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COMMENTARY

Using PET imaging to track STING-induced interferon signaling

Enitome E. Bafor^{a,1} and Howard A. Young^{a,1}

In the 19th century, Ilya Metchnikoff defined and broadened our initial understanding of phagocytosis and, in 1908, went on to show that a cellular component (i.e., DNA) stimulates immune responses (1). About a century later, Janeway conceptualized pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) (2). Since then, we know that PAMP recognition by innate immune cells via PRRs activates the production of several cytokines, including interferons (IFNs), with the eventual recruitment of lymphocytes. Subsequent research discovered that innate immune recognition of double-stranded DNA (dsDNA) triggers autoimmune diseases (3). However, before the discovery of dsDNA sensors, an endoplasmic reticulum (ER) protein encoded by the *TMEM173* gene,

known as a stimulator of IFN genes (STING), was identified as a significant factor involving DNA recognition in innate immunity (4). Subsequently, scientists discovered that the direct cytosolic DNA sensor (cGAS) activates the expression of type 1 IFNs (5). Today, it is well known that activation of cGAS-cGAMP-STING signaling is triggered by cytosolic DNA and is necessary for antimicrobial and antitumor immune responses. In PNAS, Liang et al. (6) describe an innovative process for monitoring STING-induced IFN signaling. Induction of IFN signaling by STING occurs on detection of cytoplasmic DNA, which may be tumor- or microbial-derived. STING is activated when cGAS catalyzes the synthesis of cyclic dinucleotides (CDNs) including 2'3'-cGAMP (7). STING is translocated from the ER to the perinucleus,

Fig. 1. Proposed noninvasive tracking of STING-induced IFN signaling using [¹⁸F]FLT PET by Liang et al. (6) (created using Biorender).

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phosphorylated by TANK-binding kinase 1 (TBK1) and recruits IFN regulatory factor 3 (IRF3), which in turn is phosphorylated by TBK1, forms a dimer, and enters the nucleus. Phosphorylated IRF3 activates transcription of type 1 IFNs and other relevant cytokines (8) which bind to the heterodimeric IFN receptors (IFNAR1 and IFNAR2) and recruits Janus family kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2), which in turn phosphorylates and activates IFNAR1 and IFNAR2. Activated IFNAR1 and 2 phosphorylate signal transducers and activators of transcription (STAT) proteins (STAT1 and STAT2), which are transferred to the nucleus along with IFN regulatory factor 9 (IRF9), to enhance transcription of IFN target genes (9) and subsequently activate the innate and adaptive immune responses.

The discovery of the STING signaling pathway led to further investigation into the antitumor effects of STING. As a majority of cancer patients fail to respond to immune checkpoint inhibitors, the STING–IFN signaling pathway has become an alternative immunotherapy target. Expression of type 1 IFN and related genes in tumors correlates positively with infiltration of T cells into the tumor microenvironment and support an antitumor role for type 1 IFNs (10). STING agonists are therefore currently being investigated for cancer immunotherapies. However, since most STING agonists are based on CDNs, they are delivered intratumorally due to the poor metabolic stability of these agents. In addition, there are recent concerns about the potential inhibitory impact of STING on antitumor responses, with inhibition of T cell proliferation and promotion of T cell death reported (11). Thus, STING can also potentially impair the adaptive immune response. Taken together, the effective in vivo monitoring and tracking of STING–IFN signaling on treatment with STING agonists is vital.

In PNAS, Liang et al. describe an innovative process for monitoring STING-induced IFN signaling.

Liang et al. (6) used a non-CDN STING agonist (diABZI-compound 3 trihydrochloride of the amidobenzimidazoles family) to promote STING up-regulation and proceeded to noninvasively track downstream metabolic IFN signaling in a pancreatic ductal adenocarcinoma (PDAC) model via positron emission tomography (PET). PET is utilized in imaging the systemic distribution of radiolabeled compounds such as fluoropyrimidines (12) and, therefore, is an innovative addition for PDAC and tumor imaging in general. The authors show, among other dysregulated metabolites, that type 1 IFNs triggered the up-regulation of genes associated with nucleoside breakdown, specifically thymidine phosphorylase (TYMP). TYMP catalyzes the reversible phosphorylation of thymidine and deoxyuridine and is essential for the stability of mitochondrial DNA (13). TYMP is identical to platelet-derived endothelial growth factor, a tumor angiogenic factor (14) that is increased in several tumors and associated with increased tumor invasiveness and decreased survival (15). Type 1 IFNs induce TYMP in patients with advanced malignancies, a finding that was also observed by Liang et al. (6) with type 1 IFN–TYMP elevation in PDAC. This effect was relevant as uptake of nucleoside analog PET probes is dependent on nucleoside levels (16) and it provided further support for the study's application. The consistency of TYMP expression in tumors also makes it a reliable biomarker for IFN signaling.

Since TYMP expression promotes tumor 3'-deoxy-3'-fluorothymidine (FLT) PET probe accumulation in vivo (17), the authors

considered that IFN-induced TYMP could be leveraged for detecting STING-induced IFN signaling, and these processes can be monitored via PET imaging. In PET imaging, where long-term pharmacodynamic parameters are required, compounds resistant to TYMP and with long half-lives should be utilized. As such, TYMP-resistant fluoropyrimidines labeled with [^{18}F] are currently in use for imaging purposes. For instance, FLT, as shown in Liang et al. (6), is a specific substrate of thymidine kinase 1 (TK1) and has been successfully used in tumor studies (18). Its tissue uptake is correlated with cellular proliferation (18), thus making it a valuable tool for a tumor proliferation rate measurement. The authors showed that type 1 IFNs promote [^{18}F]FLT PET probe accumulation in PDAC cells in vitro through the transcriptional up-regulation of TYMP and depletion of the sterile alpha motif and HD domain-containing protein 1 (SAMHD1)–produced thymidine (dT), an endogenous inhibitor of [^{18}F]FLT uptake. However, the use of FLT is limited in its application to detect metastasis in tissues with a high proliferation rate, such as the bone marrow (19). Other analogs such as [1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-thymine, FMAU] can be utilized to overcome this limitation with FLT. It would have been an added strength if the authors had compared FLT and FMAU, considering the broader implications of the proposed procedure. Nonetheless, it does not detract from the usefulness of the procedure.

Induction of cellular perturbations and cytosolic DNA accumulation can trigger STING induction and IFN expression (20). The authors utilized this phenomenon when they showed that STING-induced IFN signaling triggered in vitro and in vivo TYMP expression as well as up-regulation of *IFNB1* transcripts, suggesting a comparable transcriptional alteration in PDAC cell-autonomous IFN signaling downstream of STING activation. The authors also demonstrated a role for STING in regulating PDAC IFN signaling in vivo using SUIT2 cells engineered to express an active STING mutant (SUIT2-TetR-STING^{R284M}) under doxycycline-inducible promoter control. Their results showed impairment of PDAC cell proliferation and TYMP expression, which was overcome by inhibiting JAK1/2. Furthermore, the authors showed that STING could induce metabolic alterations that can be visualized by [^{18}F]FLT PET, a relevant addition to the procedure's usefulness. Immunometabolism is associated with the polarization of immune cells, and metabolites such as succinates or glucocorticoids link directly to different inflammatory statuses. Therefore, tracking metabolites specific for STING-induced IFN immunometabolism will prove relevant as pharmacodynamic markers of in vivo IFN responses induced by STING agonists. To merge their findings, the authors utilized the concept of STING agonist-driven TYMP expression to promote the accumulation of [^{18}F]FLT in vitro and in vivo. They used the diABZI-compound 3 to trigger a dose-dependent increase of *IFNB1* transcripts and TYMP in SUIT2 PDAC cells. As shown in this article (6), STING-associated immunometabolism within tumors can be effectively tracked using radiolabeled compounds that accumulate within tumors. While the use of PET is not new in tumor studies, utilizing PET for STING agonist-induced IFN signaling in tumors can provide useful insight for tumor immunotherapy. Worthy of mention are reports of other novel non-CDN systemic STING agonists (SR-717 and MSA-2) which show favorable pharmacokinetic improvements over earlier CDN-based STING agonists (13, 21). These novel systemic non-CDN STING agonists provide an alternative approach for targeting the STING–IFN pathway. As such, it would therefore be worthwhile to utilize the [^{18}F]FLT PET imaging to evaluate their pharmacodynamic profiles for an

effective comparison. A schematic of the proposed utilization of PET imaging in IFN signaling is shown in Fig. 1.

The authors, therefore, provide an interesting noninvasive procedure for monitoring STING agonist induced IFN signaling, which can prove relevant in tracking local and systemic IFN activities and enhance tumor immunotherapy.

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