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CP-240766

European Journal of Immunology

Letter to the Editor

[DOI: 10.1002/eji.202049158]

Tumor cell-intrinsic RIG-I signaling governs synergistic effects of immunogenic cancer therapies and checkpoint inhibitors in mice

Novel immunotherapeutic concepts, including immune checkpoint inhibitors of PD-1 and CTLA-4, have dramatically changed clinical practice in cancer treatment. These therapies (re)invigorate T-cell-based tumor immune responses. However, for many patients these therapies fail, as the immunosuppressive tumor milieu often compromises initial development of spontaneous immune responses. To initiate adaptive immune responses, APCs—particularly dendritic cells (DCs)—have to engulf, process, and present tumor-associated antigens to T cells. This cross-priming of tumor-reactive T cells is dependent on DC maturation by type I interferon (IFN-I) signaling [1]. In contrast to infections, lack of proinflammatory signals in the tumor microenvironment often results in suboptimal DC activation. However, under certain circumstances, tumor cells can undergo forms of programmed cell death that favor recognition by the immune system. Such immunogenic cell death (ICD) has been observed after exposure to certain chemotherapeutic agents or radiation. A characteristic of ICD is the release of proinflammatory factors—so-called danger-associated molecular patterns—that lead to DC maturation via stimulation of innate pattern recognitions receptors.

We have recently shown that activation of the RNA receptor RIG-I (retinoic acid-inducible gene 1) in tumor cells is a prerequisite for successful immune checkpoint inhibitor therapy [2]. RIG-I detects the aberrant cytosolic localization of RNA structural motifs that are normally restricted to the nucleus [3]. We found that tumor-intrinsic RIG-I signaling induces caspase-3-mediated programmed tumor cell death, cross-presentation of tumor-associated antigen by DCs, and finally activation of tumor-specific $CD8^+$ T cells. High gene expression of *DDX58* (encodes RIG-I) in human melanoma was associated with beneficial patient responses to anti-CTLA-4 checkpoint inhibition. The molecular mechanisms mediating RIG-I-induced ICD remain incompletely defined but seem to involve proapoptotic proteins and activation of caspase-3 [4, 5]. Whether tumor-intrinsic RIG-I signaling plays a role for other immunogenic cancer treatments and which endogenous RNAs active RIG-I in this context, remain unclear.

Radiation therapy (RTx)-induced cell death can be proinflammatory and synergize with anti-CTLA-4 treatment in immunogenic murine tumor models [6]. To address the role of tumor-intrinsic RIG-I signaling in the context of RTx-induced ICD, we used either wild-type (WT) or RIG-I-deficient ($RIG\text{-}I^{-/-}$) immunogenic B16.OVA melanoma cells in a bilateral flank tumor model. We locally irradiated right-sided tumors and evaluated "local" and "systemic" (contralateral, nonirradiated tumors) responses to anti-CTLA-4 immunotherapy (Fig 1A). As described in Ref. [2], anti-CTLA-4 delayed tumor growth, but was significantly less potent in mice bearing $RIG\text{-}I^{-/-}$ tumors, resulting in faster tumor growth and shortened survival compared to WT tumors (Fig 1B and C). In animals-bearing WT tumors, RTx of right flank tumors resulted in their total regression, but not of contralateral tumors (Fig 1B and C). However, combining RTx with systemic anti-CTLA-4 led to growth control of distant tumors, associated with long-term survival in most animals-bearing WT tumors (Fig 1C and D). In contrast, RTx of $RIG\text{-}I^{-/-}$ tumors combined with anti-CTLA-4 failed to prevent rapid outgrowth of distant $RIG\text{-}I^{-/-}$ tumors associated with poor survival (Fig 1C and D). Local tumor control by RTx seemed impaired in mice-bearing $RIG\text{-}I^{-/-}$ tumors (Fig 1B). Previous in vitro studies suggested that genotoxic stress in tumor cells during chemotherapy or RTx can result in leakage of small nuclear RNAs (snRNA) into the cytoplasm, where they activate RIG-I, IFN-I production, and programmed cell death [7]. Overall, these data suggest that activation of the RIG-I pathway within tumor cells facilitates RTx-triggered ICD, thus promoting anti-CTLA-4 immunotherapy, and that this might be mediated via translocation of snRNAs into the cytosol.

Hints for alternative tumor-intrinsic RIG-I ligands which may contribute to anti-CTLA-4 efficacy, came from a study that linked hypomethylating agents such

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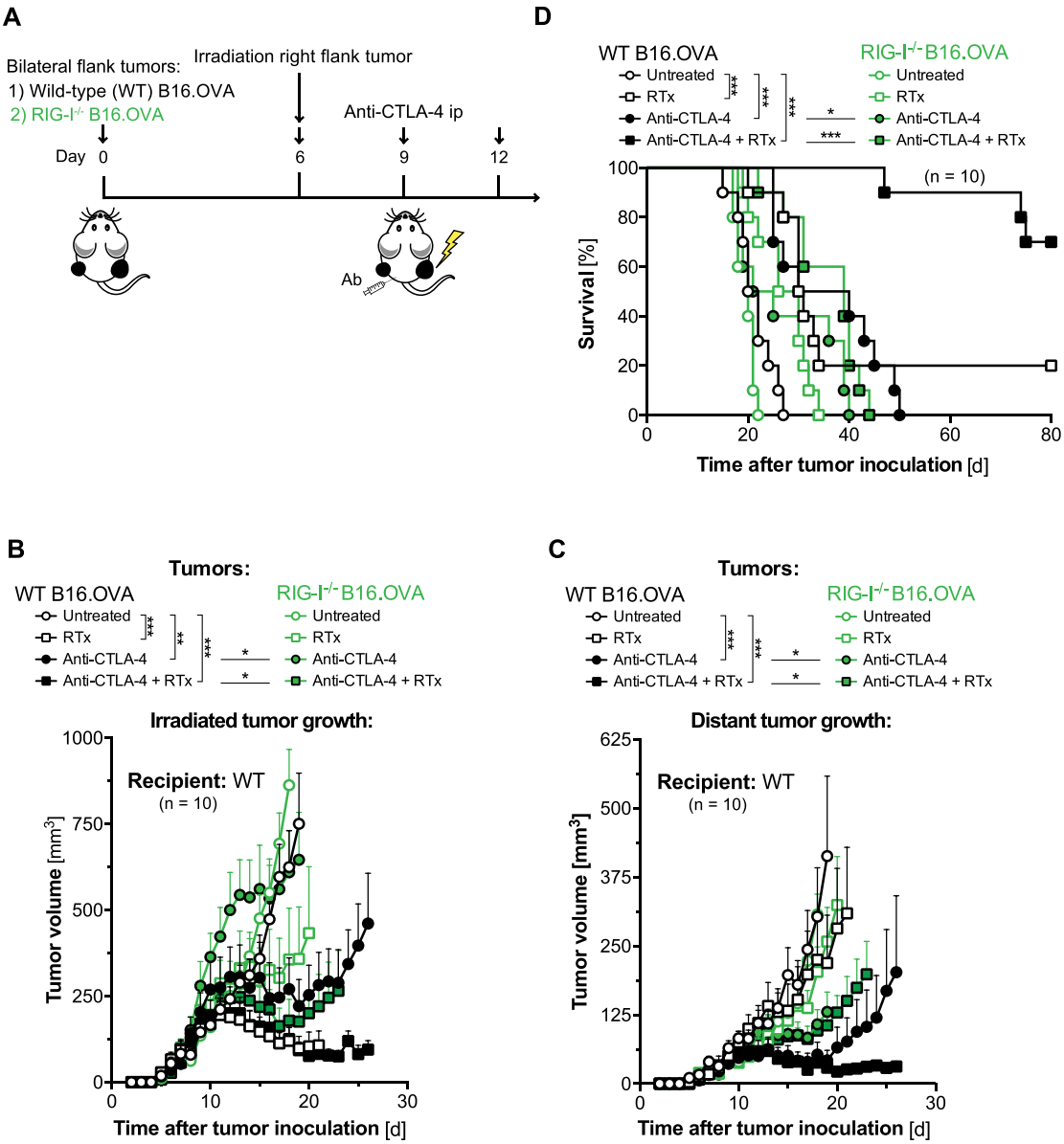

Figure 1. Synergistic immunogenic effects of radiation therapy and checkpoint inhibition depend on tumor cell-intrinsic RIG-I. (A) Mice were subcutaneously implanted with either WT or RIG-I-deficient (RIG-I$^{-/-}$) B16.OVA cells in both flanks. Right-sided tumors were induced with more cells to facilitate faster growth dynamic in comparison to left-sided tumors. Tumor-bearing animals were injected intraperitoneally with anti-CTLA-4 or isotype control antibodies. On day 6, in some mice right-sided tumors were irradiated (RTx, 20 Gy). **(B-C)** Mean tumor growth ± SEM of **(B)** irradiated **(C)** and nontreated ("distant") B16.OVA tumors. **(D)** Survival of mice bearing WT or RIG-I$^{-/-}$ tumors. Data of n = 10 individual mice were pooled from two independent experiments. Experiments were analyzed using unpaired *t*-test or one-way ANOVA with Bonferroni's post-test (tumor growth) or Log-rank test (survival). Significance was set at p values < 0.05, $p < 0.01$, and $p < 0.001$ (*, **, and ***).

as the DNA methyltransferase inhibitor 5-azacytidine (Aza) to IFN-I responses in tumors [8]. Aza is a standard therapy for acute myeloid leukemia in elderly patients. Apart from epigenetic reprogramming of gene expression in malignant cells, Aza facilitates antineoplastic immunity by increasing mRNA transcripts of otherwise DNA-hypermethylated and thus silenced endogenous retroviruses (ERVs) in tumor cells. This induces a RIG-I-like helicase-dependent IFN-I response and synergizes with anti-CTLA-4 blockade for antitumor immunity [8]. We found that in melanoma cells, Aza upregulated expression of ERVs—independent of RIG-I signaling (Fig 2A). Aza treatment subsequently induced RIG-I-mediated melanoma cell death (Fig 2B). This was independent of downstream transcription factors IRF3/IRF7 and associated IFN-I production. Using our bilateral melanoma model with localized, intratumoral Aza administration (Fig 2C), we observed that treatment efficacy of Aza and/or anti-CTLA-4 was governed by tumor-intrinsic RIG-I activity in vivo (Fig 2D-F). Particularly, systemic tumor control and associated host survival were impaired in animals bearing RIG-I$^{-/-}$ tumors.

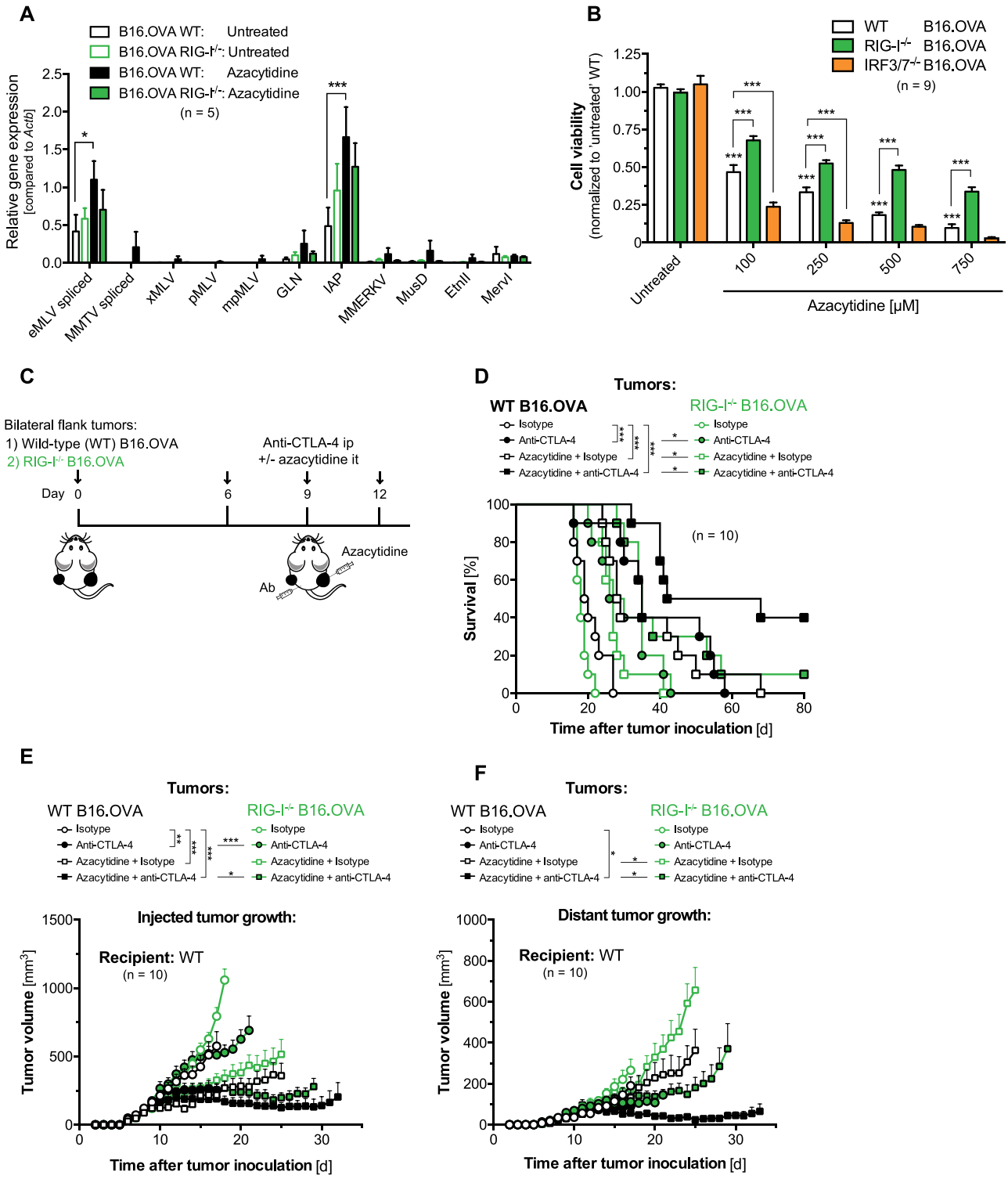

Figure 2. ERV transcript-upregulating 5-azacytidine induces RIG-I-dependent melanoma cell death and enhanced checkpoint inhibitor-mediated antitumor immunity. (A-B) WT, RIG-I$^{-/-}$, and IRF3/7-deficient (IRF3/7$^{-/-}$) B16.OVA cells were treated with 5-azacytidine. **(A)** Expression of endogenous retroviral (ERV) gene mRNA transcripts in WT and RIG-I$^{-/-}$ B16.OVA cells (normalized to *Actb1*) and **(B)** cell viability were determined. **(C)** Mice were inoculated with either WT or RIG-I$^{-/-}$ B16.OVA cells and were treated with anti-CTLA-4 as described in Figure 1A. Some mice were simultaneously injected with 5-azacytidine into the right-sided tumor. **(D)** Survival of mice-bearing WT or RIG-I$^{-/-}$ tumors. **(E and F)** Tumor growth of **(E)** Aza-treated and **(F)** nontreated ("distant") B16.OVA tumors. Tumor growth curves show mean tumor volume ± SEM. Data of n = 10 individual mice were pooled from two independent experiments. Experiments were analyzed using unpaired *t*-test or one-way ANOVA with Bonferroni's post-test (in vitro assays, tumor growth) or Log-rank test (survival). Significance was set at p values < 0.05, $p < 0.01$, and $p < 0.001$ (*, **, and ***).

Based on our findings, we hypothesize that continuous low-level detection of RIG-I ligands—presumably ERVs or snRNAs—within tumor cells may be responsible for basal, persistent pathway activation, thus, contributing to the efficacy of anti-CTLA-4-based therapy. Enhanced exposure to endogenous RIG-I ligands induced by RTx or Aza additionally fosters the immunogenicity of these cancer treatments and consequently synergism with checkpoint inhibitors. Nonetheless, environmental factors, such as microbiota, can influence efficacy of checkpoint blockade [9, 10]. Commensal bacteria may provide sources of exogenous ligands that also mediate RIG-I pathway activation in tumors, enhancing the potency of cancer immunotherapies.

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Acknowledgments: This study was supported by Deutsche Forschungsgemeinschaft–Projektnummer 360372040–SFB 1335 (to H.P. and F.B.) and 395357507–SFB 1371 (to H.P), a Mechtild Harf Research Grant (DKMS Foundation for Giving Life to H.P), European Research Commission (project BCM-UPS, grant #682473 to F.B.), a Young Investigator Award (Melanoma Research Alliance to S.H.), and Lady Tata Memorial Trust (to S.H.), the German Cancer Aid (111620) (to H.P. and S.H.), European Hematology Association (to H.P.). H.P. is supported by the EMBO Young Investigator Program.
Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest: The authors declare no commercial or financial conflict of interest.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202049158.

Data availability statement: Data available on request from the authors.

References

1 **Diamond, M. S. et al.**, *J Experiment Med*. 2011. **208**: 1989–2003.
2 **Heidegger, S. et al.**, *Sci Immunol*. 2019. **4**:eaau8943.
3 **Hornung, V. et al.**, *Science*. 2006. **314**: 994–997.
4 **Besch, R. et al.**, *J Clin Investigat*. 2009. **119**: 2399–2411.
5 **Duewell, P. et al.**, *Cell Death Differentiat*. 2014. **21**: 1825–1837.
6 **Vanpouille-Box, C. et al.**, *Vaccine*. 2015. **33**: 7415-7422.
7 **Ranoa, D. R. et al.**, *Oncotarget*. 2016. **7**: 26496–26515.
8 **Chiappinelli Katherine, B. et al.**, *Cell*. 2015. **162**: 974–986.
9 **Gopalakrishnan, V. et al.**, *Science*. 2018. **359**:97–100.
10 **Routy, B. et al.**, *Science*. 2018. **359**:91–97.

Abbreviations: **Aza**: 5-azacytidine · **ERVs**: endogenous retroviral elements · **ICD**: immunogenic cell death · **IFN-I**: type I interferon · **RTx**: radiation therapy · **RIG-I**: retinoic acid-inducible gene 1 · **snRNA**: small nuclear RNAs · **WT**: wild-type

Keywords: Cancer immunotherapy • Checkpoint inhibitors • Epigenetic therapy • Nucleic acid receptors • RIG-I

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Received: 28/12/2020
Revised: 28/12/2020
Accepted: 4/3/2021
Accepted article online: 18/3/2021

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