Correlation of MET and PD-L1 expression in malignant melanoma

Kyu Young Song
University of Minnesota
Sabina Desar
University of Minnesota
Thomas Pengo
University of Minnesota
Ryan Shanley
University of Minnesota
Alessio Giubellino (agiubell@umn.edu)
University of Minnesota https://orcid.org/0000-0002-5352-0662

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Abstract

Background: The proto-oncogene MET, the hepatocyte growth factor (HGF) receptor, is a transmembrane receptor tyrosine kinase (RTK) with a prominent role in tumor metastasis and resistance to anti-cancer therapies. Melanoma demonstrates relatively frequent MET aberrations, including MET gene amplification. Concurrently, PD-L1, with its ability to evade anti-tumor immune responses, has emerged as a prominent therapeutic target in melanoma and other malignancies and its expression is used as a predictive biomarker of response to immunotherapy.

Methods: We performed immunohistochemistry analysis of MET and PD-L1 in 18 human melanoma cell lines derived from both primary and metastatic lesions; we then performed the same analysis in a human melanoma tissue microarray (TMA) containing 100 melanocytic lesions, including 42 cutaneous malignant melanomas, 20 mucosal melanoma, 21 metastatic melanomas and 17 benign melanocytic nevi as controls. After color deconvolution, TMA cores were identified and segmented to isolate staining and calculate the percentage of positive cells in each core.

Results: Overall, MET expression was higher in metastatic lesions and it was higher in tumors with increased PD-L1 expression. Moreover, a positive correlation between MET and PD-L1 expression was found in metastatic melanoma.

Conclusions: These data suggest that testing for expression of these markers should be conducted in patients with melanoma with metastatic disease and selective therapies targeting these proteins should be considered for advanced disease.

Background

Melanoma is the leading cause of death in patients with cutaneous malignancies and its incidence has been rapidly rising over the past 30 years. According to the American Cancer Society, 91,270 new cases of melanoma and 9,320 deaths related to melanoma were estimated in 2018 [1]; moreover, the incidence of melanoma is estimated to increase by 7.7% in 2019. While the five-year survival rate is up to 98% if melanoma is diagnosed at an early stage, this rate drops to about 20% if the disease is diagnosed at a late stage and has metastasized to distant organs. While a better understanding of the molecular basis of this cancer and its microenvironment has resulted in relatively successful novel therapeutic options, resistance ensues and there is still need for a better way to select for patients who will more likely benefit from these novel therapies.

The proto-oncogene MET, the hepatocyte growth factor (HGF) receptor, is a transmembrane receptor tyrosine kinase (RTK) with a prominent role in tumor metastasis and resistance to anti-cancer therapies [2], and dysregulation of the HGF/MET signaling pathway has been demonstrated in a wide range of malignancies, including malignant melanoma [3, 4]. A recent large whole genome sequencing (WGS) analysis of melanomas has demonstrated relatively frequent MET aberrations, including MET gene amplification, single nucleotide variations/deletions, and structural variants [5].

The pleiotropic effect of the HGF/MET signaling pathway include also a role in modulation of the immune response, including involvement in dendritic cell function [6] and neutrophilic antitumoral response [7]. This function has been postulated to be involved in the potential acquisition of resistance to immunotherapy treatments [6].

Programmed death-ligand 1(PD-L1) is a transmembrane protein encoded by the CD274 gene, located on chromosome 9, and is expressed by antigen presenting cells (APCs) and tumor cells [8]. PD-L1, with its ability to evade the anti-tumor immune response, has emerged as a prominent therapeutic target in melanoma and other malignancies and its expression is used as a predictive biomarker of response to immunotherapy [9]. PD-L1 expression can be induced either by cytokines (INF-gamma) or by activation of an oncogene. For example mutations in receptor tyrosine kinase pathways, such as epidermal growth factor receptor (EGFR), have been shown to induce PD-L1 expression in lung tumors [10] and overexpression of PD-L1 on cancer cells can block anti-tumor immunity, resulting in immune escape [11], which can be overcome by PD-1/PD-L1 inhibition, restoring tumor-specific T-cell immunity.

MET expression has been shown to promote upregulation of PD-L1 in renal cancer cells [12] and expression of PD-L1 and PD-L2 are upregulated in MET-amplified gastric and lung tumor cells [13]. However, the relationship between MET and PD-L1 expression in human malignant melanoma is not well characterized.

In the present study, we surveyed by immunohistochemistry MET and PD-L1 expression in melanoma cell lines and in a human tissue microarray of cutaneous melanomas, mucosal melanoma, and metastatic melanomas, with the goal of analyzing the expression pattern and to explore a possible correlation between expression of these two proteins involved in tumor progression and immune evasion.

Methods

Cell Culture and Cell Microarray

Human (A2058, A-375, G-361, RPMI-7951, SH-4, SK-MEL-1, SK-MEL-3, SK-MEL-24, and SK-MEL-28) cell lines were obtained directly from ATCC and were cultured following ATCC's recommendations. The WM35, WM115, WM164, WM278, WM793, WM852, WM1341D, 451Lu, and 1205Lu cell lines were obtained from the Wistar Institute and cultured with tumor specialized media containing 2% FBS. The characteristics of these melanoma cell lines are highlighted in **Supplemental Table 1**.

To prepare cell pellet blocks, cells were grown in T-75 flasks to near confluence then washed 3 times with PBS and fixed with 10% neutral-buffered formalin (NBF) in flasks overnight. After fixation, cells were gently scraped and transferred to a conical tube and centrifuged to ~300 x *g* for 5 min at room temperature (RT). Formalin supernatant was then removed and pellets were washed with PBS. Finally, cell pellets were re-suspended in 80% ethanol and paraffin embedded in cell blocks. A tissue microarray (TMA), with 1.5 cm cores for each cell line, was then derived from the cell blocks. Two TMA slides were

stained for MET and two were stained for PD-L1. The average percentage of positively stained cells and FastRed mean intensity were calculated.

Tissue Microarray and Patient Characteristics

TMA slides with 100 cores were purchased from Biomax (Cat. # ME1004g; US Biomax, Inc., Derwood, MD). Available clinicopathological characteristics are summarized in **Supplemental Table 2**. The mean age of the patients from this TMA was 50 years (range 0.5 - 84 years). The study included 58% (58) males and 42% (42) females. Staging (TNM and clinical staging) was only provided for 48 patients which include 42 cases of primary cutaneous melanoma and 6 cases of mucosal melanoma. Overall, the TMA included 42 cases with cutaneous malignant melanoma, 20 mucosal melanomas (including malignant melanoma from vulva, rectum, stomach and esophagus), 21 cases were obtained from metastatic sites including lymph nodes and 17 cases were benign melanocytic nevi.

Two TMA slides were stained for MET and two were stained for PD-L1. The average percentage of positively stained cells and FastRed mean intensity were calculated.

Immunohistochemistry (IHC)

Unstained TMA sections (4 µm) were de-paraffinized and rehydrated using standard methods. For antigen retrieval, slides were incubated in 6.0 pH buffer (Reveal Decloaking reagent, Biocare Medical, Concord, CA) in a steamer for 30 min at 95-98°C, followed by a 20 min cooldown period. Subsequent steps were automated using an immunohistochemical staining platform (Intellipath, Biocare). Endogenous peroxidase activity was quenched by slide immersion in 3% hydrogen peroxide solution (Peroxidazed, Biocare) for 10 min followed by TBST rinse. A serum-free blocking solution (Background Sniper, Biocare Medical, Concord, CA) was placed on sections for 10 min. Blocking solution was removed and slides were incubated in primary antibody diluted in 10% blocking solution/90% TBST for 60 minutes at room temperature. Rabbit monoclonal anti-MET (clone D1C2 XP(R)(Cell Signaling, Denver, MA;1:50), followed with a TBST rinse and detection with Novocastra Novolink Polymer Kit (Leica Microsystems Inc., Buffalo Grove, IL) using the manufacturer's specifications. Slides then proceeded with TBST rinse and detection with diaminobenzidine (DAB) (Covance, Dedham, MA). Slides were incubated for 5 min followed by TBS rinse then counterstained with CAT Hematoxylin (Biocare, Concord, CA) for 5 min. Finally, slides were dehydrated and coverslipped.

Rabbit monoclonal anti-PD-L1 (clone 28-8, Cell Marque, Rocklin, CA, 1:200) was followed by a TBST rinse and biotinylated anti-rabbit (Vector Labs, Burlingame, CA,1:200) was applied for 30 minutes. The slides were again rinsed with TBST and 4+ Streptavidin- Alkaline Phosphatase label (4+SA-AP) (Biocare Medical, Concord, CA, RTU) was applied for 30 minutes at room temperature. Slides proceeded to a TBST rinse and detection with WARP Red Chromagen (Biocare Medical, Concord, CA) according to manufacturers' specifications. Following detection, slides were rinsed well in running tap water and counterstained for 1 minute in CAT Hematoxylin (1:2) (Biocare Medical, Concord, CA). Tap water rinse and 2 minutes in PureView PH Blue (Cancer Diagnostics, Durham, NC), 5 minute tap water rinse and then

slides were air dried. When completely dry, slides were dipped in xylene and coverslipped with Permount mounting medium (Fisher, Fair Lawn, NJ).

Image analysis

TMA slides were scanned using an Aperio scanner, with a 40X objective. The high resolution images were analyzed using QuPath [14] version 0.1.2. The workflow consisted of (i) color deconvolution, (ii) identifying the TMA cores, (iii) segmenting the tissue region in each core, (iv) isolating nuclear and cytoplasmic regions of interest, (v) estimating the abundance of the deconvolved red component in each cell and finally (vi) calculating the percentage of positive cells in each core. 3-color deconvolution was performed using vectors calibrated visually on the image data, following the procedure outlined in the software documentation. All images were deconvolved using the same stain vectors. Steps (iii) to (v) were performed using the default algorithms in QuPath. For step (vi), each cell was considered positive if the red component intensity was above a threshold, calculated independently for each TMA slide as the average between the 5th and 95th percentile of red intensities across the slide. All algorithms and parameters for the analysis in QuPath were recorded in a script for repeatability (see **Supplementary Material**).

Statistical analysis

Statistical analysis was primarily descriptive. Correlation between percentage of MET- and PD-L1-positive cells was calculated using Pearson's correlation coefficient (r) and its 95 % confidence interval calculated by the 2.5 and 97.5 percentiles of 500 bootstrap resamplings.

Results

Expression of MET and PD-L1 in melanoma cell lines

We analyzed the expression of MET and PD-L1 in eighteen human melanoma cell lines in a tissue microarray derived from seven primary and eleven metastatic lesions. Immunohistochemical staining for MET and PD-L1 revealed a wide range of expressions in these cell lines. Expression of PD-L1 was higher in metastatic melanoma cell lines compared to primary melanoma cell lines (**Figure 1A**). Median (interquartile range) PD-L1 was 32 (29, 50) for metastatic and 24 (19, 27) for primary cell lines (Wilcoxon rank sum test p=0.02), and the highest value was in a metastatic cell line. Expression of MET was more similar, with a median of 24 for both primary and metastatic, although the interquartile range was slightly higher for metastatic (17, 41) than primary (11, 29) (Wilcoxon rank sum test p=0.44), and the highest value of MET was in a metastatic cell line.

MET and PD-L1 values were correlated among primary melanoma cell lines, with Pearson's r = 0.73, with a notable wide confidence interval. For metastatic cell lines, the Pearson's correlation score was higher, with a r = 0.89 (**Figure 1B**)

Expression of MET and PD-L1 in a human melanoma tissue microarray

One-hundred melanocytic lesions were evaluated for expression of MET and PD-L1 in a human TMA with benign nevi (17 patients), cutaneous melanomas (42 patients), mucosal melanomas (20 patients), and metastatic melanomas (21 patients). The characteristics of these patients are summarized in **Supplemental Table 2**. Briefly, the median age was 26 for nevi , 52 years for cutaneous melanoma, 57 years for mucosal melanoma and 51 years for metastatic melanoma (range 0.5 ~ 84 years across all lesions).

Membranous MET expression by more than 20% of cells was present in 40% (17 of 42) of primary cutaneous melanoma, 45% (9 of 20) of mucosal melanoma, and 33% (7 of 21) of metastatic melanoma , while no nevi had MET expression above the threshold of 20% positive cells and only one (6%) was above a threshold of 10% of positive cells. At the same time, 12% (2 of 17) of nevi, 70 % (30 of 42) of primary cutaneous melanoma, 80% (16 of 20) of mucosal melanoma, and 50% (11 of 21) of metastatic melanomas demonstrated membranous PD-L1 expression by more than 20% of cells.

As in the cell lines, MET and PDL1 expression varied widely across the lesions in the human samples TMA, and the lowest levels of both MET and PD-L1 expression were detected in benign nevi (**Figure 2**), as expected. Cutaneous melanoma, mucosal melanoma and metastatic melanomas showed comparable levels of both MET and PDL1 expression.

We then calculated the correlation between MET and PDL1 expression in each category of melanocytic lesions. As shown in **Figure 3**, there was modest correlation for benign nevi and cutaneous primary melanoma, with Pearson's correlation coefficients of 0.46 and 0.49, respectively, and no correlation in mucosal melanoma (r = -0.02). In contrast, MET and PD-L1 expressions were highly correlated in metastatic melanoma (r = 0.74).

Discussion

Malignant melanoma remains a major cause of death among patients with cutaneous malignancies, despite the introductions of novel therapeutic approaches such as immunotherapy. The PD-1/PD-L1 axis [15] have emerged as a major immune checkpoint target explored successfully for immunotherapy. Resistance to drugs targeting these proteins eventually emerges [16] through several mechanisms, some of which have been characterized. For example, activation of canonic oncogenic signaling pathways, such as those driven by receptor tyrosine kinases, are well known. Thus, a viable strategy to overcome resistance is to combine immunotherapy with conventional targeted therapies, such as inhibitors of receptor tyrosine kinases. Assessment of expression of these targets in tumor tissue sections is an important strategy to improve patient selection and increase efficacy of potential drug combinations.

Several lines of evidence point out a prominent role of the MET/HGF axis in tumor progression and resistance to therapy of several malignancies, including malignant melanoma. For example, in a case of acral melanoma with KIT mutation, targeting MET with a selective inhibitor successfully overcame resistance to KIT inhibition, as confirmed also in cell line studies [17]. Another study has established that MAPK pathway inhibition following BRAF inhibitor treatment induced rapid increases in *MET* and *GAB1*

expression [18] and *MET* amplification was also observed to co-exist with *BRAF* hotspot mutations [5]. Moreover, MET appears to have a role in the regulation of immunity, as demonstrated by the key role of its ligand, HGF, in the regulation of autoimmunity and inflammation [19]. More recently a subpopulation of CD8 positive cytotoxic T-cell has been found to express MET, further linking its pathway to a role in tumor immunity [20]. Moreover, HGF has been linked to increased expression of PD-L1 in dendritic cells and CTLA-4 in T-cells, with a role in the induction of immune tolerance [6, 21].

In the era of personalized medicine, the prediction of patient's drug response has become an important prerequisite for administration of targeted therapies[22] [23]. Previous reports demonstrated PD-L1 expression to be associated with better prognosis in patients with lung, colorectal, breast, and malignant melanoma cancers [24-29]. Our study demonstrates that both MET and PD-L1 expressions are increased in metastatic melanocytic lesions when compared with primary melanoma and benign melanocytic nevi in patients' tissue samples, suggesting that in advanced lesion both proteins are potential biomarkers and viable targets for selective combined inhibition.

Currently, no definitive cut-off for PD-L1 positivity is universally recognized, in part because of variability between different antibody clones and staining platform used. In our current study we used clone 28.8 (from Cell Marque). This antibody was developed in conjunction with the first immune checkpoint inhibitor evaluation in patients with cancer, namely nivolumab, a human monoclonal antibody targeting PD-1 binding to PD-L1 and PD-L2. Measurable positive effects on overall survival were observed in patients with melanoma, NSCLC, renal cell carcinoma and ovarian cancer. This is a "complementary" diagnostic, so it has not been included as a mandatory test in their regulatory labeling, and many studies have validated its use in PD-L1 immunohistochemistry assays.

In our study we took advantage of software analysis to avoid a qualitative or semiquantitative assessment of percentage of positive cells. High resolution images were used in the assessment with a recently described open source software (QuPath) for digital pathology and image analysis.

The methodology used in our assessment, which includes color deconvolution of high resolution images, allows for a sensitive assessment of each individual staining and is able to detect low levels of signal that may be otherwise below the limit of detection by the human eye in a qualitative or semiquantitative assessment. Hence, he use of deconvolution allows for a more sensitive and quantitative assessment of the immunohistochemistry staining, in conjunction with the use of digital imaging analysis.

Our results in the established cell lines show a good correlation between MET and PD-L1 expression, meaning that degree of MET and PD-L1 expressions follow a similar trend, in both primary and metastatic cell lines. In contrast, our data on tumor biopsies from patients show a relatively modest correlation between these two proteins in primary lesions, but a much higher correlation in metastatic disease. These finding further highlights discrepancies we frequently see experimentally between cell lines and patient's samples, stressing the importance to always correlate *in vitro* findings with actual clinical specimens. However, one limitation of our study is the lack of outcomes and follow-up clinical information which was not available for the current TMA.

Conclusions

Given the higher expression of MET and PD-L1 in metastatic melanoma when compared with primary tumor and a trend in higher expression for these proteins in higher grade lesion, these data suggest that testing by immunohistochemistry for expression of these markers should be conducted in patients with metastatic disease when considering selective therapies targeting these proteins.

Moreover, given the increased expression of both MET and PD-L1 and their correlation in metastatic lesions, combinations therapies targeting these proteins should be explored as a viable therapeutic options for patients with advanced disease.

Abbreviations

HGF: hepatocyte growth factor

RTK: receptor tyrosine kinase

TMA: tissue microarray

PD-L1: Programmed death-ligand 1

APCs: antigen presenting cells

EGFR: epidermal growth factor receptor

Declarations

Ethical approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors's contributions

A.G., K.Y.S , S.D., T.P. and R.S. contributed to the design and implementation of the research, and to the analysis of the results. A.G. and K.Y.S. wrote the main manuscript. K.Y.S., S.D. carried out most of the experiments. All authors read and approved the final manuscript.

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References

1. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2019**. *CA: a cancer journal for clinicians* 2019, **69**(1):7-34.
2. Matsumoto K, Umitsu M, De Silva DM, Roy A, Bottaro DP: **Hepatocyte growth factor/MET in cancer progression and biomarker discovery**. *Cancer science* 2017, **108**(3):296-307.
3. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G: **Targeting MET in cancer: rationale and progress**. *Nature reviews Cancer* 2012, **12**(2):89-103.
4. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF: **Met, metastasis, motility and more**. *Nature reviews Molecular cell biology* 2003, **4**(12):915-925.
5. Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, Patch AM, Kakavand H, Alexandrov LB, Burke H *et al*: **Whole-genome landscapes of major melanoma subtypes**. *Nature* 2017, **545**(7653):175-180.
6. Hubel J, Hieronymus T: **HGF/Met-Signaling Contributes to Immune Regulation by Modulating Tolerogenic and Motogenic Properties of Dendritic Cells**. *Biomedicines* 2015, **3**(1):138-148.
7. Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AA, Wauters E, Walmsley S, Prenen H, Granot Z *et al*: **MET is required for the recruitment of anti-tumoural neutrophils**. *Nature* 2015, **522**(7556):349-353.
8. Gibbons Johnson RM, Dong H: **Functional Expression of Programmed Death-Ligand 1 (B7-H1) by Immune Cells and Tumor Cells**. *Frontiers in immunology* 2017, **8**:961.
9. Teng F, Meng X, Kong L, Yu J: **Progress and challenges of predictive biomarkers of anti PD-1/PD-L1 immunotherapy: A systematic review**. *Cancer letters* 2018, **414**:166-173.
10. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, Mikse OR, Cherniack AD, Beauchamp EM, Pugh TJ *et al*: **Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors**. *Cancer discovery* 2013, **3**(12):1355-1363.
11. Blank C, Mackensen A: **Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion.** . *Cancer Immunol Immunother* 2007, **56**(5):739-745.

12. Balan M, Mier y Teran E, Waaga-Gasser AM, Gasser M, Choueiri TK, Freeman G, Pal S: **Novel roles of c-Met in the survival of renal cancer cells through the regulation of HO-1 and PD-L1 expression**. *The Journal of biological chemistry* 2015, **290**(13):8110-8120.
13. Martin V, Chiriaco C, Modica C, Acquadro A, Cortese M, Galimi F, Perera T, Gammaitoni L, Aglietta M, Comoglio PM *et al*: **Met inhibition revokes IFNgamma-induction of PD-1 ligands in MET-amplified tumours**. *British journal of cancer* 2019, **120**(5):527-536.
14. Bankhead P, Loughrey MB, Fernandez JA, Dombrowski Y, McArt DG, Dunne PD, McQuaid S, Gray RT, Murray LJ, Coleman HG *et al*: **QuPath: Open source software for digital pathology image analysis**. *Scientific reports* 2017, **7**(1):16878.
15. Sharma P, Allison JP: **Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential**. *Cell* 2015, **161**(2):205-214.
16. Fares CM, Van Allen EM, Drake CG, Allison JP, Hu-Lieskovan S: **Mechanisms of Resistance to Immune Checkpoint Blockade: Why Does Checkpoint Inhibitor Immunotherapy Not Work for All Patients?** *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting* 2019, **39**:147-164.
17. Oba J, Kim SH, Wang WL, Macedo MP, Carapeto F, McKean MA, Van Arnam J, Eterovic AK, Sen S, Kale CR *et al*: **Targeting the HGF/MET Axis Counters Primary Resistance to KIT Inhibition in KIT-Mutant Melanoma**. *JCO precision oncology* 2018, **2018**.
18. Caenepeel S, Cooke K, Wadsworth S, Huang G, Robert L, Moreno BH, Parisi G, Cajulis E, Kendall R, Beltran P *et al*: **MAPK pathway inhibition induces MET and GAB1 levels, priming BRAF mutant melanoma for rescue by hepatocyte growth factor**. *Oncotarget* 2017, **8**(11):17795-17809.
19. Molnarfi N, Benkhoucha M, Funakoshi H, Nakamura T, Lalive PH: **Hepatocyte growth factor: A regulator of inflammation and autoimmunity**. *Autoimmunity reviews* 2015, **14**(4):293-303.
20. Benkhoucha M, Molnarfi N, Kaya G, Belnoue E, Bjarnadottir K, Dietrich PY, Walker PR, Martinvalet D, Derouazi M, Lalive PH: **Identification of a novel population of highly cytotoxic c-Met-expressing CD8(+) T lymphocytes**. *EMBO reports* 2017, **18**(9):1545-1558.
21. Sagi Z, Hieronymus T: **The Impact of the Epithelial-Mesenchymal Transition Regulator Hepatocyte Growth Factor Receptor/Met on Skin Immunity by Modulating Langerhans Cell Migration**. *Frontiers in immunology* 2018, **9**:517.
22. Chen DS, Mellman I: **Oncology meets immunology: the cancer-immunity cycle**. *Immunity* 2013, **39**(1):1-10.
23. Nicolaides NC, O'Shannessy DJ, Albone E, Grasso L: **Co-development of diagnostic vectors to support targeted therapies and theranostics: essential tools in personalized cancer therapy**. *Front Oncol* 2014, **4**:141.
24. Ishii H, Azuma K, Kawahara A, Yamada K, Imamura Y, Tokito T, Kinoshita T, Kage M, Hoshino T: **Significance of Programmed Cell Death-Ligand 1 Expression and Its Association With Survival in Patients With Small Cell Lung Cancer**. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2015, **10**(3):426-430.

25. Yang C-Y, Lin M-W, Chang Y-L, Wu C-T, Yang P-C: **Programmed Cell Death-Ligand 1 Expression in Surgically Resected Stage I Pulmonary Adenocarcinoma and Its Correlation With Driver Mutations and Clinical Outcomes**. *Eur J Cancer* 2014, **50**(7):1361-1369.
26. Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, Herbst RS, Gettinger SN, Chen L, Rimm DL: **Programmed Death ligand-1 Expression in Non-Small Cell Lung Cancer**. *Laboratory investigation; a journal of technical methods and pathology* 2014, **94**(1):107-116.
27. Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, Zlobec I, Eppenberger-Castori S, Tzankov A, Rosso R *et al*: **Clinical impact of programmed cell death ligand 1 expression in colorectal cancer.** . *Eur J Cancer* 2013, **49**(9):2233-2242.
28. Schalper KA, Velcheti V, Carvajal D, Wimberly H, Brown J, Pusztai L, Rimm DL: **In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas.** . *Clin Cancer Res* 2014, **20**(10):2773-2782.
29. Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, Okazaki T, Tokura Y: **Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma.** . *Cancer* 2010, **116**(7):1757-1766.

Figures

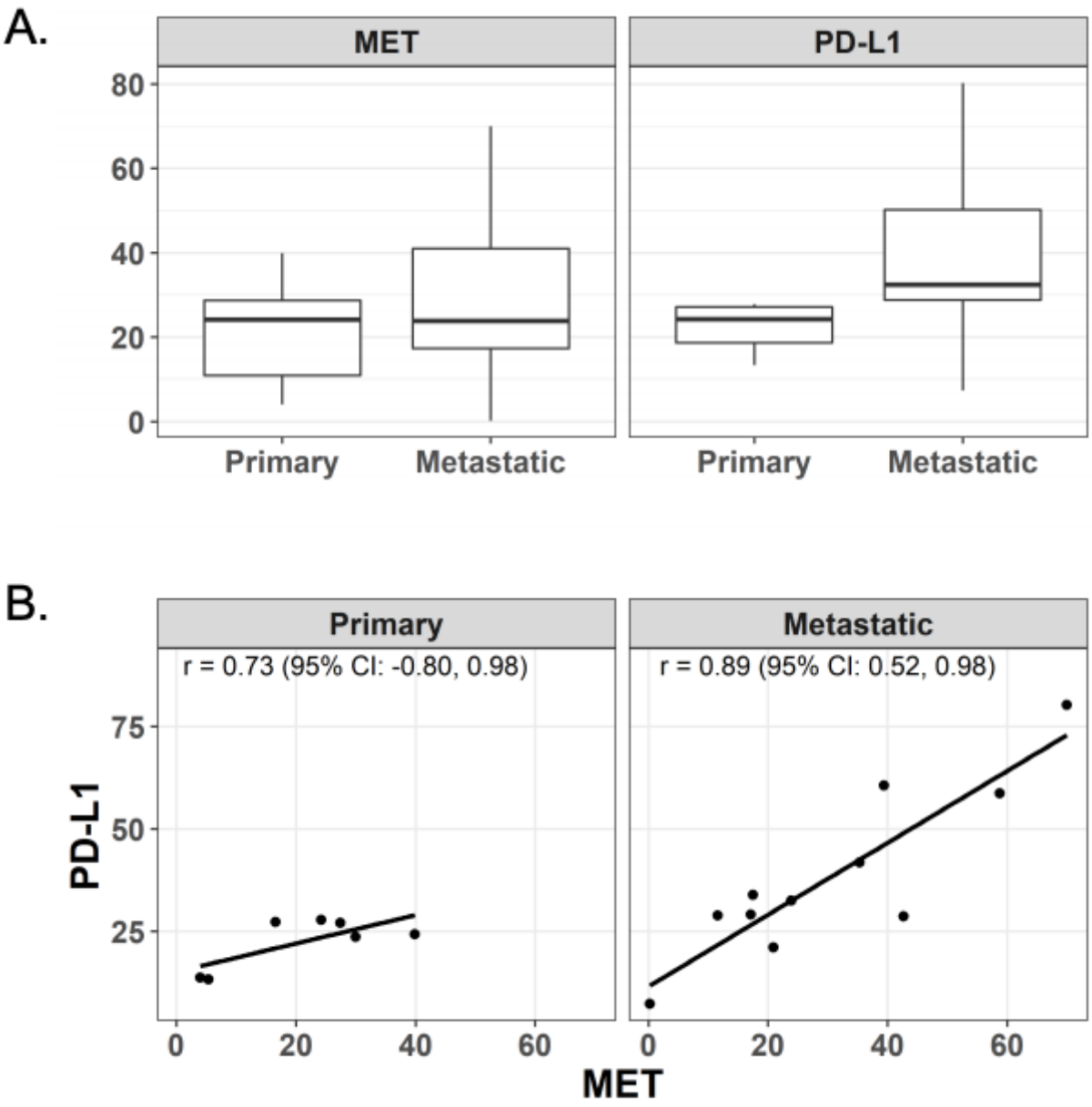

Figure 1

MET and PD-L1 expression levels in 7 primary melanomas and 11 metastatic melanomas (A). Correlation of MET and PDL1 in primary and metastatic cell lines (B).

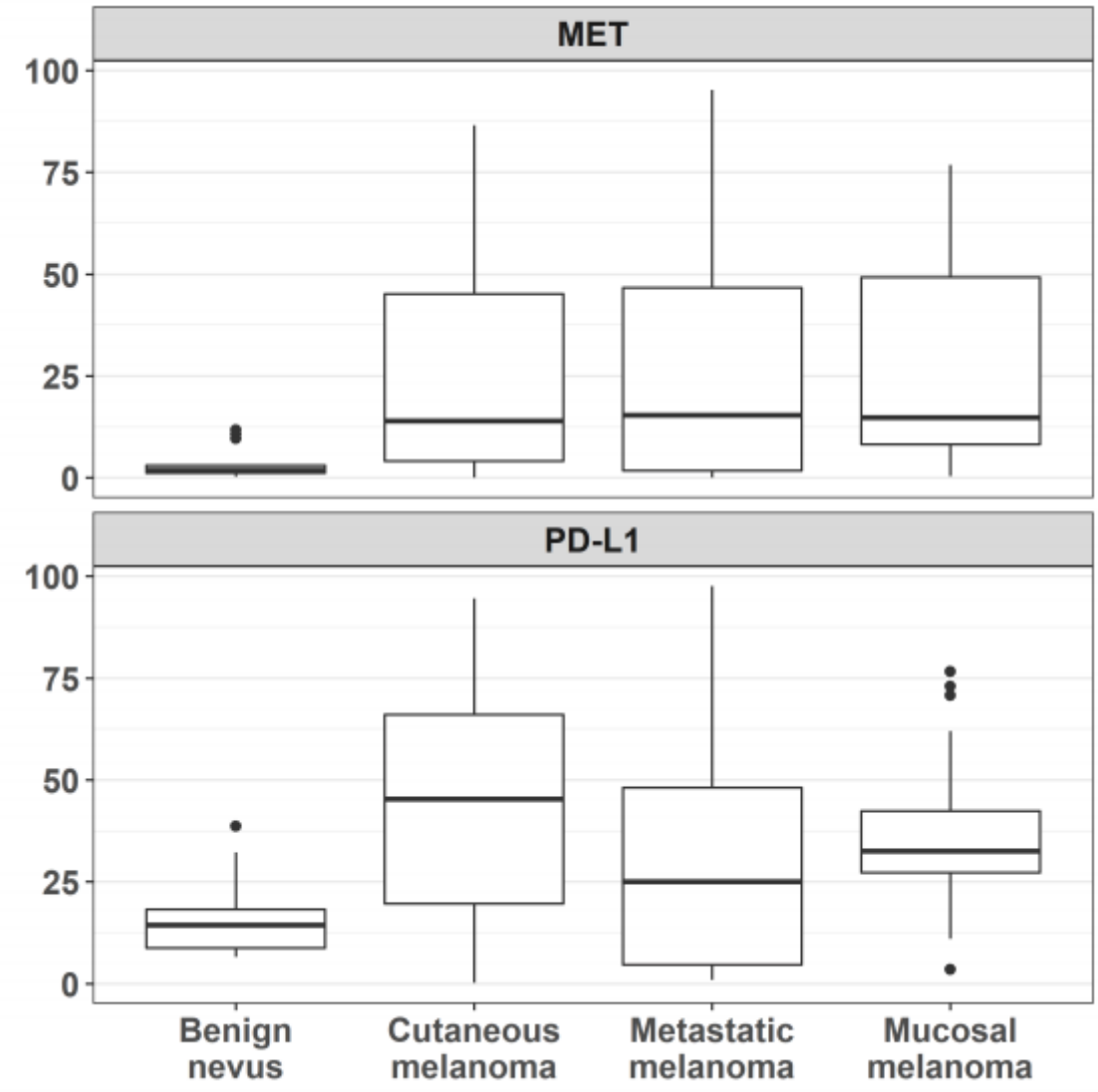

Figure 2

MET and PD-L1 expression levels in benign nevi, cutaneous melanoma, metastatic melanoma and mucosal melanoma.

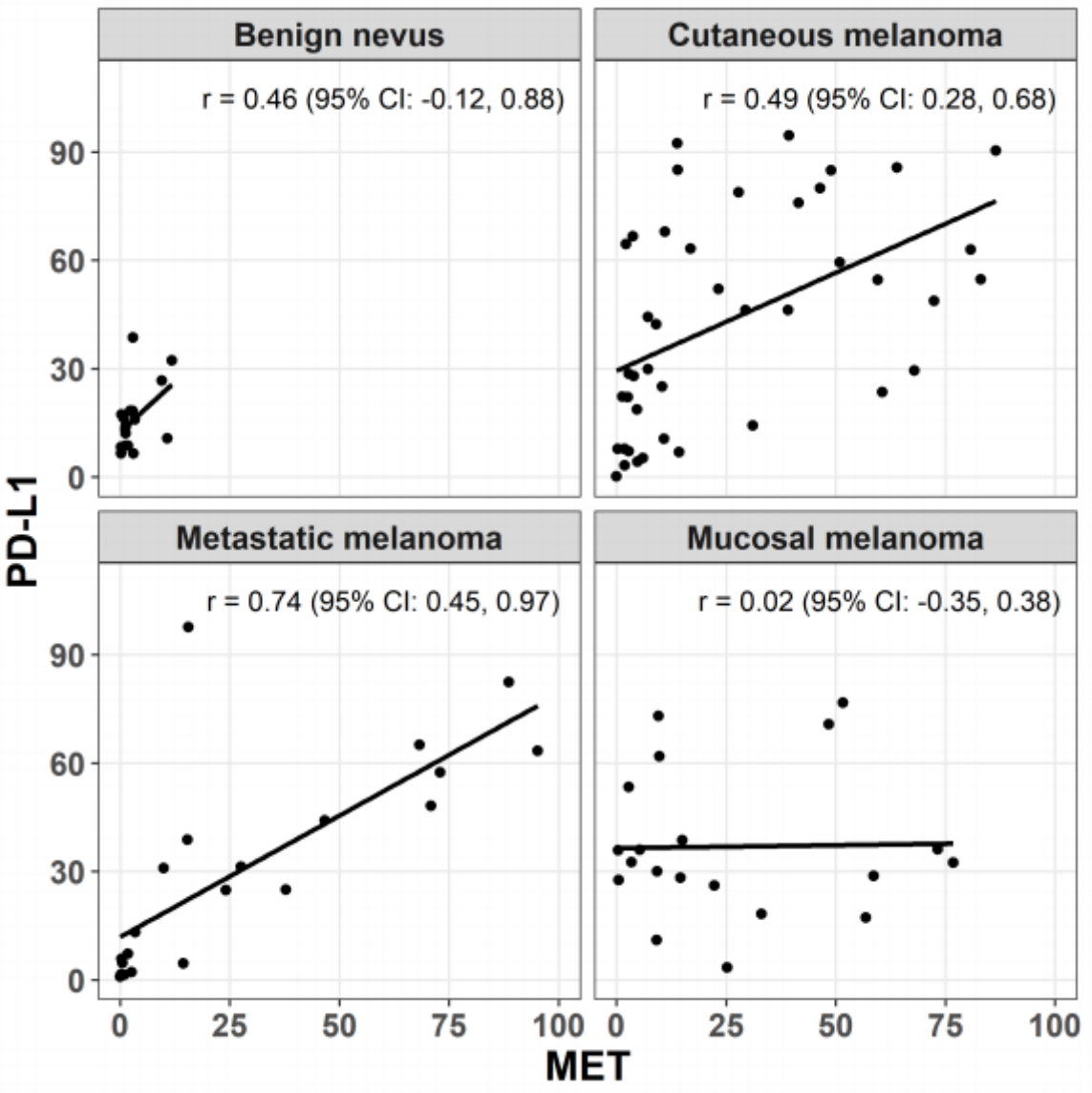

Figure 3

Correlation of MET and PDL1 in benign nevi, cutaneous melanoma, metastatic melanoma and mucosal melanoma.

Supplementary Files

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- SupplTable1v3.xlsx
- SupplTable2v3agerangeonly.xlsx