ONCOIMMUNOLOGY
2022, VOL. 11, NO. 1, e2111915 (3 pages)
https://doi.org/10.1080/2162402X.2022.2111915

Taylor & Francis
Taylor & Francis Group

AUTHOR'S VIEW

OPEN ACCESS Check for updates

PARP1 inhibition elicits immune responses against non-small cell lung cancer

Pan Juncheng[a,b,c], Antoine Lafarge[a,b,c], Guido Kroemer[a,b,d], and Maria Castedo[a,b]

[a]Equipe 11 Labellisée Par la Ligue Contre le Cancer, Université de Paris Cité, Sorbonne Université, Paris, France; [b]Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France; [c]Faculté de médecine, Université de Paris Saclay, Kremlin Bicêtre, France; [d]Institut du Cancer Paris CARPEM, Department of Biology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France

ABSTRACT

High levels of intracellular poly(ADP-ribose) (PAR) resulting from an elevated activity of PAR polymerase-1 (PARP1) correlate with poor infiltration of non-small cell lung cancers by cytotoxic T lymphocytes and dismal patient prognosis. Preclinical experimentation now demonstrates that PARP1 inhibition in cancer cells mediates strong immunostimulatory effects.

ARTICLE HISTORY
Received 22 July 2022
Revised 08 August 2022
Accepted 8 August 2022

KEYWORDS
Anticancer immune response; metabolism; tumor microenvironment; immune escape; non-small cell lung cancer

Poly(ADP-ribose) (PAR) is a polymer that is synthesized by the enzyme PAR polymerase-1 (PARP1), which is activated by DNA damage. PAR is covalently attached to chromatin-binding proteins and facilitates the recruitment of repair enzymes to the damaged double strain.[1,2] For this reason, upregulation of PARP1 may reduce the susceptibility of cancer cells to DNA-damaging chemotherapeutics such as cisplatin, while its inhibition sensitizes to cisplatin.[3–5] The mRNA levels transcribed from the *PARP1* gene are more elevated in non-small cell lung cancers than in adjacent normal tissues, perhaps reflecting an intrinsic resistance of malignant cells against DNA damage.[6] The levels of PAR can be easily assessed by immunohistochemistry. Both in non-small cell lung cancer (NSCLC) and in cervical cancer, high levels of PAR indicate poor prognosis, correlating with a reduced infiltration of the tumors by $CD8^+$ T lymphocytes.[7,8] Indeed, there is an expanding literature indicating that scarce infiltration of NSCLC by $CD8^+$ T cells is associated with dismal prognosis, as well as with poor responses to immunotherapy.[9] It is important to note that there is little if any correlation between the levels of PARP1 protein expression and the abundance of intracellular PAR,[8] likely reflecting the fact that PARP1 is usually enzymatically inactive and that it is only its activation that leads to PAR accumulation. Indeed, the expression of PARP1 protein has no prognostic value in NSCLC.[8]

Recently, we decided to determine the mechanism through which PAR expression anticorrelates with $CD8^+$ T cell infiltration in NSCLC. Theoretically, the activation of PARP1 yielding PAR in malignant cells could result from T lymphocyte-mediated immunoselection. Alternatively, or in addition, exaggerated PARP1 activation might have local immunosuppressive effects. For this reason, we decided to modulate PARP1 expression/activation in NSCLC cells and to investigate the capacity of PAR^{high} and PAR^{low} NSCLC cells to elicit T cell-mediated immune responses in mice.[6]

First, we created two PAR^{high} NSCLC cell lines (Lewis lung cancer [LLC] and tissue culture number one [TC1]) by selecting them by long-term culture in the presence of low-dose cisplatin (Figure 1a). The resulting PAR^{high} NSCLC cell lines maintained their elevated PAR level even after several months of culture in the absence of cisplatin, likely due to an increased level of spontaneous DNA damage.[6] PAR^{high} NSCLC cells were cloned to reduce their heterogeneity and then subjected to the knockout (KO) of PARP1 by CRISPR/Cas9 technology, yielding cells that lacked any signs of PAR accumulation (which demonstrates that PARP1 is the sole PARP isoform generating PAR in these cells) and became sensitive to cisplatin (which demonstrates that PARP1 was indeed responsible for chemotherapy resistance). Of note, PAR^{high} cells exhibited strong responses to the cytostatic and cytotoxic effects of niraparib, a specific PARP1 inhibitor which is clinically approved.[10] Conversely, $PARP1^{KO}$ cells became resistant to niraparib, as expected (Figure 1b).

Next, we inoculated PAR^{high} and $PARP1^{KO}$ NSCLC cells into immunodeficient mice, from which T lymphocytes had been depleted by injection of antibodies specific for CD4 or CD8. PAR^{high} and $PARP1^{KO}$ LLC or TC1 cells indistinguishably formed tumors in these T cell-depleted animals. In sharp contrast, $PARP1^{KO}$ TC1 cells failed to develop tumors in immunocompetent histocompatible mice, in conditions in which PAR^{high} TC1 cells readily proliferated, forming tumors with similar growth kinetic in immunocompetent and immunodeficient mice. When immunocompetent recipient mice had rejected the inoculation of $PARP1^{KO}$ TC1 cells, they subsequently became resistant against PAR^{high} TC1 cells implanted into the opposite flank. These findings indicate that $PARP1^{KO}$ TC1 cells cause a durable protective anticancer immune response mediated by T cells. We also found that $PARP1^{KO}$ LLC cells came under immunosurveillance. PAR^{high} LLC cells similarly grew on T cell-depleted and control

Guido Kroemer kroemer@orange.fr Equipe 11 labellisée par la Ligue contre le Cancer, Université de Paris, Sorbonne Université, INSERM U1138, Centre de Recherche des Cordeliers, Paris 75006, France;; CONTACT Maria Castedo maria.castedo-delrieu@sorbonne-universite.fr;

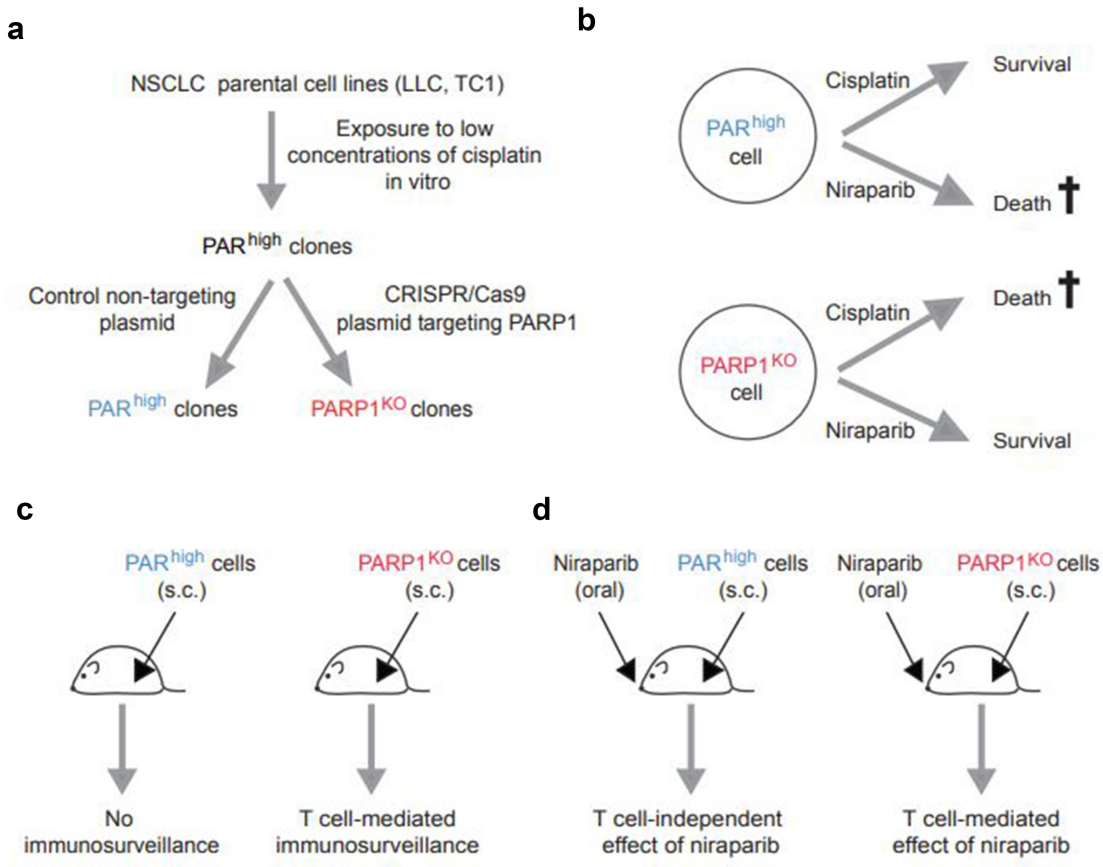

Figure 1. Modulation of PARP1 activity and immune response in mouse NSCLC models. A. Multistep generation of PAR^{high} and PARP1^{KO} NSCLC cell lines. LLC and TC1 PAR^{high} cell lines were obtained by exposure to sublethal cisplatin doses. PAR^{high} cells were cloned and transfected with a CRISPR/Cas9 vector that lacks a guidance DNA or that target PARP1. Then, LLC and TC1 clones were derived that possess a high PARP1 activity or that lack PARP1 expression and activity. B. Characteristics of PAR^{high} and PARP1^{KO} NSCLC cells in vitro. While PAR^{high} cells were largely resistant to cisplatin but died in the presence of niraparib, PARP^{KO} cells died when treated with cisplatin but only showed a marginal response to niraparib. C. Immunosurveillance of PARP1^{KO} NSCLC in vivo. PAR^{high} cells proliferated similarly in immunocompetent and T cell-deficient mice while the proliferation of PARP^{KO} cells is strongly controlled by T cells. D. Niraparib effects on PAR^{high} and PARP1^{KO} NSCLC in vivo. Whereas PAR^{high} tumors implanted in mice reduced their growth in response to niraparib in both immunocompetent and T-cell deficient mice, niraparib decreased PARP^{KO} tumor growth only in the presence of T cells. LLC, Lewis-lung cancer; NSCLC, non small cell lung cancer; PAR, poly(ADP-ribose); PARP1, Poly(ADP-ribose) polymerase-1; s.c., subcutaneous; TC1, Tissue culture number one.

mice.[6] However, PARP1^{KO} LLC cells experienced a considerable growth disadvantage when implanted into immunocompetent mice. The immune infiltrate of such PARP1^{KO} LLC cells contained more dendritic cells and more activated, ICOS-expressing CD8$^+$ T cells, but less activated regulatory T cells, coupled to the down-modulation of the exhaustion marker PD-1 on CD4$^+$ and CD8$^+$ T cells.[6] These findings confirm the capacity of active PARP1 to locally suppress the anticancer immune response (Figure 1c).

Next, we investigated whether pharmacological (rather than genetic) inhibition of PARP1 would stimulate an anticancer immune response. Of note, PAR^{high} LLC tumors similarly responded to niraparib treatments in immunocompetent and T cell-deficient mice, suggesting that niraparib effects on cells possessing the niraparib target (which is activated PARP1) do not require any major contribution by the cellular immune system. In sharp contrast, PARP1^{KO} LLC cancers only responded to niraparib when T lymphocytes were present in the system. PARP1^{KO} LLC tumors evolving in T cell-depleted mice did not respond to niraparib. However, PARP1^{KO} LLC tumors developing in immunocompetent mice reduced their growth upon systemic injection of niraparib (Figure 1d). These findings point to the possibility that PARP1 inhibitors can act through the stimulation of an anticancer immune response even when the malignant cells lack this target. However, the mechanisms of this immunostimulatory effect remain to be elucidated.

Disclosure statement

GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Samsara, Sanofi, Sotio, Vascage and Vasculox/Tioma. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio.

Funding

GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association "Ruban Rose"; Cancéropôle Ile-de-France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; Equipex Onco-Pheno-Screen; European Joint Programme on Rare Diseases (EJPRD); Gustave Roussy Odyssea, the European Union Horizon 2020 Projects Oncobiome and Crimson; Fondation Carrefour; Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LabEx Immuno-Oncology [ANR-18-IDEX-0001]; the Leducq Foundation; a Cancer Research ASPIRE Award from

the Mark Foundation; the RHU Torino Lumière; Seerave Foundation; SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE).; and SIRIC Cancer Research and Personalized Medicine (CARPEM). This study contributes to the IdEx Université de Paris ANR-18-IDEX-0001. AL was supported by a fellowship from Institut Thématique Multi-Organisme Cancer (ITMO Cancer) du Plan Cancer 2014-2019.

ORCID

Maria Castedo http://orcid.org/0000-0001-6855-3451

Data availability statement

This is a commentary on a paper published in Journal for ImunoTherapy of Cancer. All original data are available in this paper.

References

1. Krishnakumar R, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell. 2010 Jul 9;39(1):8–24. doi:10.1016/j.molcel.2010.06.017.
2. Ray Chaudhuri A, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodeling. Nat Rev Mol Cell Biol. 2017 Oct;18(10):610–621. doi:10.1038/nrm.2017.53.
3. Michels J, Vitale I, Galluzzi L, Adam J, Olaussen KA, Kepp O, Senovilla L, Talhaoui I, Guegan J, Enot DP, et al. Cisplatin resistance associated with PARP hyperactivation. Cancer Res. 2013 Apr 1;73(7):2271–2280. doi:10.1158/0008-5472.CAN-12-3000.
4. Michels J, Vitale I, Senovilla L, Enot DP, Garcia P, Lissa D, Olaussen KA, Brenner C, Soria JC, Castedo M, et al. Synergistic interaction between cisplatin and PARP inhibitors in non-small cell lung cancer. Cell Cycle. 2013 Mar 15;12(6):877–883. doi:10.4161/cc.24034.
5. Olaussen KA, Adam J, Vanhecke E, Vielh P, Pirker R, Friboulet L, Popper H, Robin A, Commo F, Thomale J, et al. PARP1 impact on DNA repair of platinum adducts: preclinical and clinical read-outs. Lung Cancer. 2013 May;80(2):216–222. doi:10.1016/j.lungcan.2013.01.014.
6. Juncheng P, Joseph A, Lafarge A, Martins I, Obrist F, Pol J, Saavedra E, Li S, Sauvat A, Cerrato G, et al. Cancer cell-autonomous overactivation of PARP1 compromises immunosurveillance in non-small cell lung cancer. J Immunother Cancer. 2022 Jun;10(6):e004280. doi:10.1136/jitc-2021-004280.
7. Joseph A, Juncheng P, Mondini M, Labaied N, Loi M, Adam J, Lafarge A, Astesana V, Obrist F, Klein C, et al. Metabolic features of cancer cells impact immunosurveillance. J Immunother Cancer. 2021 Jun;9(6):e002362. doi:10.1136/jitc-2021-002362.
8. Michels J, Adam J, Goubar A, Obrist F, Damotte D, Robin A, Alifano M, Vitale I, Olaussen KA, Girard P, et al. Negative prognostic value of high levels of intracellular poly(ADP-ribose) in non-small cell lung cancer. Ann Oncol. 2015 Dec;26(12):2470–2477. doi:10.1093/annonc/mdv393.
9. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019 Jun;19(6):307–325. doi:10.1038/s41568-019-0144-6.
10. Mateo J, Lord CJ, Serra V, Tutt A, Balmaña J, Castroviejo-Bermejo M, Cruz C, Oaknin A, Kaye SB, de Bono JS. A decade of clinical development of PARP inhibitors in perspective. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2019 Sep 1;30(9):1437–1447. doi:10.1093/annonc/mdz192.