

## IFI16 PROMOTES IMMUNE RESPONSES THROUGH IFN- $\gamma$ PATHWAY ACTIVATION IN METASTATIC CUTANEOUS MELANOMAS

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**Background** IFN- $\gamma$ -inducible protein 16 (IFI16) is known to initiate STING pathway cascade, which leads to the downstream activation of various cytokines and chemokines that play important roles in enhancing tumor microenvironment immune responses. IFI16 activates STING signaling through the cooperation with cyclic GMP-AMP synthase (cGAS) through the detection of cytoplasmic DNA. Recently, we have shown that IFI16-dependent STING signaling upregulates effective anti-HER2 cellular immune responses in HER2 (+) breast cancer.<sup>1</sup> The aim of this study was to assess the role of *IFI16* levels with immune responses in metastatic cutaneous melanomas.

**Methods** Cutaneous melanoma datasets for mRNA expression were obtained from TCGA SKCM, GTEx, GSE7553, GSE15605, and GSE46517. The fraction of tumor-infiltrated immune cells (TIIC) was estimated by CIBERSORT. Patients from TCGA were divided into high- and low-*IFI16* mRNA level groups, based on the highest and lowest quartiles. Extraction of Expression Module (EEM) analysis was performed. Correlations between variables were calculated with the Mann-Whitney U test with FDR corrections.

**Results** *IFI16* mRNA levels were significantly upregulated in metastatic cutaneous melanomas (AJCC III/IV, n = 193) compared to normal skin (figure 1A, p < 0.0001), but significantly downregulated in metastatic versus primary melanomas in GSE7553 dataset (figure 1B, p = 0.016). High levels of *IFI16* were significantly associated with poor prognosis in stage III/IV melanoma patients (figure 1C, p < 0.0001). The analysis of TIIC showed that M1 macrophage subset levels were significantly higher in the high-*IFI16* vs low-*IFI16* group (figure 1D, p = 0.0028). IFN- $\gamma$  score was significantly higher in the high- vs low-*IFI16* group in TCGA (figure 1E, p = 0.00051), GSE46516 (p = 0.00068), and GSE15605 (p = 0.026). EEM analysis showed that the activity scores of IFN- $\gamma$  response were significantly higher in high-*IFI16* vs low-*IFI16* groups (p = 0.0048). Furthermore, data integration from EEM and differential expressed genes analysis identified 34 immune-related genes, of which chemokines *CXCL10* and *CXCL11* were significantly upregulated by the IFN- $\gamma$  response pathway. The elevated *CXCL10* and *CXCL11* are known to enhance tumor immune microenvironment activity. *CXCL10* and *CXCL11* were significantly higher in high-*IFI16* group in both the TCGA (figure 1F, p < 0.0001, p < 0.0001, respectively) and GSE46517 (p = 0.00016, p = 0.00062, respectively).

**Conclusions** High-*IFI16* mRNA levels may represent predictive biomarkers of good prognosis in metastatic melanomas, whereby *IFI16* mRNA levels were associated with significant immune responses through the upregulation of the IFN- $\gamma$  pathway, M1 macrophage subset, and *CXCL10/CXCL11*.

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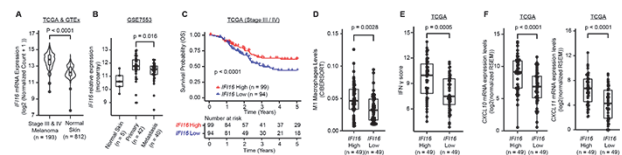
### REFERENCE

- Ong LT, Lee WC, Ma S, Oguz G, Niu Z, Bao Y, Yusuf M, Lee PL, Goh JY, Wang P et al. IFI16-dependent STING signaling is a crucial regulator of anti-HER2

immune response in HER2+ breast cancer. *Proc Natl Acad Sci U S A* 2022;119(31):e2201376119.

**Ethics Approval** This study followed the principles in the Declaration of Helsinki. All human samples and clinical information for this study were obtained according to the protocol guidelines approved by Providence SJHC under SJHC Joint Institutional Review Board (IRB): IRB: JWCI-18-0401) and IRB: MORD-RTPCR-0995.

**Consent** Written informed consent was obtained from the patient for publication of this abstract. A copy of the written consent is available for review by the Editor of this journal.



**Abstract 962 Figure 1** IFI16 expression in metastatic cutaneous melanomas

A. IFI16 mRNA levels in tumor tissues (TCGA SKCM dataset) and normal skin tissues (GTEx dataset). B. IFI16 mRNA levels in normal skin, primary tumors, and metastatic tumors (GSE7553). C. Kaplan-Meier curves for melanoma patients of AJCC stage III&IV according to IFI16 mRNA expression in TCGA dataset. D. Infiltration levels of M1 macrophages estimated by CIBERSORT using TCGA dataset. E. IFN- $\gamma$  signature score in TCGA. F. *CXCL10*, and *CXCL11* mRNA expression levels in TCGA

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