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Predicting PD-L1 expression on human cancer cells using next-generation sequencing information in computational simulation models

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Abstract

Purpose—Interaction of the programmed death-1 (PD-1) co-receptor on T-cells with the programmed death-ligand 1 (PD-L1) on tumor cells can lead to immunosuppression, a key event in the pathogenesis of many tumors. Thus, determining the amount of PD-L1 in tumors by immunohistochemistry (IHC) is important as both a diagnostic aid and as a clinical predictor of immunotherapy treatment success. Because IHC reactivity can vary, we developed computational simulation models to accurately predict PD-L1 expression as a complementary assay to affirm IHC reactivity.

Methods—Multiple myeloma (MM) and oral squamous cell carcinoma (SCC) cell lines were modeled as examples of our approach. Non-transformed cell models were first simulated to establish non-tumorigenic control baselines. Cell line genomic aberration profiles, from next generation sequencing (NGS) information for MM.1S, U266B1, SCC4, SCC15, and SCC25 cell lines were introduced into the workflow to create cancer cell line-specific simulation models.

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Conflict of interest

Kim A. Brogden has had a Cooperative Research and Development Agreement with Cellworks Group Inc., San Jose, CA. Emily A. Lanzel, M. Paula Gomez Hernandez, Amber M. Bates, Christopher N. Treinen, Emily E. Starman, Carol L. Fischer, Janet M. Guthmiller, Georgia K. Johnson, and Kim A. Brogden declare no competing financial interests in the findings of this study or with Cellworks Group Inc., San Jose, CA USA or Cellworks Research India Ltd, Whitefield, Bangalore, India. Taher Abbasi and Shireen Vali are employed by Cellworks Group Inc., San Jose, CA USA and Deepak Parashar is employed by Cellworks Research India Ltd, Whitefield, Bangalore, India.

Percentage changes of PD-L1 expression with respect to control baselines were determined and verified against observed PD-L1 expression by ELISA, IHC, and flow cytometry on the same cells grown in culture.

Results—The observed PD-L1 expression matched the predicted PD-L1 expression for MM. 1S, U266B1, SCC4, SCC15, and SCC25 cell lines and clearly demonstrated that cell-genomics play an integral role by influencing cell signaling and downstream effects on PD-L1 expression.

Conclusion—This concept can easily be extended to cancer patient cells where an accurate method to predict PD-L1 expression would affirm IHC results and improve its potential as a biomarker and a clinical predictor of treatment success.

Keywords

Computational modeling; Simulation modeling; PD-L1; Multiple myeloma; Oral squamous cell carcinoma; Immunotherapy

Introduction

Programmed death-ligand 1 (PD-L1) is a member of the B7 family of molecules and is present on the surface of many hematopoietic and non-hematopoietic cells [1–3]. It is part of the programmed death pathway, binding to the co-receptor programmed death-1 (PD-1) on the surface of activated T-cells, natural killer cells, B-cells, monocytes, and dendritic cells as one of the checkpoints for normal immune homeostasis [1, 2]. PD-L1 is also found on melanoma cells, renal cell carcinoma cells, multiple myeloma (MM) cells, oral squamous cell carcinoma (SCC) cells, gastrointestinal cancer cells, bladder cancer cells, ovarian cancer cells, and hematological cancer cells [4–6]. In cancer pathogenesis, overexpression of PD-L1 by tumor cells increases immunosuppression by inhibiting T-cell proliferation, reducing T-cell survival, inhibiting cytokine release, and promoting T-cell apoptosis [4, 7].

Immunohistochemistry (IHC) is currently used to detect PD-L1 on tumor cells after biopsy. However, its detection varies depending upon differences in antibody specificities, affinities, and commercial sources [8, 9]. This variability results in challenges using PD-L1 reactivity to select patients for PD-L1 immunotherapy and to predict clinical treatment outcomes. For example, in a study of 1,400 patients, ~45% patients with PD-L1⁺ tumor cells and ~15% patients with PD-L1[−] tumor cells had objective responses [10]. The high proportion of patients with PD-L1[−] tumor cells that had objective responses argues against the use of IHC as a sole method to determine PD-L1 expression for patient selection for treatment.

Complicating the situation is the presence of soluble PD-L1 (sPD-L1) in serum and plasma [11]. In normal individuals, sPD-L1 concentrations vary with age and range from 725.0 ± 181.0 pg/ml (children 3–10 years of age), 766.0 ± 253.0 pg/ml (young adults), and 889.0 ± 270.0 (adults) to 1,040 ± 681.0 pg/ml (older adults 51–70 years of age) [12]. In patients with cancer, sPD-L1 concentrations are elevated and may play an important role in tumor immune evasion and patient prognosis [12]. For example, elevated sPD-L1 concentrations are associated with poor post-cryoablation prognosis in patients with hepatitis B virus-related hepatocellular carcinoma [13], poor prognosis in patients with advanced gastric cancer [14], correlated with differentiation and lymph node metastasis, and associated with

diffuse large B-cell lymphoma [15]. Patients with elevated sPD-L1 experienced a poorer prognosis with a 3 year overall survival of 76% versus 89% concluding that sPD-L1 is a potent predicting biomarker in this disease.

There is a critical need to improve methods to accurately affirm PD-L1 expression. Since determining PD-L1 reactivity in tumors using IHC and determining sPD-L1 in serum or plasma can both be variable [15], we created predictive computational simulation models containing cell line genomic signatures to fill this gap and to predict PD-L1 expression with a validated, cancer network model. Simulation models using a computational approach can accurately predict and reproduce the behavior of interacting and interdependent biological systems, like that of signaling pathways in cancer cells. This knowledge is useful in determining the parameters important in the expression of cell-associated immunosuppressive biomarkers. PD-L1 expression is regulated by signaling pathways, transcription factors, and epigenetic factors modulated by tumor genomics [2, 16, 17], which makes PD-L1 an ideal molecule to predict via computational simulation modeling. In this study, we constructed computational simulation models of PD-L1 expression in MM and oral SCC cell lines. We used these models to accurately show that cancer cell genomics plays an integral role in influencing cell signaling and downstream effects on PD-L1 expression. We show differences in PD-L1 expression among cell lines and these results were verified against PD-L1 concentrations detected on the same cells grown in culture using ELISA, IHC, and flow cytometry. Thus, predictions of PD-L1 expression may be a method to affirm diagnostic IHC results and support the role of PD-L1 as a biomarker and clinical predictor of treatment success.

Material and methods

Cell line mutational profiles

MM and SCC cell line-specific mutational profiles were first created. MM. 1S is a peripheral blood B lymphoblast from a 42-year-old adult with immunoglobulin A lambda myeloma [18, 19], and U266B1 is a peripheral blood B lymphocyte from a 53-year-old adult with a plasma cell myeloma [20]. Next generation sequencing (NGS) information containing mutations for MM. 1S and U266B1 (Fig. 1a) were taken from the cBioPortal for Cancer Genomics database [21, 22], the TCGA Research Network (<http://cancergenome.nih.gov/>), and published information [23–25]. SCC cell lines SCC4 (tongue), SCC15 (tongue), UM-SCC19 (tongue), SCC25 (tongue), UM-SCC84 (tongue), UM-SCC92 (tongue), and UM-SCC99 (tonsil) were also used [25, 26]. NGS information containing mutations (Fig. 1a) and copy number variations (Fig. 1b) were taken from the cBioPortal for Cancer Genomics database [21, 22] and the Sanger sites for SCC4 (http://www.cbioportal.org/case.do?sample_id=SCC4_UPPER_AERODIGESTIVE_TRACT&cancer_study_id=cell_line_ccle_broad, http://cancer.sanger.ac.uk/cell_lines/sample/overview?id=910904); SCC15 (http://www.cbioportal.org/case.do?sample_id=SCC15_UPPER_AERODIGESTIVE_TRACT&cancer_study_id=cell_line_ccle_broad, http://cancer.sanger.ac.uk/cell_lines/sample/overview?id=910911); and SCC25 (<http://www.cbioportal.org/case.do?>

sample_id=SCC25_UPPER_AERODIGESTIVE_TRACT&cancer_study_id=ce_llline_ccle_broad, http://cancer.sanger.ac.uk/cell_lines/sample/overview?id=910701).

All cell line-specific mutation and copy number variation profiles were assessed for changes in gene sequence that altered gene function (Fig. 1c). Mutated genes were first compared to the Cellworks Group, Inc. Learnt Mutational Library. This is a library of genes whose mutations are known or previously determined to effect gene function. If the mutation was listed in the Library, then that result was used. If the mutation was not listed in the Library, then the effect of gene mutation on gene function was determined using cancer mutation effect prediction algorithms [27]. These algorithms include SIFT [28], Polyphen [29], FATHMM [27], Mutation Assessor [30], and PROVEAN [31]. Final results were recorded as an effect of unknown signature, neutral to gene function, or deleterious to gene function (Fig. 1c).

Predictive computational simulation models

Computational simulation model creation (Fig. 2a–e), prediction (Fig. 2f), and validation (Fig. 2g,h) for MM cell lines (MM.1S, U266B1) and oral SCC cell lines (SCC4, SCC15, and SCC25) were created in a series of successive steps. Non-transformed cell models (e.g., models not containing cell line-specific mutations and copy number variations) were used that contained integrated cancer cell networks created as previously described [32, 33]. These networks were created from published reports on cell receptors, signaling pathways, pathway signaling intermediates, activation factors, transcription factors, and enzyme kinetics. Information on gene functionality and links between different genes, proteins, and pathways were manually researched, analyzed, curated, and aggregated to construct the integrated network maze. Reactions were modeled mathematically using Michaelis Menton kinetics, mass action kinetics, and variations of these representations. Modeled events included, but were not limited to interactions at the cell surface (e.g., binding of agonists to receptors, etc.), metabolic and cell signaling pathways (e.g., signal pathway events, cross-talk interactions among pathways, feedback control, etc.), activation and regulation of genes (e.g., activation links of transcription factors, etc.), intracellular processes such as proteasomal degradation, endoplasmic reticulum stress, oxidative stress, DNA damage and repair pathways, and cell cycle pathways. Time-dependent changes in signaling pathway fluxes of biological reactions were modeled utilizing modified ordinary differential equations solved with a proprietary solver. At this level, data input and output were validated with a series of internal control analysis checks on predictive expression. Analyses included assessing the effects of pathway molecule over-expression or knockdown on predictive pathway responses; effects of drugs on predictive pathway responses; and activation, regulation, and cross-talk interactions among pathway intermediates on predictive pathway responses.

To start, each non-transformed cell model (Fig. 2c) was simulated until the system reached homeostatic steady state aligning it to a normal cell non-tumorigenic physiology. This established the control baseline for PD-L1 expression. Cell line-specific mutation profiles (Fig. 2a,b) were annotated into the cancer physiology network model (Fig. 2d) and it was simulated to induce the cell line-specific states (Fig. 2e) and to predict cell line-specific

dysregulated pathways. The time required to achieve a network varied depending upon on the complexity of the profile definition.

Simulation model predictions

Cell line-specific PD-L1 expression (Fig. 2f) was reported as a percent change with respect to non-tumorigenic baseline controls and calculated within each cell line separately. The percent change was calculated as $((D/C)-1)*100$, where C is the absolute value of the non-tumorigenic baseline control (μM) and D is the absolute value of the biomarker obtained in the cell line-specific network (μM).

Cell culture

Predicted PD-L1 expression was verified against observed PD-L1 expression on the same cell lines grown in culture (Fig. 2g,h). All cell lines were grown and maintained in a humidified atmosphere of 5% CO_2 at 37°C in media containing 100 units/ml penicillin (Life Technologies, Madison, WI) and 100 units/ml streptomycin (Life Technologies). MM.1S and U266B1 were grown in high glucose RPMI-1640 with L-glutamine, HEPES (ATCC, Manassas, VA), and 10% (MM.1S) or 15% (U266B1) fetal bovine serum (FBS) (ATCC).

SCC4 was grown in Dulbecco's Modified Eagle's Medium: F-12 (DMEM:F-12) containing 2 mM L-glutamine, 1% nonessential amino acids (ATCC), 400 ng/mL hydrocortisone (Sigma-Aldrich Corp., St. Louis, MO), and 10% FBS (ATCC). SCC15, SCC25, and UM-SCC84 were grown in Lymphocyte Growth Media-3 (LGM-3) (Lonza, Walkersville, MD), and 10% FBS (ATCC). UM-SCC19, UM-SCC92, and UM-SCC99 were grown in DMEM containing 2 mM L-glutamine, 1% nonessential amino acids (ATCC), and 10% FBS (ATCC).

PD-L1 reactivity has been observed by IHC on oral keratinocytes [33] and primary gingival epithelial (GE) keratinocyte cell lines GE365 and GE376 were used as PD-L1⁺ expression controls. GE keratinocytes, prepared in a previous study [34], were grown in keratinocyte-SFM with L-glutamine, human recombinant epidermal growth factor (EGF 1–53), bovine pituitary extract (BPE) (Gibco Life Sciences, Grand Island, NY), and 10% FBS (ATCC).

PD-L1 ELISA

Whole cell PD-L1 concentrations were first determined. 1.0 ml of cultured cells (1.0×10^6 viable cells/ml) in high glucose RPMI-1640 with L-glutamine, HEPES (ATCC), 100 units/ml penicillin (Life Technologies), and 100 units/ml streptomycin (Life Technologies) was added to wells of a 12-well culture plate (Corning Inc., Corning, NY). Cell lines suspended in this minimal media have higher computational and experimental correlation rates. The effects of different cell culture media and additives on cell biomarker responses are dramatically reduced.

At 24 hours, the 1.0 ml of RPMI-1640 was removed, the cells were lysed with 1.0 ml cell lysis buffer (Cell Signaling Technologies, Danvers, MA) containing 1.0 mM phenylmethanesulfonyl fluoride (Cell Signaling Technologies), and the lysate was stored at -80°C . PD-L1 concentrations (3 replications) were then determined on MM, SCC, and GE

keratinocyte lysates (ELISA, Cusabio Biotech Co., Ltd., Wilmington, DE) using the manufacturers protocol.

PD-L1 IHC

Cell surface PD-L1 expression was determined by IHC. 1.0 ml of cultured cells (1.0×10^6 viable cells/ml) in the minimal media described above was incubated in a humidified atmosphere of 5% CO₂ at 37°C. At 24 hours, cells were pelleted by centrifugation (400 RCF, Eppendorf 5810R, Brinkmann Instruments, Inc., Westbury, NY) at 4°C for 5 minutes, fixed in 10.0% neutral buffered formalin at room temperature, and suspended in 1.0% BactoAgar (Becton, Dickinson, and Co., Sparks, MD)-1.25% gelatin (Electron Microscopy Sciences, Hatfield, PA). BactoAgar-gelatin punches were made and plugs containing cells were immobilized together in a 12-plug array using molten BactoAgar-gelatin [34] and processed for microtomy as recently described [35].

IHC was performed by the University of Iowa Diagnostic Laboratories (University of Iowa, Iowa City, IA). Briefly, sections were deparaffinized and rehydrated. Cell antigens were unmasked in Citrate Buffer, pH 6.0 at 125°C for 5 minutes (Decloaker, Biocare Medical, Concord, CA) and sections were treated in 3% H₂O₂ for 8 minutes to quench endogenous peroxidase activity. Primary PD-L1 antibody (17952-1-AP CD274, Proteintech Group, Inc., Rosemont, IL) diluted 1:250 in Dako diluent (Dako North America, Inc., Carpinteria, CA) was added and sections were washed with Dako Wash Buffer (Dako North America, Inc.). Sections without primary PD-L1 antibody were included as negative section controls. After 60 minutes, PD-L1 reactivity on all sections was developed using Dako EnVision+System-HRP Labeled Polymer Anti-Rabbit (Dako North America, Inc.) and enhanced with Dako DAB Enhancer (Dako North America, Inc.). Sections were counterstained with Leica Hematoxylin. On negative antibody control slides, the same secondary antibody was used on the positive and negative control slides for 30 minutes at room temperature (Dako EnVision +System-HRP Labelled Polymer Anti-Rabbit, Dako North America, Inc.). The percent of PD-L1⁺ cells/total cells were counted on each section. Human pancreas and human tonsil tissues were used as IHC positive and negative controls, respectively.

PD-L1 flow cytometry

Flow cytometry was performed as recently described [35] to confirm cell surface expression of PD-L1. Briefly, 0.5 ml containing 0.5×10^5 viable cells in their respective media were incubated without and with IFN γ (10 ng/ml) for 24 hours. Cells were stained with anti-human APC-CD274 (563741 PD-L1, BD Pharmingen, San Jose, CA), stained with Live/Dead Fixable Green Dead Cell Stain (BD Biosciences, San Jose, CA), and examined using an LSR II Violet Flow Cytometer (BD Biosciences). Samples stained also with an isotype control (APC mouse IgG1 K, BD Pharmingen, San Jose, CA). Additional controls included unstained cells. Flow cytometric data was analyzed using FlowJo software (Tree Star, Inc., Ashland, OR).

Statistical analysis

Analogous two-way fixed effect ANOVA was fit to the PD-L1 concentrations determined by ELISA (JMP10, Version 10.0, SAS, Cary, NC). Pairwise comparisons were conducted using the T-test. A 0.05 level was used to determine statistically significant differences.

Results

Predictive computational simulation models

Simulation models were constructed to predict PD-L1 expression in MM and SCC cell lines. Cancer cell line mutations were first identified, assessed for mutational effects on gene function, converted into a computational format, and imported into the simulation workflow converting non-transformed cell models into dynamic cancer cell line-specific simulation models. Results were reported as percentage change with respect to the normal cell nontumorigenic state and PD-L1 expression varied among MM and SCC cell line-specific simulation models. In MM cell lines, the percentage change of U266B1 (39.2%) was greater than the percentage change of MM.1S (11.1%) (Fig. 3a). In SCC cell lines, the percentage change of SCC25 (91.3%) was greater than the percentage changes of SCC15 (49.3%) and SCC4 (11.1%) (Fig. 3a).

PD-L1 ELISA

Total whole cell PD-L1 concentrations were determined in MM (Fig. 3b), SCC (Fig. 3c,d), and GE keratinocyte (Fig. 3d) cell lysates using an ELISA. MM cell lysates ranged from 275.2 to 528.8 pg/ml PD-L1. U266B1 cell lysates contained more PD-L1 (482.4 ± 24.4 standard error pg/ml) than MM.1S (301.1 ± 25.9 standard error pg/ml) (Fig. 3b). SCC cell lysates ranged from 57.5 to 539.8 pg/ml PD-L1 (Fig. 3c). SCC25 cell lysates contained the most PD-L1 (387.6 ± 78.3 standard error pg/ml) and UM-SCC19 cell lysates contained the least (92.7 ± 8.7 standard error pg/ml). GE keratinocyte lysates ranged from 29.6 to 126.7 pg/ml PD-L1. GE365 cell lysates contained more PD-L1 (68.2 ± 29.3 standard error pg/ml) than GE376 (56.0 ± 15.2 standard error pg/ml) (Fig. 3d).

The concentrations of PD-L1 in MM and SCC cell lysates matched the percent of PD-L1 expression predicted to occur in these cells based on their mutational profile signatures (Table 1). The PD-L1 expression for U266B1 was greater than MM.1S, the PD-L1 expression for SCC25 was greater than SCC4, and the PD-L1 expression for SCC25 was greater than SCC15. However, the observed and predicted expression for SCC4 and SCC15 did not match. The mismatch was simply due to the lack of significant difference between the concentrations of PD-L1 in SCC4 and SCC15 cell lysates ($p < 0.05$) and a match could not be made.

PD-L1 IHC

PD-L1 reactivity was seen in the cytoplasm of some cells and on the surface of other cells (Fig. 4a–e). PD-L1 reactivity was specific. It was absent in the primary and secondary antibody negative controls. The percent of PD-L1⁺ MM and SCC cells matched the percent of PD-L1 expression predicted to occur in these cells based on their mutational profile signatures (Table 1). The numbers of PD-L1⁺ cells varied depending upon the cell line and

ranged from 4.0 to 23.8% (Fig. 4f). PD-L1 reactivity on U266B1 was greater than MM.1S, the reactivity of PD-L1 on SCC25 was greater than SCC4, the reactivity of PD-L1 on SCC25 was greater than SCC15, and the reactivity of PD-L1 on SCC15 was greater than SCC4 (Table 1).

PD-L1 flow cytometry

Cell surface PD-L1 is the major factor in influencing anti-tumor immune responses. Here, flow cytometry was used to validate cell surface expression of PD-L1 in MM and SCC cell lines (Fig. 4a–e). PD-L1 reactivity was specific. The percent PD-L1⁺ cells increased in MM and SCC cell lines treated with IFN γ (10 ng/ml) as a positive control (data not shown) compared to MM and SCC cell lines not treated. The percent of PD-L1⁺ MM and SCC cells (Fig. 4g) matched the percent of PD-L1⁺ expression predicted to occur in these cells. PD-L1 reactivity a) on U266B1 was greater than MM.1S, b) on SCC25 was greater than SCC4, c) on SCC25 was greater than SCC15, and d) on SCC15 was greater than SCC4 (Table 1).

Discussion

Detecting PD-L1 on cancer cells is important in cancer diagnosis and as a clinical predictor of cancer immunotherapy treatment success for both anti-PD-1 and anti-PD-L1 antibodies [36]. For better patient outcomes, there is a critical need to improve methods to accurately affirm PD-L1 expression. Determining PD-L1 reactivity in tumors using IHC can be variable. Determining sPD-L1 in serum or plasma can also be variable with differences associated with the collection methods, the samples (e.g., serum or plasma), and the assay methods [15]. Here, we created predictive computational simulation models containing cell line-specific genomic signatures to fill this gap and to predict PD-L1 expression with a validated, cancer network model. We found that computational models predicting PD-L1 expression correlated with observed PD-L1 expression by ELISA, IHC, and flow cytometry on the same cells grown in culture.

These simulation models have a variety of applications. They could be used a) after cancer diagnosis to aid in selecting patients for PD-L1 immunotherapy, b) to predict clinical immunotherapy treatment success, c) to predict the influence of various factors on the production and regulation of PD-L1, d) to differentiate PD-L1 drug responders from PD-L1 drug nonresponders, and e) to show the intermediate steps in cell signaling pathways where genomic signatures influence the downstream effects on PD-L1 expression (Fig. 5). As per the network representation, PD-L1 expression is regulated by the different transcription factors that are modulated by the upstream signals and the translated PD-L1 is then present as a membrane bound fraction.

MM is a hematologic disease characterized by the infiltration, expansion, and survival of malignant plasma cells in the bone marrow [37]. There are 30,330 estimated new cases of MM in the USA in 2016 (1.8% of all new cancer cases) and 12,650 estimated deaths (2.1% of all cancer deaths) [38]. The 5 year survival rate is 48.5% [38]. Clinical trials suggest that MM responds to programmed death immunotherapy. Current anti-PD-1 trials for MM include pembrolizumab (NCT01953692), nivolumab (NCT01592370), and pidilizumab (NCT02077959) and current anti-PD-L1 trials for MM include BMS-936559

(NCT01452334, withdrawn), atezolizumab (NCT02431208), and MEDI4736 (NCT01693562).

SCCs are common neoplasms of oral tissues causally associated with genetic factors, human papillomavirus infections, and behavioral exposures to environmental carcinogens, such as alcohol and tobacco consumption [39–41]. There are 48,330 estimated new cases of oral cavity and pharynx cancer in the USA in 2016 (2.9% of all new cancer cases) and 9,570 estimated deaths in 2016 (1.6% of all cancer deaths) [42]. The 5 year survival rate, regardless of tumor stage/grade of tumor differentiation, is 64.0% [42]. Clinical trials suggest that SCC responds to programmed death immunotherapy. Current anti-PD-1 trials for SCC include pembrolizumab (NCT01848834) and nivolumab (NCT02105636) and current anti-PD-L1 trials for SCC include atezolizumab (NCT01375842), avelumab (NCT01772004), and MEDI4736 (NCT01693562, NCT02207530).

Computational models to predict PD-L1 expression could enhance the clinical outcomes of these trials and improve treatment success rates. Recent studies have confirmed that tumor cell PD-L1 expression is highly correlated with objective responses [43]. In a phase I trial with nivolumab, patients with 5% IHC PD-L1⁺ tumor cells had an objective response rate of 36% whereas patients with IHC PD-L1[−] tumor cells did not have any objective response rate [44]. In another phase I trial with nivolumab, patients with IHC PD-L1⁺ tumor cells had an objective response rate of 44% whereas patients with IHC PD-L1[−] tumor cells had an objective response rate of 17% [45]. In another trial with MPDL3280A, patients with 5% IHC PD-L1⁺ bladder cancer cells had an objective response rate of 43.3% whereas patients with IHC PD-L1[−] tumor cells had an objective response rate of 11.4% [46]. Finally, utilizing MPDL3280A with advanced or metastatic solid tumors, patients with 5% IHC PD-L1⁺ tumor cells had an objective response rate of 39% whereas patients with IHC PD-L1[−] tumor cells had an objective response rate of 13.0% [47].

SCC cell lines do not uniformly express PD-L1 [4, 48, 49]. PD-L1 has a focal or diffuse distribution on the cell membrane and/or in the cell cytoplasm, which may be influenced by environmental stimuli [16]. We observed similar trends and PD-L1 reactivity was focal or diffuse on the cell membrane and in the cell cytoplasm (Fig. 4a–e). PD-L1 has also been detected at different concentrations with flow cytometry in unstimulated and stimulated SCC cell lines [48, 49]. Here, we detected differences in PD-L1 concentrations with ELISA, IHC, and flow cytometry in unstimulated MM and SCC cell lines (Fig. 3b, c). Observed PD-L1 expression matched the predicted PD-L1 expression (Table 1). The one mismatch seen between the predicted and the observed expression was due to the lack of significant difference between the observed concentrations of PD-L1 in SCC4 and SCC15 cell lysates.

In our models, intrinsic cellular control of PD-L1 expression was influenced via a number of different signaling pathways (Fig. 5). This is supported by other findings in the literature and the signaling pathways modeled for PD-L1 expression for MM and SCC by our simulated models, which are similar to that proposed by Ritprajak and Azuma for both extrinsic and intrinsic control of PD-L1 in a variety of cell types [16]. PD-L1 expression is induced in SCC cell lines and GE keratinocytes by IFN γ , TNF α , IL-1 α , and IL-1 β [48–50]. These and other induction stimuli likely act via toll-like receptors (TLRs) or interferon (IFN) receptors

to modulate the expression and activation of PD-L1 expression through various downstream signaling molecules, such as nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K), mechanistic target of rapamycin (mTOR), and janus kinase/signal transducers and activators of transcription (JAK/STAT) that affect cell cycle progression, cell proliferation, and activation or regulation of transcription factors [51–53]. These further regulate the nuclear translocation of transcription factors to the PD-L1 promoter [16].

Induction stimuli are processed through extracellular signal-regulated kinase (ERK) signaling pathways via epidermal growth factor receptor (EGFR), B-raf proto-oncogene, serine/threonine kinase (BRAF-V600E), mitogen-activated protein kinase kinase 1/2 (MEK1/2) (e.g., mitogen-activated protein kinase kinase 1 (MAP2K1) and MAP2K2); ERK1/2 (e.g., MAPK3 and MAPK1); and Jun proto-oncogene (c-Jun). High levels of PD-L1 expression are processed through signal transducer and activator of transcription (STAT) 3 and ERK signaling pathways [54, 55]. Induction stimuli are also processed through the epidermal growth factor (EGF) receptor (Erb) signaling pathway via neuroblastoma RAS viral oncogene homolog (NRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), V-akt murine thymoma viral oncogene homolog (AKT), mTOR, and STAT3 as well as the IFN γ pathway via IFNG, interferon gamma receptor 1 (IFNGR1), STAT1, and interferon regulatory factor 1 (IRF1). Pathway signals converge to activation factors activator protein-1 (AP-1), STAT1, STAT3, and IRF1 leading to transcription of PD-L1 genes. Tumors are driven by multiple aberrations in many of these genes that can in turn influence the expression levels of PD-L1 on the tumor cell and immunosuppressive tumor microenvironment.

In conclusion, we created computational simulation models containing cell line-specific genomic signature profiles to accurately predict PD-L1 expression on MM and SCC cell lines. Results from these models show that cell-genomics play an integral role by influencing cell signaling and downstream effects on PD-L1 expression. Computation-based prediction of PD-L1 expression could add value to analysis of PD-L1 expression in human cancer tissues. However, analysis of tumor cell lines could be misleading in predicting PD-L1 expression in tumor tissues where multiple inflammatory factors are involved in regulation of PD-L1. Given that PD-L1 expression by tumor cells has been considered a hallmark of adaptive resistance of cancers, conclusion based on tumor cells alone might be less significant, and future prediction models should be addressed in context with immune cells. This includes the cell-to-cell contact that occurs among cancer cells, T-cells, and dendritic cells in the tumor microenvironment. In the future, we anticipate that these models will also include the roles of other immunosuppressive chemokines and cytokines and the roles of immune cells. Together these models will be able to accurately predict PD-L1 expression of cancer cells, improve the potential of PD-L1 as a biomarker, and retain PD-L1 as an important clinical predictor of treatment success.

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Abbreviations

AKT	V-akt murine thymoma viral oncogene homolog
AP-1	Activator protein-1
BRAF-V600E	B-Raf proto-oncogene, serine/threonine kinase
c-Jun	Jun proto-oncogene
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FBS	Fetal bovine serum
GE	Gingival epithelial
IFNG	Interferon gamma
IFNGR1	Interferon gamma receptor 1
IHC	Immunohistochemistry
IRF1	Interferon regulatory factor 1
JAK/STAT	Janus kinase/signal transducers and activators of transcription (JAK/STAT)
MAP2K1	Mitogen-activated protein kinase kinase 1
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
MM	Multiple myeloma
mTOR	Mechanistic target of rapamycin
NF-κB	Nuclear factor kappa B
NGS	Next generation sequencing
NRAS	Neuroblastoma RAS viral oncogene homolog
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1

PI3K	Phosphatidylinositol 3 kinase
PIK3CA	Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha
SCC	Squamous cell carcinoma
sPD-L1	soluble PD-L1
STAT1	Signal transducer and activator of transcription 1
TLR	Toll-like receptor

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Fig. 1.

Cell line mutations **(a)** and copy number variations **(b)** for MM cell lines MM. 1S and U266B1 and oral SCC cell lines SCC4, SCC15, and SCC25 that were used to create cancer cell line-specific computational simulation models. Mutations and copy number variations were assessed for changes in gene sequence **(c)**. Mutated genes were identified and first compared to the Cellworks Group, Inc. (CWG) Learnt Mutational Library **(c)**. If the effect of gene mutation on gene function was listed in the CWG Library, then that result was used. If not, the effect of gene mutation on gene function was determined using the cancer mutation

effect prediction algorithms FannsDB, SIFT, Polyphen, FATHMM, Mutation Assessor (MA), and PROVEAN. Final results were recorded as an effect of unknown signature, neutral to gene function, or deleterious to gene function.

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Fig. 2.

A schema showing computational simulation model creation (**a–e**), prediction (**f**), and validation (**g,h**) for MM cell lines (MM.1S, U266B1) and oral SCC cell lines (SCC4, SCC15, and SCC25). Cell specific mutational profiles (**a**) were determined and the presence and impact of deleterious gene mutations and copy number variations on gene function were assessed (**b**). Information was imported into nontransformed predictive computational simulation models (**c, d**) to create cell line-specific computation models (**e**). These models were then used to predict PD-L1 expression (**f**). Predicted PD-L1 expression was verified against observed PD-L1 expression on the same cells grown in culture (**g**). The match rates between the predicted PD-L1 expression and the experimental PD-L1 concentrations were then determined (**h**).

Fig. 3.

The predicted PD-L1 percent expression with respect to (w.r.t.) controls (**a**) and the observed ELISA PD-L1 pg/ml concentration (**b, c**) in MM cell lines MM.1S and U266B1 and oral SCC cell lines SCC4, SCC15, and SCC25. * = $p < 0.05$. For comparison, the ELISA PD-L1 pg/ml concentrations are shown for other oral SCC cell lines SCC19, SCC84, SCC92, and SCC99 and GE keratinocytes GE376 and GE365 (**d**).

Fig. 4.
 The detection of PD-L1 by IHC and flow cytometry on MM cell lines MM.1S (a) and U266B1 (b) and on oral SCC cell lines SCC4 (c), SCC15 (d), and SCC25 (e). The percentage of PD-L1⁺ cells in IHC (f) and flow cytometry (g) are compared.

Fig. 5.

Computational simulation models of MM and oral SCC cell lines clearly demonstrate that cell line-specific genomics influence cell signaling and downstream effects on PD-L1 expression. In these models, production of PD-L1 is influenced via a number of intermediate proteins in the signaling pathways.

Table 1

Percentage changes of PD-L1 expression with respect to control baselines were determined and then verified against observed PD-L1 expression by ELISA, IHC, and flow cytometry on the same multiple myeloma (MM) and oral squamous cell carcinoma (SCC) cell lines grown in culture.

Predicted PD-L1⁺ expression (%)	Observed PD-L1⁺ expression (ELISA)	Observed PD-L1⁺ expression (IHC)	Observed PD-L1⁺ expression (Flow cytometry)	Mismatch/Match
U266B1 (39.2%) > MM.1S (11.1%)	U266B1 > MM.1S (p < 0.05)	U266B1 (9.0%) > MM.1S (7.7%)	U266B1 (0.2%) > MM.1S (0.1%)	Match
SCC15 (49.3%) > SCC4 (11.1%)	SCC4>SCC15 (p>0.05) ns	SCC15 (17.5%) > SCC4 (4.0%)	SCC15 (7.4%) > SCC4 (1.8%)	Mismatch/Match
SCC25 (91.3%) > SCC4 (11.1%)	SCC25 > SCC4 (p < 0.05)	SCC25 (23.8%) > SCC4 (4.0%)	SCC25 (24.0%) > SCC4 (1.8%)	Match
SCC25 (91.3%) > SCC15 (49.3%)	SCC25 > SCC15 (p < 0.05)	SCC25 (23.8%) > SCC15 (17.5%)	SCC25 (24.0%) > SCC15 (7.4%)	Match