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Analysis of PD-L1 expression in non-small cell lung cancer microenvironment and its role as a potential predictive biomarker

Análise da expressão de PD-L1 no microambiente do câncer de pulmão de não pequenas células e de seu papel como potencial marcador preditivo

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ABSTRACT: ***Objective***: To analyse the recent findings regarding programmed-death ligand 1(PD-L1) expression on tumor infiltrating immune cells in NSCLC and its potential role as a predictive biomarker for clinical outcomes and for successful PD-1/PD-L1 blocking immunotherapy. ***Methods***: 5 databases were accessed for search: PubMed, Web of Science, Scopus, Lilacs, and Clinical Trials.gov. Articles were selected if written in English, Portuguese or Spanish and if available via institutional access. ***Results***: 15 articles were selected. PD-L1 expression was found to be related to the presence of immature DCs and had also constitutive expression on fibroblasts derived from NSCLC specimens. PD-L1 expression in tumor infiltrating immune cells was observed to be correlated with overall survival benefit and improved tumor response after atezolizumab therapy. A significant correlation between PD-L1 expression in peripheral T cells and clinical outcomes was also detected, besides the finding of significant correlation between an increased PD-L1 expression and clinical benefits in anti-PD-1 therapy. ***Discussion***: Preliminary observations showed that PD-L1 expression in immune cells is related to an immunosuppressive milieu in NSCLC and to clinical benefits of immunotherapy.

Keywords: Immunotherapy; Carcinoma, non-small-cell lung; Antigens, CD274; Biomarkers.

RESUMO: ***Objetivo***: Analisar a literatura científica para a expressão de PD-L1 no infiltrado de células imunes de tumores do tipo CPCNP, além de seu potencial uso como biomarcador preditivo de desfechos clínicos e de resposta à imunoterapia com drogas anti PD-1 e anti PD-L1. ***Métodos***: 5 bases de dados foram consultadas para buscas (PubMed, Web of Science, Scopus, Lilacs e Clinical Trials.gov.). Artigos foram incluídos se pertinentes, disponíveis através de acesso institucional e se escritos em Português, Inglês ou Espanhol. Não houve restrição na seleção quanto tipo de estudo ou ano de publicação. ***Resultados***: 15 artigos foram selecionados. Foi observado relação entre o nível de expressão de PD-L1 e a presença de células dendríticas imaturas, além de expressão constitutiva da molécula em fibroblastos de pacientes com CPCNP. A expressão de PD-L1 nas células imunes infiltradas correlacionou-se com sobrevida aumentada e resposta tumoral melhor após terapia com atezolizumab, além de benefícios clínicos na terapia anti-PD-1. Outros artigos demonstram correlação significativa entre a expressão de PD-L1 em linfócitos T periféricos e desfechos clínicos. ***Discussão***: Observações preliminares demonstraram que a expressão de PD-L1 nas células imunes estão relacionadas ao sucesso clínico da imunoterapia e ao microambiente imunossupressor visto no CPCNP.

Descritores: Imunoterapia; Carcinoma pulmonar de células não pequenas; Antígenos CD274; Biomarcadores.

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INTRODUCTION

Non-Small Cell Lung Cancer (NSCLC) accounts for approximately 85%[1,2] of all lung cancer cases worldwide and is considered nowadays the most important cause of cancer-related deaths around the globe[3].

It is worth noting that oncologic treatment with a wide range of conventional therapies has been available and adopted for decades[2], but overall prognosis of NSCLC still remains unfavorable. In fact, the development of new and alternative therapies for NSCLC was reinforced lately by the promise of significant survival benefits for patients and the prospects of improved management in treatment toxicities[4].

Anti-tumor interventions based on immune system responses are not an innovative anti-cancer strategy, with studies dating back to mid -1970s[5]. Indeed, the accumulated comprehension of immune system's ability to fight cancer culminated more recently in the development of new immunotherapeutic agents, as therapeutic vaccines and immune checkpoint inhibitors[4,6].

Immune checkpoints, elected as object of great interest in last years, consist of various signaling pathways responsible for immunomodulation effects. Under physiological conditions, they are essential for providing self-tolerance and avoiding tissue damage, while in tumors they contribute to immune editing and tolerance[7].

The axis PD-1/PD-L1 is an important mechanism for inducing immune escape, leading to anergy and exhaustion of T-cells. PD-L1 is found to be overexpressed in several types of cancer, including NSCLC[7].

Examples of immunotherapy concerning checkpoint inhibitors are monoclonal antibodies that block the interaction of PD-1 on lymphocytes and PD-L1 on Antigen Presenting Cells (APCs) and tumor cells. They have already been applicable to melanoma, Hodgkin´s lymphoma, bladder cancer and non-small cell lung cancer (NSCLC), among others[8].

Nonetheless, not all patients with NSCLC will have significant benefits from immune checkpoint blocking drugs. Determining predictive biomarkers related to tumor response and clinical efficacy of these drugs is therefore essential to select those best candidates to immunotherapy.

The expression of PD-L1 in tumor cells has been largely considered as a potential predictive biomarker for clinical outcomes by many authors[9,10,11]. The same correlation between PD-L1 expression on tumor infiltrating immune cells and clinical outcomes has been, however, less frequently explored in scientific literacy.

In fact, studies approaching the issue resulted in the suggestion that increased expression of this molecule in tumor microenvironment could be possibly related to better clinical responses[12,13,14].

Therefore, we intend to review here the current understanding of PD-L1 expression on infiltrating immune cells in NSCLC, as well its potential as a predictive biomarker for clinical outcomes and for PD-1/PD-L1 immunotherapy response.

METHODS

Five databases were selected for search (PubMed, Web of Science, Clinical Trials.gov, Lilacs and Scopus).

To be included, articles should have adequate content, i.e., the expression and role of the molecule PD-L1 in the microenvironment (stroma and/or immune cells) in the context of non-small-cell lung cancer.

Criteria for exclusion were:

1. The language of articles (exclusion occurred if articles were not written in Portuguese, Spanish or English);
2. The availability of access;
3. Presence of abstracts/titles reporting PD-L1 expression in infection, other types of cancer or autoimmunity as main theme.

There was no restriction about the type of articles. Papers reporting experimental models should describe the cell lines used in tests. References were manually checked.

Search Strategy

Two search strategies for the retrieval of manuscripts were designed: one for Clinical Trials.gov and another one for the others 4 databases.

In Clinical Trials.gov, searches were made with the following combined terms:

- ''MPDL3280A'' and ''Non Small Cell Lung Cancer'';
- ''MEDI4736'' and '' Non Small Cell Lung Cancer'';
- ''MSB0010718C'' and ''Non Small Cell Lung Cancer''.

On the remaining databases, terms were combined as below to guarantee an adequate retrieval of articles. Controlled vocabulary was also adopted, specially as MeSH terms.

- PD-L1 and tumor infiltrating and biomarker
- PD-L1and tumor microenvironment AND biomarker
- PD-L1and tumor infiltrating and immunotherapy
- PD-L1and tumor microenvironment AND immunotherapy
- PD-L1 and myeloid cells and non small cell lung cancer
- PD-L1 and dendritic cells and non small cell lung cancer
- PD-L1 and macrophages and non small cell lung cancer
- PD-L1 and lymphocytes and non small cell lung cancer
- antigens, cd274 [mesh]) and myeloid cells [mesh]

- antigens, cd274 [mesh]) and dendritic cells [mesh]
- antigens, cd274"[mesh]) and carcinoma, non-small-cell lung [mesh]) and tumor microenvironment [mesh]
- tumor microenvironment [mesh]) and antigens, cd274 [mesh]
- lymphocytes, tumor-infiltrating [mesh]) and antigens, cd274 [mesh]) and carcinoma, non-small-cell lung [mesh]

RESULTS

Fifteen articles were selected after search, as described in Table 1.

Papers were then examined according to the type of cells studied, experimental designs and assessment methodology for PD-L1 expression (Table 2).

These points usually vary in scientific reports concerning PD-L1 expression, and have already been considered, among other factors, as obstacles for use of this molecule as a predictive biomarker in clinical outcomes[14].

For systematization, articles were also clustered in 3 different groups and graphically organized (Table 2).

Table 1. Description of number of articles retrieved and selected for each database

Data Base	Number of articles retrieved in search	Number of articles selected for review
Pubmed	993	14
Scopus	460	9
Web of Science	432	7
Lilacs	0	0
Clinical Trials.Gov	56	0
Articles retrieved after manual search	1	1
Full amount of articles selected for review after removal of duplicates and correction	15	

Table 2. Main findings of the articles included in the review

	PD-L1 expression in cells culture and experimental models	PD-L1 expression on tumor infiltrating cells extracted from oncologic patients	Clinical implications of PD-L1 expression as prognostic and predictive biomarker
Articles	Deng et al.[15]	Nazareth et al.[20]	Herbst et al.[13]
	Chen et al.[16]	Mu et al.[21]	Müller et al.[25]
	Noman et al.[17]	Perrot et al.[22]	Fehrenbacher et al.[26]
	Ni et al.[18]	Ilie et al.[23]	Taube et al.[27]
	Akbay et al.[19]	Yang et al.[24]	Meniawy et al.[28]
Types of cells studied	Myeloid-derived suppressor cells, DCs and macrophages[15-17]	Fibroblasts derived from NSCLC biopsies[20]	Macrophages, DC and lymphocytes[13]
	Murine DC[18]	TIDC (tumor-infiltrating dendritic cell)[21,22]	Tumor-infiltrating myeloid cells[25]
	Macrophages infiltrated in tumor and associated hematopoietic cells[19]	Tumor-infiltrating immune cells (no further specification)[23,24]	Tumor-infiltrating immune cells (no further specification)[26]
			Tumor-infiltrating lymphocytes, histiocyte/macrophages and native stroma[27]
			Peripheral blood T lymphocytes[28]
Experimental design of the study	Injection of varied tumor cells lines in murine model followed by cells collection and molecular analysis of PD-L1 expression[15-17]	Tumor associated fibroblasts and tumor associated T cells isolation with following growth of fibroblasts[20]	Pretreatment tumor specimens collected for PD-L1 study, followed by correlation with clinical outcomes[13,27]
	Co-incubation of labeled Lewis lung cancer cells, fibroblasts and DCs, followed by analysis of TGF-β and PD-L1 expression on DCs[18]	Biopsy sections deparaffinized and analysed for PD-L1 expression on tumor and TIDC, as well as for maturation status of these immune cells[21]	Patients assessed for PD-L1 tumor-infiltrating immune cell status and randomly allocated to receive atezolizumab or docetaxel. Clinical following until death of patients[26]
	Microenvironment analysis of EGFR mutated tumor[19]	Study of TIDC obtained from pulmonary mononuclear cell suspension[22]	Retrospective comparison of PD-L1 expression on stroma and tumor cells in primary lesions versus metastatic lesions[25]
		PD-L1 expression on surgical tissue sections versus previous lung biopsies[23]	Collection of blood samples before and after therapy with EGFR inhibitors, followed by correlation between PD-L1 expression and clinical outcomes[28]
		PD-L1 expression on immune and tumor cells from stage I pulmonary squamous cell carcinoma[24]	
Assessment methodology for PD-L1 expression	Flow cytometry[15-19]	Flow cytometry[20,22]	IHC[13,25-27]
	IHC[19]	Immunofluorescence staining and confocal microscopy for PD-L1 expression in DC[21]	Flow cytometry[28]
		IHC[23,24]	

Articles approaching PD-L1 expression in cells culture and experimental models composed the first group. Here, Dendritic Cells (DCs) and myeloid cells were the cells most frequently used for study, as well as flow cytometry for assessment of PD-L1 expression. One of the articles adopted both immunohistochemistry and flow cytometry for PD-L1 assessment[19].

Experimental designs chosen for studies presented great variability, including injection of varied tumor cells lines in mice followed by immune cells collection and the establishment of an in vitro[18] and an in vivo[19] lung cancer microenvironment.

The main results concerning PD-L1 expression showed increased presence on DCs and tumor cells after tumor radiation[15], on aged mice when compared to young animals[16], on tumor infiltrating Myeloid Derived Supressor Cells (MDSCs), macrophages and DC of tumor cultured under hypoxia[17] and TGF-β[18] and on hematopoietic cells associated to EFGR mutated tumors[19].

Articles approaching PD-L1 expression on tumor infiltrating cells extracted from oncologic patients composed the second group. Here, tumor-infiltrating dendritic cells were the cell type most frequently used for study. Fibroblasts, considered as important components of tumor stroma, were object of study in just one of the articles selected for this review[20]. Flow cytometry and immunohistochemistry were equally used for assessment of PD-L1 expression, with authors electing immunofluorescence staining and confocal microscopy for PD-L1 expression in one of the articles[21].

Results of this section of works explored the finding of immature dendritic infiltrating DCs in tumoral and peritumoral tissues, with increased expression of PD-L1 when compared to mature DCs[21,22]. It was also demonstrated the constitutive expression of PD-L1 on fibroblasts derived from NSCLC specimens, as well the up-regulation of PD-L1 in these stroma cells after IFN-γ treatment[20].

In a retrospective study, Ilie et al.[23] presented an overall discordance rate of 48% between PD-L1 expression on surgical resected and matched biopsy specimens of patients diagnosed with NSCLC and not treated with Radiotherapy (RT) or Chemotherapy (CT). For the authors, 75% of the discordant cases could be credited to PD-L1 positivity of immune infiltrate cells in resection specimens and previous negative results for ICs on diagnostic biopsies[23].

Articles approaching clinical implications of PD-L1 expression as prognostic and predictive biomarker composed the third and last group. Here, diverse types of immune cells were used for study, without any clear trend. All researchers, with exception in one paper[28], worked with IHC to assess PD-L1 expression.

Results of this last section included:

- Statistical significant association of response to MPDL3280A treatment and tumor-infiltrating immune cell PD-L1 expression in NSCLC[13];
- No significant difference in PD-L1 expression on tumor or myeloid cells when comparing primary tumors and corresponding metastases[25];
- Correlation of overall survival benefit from atezolizumab therapy with PD-L1 expression on tumor cells, tumor infiltrating immune cells, or both[26];
- No statistical significance with objective clinical response (p=0.14), but significant correlation with clinical benefit after anti PD-1 therapy. (p=0.038)[27];
- Significant correlation between PD-L1 expression on peripheral T cells and clinical outcomes in EGFR-TKI-treated NSCLC[28].

DISCUSSION

PD-L1 expression in tumor infiltrating immune cells was considered in a reduced number of articles. In fact, questions addressing its clinical implication or specifically its expression in the context of NSCLC also seemed slightly explored in literature, indicating a potential direction for future researches.

Another important aspect to be considered was the heterogeneity between articles selected. There was no consensus about the type of cells used, about experimental design of study or methodological assessment for PD-L1 expression. Articles containing experimental model worked with different cell lines, with no correlation to NSCLC seen in patients. The development of cells cultures resembling the tumor microenvironment was also a difficult task.

Articles approaching clinical implications had also registered different experimental designs in each study, with different results obtained in this section. PD-L1 expression was, however, only implicated with clinical benefits or prognostic factor in interventional studies with therapies directed to immune checkpoints. We did not find any article approaching the relationship between PD-L1 expression and clinical outcomes derived from natural history or traditional oncologic treatments. Our results do not support an increased risk for metastasis occurrence in PD-L1 positive patients.

The finding of immature infiltrating DCs was frequently pointed in articles selected for this review. This observation is consistent with the described PD-L1 function on immune system, and can become a future point of interest and clinical intervention.

In fact, the data here collected point to the need for further investigations, since that preliminary observations showed PD-L1 expression on immune cells related to clinical success in immunotherapy and to an immunosuppressive milieu in the context of NSCLC.

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