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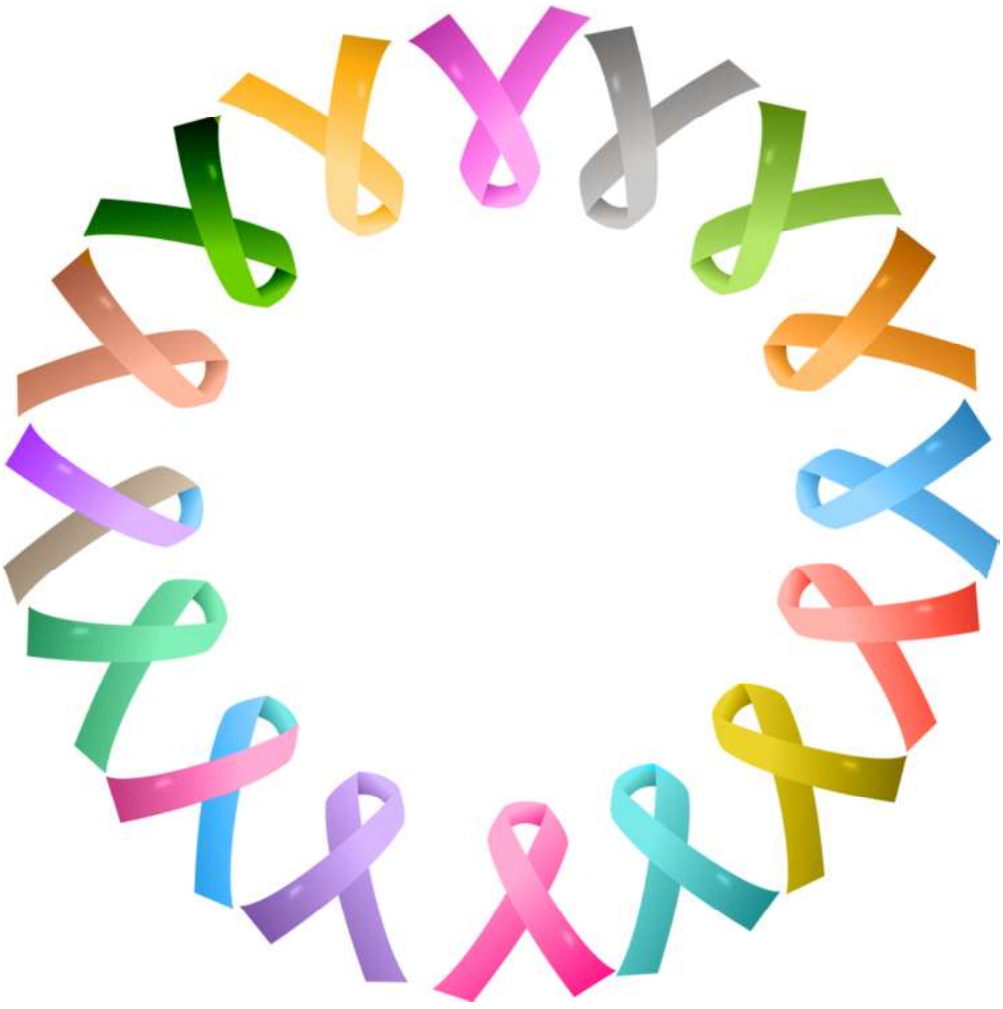

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2. Trama A, Stark D, Bozovic-Spasojevic I, Gaspar N, Peccatori F, Toss A, Bernasconi A, Quarello P, Scheinemann K, Jezdic S, Blondeel A, Mountzios G, Bielack S, Saloustros E, Ferrari A. Cancer burden in adolescents and young adults in Europe. ESMO Open. 2023;8(1):100744. doi: 10.1016/j.esmoop.2022.100744

3. Bozovic Spasojevic I, Ferrari A, De Munter J, Gamble A, Konsoulova-Kirova A, Rizvi K, Schneider C, Balsat M, Castleton A, Gofti-Laroche L, Kienesberger A, Timmermann B, Vormoor J, Saloustros E, Stark DP. Have we made progress in taking care of adolescents and young adults with cancer? Results of a European multi-professional survey. Tumori. 2023:3008916231183477. doi: 10.1177/03008916231183477.

4. Saloustros E, Stark DP, Michailidou K, Mountzios G, Brugieres L, Peccatori FA, Jezdic S, Essiaf S, Douillard JY, Bielack S. The care of adolescents and young adults with cancer: results of the ESMO/SIOPE survey. ESMO Open. 2017;2(4):e000252. doi: 10.1136/esmoopen-2017-000252.

5. Ferrari A, Stark D, Peccatori FA, Fern L, Laurence V, Gaspar N, Bozovic-Spasojevic I, Smith O, De Munter J, Derwich K, Hjorth L, van der Graaf WTA, Soanes L, Jezdic S, Blondeel A, Bielack S, Douillard JY, Mountzios G, Saloustros E. Adolescents and young adults (AYA) with cancer: a position paper from the AYA Working Group of the European Society for Medical Oncology (ESMO) and the European Society for Paediatric Oncology (SIOPE). ESMO Open. 2021;6(2):100096. doi: 10.1016/j.esmoop.2021.100096.

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Control of IFN-γ Responsiveness and Metastatic Potential in Melanoma by GSTA4

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Background: Interferon-gamma (IFNγ) is a crucial effector molecule of antitumor immunity. This cytokine promotes the excessive production of reactive oxygen species (ROS) in tumor cells, which leads to DNA damage and senescence [1]. Recently it has been discovered that IFNγ can also trigger cancer cell ferroptosis by fostering lipid peroxidation [2]. Cancers often evade antitumor immunity by losing their responsiveness to IFNγ. Consequently, IFNγ becomes a critical player in the immunoediting process, selecting tumor cells with immunoevasive properties [3]. Defects in responsiveness to IFNγ in cancer cells significantly contribute to the limited success of cancer immunotherapy in clinics [4], emphasizing the importance of understanding the mechanism behind the IFNγ-mediated immunoediting process. To address this issue, we investigated how tumor cells escape IFNγ-dependent immune response through immunoediting by analyzing originally established immune-escape variants of melanoma cells. **Material and Methods**: We used a previously established in vivo model in which antitumor immunity was IFNγ dependent [5]. Mouse B16 melanoma cells expressing ovalbumin as a tumor-specific antigen (B16OVA) were subcutaneously inoculated in OVA-immunized B6 mice. In this model, tumor growth suppression by host IFN-γ lasts for a limited time, after which all tumors progress. Next, we established cancer cell lines with different in vivo immunological experiences. Tumor cells were isolated from same-sized tumors from wild-type (WT) untreated mice (established cell lines were named "NIMM"), from WT OVA-immunized mice after the cessation of immune control of tumor growth (established cell lines were named "IMM"), or from IFNγ knockout (IFNγ KO) OVA-immunized mice (established cell lines were named "GKO-IMM"). IMM, NIMM, and GKO-IMM cells were re-challenged in OVA-immunized mice to test their ability to provoke antitumor immunity. Instead of immunization with OVA antigen, in some experiments, the anti-PD-1 antibody was administered intraperitoneally to initiate tumor-specific immunity in vivo. To examine changes in phenotype resulting from the IFNγ immunoediting process, total RNA was extracted from parental B16OVA cells and immune-escaped IMM cells. Gene expression was analyzed using a GeneChip system with GeneChip Mouse Gene 2.0 ST Array. mRNA and protein expression of selected genes was quantitatively determined by real-time PCR and western blotting, respectively. GSTA4 overexpression or knockdown was performed to determine its functional role in the immunoevasive phenotype of IMM cells. Cell sensitivity to IFNγ and 4–hydroxynonenal (4–HNE), a lipid peroxidation product, was estimated by WST–8 cell viability assay. CellROX Deep Red reagent was used to detect IFNγ-induced intracellular ROS accumulation. Transwell invasion assay was used to assess melanoma cells' in vitro metastatic potential. In the in vivo experimental lung metastasis model, cells were injected into the tail vein and metastasized tumor colonies on the surface of the lungs were counted. The correlation of GSTA4 expression in human melanoma patients with tumor-free survival rates, and response to anti–PD1 treatment

in correlation with GSTA4 expression and survival rates were obtained from publicly available databases. **Results:** Upon re-challenging into OVA-immunized mice, IMM cells showed unrestrained progression, while the growth of NIMM and GKO-IMM tumors was suppressed. In addition, only IMM cells specifically lost OVA antigen expression, indicating that these cells gained the ability to evade the OVA-specific antitumor immune response. In line with in vivo data, IFNγ treatment in vitro reduced the viability of parental B16OVA, NIMM, and GKO-IMM cells, while the viability of IMM cells was intact. Interestingly, IFNγ upregulated the expression of MHC class I (H-2Kd) and PD-L1in IMM cells, suggesting that these cells did not have the defect in IFNγ signaling. We found that the lack of IMM cell responsiveness to the IFNγ-induced cytostatic effect was due to the acquisition of resistance to the IFNγ-induced oxidative stress response. Gene expression analysis using DNA microarray revealed that the most upregulated gene in immunoevasive IMM cells was glutathione-S-transferase-4 (GSTA4). GSTA4 is a member of a family of detoxification enzymes that play an essential protective role in cellular oxidative stress responses [6]. GSTA4 overexpression in parental B16OVA cells reduced ROS production and increased their resistance to the IFNγ-induced cytostatic effect in vitro. Consequently, the growth of B16OVA cells overexpressing GSTA4 was more aggressive in OVA-immunized mice than that of parental B16OVA cells. In parallel, the knockdown of GSTA4 in IMM cells led to increased intracellular ROS levels and decreased viability upon in vitro IFNγ treatment. IMM tumors were resistant to anti-PD1 treatment in vivo, and the knockdown of GSTA4 reinvigorated their responsiveness. In addition to the role in acquired resistance to IFNγ, we found that the upregulation of GSTA4 was also responsible for the higher metastatic potential of IMM tumors. Next, we confirmed the results from the mouse model in human melanoma. GSTA4 expression levels in Malme3M, UACC 62, and MeWo melanoma cell lines inversely correlated with their sensitivity to in vitro IFNγ treatment. Database analysis revealed a significant correlation between the expression of GSTA4 and the metastasis-free survival rate of human melanoma patients. Melanoma patients with low GSTA4 expression were better responders and showed a better progression-free survival rate to anti-PD1 therapy, further supporting the clinical relevance of our findings. **Conclusion**: In this study, we uncovered a new mechanism through which cancer cells evade immune surveillance and enhance their ability to metastasize by developing resistance to oxidative stress responses through GSTA4 upregulation. Our results suggest that targeting the oxidative stress response in cancer cells emerges as a promising therapeutic strategy to overcome immune resistance and regulate the progression of metastasis [7].

Keywords: Immunotherapy, Interferon-gamma, Melanoma, Neoplasm Metastasis, Oxidative Stress

References

1. Hubackova S, Kucerova A, Michlits G, Kyjacova L, Reinis M, Korolov O, et al. IFNγ induces oxidative stress, DNA damage and tumor cell senescence via TGFb/SMAD signaling-dependent induction of Nox4 and suppression of ANT2. Oncogene 2016;35:1236–49.

2. Wang W, Green M, Choi JE, Gijon M, Kennedy PD, Johnson JK, et al. CD8+ T cells regulate tumourferroptosis during cancer immunotherapy. Nature 2019; 569:270–4.

3. Mojic M, Takeda K, Hayakawa Y. The Dark Side of IFN-γ: Its Role in Promoting Cancer Immunoevasion. Int J Mol Sci. 2017;19(1):89.

4. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 2017;168:707–23.

5. Mojic M, Shitaoka K, Ohshima C, Ucche S, Lyu F, Hamana H, Tahara H, Kishi H, Hayakawa Y, NKG2Ddefines tumor-reacting effector CD8+ T cells within the tumor microenvironment. Can Science 2021;112(9):3484–3490.

6. Balogh LM, Atkins WM. Interactions of glutathione transferases with 4-hydroxynonenal. Drug Metab Rev 2011;43:165–78.

7. This work is published: Ucche S, Yokoyama S, Mojic M, Kohei O, Ohshima C, Tsuihiji H, Takasaki I, Tahara H, Hayakawa Y. GSTA4 Governs Melanoma Immune Resistance and Metastasis. Mol Can Research 2023;21(1):76–85.