DOI: 10.23925/1984-4840.2019v21i1a2 **Review /** *Revisão*

Inhibition of PD-1 protein by the CRISPR-Cas9 method as antitumor therapy of non-small cell lung cancers

Inibição da proteína PD-1 pelo método CRISPR-Cas9 como terapia antitumoral para tumores de pulmão de não pequenas células

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ABSTRACT

Lung carcinoma is the second most common type of tumor in the world. Among them, 85% of the cases are of non-small cell lung cancer (NSCLC). It is known that, in general, NSCLC tumor cells proliferate due to a reduction in the cytotoxic T lymphocyte response. In the immune response to tumors, the interaction of the programmed death ligand 1 (PD-L1), expressed in tumor cells and the programmed cell death protein 1 (PD-1), expressed in cytotoxic T lymphocytes, promotes suppression of the immune response, leading to inhibition of the activation of cytotoxic T lymphocytes. Despite the biological therapies that have proven effective for the treatment of lung tumors, studies seek a genetic treatment option, such as the CRISPR/Cas9 method. This review aims to provide an update of the CRISPR-Cas9 method and its application as a therapeutic tool in NSCLC to deactivate the gene encoding the PD-1 protein. The genetic alteration of PD-1 protein by CRISPR-Cas9 can affect the interaction between receptor and ligand, allowing cytotoxic T lymphocytes to recognize and exert an antitumor response to NSCLC tumors.

Keywords: carcinoma, bronchogenic; CRISPR-Cas Systems; genetic therapy.

RESUMO

O carcinoma de pulmão é o segundo tipo de tumor de maior incidência em todo o mundo, sendo 85% deles carcinomas de pulmão de não pequenas células (CPNPC). As células tumorais do CPNPC proliferam em razão de um bloqueio da resposta de linfócitos T citotóxicos. Na resposta imune a tumores, a interação do ligante-1 do receptor de morte celular programada (PD-L1), expresso em células tumorais com a proteína de morte celular programada 1 (PD-1), expressa em linfócitos T citotóxicos, promove a supressão da resposta imune, levando à inibição da ativação de linfócitos T citotóxicos. Apesar de as terapias biológicas mostrarem-se eficazes para o tratamento de tumores pulmonares, estudos buscam uma opção de tratamento genético, como o método CRISPR/Cas9. O objetivo desta revisão é fornecer uma atualização do método CRISPR-Cas9 e a aplicação dele como ferramenta terapêutica buscando desativar o gene que codifica a proteína PD-1 em casos de CPNPC. A alteração genética da proteína PD-1 pelo CRISPR-Cas9 pode interromper a interação entre receptor e ligante, permitindo que linfócitos T citotóxicos reconheçam e exerçam uma resposta antitumoral contra tumores de CPNPC.

Palavras-chave: carcinoma broncogênico; sistemas CRISPR-Cas; terapia genética.

BACKGROUND

Lung cancer is one of the most aggressive types of neoplasia, and its incidence has increased in the world. Its high mortality is due to late diagnosis, since, in general, it does not present signs and symptoms in its initial stage. Histological types of lung cancer are adenocarcinoma, squamous cell carcinoma (SCC), large cell carcinoma, and small cell carcinoma. Lung cancer can be classified into small cell lung cancer (SCLC), and non-small cell lung cancer (NSCLC). Although conventional therapies such as chemotherapy, radiotherapy and lung resection remain the first line of treatment for NSCLC, new therapies based on monoclonal antibodies and biological inhibitors have been shown to be effective as alternative therapies, generating results with fewer side effects.[1-5]

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Received on 02/27/2018. Accepted for publication on 08/23/2018.

Among these therapies, anti-PD-1 and anti-PD-L1 monoclonal antibodies, such as nivolumab and pembrolizumab, have shown effective responses in the treatment of NSCLC[1]. The programmed cell death protein 1 (PD-1) is an immune system inhibitory receptor found primarily in cytotoxic T lymphocytes, which interacts with two ligands, the programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD- L2), the former being expressed in tumor cells and the latter in inflammatory cells. These interactions between receptors and ligands are also active forms of control of autoimmune responses by the inhibition of the activation of cytotoxic T lymphocytes.[6-8]

Experimental studies have shown that increased expression of PD-L1 results in unfavorable responses in patients with certain types of tumors, and this expression affects the T lymphocyte response, resulting in the exhaustion or even apoptosis of this population.[9] Therefore, the interaction of PD-L1 with the PD-1 receptor promotes an escape of the tumor cells because of the blockade of the T lymphocyte response. These results enabled the search for strategies for blocking the PD-1/PD-L1 interaction as an immunological therapy promising for tumors.[6,10,11]

Clustered regularly-interspaced short palindromic repeats (CRISPR) is a system that allows bacteria to recognize and fight viral infections when combined with CRISPR-associated proteins (Cas). The use of the CRISPR method associated with Cas9 (CRISPR-Cas9) has been shown to be a promising tool in biomedical and biological research as gene therapy against infectious, cancerous, and other diseases. This is due to its ability to correct errors found in the genome, by activating or deactivating genes, easily and non-expensively.[12-14]

Currently, the application of the CRISPR-Cas9 method to deactivate the gene coding for PD-1 is a therapeutic possibility in the generation of cytotoxic T lymphocytes that can act on antitumor response to the NSCLC. This work aimed to perform a literature review on NSCLC, comparing its biological treatment with the conventional one, and how the use of genetic editing using the CRISPR-Cas9 method can serve as antitumor therapy for this type of tumor.

NON-SMALL-CELL LUNG CANCER

Only about 16% of patients with lung tumors have a 5-year survival rate. Although smoking is one of the major triggers of lung cancer, carcinogenic and gas emissions from coal combustion are also considered risk factors for the development of this type of tumor, as well as frequent pulmonary infections, history of tuberculosis and deficiency or excess of vitamin A. Lung tumors are divided into SCLC and NSCLC. NSCLC includes different histological types, in which the main ones are large cell carcinoma, squamous cell carcinoma and adenocarcinoma, the latter being responsible for most cases of NSCLC, and the most common type of lung carcinoma in women and nonsmokers.[4,8,15-18]

Oncogenes and tumor suppressor genes (TSGs) that are overexpressed or containing modifications are the most common molecular alterations involved in lung carcinomas. Some of the most activated oncogenes in NSCLC include epidermal growth factor receptor (EGRF) and KRAS, while the most common tumor suppressor genes include TP53, p16, and TSG of chromosome 3p.[19]

KRAS is an oncogene responsible for signaling the production of GTPase transducing proteins, K-ras proteins, involved in the regulation of cell division. K-ras proteins signal to the cell nucleus, directing the processes of cell growth, division and differentiation. Mutations in the KRAS gene may result in uncontrolled proliferation, leading to the formation of cancer cells.[20] Mutations in KRAS are common in patients with NSCLC, but rare in cases of SCLC. These mutations are strongly associated with smoking and have a worse prognosis in patients with adenocarcinoma, including in cases of resected tumor.[21]

Tyrosine kinase receptors, when interacting with its ligands, trigger various cellular processes mediated by signaling pathways of growth factors important for cell growth and division, among other functions. EGFR belongs to the family of tyrosine kinase receptors present in various cellular processes, such as differentiation and proliferation. These receptors may be dysregulated due to mutations leading to their overexpression, or overproduction of the ligands and may trigger the development of tumor cells.[22-24]

PROGRAMMED DEATH RECEPTOR 1/ PROGRAMMED DEATH LIGAND

PD-1 is an immunoinhibitory cellular receptor that belongs to the CD28 family and is expressed on T lymphocytes, B lymphocytes, monocytes, among other cells of the immune system. The main function of PD-1 is to control autoimmunity during inflammatory, infectious and carcinogenic processes. It has two ligands, PD-L1 and PD-L2, which have distinct functions and are expressed on several different cell types, including tumor cells. PD-L1 is expressed on T cells, B cells, macrophages, NK cells, dendritic cells, and in non-hematopoietic tissues such as lung tissue, while PD-L2 is expressed predominantly in dendritic cells, macrophages and mast cells. The interaction of PD-1 with its ligands (PD-1/PD-L) controls the peripheral tolerance of T lymphocytes by different mechanisms, reducing the activation of these lymphocytes and decreasing the production of cytokines, thus preventing autoimmunity. However, this has antagonistic response by providing an escape of the tumor cells against the response of the T cells.[25-29]

In addition to its expression on immune cells, PD-L1 protein is expressed in several types of tumor cells, including those of NSCLC, and its high expression on tumor cells may be related to tumor development, degree of aggressiveness and patient survival. This is due to the interaction of PD-L1 present in tumor cells with PD-1 present in tumor infiltrating lymphocytes, resulting in T lymphocyte exhaustion and reduced cytokine production against tumor cells. Thus, the use of monoclonal antibodies (mAbs) to block PD-1/PD-L1 interaction has become an option to improve anti-tumor response of T lymphocytes. Studies using anti-PD-1 and anti-PD-L1

mAbs have shown that the blockade of this interaction increases T-cell activity and the production of cytokines in the tumor environment, suggesting that this mechanism may be efficient in regulating tumor growth.[30,31]

GENE THERAPY

With the advancement of gene therapy using vectors, genetic editing methods have emerged capable of inducing highly accurate changes in the genome. The use of genetic editing, in contrast to conventional methods of gene therapy that are temporary, makes it possible to definitively treat disease by the induction of molecular alterations. The most commonly used methods for genetic editing are Zinc-finger nucleases (ZFNs), Transcription Activator-like effector-based Nucleases (TALENs) and CRISPR, which uses in *vitro* modified nucleases to induce precisely double-stranded breaks (DSBs) at specific sites in the genome, and trigger repair mechanisms such as non-homologous end joining (NHEJ) and homology-directed repair (HDR). CRISPR differs from ZFNs and TALENs because of its simplicity. Furthermore, it can target low nucleotide sequences, whereas ZFN and TALEN require large segments of DNA for insertion.[32,33]

CLUSTERED REGULARLY-INTERSPACED SHORT PALINDROMIC REPEATS (CRISPR)/ CRISPR-ASSOCIATED (CAS) 9

CRISPR, when combined with Cas, is a defense system found in several species of prokaryotic bacteria that is used to recognize and fight invasive genetic material, usually DNA from viral infections. The system has a locus that contains short repeats of DNA, interspaced with single and variable sequences called spacers, derived from DNA of bacteria-invading viruses, that protects the bacteria against future infections with that same DNA sequence.[34] The sequence of invasive genetic material that corresponds to the sequence of the spacers in the CRISPR locus is called protospacer and is adjacent to a sequence called protospacer adjacent motif (PAM).[35] The PAM sequence identifies the invading DNA and signals where the Cas protein must bind to cleave the DNA. Mutations in the PAM sequence prevent the Cas protein from cleaving the invading DNA, because without this sequence the CRISPR system may recognize its own spacer as an invasive material. Many viruses contain mutations in PAM sequence as a way of escaping immunity by CRISPR in bacteria.[36]

When the invasive material is injected into the bacterium, the CRISPR system transcribes and translates a Cas protein, and transcribes the DNA sequence that corresponds to the protospacer creating the CRISPR RNA (crRNA) that fits into a Cas. Cas binds in the PAM sequence of the invading DNA, which is after the complementary sequence to the crRNA, and cleaves the invading DNA into small fragments. If it is a new infection, CRISPR will transcribe and translate another Cas protein that will break the invading DNA and copy its sequence into the locus of the CRISPR system, creating a new spacer.[34,37,38]

There are three types of CRISPR. CRISPR type I and III have in common the use of a Cas protein complex to recognize and cleave the target DNA, whereas CRISPR type II uses only one type of Cas to perform these processes, Cas 9. In bacteria, Cas 9 uses two RNAs to operate their function, the crRNA that identifies the target DNA sequence, and trans-activating crRNA (tracrRNA) that activates the double strand break (DSB) of the target DNA by the Cas. A group of scientists were able to create a synthetic RNA by the junction of the crRNA and tracrRNA, generating a single RNA guide called single guide RNA (sgRNA) that succeeded in performing the functions of crRNA and tracrRNA separately, making CRISPR-Cas9 system even simpler.[38]

When DSB occurs, cell will trigger its repair mechanisms. The most common mechanisms are non-homologous end joining (NHEJ) and homologous recombination (HR). In the NHEJ mechanism, the damaged DNA ends bind together, losing DNA sequences, deactivating the target gene and generating insertions and/or deletions leading to mutations. In the HR mechanism the repair is done using a DNA sequence that will serve as a copy to fulfill the space where the damage occurred. In the latter context, scientists have developed a repair mechanism called homology-directed repair (HDR) using a donor DNA that will be inserted into the desired sequence producing precise insertions, deletions, and base substitutions at the DSB site.[39]

The editions performed by CRISPR-Cas9 can correct errors in the genome and activate or deactivate genes.[40] Despite its accuracy in identifying target sequences, Cas9 tolerates incompatibility of some nucleotides of the desired sequence, which can generate several off-target mutations, which can only be identified with whole genome sequencing (WGS).[37] The reasons that can lead Cas9 to cleave off-target sequences may be the incompatibility of some bases in off-target sequences that are the same size as the target sequence, and/or the off-target sequence has different number of bases than the target sequence.[41] These off-target cleavages may result in undesired mutations of the genome and in chromosomal changes. Although these effects have raised concerns about the applicability of the system, several advances have been made to minimize off-target effects, including a method using a Cas9 variant, SpCas9-HF1, which reduced off-target changes of the genome to not-detectable levels.[41,42]

APPLICATION OF CLUSTERED REGULARLY-INTERSPACED SHORT PALINDROMIC REPEATS IN THE DEVELOPMENT OF TUMOR MODELS

Processes that lead to the development of tumors in humans are usually caused by genetic alterations, such as translation, duplication, deletion, or inversions of chromosomes, and by mutations that inactivate tumor suppressor genes, activate oncogenes or alter genes due to repair processes.

The CRIPSR/Cas9 system helps not only to treat tumors but also to better understand the primary processes that lead to carcinogenesis by inducing gene alterations using the method to create tumor models.[43]

With this approach, scientists succeeded in inactivating tumor suppressors in rat lungs, obtaining a model like tumors induced by other methods, injected plasmids that led to the formation of hepatic carcinomas, and injected lentiviral vectors that resulted in pancreatic adenocarcinoma. The use of CRISPR to model and test these mutations *in vitro* and *in vivo* could provide information on the consequences of these changes, aiding in the choice of customized alternative treatments for patients.[44]

Chimeric antigen receptors (CAR) are synthetic receptors that redirect the specificity of T lymphocytes. They are currently being used as a form of immunotherapy for various diseases, including neoplasia. In antitumor therapy, T lymphocytes are genetically engineered to express specific antigens, the CARs, against tumor cells with high affinity and without MHC restriction. CAR T lymphocytes may also enhance T lymphocyte expansion, resistance and activation in the tumor environment, and may overcome tumor cells escape mechanisms.[45,46]

Therapy using CAR T-lymphocytes can become an allogeneic T lymphocyte bank of universal donors for patients who are suited for its use as treatment. It is known that in transplants, endogenous T cell receptors (TCR) in allogeneic T lymphocytes can recognize the receptor alloantigen and induce graft versus host disease (GVHD) and rejection of transplanted tissue. The use of gene therapy, such as CRISPR/Cas9, can prevent these reactions by promoting deactivation of endogenous T-cell TCR genes and by decreasing the expression of major histocompatibility complex (MHC) proteins from T lymphocytes derived from donors. In T lymphocytes, CRISPR-Cas9 can also be used to deactivate genes encoding T lymphocyte inhibitory receptors, such as PD-1, along with endogenous TCR, as was done in a clinical trial with positive results.[47]

CLUSTERED REGULARLY-INTERSPACED SHORT PALINDROMIC REPEATS IN NON-SMALL-CELL LUNG CANCER

In NSCLC, tumor cells escape the immune system due to PD-1/PD-L1 interaction. Currently, various clinical trials assess the efficacy of inhibition of this interaction using gene therapy. A clinical trial using T-lymphocytes from patients with carcinoma and from healthy donors showed that the expression of the PD-1 receptor can be effectively deactivated in T lymphocytes using the CRISPR-Cas9 method without the proliferation of these cells being altered. T cell cytokine production with deactivated PD-1 was also tested, and the results showed that the deactivation of PD-1 by Cas9 increases cytokine production by T lymphocytes from carcinoma patients and from healthy donors, increasing antitumor response.[48]

Another clinical trial also showed the antitumor efficacy of CAR T lymphocytes, with inhibited PD-1 using the CRISPR-Cas9 system. In this study, genetic editing and lentiviral vectors was combined to produce PD-1 deficient CAR T lymphocytes. With this method, the reduction of more than 50% of CAR T-lymphocytes expressing PD-1 was observed. Deletion of PD-1 did not alter the activation of CAR T lymphocytes. The antitumor efficiency of PD-1 CAR T lymphocytes was also tested in vivo using murine model. The results showed that mice with initial tumor burden that received the CAR T-lymphocytes with PD-1 deactivated were free of the tumor. However, the results were dependent on the number of CAR T lymphocytes transferred. These results indicated that the deactivation of PD-1 in CAR T lymphocytes using the CRISPR-Cas9 method increases the antitumor response of these cells.[49]

A group of scientists are performing the first test of the CRISPR-Cas9 method for the treatment of NSCLC in humans. Scientists collected T lymphocytes from the patient with metastatic NSCLC and edited these cells by turning off the gene encoding the PD-1 protein using the CRISPR-Cas9 method. The lymphocytes were cultured *in vitro*, injected into the patient, and monitored for six months for possible side effects, but there are no sufficient results yet.[50]

FINAL CONSIDERATIONS

With advances in gene therapy methods, genetic treatment for NSCLC has become a more promising and long-lasting option. Based on the knowledge that NSCLC tumor cells can inhibit the activation of the immune response mediated by the PD-1/PD-L interaction, clinical trials have been initiated to evaluate the effectiveness of the blocking therapies for this interaction. Inactivation of the gene encoding the PD-1 protein in T lymphocytes using the CRISPR-Cas9 method is expected to provide a more efficient therapeutic possibility.

It is expected that, by using T lymphocytes without the expression of PD-1, tumor cells will not have an escape mechanism due to PD-1/PD-L interaction, and thus allowing T lymphocytes to fight tumor cells more efficiently and for a longer term. Although clinical trial results in humans are still inconclusive, this is a promising therapeutic possibility, and further testing should be performed to prove the efficacy of this therapy in NSCLC.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Nakamura H, Saji H. A worldwide trend of increasing primary adenocarcinoma of the lung. Surg Today. 2014;44(6):1004-12. https://doi.org/10.1007/s00595-013-0636-z
2. Garon EB. Current perspectives in immunotherapy for non-small cell lung cancer. Semin Oncol. 2015;42 Suppl 2:S11-8. https://doi.org/10.1053/j.seminoncol.2015.09.019

3. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med. 2008;359(13):1367-80. https://doi.org/10.1056/NEJMra0802714
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11-30. https://doi.org/10.3322/caac.21166
5. Zer A, Leighl N. Promising targets and current clinical trials in metastatic non-squamous NSCLC. Front Oncol. 2014;4:329. https://doi.org/10.3389/fonc.2014.00329
6. Kim JW, Eder JP. Prospects for targeting PD-1 and PD-L1 in various tumor types. Oncology (Williston Park). 2014;28 Suppl 3:15-28.
7. Sunshine J, Taube JM. PD-1/PD-L1 inhibitors. Curr Opin Pharmacol. 2015;23:32-8. https://doi.org/10.1016/j.coph.2015.05.011
8. Silva APS, Coelho PV, Anazetti M, Simioni PU. Targeted therapies for the treatment of non-small-cell lung cancer: monoclonal antibodies and biological inhibitors. Hum Vaccin Immunother. 2017;13(4):843-53. https://doi.org/10.1080/21645515.2016.1249551
9. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-75. https://doi.org/10.1200/JCO.2009.26.7609
10. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov. 2013;3(12):1355-63. https://doi.org/10.1158/2159-8290.CD-13-0310
11. Kim JM, Chen DS. Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). Ann Oncol. 2016;27(8):1492-504. https://doi.org/10.1093/annonc/mdw217
12. Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol. 2011;9(6):467-77. https://doi.org/10.1038/nrmicro2577
13. Bondy-Denomy J, Garcia B, Strum S, Du M, Rollins MF, Hidalgo-Reyes Y, et al. Multiple mechanisms for CRISPR-Cas inhibition by anti-CRISPR proteins. Nature. 2015;526:136-9. https://doi.org/10.1038/nature15254
14. Richter C, Chang JT, Fineran PC. Function and regulation of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) systems. Viruses. 2012;4(10):2291-311. https://doi.org/10.3390/v4102291
15. Cohen MH, Johnson JR, Chattopadhyay S, Tang S, Justice R, Sridhara R, et al. Approval summary: erlotinib maintenance therapy of advanced/metastatic non-small cell lung cancer (NSCLC). Oncologist. 2010;15(12):1344-51. https://doi.org/10.1634/theoncologist.2010-0257
16. Carvalho L. Reclassifying bronchial-pulmonary carcinoma: differentiating histological type in biopsies by immunohistochemistry. Rev Port Pneumol. 2009;15(6):1101-19. https://doi.org/10.1016/S0873-2159(15)30195-1
17. Scagliotti GV, Hirsh V, Siena S, Henry DH, Woll PJ, Manegold C, et al. Overall survival improvement in patients with lung cancer and bone metastases treated with denosumab versus zoledronic acid: subgroup analysis from a randomized phase 3 study. J Thorac Oncol. 2012;7(12):1823-9. https://doi.org/10.1097/JTO.0b013e31826aec2b
18. Rossi A, Maione P, Bareschino MA, Schettino C, Sacco PC, Ferrara ML, et al. The emerging role of histology in the choice of first-line treatment of advanced non-small cell lung cancer: implication in the clinical decision-making. Curr Med Chem. 2010;17(11):1030-8. https://doi.org/10.2174/092986710790820589
19. Larsen JE, Minna JD. Molecular biology of lung cancer: clinical implications. Clin Chest Med. 2011;32(4):703-40.
20. Jančík S, Drábek J, Radzioch D, Hajdúch M. Clinical relevance of KRAS in human cancers. J Biomed Biotechnol. 2010;Article ID 150960:1-13. https://doi.org/10.1155/2010/150960
21. Duarte RLM, Paschoal MEM. Molecular markers in lung cancer: prognosis role and relationship to smoking. J Bras Pneumol. 2005;32(1):56-65. https://doi.org/10.1590/S1806-37132006000100012
22. Doebele RC, Oton AB, Peled N, Camidge DR, Bunn PA Jr. New strategies to overcome limitations of reversible EGFR tyrosine kinase inhibitor therapy in non-small cell lung cancer. Lung Cancer. 2010;69(1):1-12. https://doi.org/10.1016/j.lungcan.2009.12.009
23. Reungwetwattana T, Dy GK. Targeted therapies in development for non-small cell lung cancer. J Carcinog. 2013;12:22. https://doi.org/10.4103/1477-3163.123972.eCollection 2013
24. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway : a model for targeted therapy. Clin Cancer Res. 2006;12(18):5268-72. https://doi.org/10.1158/1078-0432.CCR-05-1554
25. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit t cell responses. Immunity. 2007;27(1):111-22. https://doi.org/10.1016/j.immuni.2007.05.016
26. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol. 2007;8(3):239-45. https://doi.org/10.1038/ni1443
27. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704. https://doi.org/10.1146/annurev.immunol.26.021607.090331
28. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219-42. https://doi.org/10.1111/j.1600-065X.2010.00923.x.

29. Jin HT, Ahmed R, Okazaki T. Role of PD-1 in regulating T-cell immunity. Curr Top Microbiol Immunol. 2011;350:17-37. https://doi.org/10.1007/82_2010_116
30. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015;21(1):24-33. https://doi.org/10.1016/j.molmed.2014.10.009
31. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24(2):207-12. https://doi.org/10.1016/j.coi.2011.12.009
32. Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397-405. https://doi.org/10.1016/j.tibtech.2013.04.004
33. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Invest. 2014;124(10):4154-61. https://doi.org/10.1172/JCI72992
34. Terns MP, Terns RM. CRISPR-based adaptive immune systems. Curr Opin Microbiol. 2011;14(3):321-7. https://doi.org/10.1016/j.mib.2011.03.005
35. Barrangou R, Marraffini LA. CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity. Mol Cell. 2014;54(2):234-44. https://doi.org/10.1016/j.molcel.2014.03.011
36. Semenova E, Jore MM, Datsenko KA, Semenova A, Westra ER, Wanner B, et al. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. Proc Natl Acad Sci. 2011;108(25):10098-103. https://doi.org/10.1073/pnas.1104144108
37. Reis A, Hornblower B; New England Biolabs. CRISPR/Cas9 and targeted genome editing: a new era in molecular biology. New Engl BioLabs [Internet]. 2014;1 [acessado em 07 mai. 2019]. Disponível em: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
38. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-21. https://doi.org/10.1126/science.1225829
39. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. Elife. 2014;3:e04766. https://doi.org/10.7554/eLife.04766
40. Dance A. Core Concept: CRISPR gene editing. Proc Natl Acad Sci. 2015;112(20):6245-6. https://doi.org/10.1073/pnas.1503840112
41. Lin Y, Cradick TJ, Brown MT, Deshmukh H, Ranjan P, Sarode N, et al. CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. Nucleic Acids Res. 2014;42(11):7473-85. https://doi.org/10.1093/nar/gku402
42. Kim D, Bae S, Park J, Kim E, Kim S, Yu HR, et al. Digenome-seq: Genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. Nat Methods. 2015;12(3):237-43. https://doi.org/10.1038/nmeth.3284
43. Torres-Ruiz R, Rodriguez-Perales S. CRISPR-Cas9: a revolutionary tool for cancer modelling. Int J Mol Sci. 2015;16(9):22151-68. https://doi.org/10.3390/ijms160922151
44. Kannan R, Ventura A. The CRISPR revolution and its impact on cancer research. Swiss Med Wkly. 2015;145:w14230. https://doi.org/10.4414/smw.2015.14230. eCollection 2015
45. Jena B, Dotti G, Cooper LJN. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010;116(7):1035-44. https://doi.org/10.1182/blood-2010-01-043737
46. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3(4):388-98. https://doi.org/10.1158/2159-8290.CD-12-0548
47. Ren J, Zhao Y. Advancing chimeric antigen receptor T cell therapy with CRISPR/Cas9. Protein Cell. 2017;8(9):634-43. https://doi.org/10.1007/s13238-017-0410-x
48. Su S, Hu B, Shao J, Shen B, Du J, Du Y, et al. CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci Rep. 2016;6:20070. https://doi.org/10.1038/srep20070
49. Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-Tumor efficacy of human chimeric antigen receptor T cells. Sci Rep. 2017;7(1):737. https://doi.org/10.1038/s41598-017-00462-8.
50. Cyranoski D. CRISPR gene-editing tested in a person for the first time. Nature. 2016;539(7630):479. https://doi.org/10.1038/nature.2016.20988

How to cite this article:
Biggi AFB, Simioni PU. Inhibition of PD-1 protein by the CRISPR/Cas9 method as antitumor therapy of non-small cell lung tumors. Rev Fac Ciênc Méd Sorocaba. 2019;21(1):2-7. https://doi.org/10.23925/1984-4840.2019v21i1a2