Published in final edited form as:

Mol Carcinog. 2018 May ; 57(5): 678–683. doi:10.1002/mc.22786.

Targeting insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in metastatic melanoma to increase efficacy of BRAF^{V600E} inhibitors

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Abstract

Melanoma is one of the deadliest forms of skin cancer. Although BRAF inhibitors significantly enhance survival of metastatic melanoma patients, most patients relapse after less than a year of treatment. We previously reported that mRNA binding protein Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is overexpressed in metastatic melanoma and that expression of IGF2BP1 confers resistance to chemotherapeutic agents. Here we demonstrate that IGF2BP1 plays an important role in the sensitivity of melanoma to targeted therapy. Inhibition of IGF2BP1 enhances the effects of BRAF-inhibitor and BRAF-MEK inhibitors in BRAF^{V600E} melanoma. Also, knockdown of IGF2BP1 alone is sufficient to reduce tumorigenic characteristics in vemurafenib-resistant melanoma. These findings suggest that IGF2BP1 can be a novel therapeutic target for melanoma.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Keywords

CRD-BP; IGF2BP1; IMP1; resistance to targeted therapies; RNA-binding protein

1 | INTRODUCTION

Melanoma accounts for a majority of deaths from skin cancers. Although early stage melanomas are usually curable, the survival rate of patients with metastatic melanoma is significantly diminished. Aberrant MAPK activation is commonly found in melanoma due to missense mutations of BRAF, especially BRAF^{V600E} as the most common type of mutation.¹ Vemurafenib is an inhibitor of BRAF^{V600E} and its administration significantly increased overall and progression-free survival of melanoma patients.² However, most patients relapsed after 6 months of treatment and became resistant to second-line therapy.³ While the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib significantly improved overall survival compared with vemurafenib monotherapy in previously untreated patients with metastatic melanoma with BRAF V600E or V600 K mutations, median progression-free survival is still less than 1 year.⁴ Acquired resistance to BRAF inhibitors is caused by a variety of alterations in the MAPK and other signaling pathways,^{5–9} and targeting all possible mechanisms to overcome resistance becomes a daunting challenge. Therefore, identification of a novel target that affects pro-survival and drug resistance pathways is needed to overcome acquired resistance.

IGF2BP1 is a multifunctional RNA-binding protein. Its overexpression in metastatic melanoma affects a variety of proto-oncogenes and oncogenic signaling pathways leading to tumor progression, survival, and resistance to chemotherapy.^{10–12} In this report, we describe the role of IGF2BP1 in the sensitivity of melanoma to selective BRAF^{V600E} inhibitors.

2 | RESULTS AND DISCUSSION

In our experiments, IGF2BP1 inhibition using shRNA specific to IGF2BP1 (shIGF2BP1) significantly reduced growth of melanoma cells (Figure 1). Compared to BRAF^{WT} cells (skmel2), BRAF^{V600E} (skmel28, 451Lu, MRA6) melanoma cell lines were sensitive to vemurafenib although response to vemurafenib in the presence of IGF2BP1 inhibition occurred at higher doses in 451 Lu compared to skmel28 and MRA6. Inhibition of cell growth in skmel28 and MRA6 was significant from 0.5 μ M of vemurafenib whereas 451 Lu was significant from the 5 μ M dose level. Inhibition of IGF2BP1 also significantly suppressed cell growth in BRAF^{V600E} cell lines, and combination of vemurafenib and shIGF2BP1 further suppressed cell growth compared to single inhibition of IGF2BP1 or BRAF (Figure 1B-D), and synergistic effect of vemurafenib and IGF2BP1 knockdown was shown in 451 Lu and A375 ($P < 0.001$ and $P = 0.004$, respectively) (Figures 1C and 2A, Supplementary Figures S1).

To further investigate how expression of IGF2BP1 affects cell growth of melanoma, we studied cell proliferation, cell cycle, and apoptosis. Compared to its effects in BRAF^{WT} skmel2 cells (0.8 fold decrease), vemurafenib drastically (>three-fold) inhibited cell proliferation in BRAF^{V600E} 451 Lu cells. Knockdown of IGF2BP1 also reduced cell

proliferation by half and inhibition of both BRAF and IGF2BP1 reduced cell proliferation by 10-fold compared to the control (Supplementary Figures S2A). Similarly, inhibition of BRAF or IGF2BP1 in 451 Lu cells resulted in more cells in G1 phase and less cells in S phase, as compared to skmel2 cells (Supplementary Figures S2B). Furthermore, combination of vemurafenib and shIGF2BP led to the greatest G1 phase arrest and the least cells in S phase compared to the inhibition of either BRAF or IGF2BP1 alone. In skmel2, inhibition of IGF2BP1, regardless of vemurafenib treatment, increased apoptosis by 3-4 fold (Supplementary Figures S2C). Conversely, in skmel28, 5 μ M of vemurafenib significantly induced apoptosis (2-3 fold), and IGF2BP1 knockdown combined with the apoptotic effect of vemurafenib resulted in a six-fold increase in apoptosis. Thus, vemurafenib and IGF2BP1 downregulation respectively regulate cell proliferation, cell cycle arrest, and apoptosis, and dual inhibition of BRAF^{V600E} and IGF2BP1 leads to greater suppression of cell growth and apoptosis in BRAF^{V600E} melanoma.

To study the role of IGF2BP1 in vemurafenib-resistant melanoma we generated two vemurafenib-resistant melanoma cell lines from vemurafenib-sensitive cell lines, G361 and A375. 3-5 μ M of vemurafenib is relevant to plasma level of vemurafenib-treated patients. Thus, unaltered survival at 3 μ M was considered as a resistant cell line and resistant cells were cultured at 3 μ M of vemurafenib on every passaging to maintain the resistance. Cell growth assays showed that both resistant cell lines (G361R and A375R) survived at high concentrations of vemurafenib (Supplementary Figures S3A and S3B). Vemurafenib treatment suppressed the growth of G361 at 0.3 μ M whereas acquired resistance reduced sensitivity to vemurafenib more than 10-folds. G361 was sensitive at lower dose than A375 and it was enough to show statistical significance (Figures 2A and 2C). Additionally, MRA5, a short-term cell line intrinsically resistant to vemurafenib, was used to study the role of IGF2BP1 on intrinsic resistance to vemurafenib. Growth of vemurafenib-resistant cells (G361R, A375R, and MRA5) was not affected until the high concentration of vemurafenib was administered whereas knockdown of IGF2BP1 significantly suppressed growth of both parental and resistant cells (Figure 2).

Dual inhibition of BRAF and MEK, represented by dabrafenib and trametinib, respectively, increases the efficacy over single treatment, but resistance to combination therapy still arises.¹³ We wanted to test whether vemurafenib-resistant cell line is sensitive to dabrafenib and trametinib and whether inhibition of IGF2BP1 can sensitize melanoma cells to dabrafenib and trametinib. Based on the experimentally determined vemurafenib IC₅₀ in A375, we chose low and high concentrations (dabrafenib: 10 nM and 100 nM; trametinib: 0.1 nM and 1 nM).¹⁴ A375 was sensitive to single treatment of dabrafenib or trametinib and combination therapy whereas A375R was less sensitive to these drugs, especially to trametinib (Figure 3); knockdown of IGF2BP1 sensitized A375 to targeted therapies ($P < 0.15$ for interaction between IGF2BP1 and each of the treatments). Although inhibition of IGF2BP1 in A375R resulted in less reduction in colony formation compared to the parental cell line, knockdown of IGF2BP1 did have a strong effect ($P < 0.0001$), and appeared to sensitize A375R to targeted therapies except for the interaction between IGF2BP1 and dabrafenib 10 nM. Reduced efficacy of cell growth inhibition in resistant cell line by IGF2BP1 knockdown may involve one or more resistance mechanisms in MAPK pathway; however, most treatment groups had the number of colonies reduced by half or more, which

underscores the important role of IGF2BP1 in metastatic melanoma resistant to targeted therapy.

Knockdown of IGF2BP1 alone resulted in 4-5 fold increase in the number of cells undergoing apoptosis in A375 (Supplementary Figures S4A). Combining inhibition of IGF2BP1 with vemurafenib treatment significantly increased apoptosis by two-fold compared to vemurafenib alone, and by 10-fold compared to the control. Although apoptosis level in A375R was not affected by vemurafenib treatment, knockdown of IGF2BP1 doubled apoptosis level compared to the control (Supplementary Figures S4B). In cell cycle analysis, the low concentration (0.5 μ M) of vemurafenib was able to arrest A375 cells in G1 phase, implying that G1 phase in A375 reached maximum plateau by vemurafenib treatment alone (Supplementary Figures S4C). Additionally, inhibition of IGF2BP1 combined with either 0.5 μ M or 5 μ M of vemurafenib further reduced cells in S phase by half. A375R exhibited less G1 phase arrest with vemurafenib compared to A375; 0.5 μ M of vemurafenib barely affected cell cycle distribution of A375R (Supplementary Figures S4D). Five micromolar of vemurafenib in A375R arrested 20 percent more cells at G1 compared to the control. Moreover, knockdown of IGF2BP1 further induced G1 phase arrest in the control and vemurafenib treated groups. These results confirmed that cell growth in vemurafenib-resistant melanoma is impeded by knockdown of IGF2BP1 through induction of apoptosis and G1 phase arrest independent of vemurafenib concentration.

Our results suggest that inhibition of IGF2BP1 can significantly increase efficacy of vemurafenib in vemurafenib-sensitive BRAF^{V600E} melanoma. Moreover, although inhibition of IGF2BP1 does not sensitize vemurafenib-resistant melanoma to vemurafenib, knockdown of IGF2BP1 alone is sufficient to reduce tumorigenic characteristics in vemurafenib-resistant melanoma. Importantly, inhibition of IGF2BP1 also cooperates with combinational therapy of BRAF-MEK inhibition effectively against resistant melanoma. Effects of IGF2BP1 in vemurafenib resistance are likely pleiotropic, as IGF2BP1 was shown to target mRNAs encoding key component of several pathways reported to be involved in resistance to BRAF inhibitors. For example, IGF2BP family of proteins affects expression of multiple components of RAS-RAF-MAPK¹⁵⁻¹⁷ and PI3K-AKT^{6,18,19} signaling pathways. Other well-established IGF2BP1 targets such as CTNNB1,²⁰ c-myc,²¹ and GLI^{10,22} were shown to contribute to vemurafenib resistance. Overexpression of Gli1, the key transcription factor of Hedgehog (Hh) signaling pathway, is associated with the development of multiple tumor types. Since IGF2BP1 induces overexpression of Gli1, cell growth inhibition of vemurafenib-resistant melanoma via knockdown of IGF2BP1 may be caused by a Gli1 dependent manner. These results suggest that IGF2BP1 may be a promising therapeutic target for BRAF^{V600E} melanoma and a novel mechanism to overcome resistance to targeted therapy.

3 | MATERIALS AND METHODS

3.1 | Cell line, culture, and transfection conditions

Skmel2 and skmel28 were maintained in MEM with 10% FBS. 451 Lu was maintained in MEM with 1% nonessential amino acids, 1% sodium pyruvate and 10% FBS. A375, MRA5, and MRA6 were maintained in DMEM with 10% FBS. MRA5 and MRA6 were kindly

provided by Dr. M. Albertini (University of Wisconsin, Madison WI). G361 was maintained in McCoy's 5A with 10% FBS. All cell lines except skmel28 were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Skmel28 were transfected with Lipofectamine 3000 (Invitrogen) following the manufacturer's recommendation. Medium was replaced either 6h or 24 h post transfection depending on the sensitivity to lipofectamine. Transfection efficiency between scrambled shRNA (shScram.) and shIGF2BP1 were tested by cotranfection of Amara-GFP plasmid and significant difference in GFP expression was not observed (not shown).

3.2 | Colony formation assay

Cells were cotransfected with shScram. and pTK-puro plasmid or shIGF2BP1 and pTK-puro plasmid. 24hrs after transfection, 5×10^5 cells were seeded in six-well plates in triplicates and was treated with puromycin (skmel2: 1 μ g/mL; skmel28: 1 μ g/mL, 451 Lu: 1 μ g/mL, A375: 2 μ g/mL, G361: 1 μ g/mL, MRA5: 0.2 μ g/mL, MRA6: 0.3 μ g/mL), and vemurafenib, dabrafenib, trametinib or dabrafenib + trametinib. Drug treatment with puromycin was maintained for 2-3 weeks until colonies become visible to the naked eye. Colonies were fixed with 10% formalin for 1 h followed by staining with 1% crystal violet (w/v) in 1:1 ratio of water and methanol for 1h.

3.3 | Cell growth assay (MTS assay)

Cells were counted using Countess II FL automated cell counter (Thermo Fisher Scientific, Waltham, MA) and 1000 or 2000 cells were plated in 96-well plates. Five days later, cell growth was measured using CellTiter 96 Aqueous One Solution reagent (Promega, Madison, WI). 20 μ L of reagent was added to the plate containing cells with 100 μ L of fresh medium. Cells were incubated at 37°C for 1.5 h and absorbance was recorded at 490 nm using Synergy H1 hybrid reader (Biotek, Winooski, VT).

3.4 | Apoptosis assay

Cells were cotransfected with Amara-GFP plasmid and shIGF2BP1 or scrambled shRNA plasmid. Cells were subcultured 24h after transfection and fresh medium containing DMSO or 5 μ M of vemurafenib was added when cells adhered to the plate. After incubation for 24h with DMSO or vemurafenib, cells were harvested and labeled with Annexin V, Alexa fluor 350 conjugate (Thermo Fisher Scientific) followed by manufacturer recommendation. As a dead cell indicator, propidium iodide (final concentration 10 μ g/mL, Invitrogen) was added with annexin V conjugate and incubated at room temperature for 15 min. After the incubation period, cells were analyzed by flow cytometry using BD LSR II (BD Biosciences, San Jose, CA).

3.5 | Cell proliferation analysis

The same method as apoptosis assay was used until vemurafenib treatment. After 24 h post treatment, cells were incubated with 30 μ M of EdU for 2h and harvested to detect incorporated EdU by Click-iT Alexa Fluor 647 Azide (Invitrogen). The following steps were done by manufacturer's recommendation. To obtain cell cycle distribution, cells were

incubated with 20 µg/mL of Hoechst 33342 (Invitrogen) before proceeding to the analysis by flow cytometry using BD LSR II (BD Biosciences, San Jose, CA).

3.6 | Cell cycle analysis

The same method as apoptosis assay was used until vemurafenib treatment. After 24 h of vemurafenib or DMSO incubation, cells were collected and resuspended to a concentration of 1×10^5 cells/mL in culture medium. Hoechst 33342 (Invitrogen) was added to the cell mix at the final concentration of 20 µg/mL and the mixture was incubated for 30 min at 37°C. Cells were pelleted and resuspended to yield a concentration of 1×10^6 cells/mL. Cell cycle was analyzed by flow cytometry using BD LSR II (BD Biosciences).

3.7 | Statistical analysis

Because the data represents discrete events, we considered colony count data to have a Poisson distribution. We examined the colony count data with negative binomial regression models, because of their ability to handle overdispersion, and underdispersion well. For each model, the factors IGF2BP1 inhibition and vemurafenib dose level were tested for an interaction. In the presence of an interaction, differences from 0 µM were tested within the Control and IGF2BP1 inhibition groups; Bonferroni adjustments were used to adjust for multiplicity. In the absence of interaction, main effects were tested; if the main effect for dose was significant, comparisons were made between 0µM (Vehicle) and other doses using a Bonferroni adjustment. Fold differences in apoptosis were tested after a log transform using mixed models regression. Percent of cells at cell cycle levels were modeled using mixed model regression. For both apoptosis and cell cycle data, in the presence of an interaction, Bonferroni corrections were used to adjust for multiple comparisons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This study was supported in part by the NIH grant R01 AR063361 (V.S.S.), Dermatology Foundation Career Development Award (Y.G.X.), University of Wisconsin-Madison Institute for Clinical & Translational Research Grant UL1TR000427/Skin Disease Research Center NIAMS grant P30 AR066524 (Y.G.X.), and NCI Cancer Center Support Grant P30 CA014520 (K.M.K.). The authors also want to thank Dr Vijay Setaluri for helpful discussion, and Megan L Maguire, and Diane Bock for their administrative support.

Funding information

Dermatology Foundation Career Development Award; University of Wisconsin-Madison Institute for Clinical & Translational Research Grant, Grant number: UL1TR000427; Skin Disease Research Center NIAMS, Grant number: P30 AR066524; NCI Cancer Center Support Grant, Grant number: P30 CA014520; NIH, Grant number: R01 AR063361

REFERENCES

1. Davies H, Bignell GR, Cox C, et al. Mutations in the BRAF gene in human cancer. *Nature*. 2002;417:949–954. [PubMed: 12068308]
2. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Eng J Med*. 2011;364:2507–2516.

3. Kim KB, Kefford R, Pavlick AC, et al. Phase II study of the MEK1/MEK2 inhibitor trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. *J Clin Oncol*. 2013;31:482–489. [PubMed: 23248257]
4. Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med*. 2015;372:30–39. [PubMed: 25399551]
5. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature*. 2010;468:973–977. [PubMed: 21107323]
6. Villanueva J, Vultur A, Lee JT, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3 K. *Cancer Cell*. 2010;18:683–695. [PubMed: 21156289]
7. Poulikakos PI, Persaud Y, Janakiraman M, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF-(V600E). *Nature*. 2011;480:387–390. [PubMed: 22113612]
8. Smalley KS, Xiao M, Villanueva J, et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene*. 2009;28:85–94. [PubMed: 18794803]
9. Atefi M, von Euw E, Attar N, et al. Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AK/mTOR pathway. *PLoS ONE*. 2011; 6, e28973. [PubMed: 22194965]
10. Noubissi FK, Goswami S, Sanek NA, et al. Wnt signaling stimulates transcriptional outcome of the hedgehog pathway by stabilizing Gli1 mRNA. *Cancer Res*. 2009;69:8572–8578. [PubMed: 19887615]
11. Noubissi FK, Nikiforov MA, Colburn N, et al. Transcriptional regulation of CRD-BP by c-myc: implications for c-myc functions. *Genes Cancer*. 2010;1:1074–1082. [PubMed: 21779431]
12. Craig EA, Spiegelman VS. Inhibition of coding region determinant binding protein sensitizes melanoma cells to chemotherapeutic agents. *Pigment Cell Melanoma Res*. 2012;25:83–87. [PubMed: 21981993]
13. Long GV, Fung C, Menzies AM, et al. Increased MAPK activation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. *Not Commun*. 2014;5:5694.
14. Greger JG, Eastman SD, Zhang V, et al. Combinations of BRAF, MEK, and PI3K/mTOR inhibitors overcome acquired resistance to the BRAF inhibitor GSK2118436 dabrafenib, mediated by NRAS or MEK mutations. *Mol Cancer Ther*. 2012;11:909–920. [PubMed: 22389471]
15. Mongroo PS, Noubissi FK, Cuatrecasas M, et al. IMP-1 displays cross-talk with K-Ras and modulates colon cancer cell survival through the novel proapoptotic protein CYFIP2. *Cancer Res*. 2011;71:2172–2182. [PubMed: 21252116]
16. Stohr N, Kohn M, Lederer M, et al. IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. *Genes Dev*. 2012;26:176–189. [PubMed: 22279049]
17. Rini J, Anbalagan M. IGF2BP1: a novel binding protein of p38 MAPK. *Mol Cell Biochem*. 2017;435:133–140. [PubMed: 28497370]
18. Zhang J, Cheng J, Zeng Z, et al. Comprehensive profiling of novel microRNA-9 targets and a tumor suppressor role of microRNA-9 via targeting IGF2BP1 in hepatocellular carcinoma. *Oncotarget*. 2015;6:42040–42052. [PubMed: 26547929]
19. Suvasini R, Shruti B, Thota B, et al. Insulin growth factor-2 binding protein 3 (IGF2BP3) is a glioblastoma-specific marker that activates phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3K/MAPK) pathways by modulating IGF-2. *J Biol Chem*. 2011;286:25882–25890. [PubMed: 21613208]
20. Gu W, Wells AL, Pan F, Singer RH. Feedback regulation between zipcode binding protein 1 and beta-catenin mRNAs in breast cancer cells. *Mol Cell Biol*. 2008;28:4963–4974. [PubMed: 18490442]
21. Doyle GA, Betz NA, Leeds PF, et al. The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. *Nucleic Acids Res*. 1998;26:5036–5044. [PubMed: 9801297]

22. Faiao-Flores F, Alves-Fernandes DK, Pennacchi PC, et al. Targeting the hedgehog transcription factors GLI1 and GLI2 restores sensitivity to vemurafenib-resistant human melanoma cells. *Oncogene*. 2017;36:1849–1861. [PubMed: 27748762]

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FIGURE 1.

Effect of vemurafenib and IGF2BP1 knockdown on cell growth of melanoma cell lines. A-D, colony formation assay using melanoma cell lines. A, BRAF^{WT} melanoma. B-D, BRAF^{V600E} melanoma. Cells were selected for puromycin resistance and were treated with various concentrations of vemurafenib (0.5 μ M-15 μ M). Each value represents mean $\pm$ SD (shScram: scrambled shRNA). (* $P < 0.05$ between dosages aggregated over inhibition status; † $P < 0.05$ between dosages within shScram or shIGF2BP1 status; all differences are Bonferroni corrected)

FIGURE 2.

Effect of IGF2BP1 knockdown in vemurafenib-resistant melanoma. A-E, colony formation assay using five melanoma cell lines. (A, C: BRAF^{V600E}, vemurafenib-sensitive melanoma. B, D, and E: BRAF^{V600E}, vemurafenib-resistant melanoma). MRA5 cell line was freshly isolated from human melanoma and showed intrinsic resistance to vemurafenib. Cells were selected for puromycin resistance and were treated with various concentrations of vemurafenib (0.5 μM-10 μM). Each value represents mean ± SD. (* $P < 0.05$ between dosages aggregated over inhibition status; † $P < 0.05$ between dosages within shScram or shIGF2BP1 status; all differences are Bonferroni corrected)

FIGURE 3.

Effect of IGF2BP1 knockdown on melanoma cell lines treated with dabrafenib and trametinib. A, vemurafenib-sensitive melanoma. B, vemurafenib-resistant melanoma. Cells were selected for puromycin resistance and were treated with dabrafenib (10 nM or 100 nM), trametinib (0.1 nM or 1 nM) or dabrafenib + trametinib (10 nM + 0.1 nM or 100 nM + 1 nM). Each value represents mean $\pm$ SD. ($\ddagger P < 0.01$ between inhibition groups for applicable treatment, differences are Bonferroni corrected)