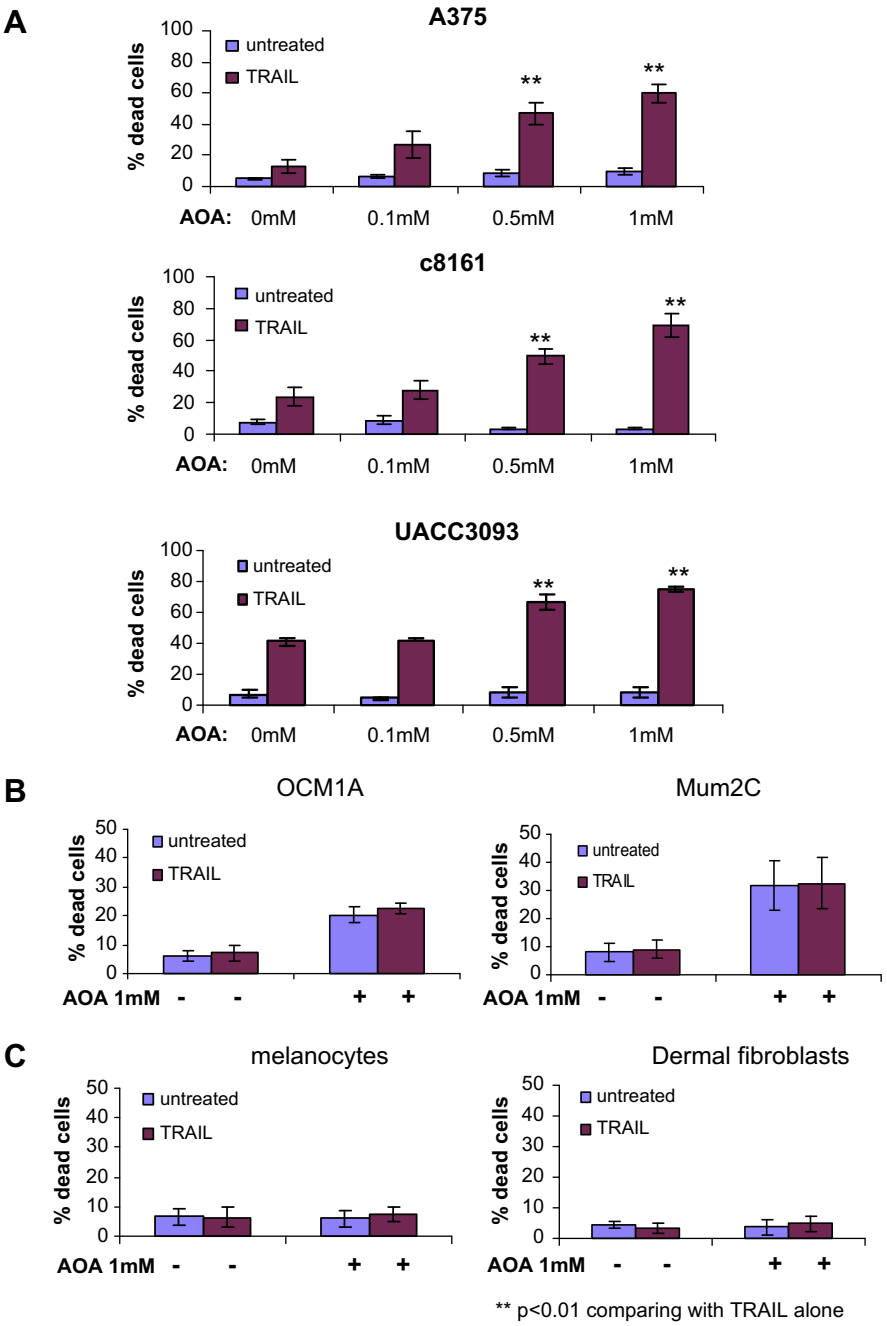

Fig. 1. Characterization of the cytotoxic response to AOA and/or TRAIL by melanoma cell lines and normal cells. (A) Three melanoma cell lines (A375, c8161, and UACC3093 cells) were not significantly killed by AOA alone treatment (0.1, 0.5, and 1 mM, 24 h), but varying levels of cell death were induced by TRAIL alone treatment (100 ng/ml, 24 h) killing between 10%, 20%, and 40% of the total cell population in A375, c8161, and UACC3093 cell lines, respectively. The presence of AOA sensitized these melanoma lines to enhanced killing by TRAIL in a concentration-dependent fashion. $^{**}p < 0.01$ comparing with TRAIL alone. (B) Two melanoma cell lines (OCM1A and Mum2C) were resistant to killing by TRAIL (100 ng/ml, 24 h) when used alone, but were sensitive to killing by treatment with AOA alone (1 mM, 24 h); however, no further increase in killing was achieved when AOA and TRAIL were combined. (C) Two normal cell types (melanocytes and fibroblasts), were not killed by either AOA (1 mM) or TRAIL (100 ng/ml) when used singly or in combination.

3.2. Characterization of mode of cell death by AOA, TRAIL, and AOA plus TRAIL

Using Annexin-V/PI staining combined with flow cytometry, two cell lines were examined (A375 and c8161 cells) before and after treatment with either TRAIL alone or AOA plus TRAIL (Fig. 2A). While there was minimal spontaneous death among A375 cells (approximately 4%) or c8161 cells (approximately 5%); addition of TRAIL alone increased killing to 7% and 12%, respectively. However, the presence of AOA increased the sensitivity to TRAIL in the absence of a caspase inhibitor (no ZVAD) in A375 cells to 34%, and in c8161 cells to 47%. When a pan-caspase inhibitor was added (plus ZVAD), the percentage of dead cells using AOA plus TRAIL was completely reverted to untreated levels. To probe mechanistically into the cell death triggered by AOA and/or TRAIL, immunoblots for caspases 8 and 3, as well as Bid levels were examined before and after treatment (Fig. 2B). Full length caspase 8 representing the inactive form was present in all three untreated melanoma lines examined (e.g. A375, c8161, and UACC3093 cells), but was reduced to the indicated levels (signifying activation) by

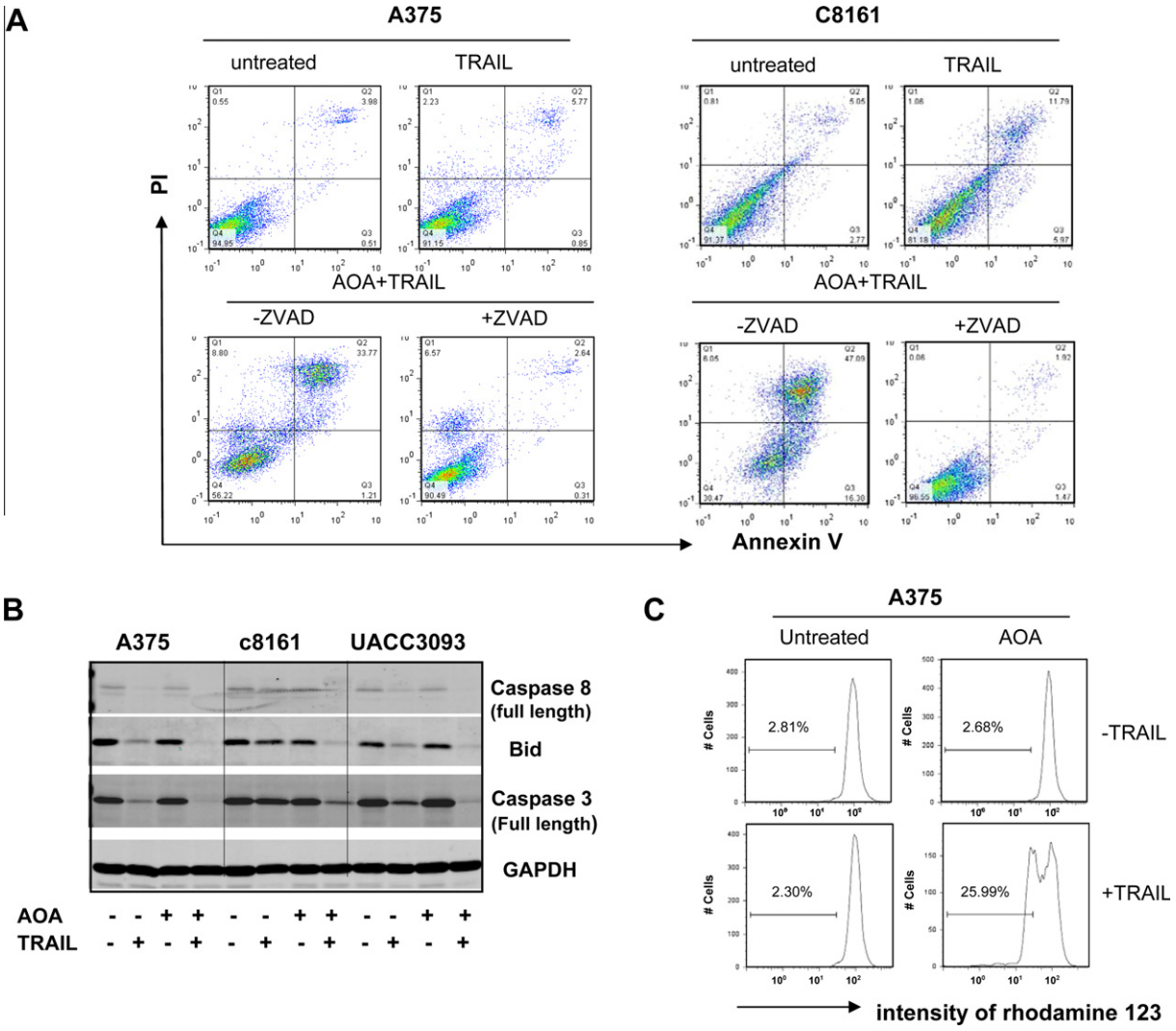

Fig. 2. Treatment of melanoma cell lines by AOA and/or TRAIL induced a caspase-dependent killing accompanied by: activation (cleavage) of caspases 8, 3, and Bid; as well as by alteration in mitochondria. (A) Representative profile of FACS analysis after Annexin-V/PI staining using two sensitive cell lines (A375 and c8161 cells) demonstrating that killing by AOA plus TRAIL (as described in Fig. 1) is caspase dependent because the presence of the pan-caspase inhibitor, ZVAD, blocked the cell killing for both cell lines. (B) Western blots of three melanoma cell lines (A375, c8161, and UACC3093 cells) reveals constitutive presence of full length (inactive) caspases 8 and 3, as well as Bid in untreated cells, but varying levels of activation (cleavage) for these pro-death mediators following treatment with AOA and/or TRAIL as described in Fig. 1 with the greatest activation occurring in all three melanoma cell lines when AOA is combined with TRAIL. GAPDH levels indicate equivalent loading. (C) The assessment of mitochondrial functional status in A375 cells reveals minimal abnormality in retention of Rhodamine 123 dye in untreated cells as well as cells treated with either AOA or TRAIL alone, but almost 10-fold increase in cells unable to retain the dye consistent with dying cells when AOA is combined with TRAIL.

the specified treatments. Similar changes in the activation patterns for Bid and caspase 3 were observed in the three melanoma lines; with the most consistent and strongest activation triggered by the combination of AOA plus TRAIL. These findings are consistent with the ability of ZVAD to block AOA plus TRAIL-mediated killing (Fig. 2A), and reveal participation of caspases 8, 3, and Bid in the killing process. To explore the integrity of mitochondrial function, A375 melanoma cells were examined before and following AOA and/or TRAIL treatment using a dye (Rhodamine 123) that is taken up and retained within intact mitochondria. Before treatment the fluorescence was clearly contained within the intact mitochondria (approximately 3% had leaked out of the mitochondria); and while AOA alone or TRAIL alone did not alter dye levels (approximately 2–3%), when AOA plus TRAIL was used, significant lack of retention of the dye was observed (26%) indicating loss of mitochondrial membrane potential. Taken together, these results indicate that AOA definitely sensitizes melanoma lines to killing which involves both activation of caspase cascades and mitochondrial damage.

3.3. Characterization of death receptors as well as pro-survival vs. pro-death molecules

To determine if AOA sensitized cells to killing by TRAIL involved modulation of the levels of the two cell surface death receptors DR4 and DR5, A375, and c8161 melanoma lines were examined by flow cytometry using specific abs to detect each receptor (Fig. 3A). Compared to isotype control cell surface staining, untreated melanoma cells expressed both DR4 and DR5; however, no significant change in the surface levels for either death receptor for TRAIL was detected by treatment with AOA. Thus, we sought to determine if AOA and/or TRAIL could impact key pro-survival proteins such as Bcl-2 and Mcl-1, or pro-death molecule, Noxa [11]. The constitutive levels for both of the pro-survival proteins (Bcl-2 and Mcl-1) was easily detectable by immunoblotting in all three melanoma lines (A375, c8161, and UACC3093 cells) as displayed in Fig. 3B. Upon exposure to AOA and/or TRAIL, variable decreases in these pro-survival proteins were observed, with the

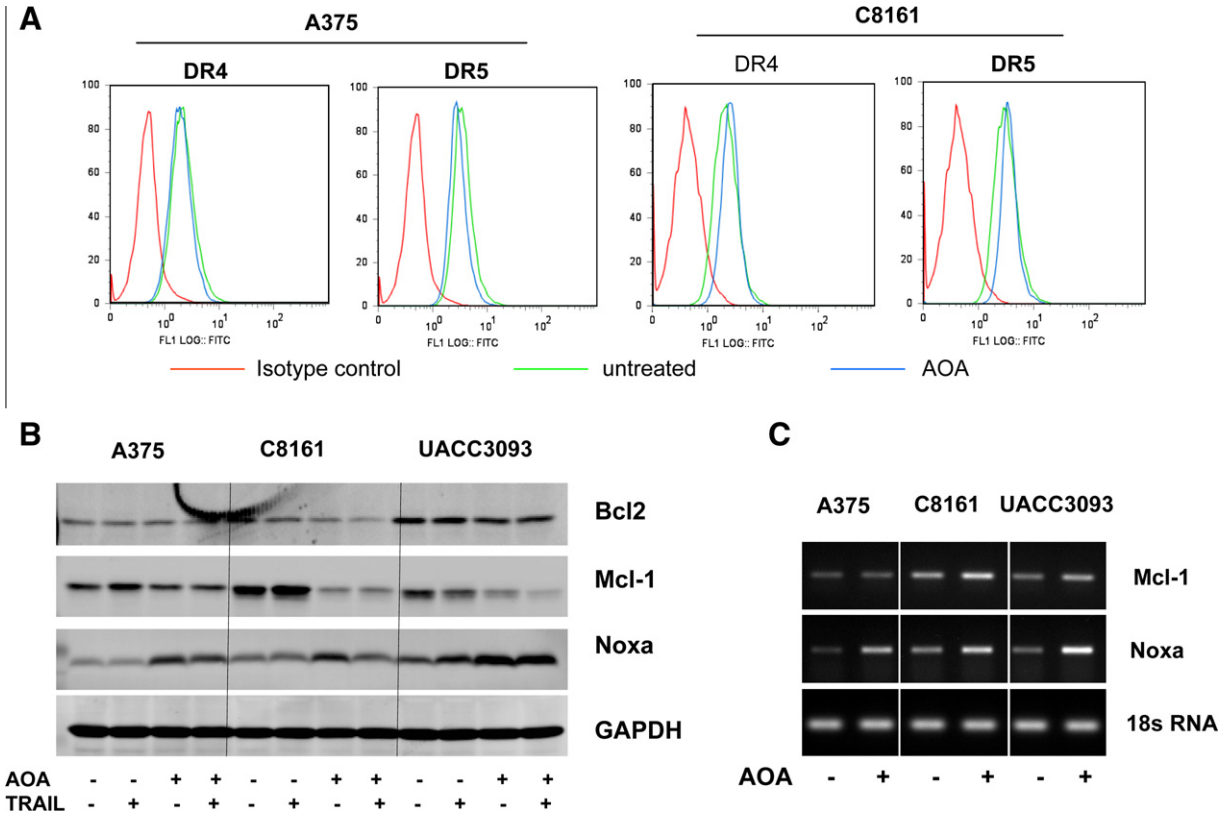

Fig. 3. Treatment of melanoma cells with AOA and/or TRAIL does not influence surface DR expression, but does alter levels of proteins regulating cell survival and cell death including induction of Noxa by AOA at the protein and mRNA level. (A) Analysis of DR4 and DR5 cell surface expression by FACS indicates constitutive levels for both TRAIL-related receptors (compare untreated profiles using isotype control) in both melanoma cell liens (A375 and c8161 cells), but no increase in either DR4 or DR5 levels following treatment with AOA (1 mM, 24 h). (B) Western blots of three melanoma cell lines (A375, c8161, and UACC3093 cells) reveals constitutive presence of pro-survival proteins Bcl-2 and Mcl-1; as well as lower levels for Noxa in untreated cells, but varying levels of reduction for the pro-survival proteins accompanied by increases in Noxa levels following treatment with AOA; compared and contrasted to levels with either TRAIL alone treatment or AOA plus TRAIL as described in Fig. 1. GAPDH levels indicate equivalent loading. (C) RT-PCR analysis of mRNA levels for Mcl-1 and Noxa in three melanoma lines reveals AOA alone treatment has minimal effects on the level of Mcl-1 mRNA, but consistently induced or increased Noxa mRNA levels. 18s RNA signal indicates equivalent reaction conditions.

greatest decreases achieved by AOA plus TRAIL. By contrast the absent to low constitutive levels for the pro-death molecule, Noxa was increased particularly by AOA alone in all three melanoma lines. Relative mRNA level encoding these proteins was determined by RT-PCR using the same three cell lines before and after AOA treatment (Fig. 3C). While the levels of mRNA for Mcl-1 did not decrease to the same levels as the protein changes (most likely reflecting the known short half-life of the protein and degradation by the proteasome), there was a good correlation between Noxa mRNA and protein levels induced by AOA.

3.4. Glutamine depletion and response to TRAIL-induced killing

To confirm and extend the role for AOA at targeting glutamine metabolism, medium devoid of glutamine was utilized and both the relative addiction to glutamine, as well as the ability of glutamine depletion to sensitize to TRAIL, was determined for both malignant and normal cells. Compared to when glutamine was present in the medium, the depletion of glutamine greatly sensitized three melanoma cell lines (A375, c8161, and UACC3093 cells) to TRAIL-induced killing (Fig. 4A, double asterisks). Since the melanocytes use a proprietary medium, we could not deplete glutamine, and hence used fibroblasts that could be grown in a glutamine deficient medium. Even when glutamine was depleted from fibroblasts (Fig. 4C), no significant increase in killing was observed, consistent with data presented in Fig. 1C. Analogous to the approach used in Fig. 3B and C, the three melanoma lines and fibroblasts were analyzed by immunoblot and RT-PCR assays, respectively, in the presence and absence of glutamine. In general, glutamine depletion caused reduction in the two pro-survival proteins less consistently than AOA, and shared with AOA differences between protein and mRNA levels for Mcl-1. However, glutamine depletion did induce Noxa in several melanoma lines, but not fibroblasts at the protein level, and induced Noxa mRNA in all three melanoma lines. To our knowledge, this is the first time either AOA or glutamine depletion has been shown to trigger Noxa induction in melanoma cells. The lack of induction in normal fibroblasts further enhances enthusiasm for pursuing this approach that results in selective killing of malignant cells, while leaving normal cells unscathed.

4. Discussion

Hearing the words "malignant melanoma" or worse "metastatic melanoma" is never good for patients. The incidence of melanoma is on the rise [12], as is the number of individuals dying from metastatic melanoma [13]. One of the contributing factors facing patients and investigators regarding poor prognosis is the difficulty in rationally designing therapeutic agents due to the complexity of genetic alterations acquired during melanoma progression [14,15]. However, by focusing on the alterations related to glutamine metabolism, we have identified an Achilles' heel [5] by which either AOA or glutamine depletion can sensitize melanoma cells to killing by TRAIL. While TRAIL-related clinical trials are numerous [16], and TRAIL can usually distinguish between normal and malig-

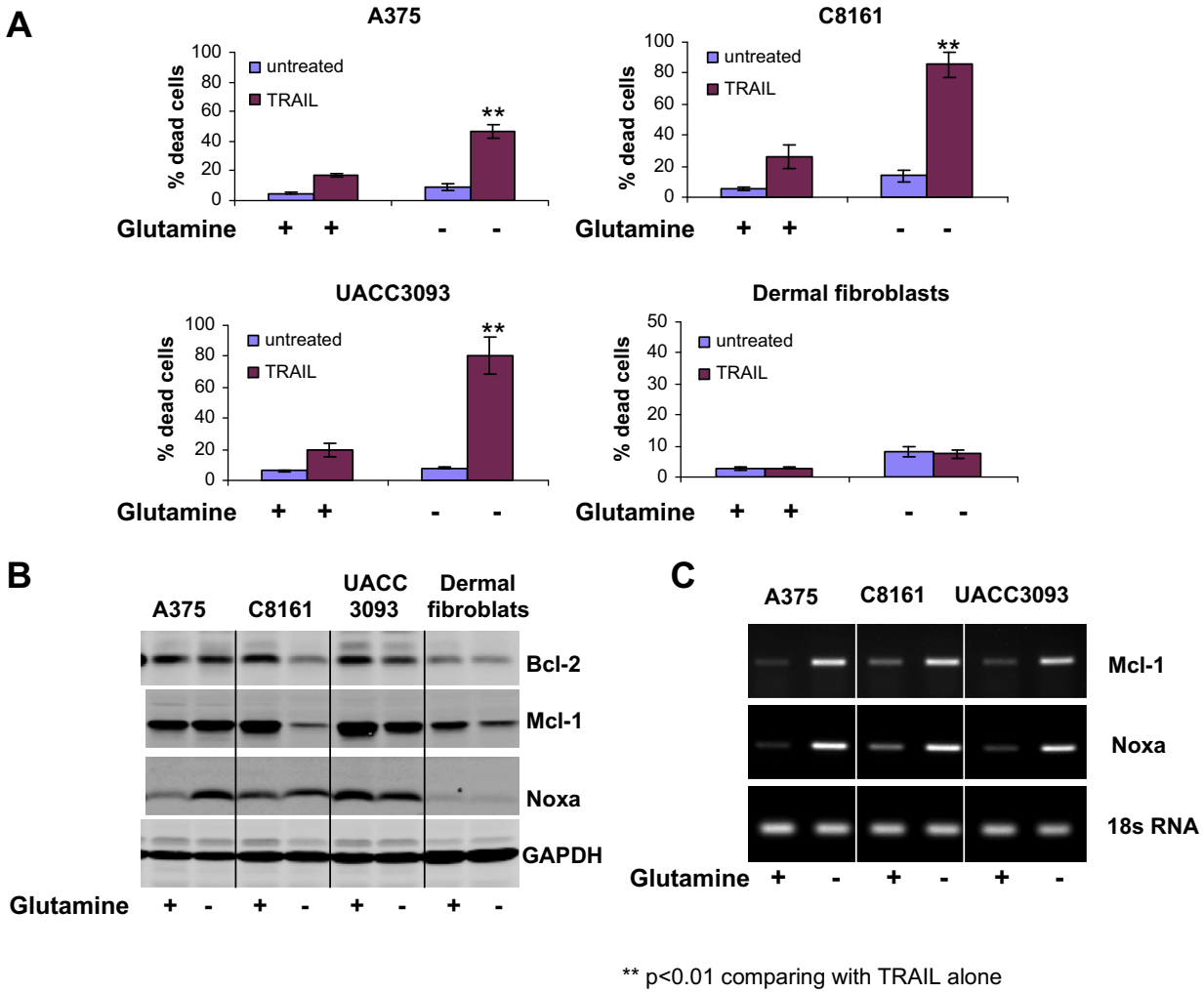

Fig. 4. Glutamine depletion also sensitized melanoma cells, but not fibroblasts to TRAIL-mediated killing, accompanied by variable decreases in Bcl-2 and Mcl-1 levels, and variable increases in Noxa levels by immunoblot and RT-PCR analysis. (A) Three melanoma cell lines (A375, c8161, and UACC3093 cells) were not significantly killed by glutamine depletion alone, but varying levels of cell death were induced by TRAIL alone treatment (as observed in Fig. 1), but glutamine depletion sensitized these melanoma lines to enhanced killing by TRAIL, with no cytotoxicity observed in fibroblasts. $^{**}p < 0.01$ comparing with TRAIL alone. (B) Relative protein levels for Bcl-2, Mcl-1, and Noxa were determined using three melanoma cell lines and fibroblasts in the presence and after depletion of glutamine from the medium reveals varying decreases in some melanoma lines for pro-survival protein levels, and varying degrees of increases in Noxa levels. *Note:* there is no induction of Noxa in the fibroblast, which are not killed by glutamine depletion. GAPDH levels confirm equivalent loading. (C) Relative mRNA levels for Mcl-1 and Noxa in three melanoma cell lines maintained in either glutamine containing or glutamine depleted medium. By RT-PCR analysis, glutamine depletion increased both Mcl-1 as well as Noxa mRNA levels. 18s RNA signal indicates equivalent reaction conditions.

nant cells via differential glycosylation events [17], the current results clearly point to a novel method to enhance the effectiveness of TRAIL by combining it with either AOA or glutamine depletion. It should be noted that first generation glutamine antagonists are neurotoxic, but other agents with better safety profiles are on the horizon [1].

The current findings provide a rationale for using AOA or glutamine antagonists in combination with TRAIL because there is a caspase-dependent killing which includes also damaging the mitochondrial selectively in melanoma cells, but not normal cells. While previous investigators, including ourselves, devoted considerable time and effort to identify agents that can induce the potent and pro-death molecule Noxa [11,18–21], the current results demonstrating the ability of AOA and glutamine depletion to induce Noxa at the mRNA and protein levels are novel.

The additional molecular mechanisms mediating these cytotoxic reactions are likely to be complex and require additional investigation, but there does not appear to be any role for changes in the levels of either of the two different cell surface death receptors for TRAIL to explain the ability of AOA to sensitize some of the melanoma lines to killing by TRAIL. This is in contrast to the ability of 2-deoxy-D-glucose (an antagonist of glucose, rather than glutamine, metabolism) to enhance TRAIL-mediated killing of melanoma cells via up regulation of TRAIL death surface receptors [22]. Another opportunity for future studies is to delineate what factors contribute to the resistance of some melanoma cell lines to becoming sensitized by either AOA or glutamine depletion to subsequent killing by TRAIL.

In conclusion, these results reinforce the concept that melanoma associated metabolomics alterations can be exploited therapeutically by focusing on glutamine metabolism in combination with TRAIL. Thus, AOA is worthy of further consideration for preclinical studies, as well as other glutamine antagonists, especially in combination with TRAIL. Overcoming the notorious resistance of melanoma cells to killing presents many challenges, but rationally designed combination therapy is most likely the path forward to provide new therapeutic windows of opportunities for patients suffering from metastatic melanoma.

Acknowledgment

This work was supported by Institutional Funding.

References

[1] W.G. Kaelin Jr., C.B. Thompson, Q&A: Cancer: clues from cell metabolism, Nature 465 (2010) 562–564.
[2] C.M. Balch, A.C. Buzaid, S.J. Soong, M.B. Atkins, N. Cascinelli, D.G. Coit, I.D. Fleming, J.E. Gershenwald, A. Houghton Jr., J.M. Kirkwood, K.M. McMasters, M.F. Mihm, D.L. Morton, D.S. Reintgen, M.I. Ross, A. Sober, J.A. Thompson, J.F. Thompson, Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma, J. Clin. Oncol. 19 (2001) 3635–3648.
[3] M. Herlyn, M. Padarathsingh, L. Chin, M. Hendrix, D. Becker, M. Nelson, Y. DeClerck, J. McCarthy, S. Mohla, New approaches to the biology of melanoma: a workshop of the National Institutes of Health Pathology B Study Section, Am. J. Pathol. 161 (2002) 1949–1957.
[4] R.G. Jones, C.B. Thompson, Tumor suppressors and cell metabolism: a recipe for cancer growth, Genes Dev. 23 (2009) 537–548.
[5] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer Cell 13 (2008) 472–482.
[6] L. Aravind, V.M. Dixit, E.V. Koonin, Apoptotic molecular machinery: vastly increased complexity in vertebrates revealed by genome comparisons, Science 291 (2001) 1279–1284.
[7] D. Pathania, M. Millard, N. Neamati, Opportunities in discovery and delivery of anticancer drugs targeting mitochondria and cancer cell metabolism, Adv. Drug Deliv. Rev. 61 (2009) 1250–1275.
[8] H. Pelicano, D.S. Martin, R.H. Xu, P. Huang, Glycolysis inhibition for anticancer treatment, Oncogene 25 (2006) 4633–4646.
[9] J.Z. Qin, H. Xin, B.J. Nickoloff, 3-Bromopyruvate induces necrotic cell death in sensitive melanoma cell lines, Biochem. Biophys. Res. Commun. 396 (2010) 495–500.
[10] J.Z. Qin, P. Bacon, J. Panella, L.A. Sitailo, M.F. Denning, B.J. Nickoloff, Low-dose UV-radiation sensitizes keratinocytes to TRAIL-induced apoptosis, J. Cell. Physiol. 200 (2004) 155–166.
[11] J.Z. Qin, L. Stennett, P. Bacon, B. Bodner, M.J. Hendrix, R.E. Seftor, E.A. Seftor, N.V. Margaryan, P.M. Pollock, A. Curtis, J.M. Trent, F. Bennett, L. Miele, B.J. Nickoloff, P53-independent NOXA induction overcomes apoptotic resistance of malignant melanomas, Mol. Cancer Ther. 3 (2004) 895–902.
[12] E. Linos, S.M. Swetter, M.G. Cockburn, G.A. Colditz, C.A. Clarke, Increasing burden of melanoma in the United States, J. Invest. Dermatol. 129 (2009) 1666–1674.
[13] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray, M.J. Thun, Cancer statistics, 2008, CA: Cancer J. Clin. 58 (2008) 71–96.
[14] C. Haqq, M. Nosrati, D. Sudilovsky, J. Crothers, D. Khodabakhsh, B.L. Pulliam, S. Federman, J.R. Miller III, R.E. Allen, M.I. Singer, S.P. Leong, B.M. Ljung, R.W. Sagebiel, M. Kashani-Sabet, The gene expression signatures of melanoma progression, Proc. Natl. Acad. Sci. USA 102 (2005) 6092–6097.
[15] A.N. Houghton, D. Polsky, Focus on melanoma, Cancer Cell 2 (2002) 275–278.
[16] A. Ashkenazi, Directing cancer cells to self-destruct with pro-apoptotic receptor agonists, Nat. Rev. Drug Discov. 7 (2008) 1001–1012.
[17] K.W. Wagner, E.A. Punnoose, T. Januario, D.A. Lawrence, R.M. Pitti, K. Lancaster, D. Lee, M. von Goetz, S.F. Yee, K. Totpal, L. Huw, V. Katta, G. Cavet, S.G. Hymowitz, L. Amler, A. Ashkenazi, Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL, Nat. Med. 13 (2007) 1070–1077.
[18] Y. Fernandez, M. Verhaegen, T.P. Miller, J.L. Rush, P. Steiner, A.W. Opipari Jr., S.W. Lowe, M.S. Soengas, Differential regulation of noxa in normal melanocytes and melanoma cells by proteasome inhibition: therapeutic implications, Cancer Res. 65 (2005) 6294–6304.
[19] C. Ploner, R. Kofler, A. Villunger, Noxa: at the tip of the balance between life and death, Oncogene 27 (Suppl. 1) (2008) S84–S92.
[20] J.Z. Qin, J. Ziffra, L. Stennett, B. Bodner, B.K. Bonish, V. Chaturvedi, F. Bennett, P.M. Pollock, J.M. Trent, M.J. Hendrix, P. Rizzo, L. Miele, B.J. Nickoloff, Proteasome inhibitors trigger NOXA-mediated apoptosis in melanoma and myeloma cells, Cancer Res. 65 (2005) 6282–6293.
[21] J.Z. Qin, H. Xin, L.A. Sitailo, M.F. Denning, B.J. Nickoloff, Enhanced killing of melanoma cells by simultaneously targeting Mcl-1 and NOXA, Cancer Res. 66 (2006) 9636–9645.
[22] H. Liu, C.C. Jiang, C.J. Lavis, A. Croft, L. Dong, H.Y. Tseng, F. Yang, K.H. Tay, P. Hersey, X.D. Zhang, 2-Deoxy-D-glucose enhances TRAIL-induced apoptosis in human melanoma cells through XBP-1-mediated up-regulation of TRAIL-R2, Mol. Cancer 8 (2009) 122.