Editorial

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Future Medicinal Chemistry

Novel non-nucleotidic STING agonists for cancer immunotherapy

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"the host STING pathway as a critical mechanism of innate immune-sensing of cancer, driving the production of type-I IFNs and promoting aggressive antitumor responses"

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STING, a novel & promising target for cancer immunotherapy

Cancer immunotherapy is showing promise in treating patients with advanced or metastatic cancers and was named 'Breakthrough of the Year' by *Science* magazine in 2013. It aims to redirect the immune system onto the tumor tissue, for which escape from immune surveillance is a common feature and constitutes a hallmark of cancer. Immune checkpoint inhibitor therapy has recently illustrated the potential effectiveness of cancer immunotherapy in a clinical setting, as well as the need for further development and novel targets to extend its impact across more patients. Within this fast moving environment, agonists of the cellular double stranded DNA sensor STING [1] have 'stung' the immuno-oncology field and raised hope for patients as powerful adjuvants for combined therapies, by further harnessing the antitumor potential of the immune system. This is a rapidly developing area and evidence from recent studies has identified the host STING pathway as a critical mechanism of innate immune-sensing of cancer, driving the production of type-I IFNs and promoting aggressive antitumor responses [2–8].

The STING pathway is physiologically activated by the presence of dinucleotides in the cytosol and constitutes an innate response to pathogens. Cytosolic nucleic acids are usually a sign of viral or bacterial infection, and therefore trigger immune response. Upon activation, STING (a 28 kDa dimeric endoplasmic reticulum membrane protein) recruits cytosolic kinases that activate transcription factors including NF-B and IRF3, which enter the nucleus and function together to induce the expression of interferons and other cytokines [9]. Among natural ligands, cyclic dinucleotides (CDNs) are especially potent activators of STING that are ubiquitous second messengers in prokaryotes [10] and within the immune system of eukaryotic species [11]. These CDNs are produced either through the cGAS pathway, which produces the noncanonical dinucleotide cyclic guanosine monophosphate-adenosine monophosphate (2′,3′-cGAMP) upon sensing of cytosolic DNA [9], or are directly found in the cytosol due to the presence of pathogens [12].

The human STING protein & its function

The human STING protein (hSTING) is composed of an N-terminal transmembrane domain (aa 1–154), a central globular domain (aa 155–341) and a C-terminal tail (aa 342–379); the structure of hSTING co-crystallized with a CDN ligand was published in 2013 [13]. This structure provides insights into the important interactions required for the binding of CDNs to STING, while the authors also demonstrated that 2′,3′-cGAMP synthesized by cGAS is the endogenous second messenger in mammalian cells. This CDN was also shown to have high affinity toward STING, although other phosphodiester isomers can also bind strongly. However, in comparison, the bacterial cyclic di-guanosine monophosphate (c-di-GMP) has a rather high K_d and is a poor interferon inducer [12].

For the purpose of medicinal chemistry, further examination of the important interactions for the binding of cGAMP to STING reveals critical features that may be needed for the design of novel synthetic agonists: the

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two nucleobases are almost parallel and the π-stacking is extended to neighboring Tyr167 and Tyr240 residues; phosphodiester groups bridging the ribose units actively participate in binding via polar contacts with the cationic Arg232 and Arg238 side chains; the ribose hydroxyl groups are solvated by water molecules except for one hydrogen-bridge with a single serine (Ser162) and the guanine binds a glutamate and threonine (Glu260 and Thr263) while the adenine is involved in a hydrogen bond with the carbonyl group of Val239.

It was also demonstrated in this seminal study that STING is mandatory for this signaling cascade to initiate an effective immune response and the production of interferon. It cannot be bypassed by other pathways that would rely on production of CDNs by cGAS, as shSTING mutants are unable to produce IFN-β in response to DNA or cGAMP stimulation [12].

STING agonists for cancer immunotherapy

Within the specific area of cancer immunotherapy, the generation of tumor-specific activated T cells is crucial, and their presence has been shown to be of prognostic and clinical benefit. More specifically, effector-memory $CD8^+$ T cells have been reported to be of powerful prognostic importance in colon, breast and ovarian cancers [14–16]. In early-stage colon cancer, intratumoral $CD8^+$ T-cell infiltration is more predictive of outcome than the Tumor, Node, Metastasis stage. Besides being of utmost importance for cancer regression and clinical outcome, generation of tumor-directed T cells is dependent on the cGAS-STING pathway and the production of interferon, that is due to the innate immune recognition of tumor-derived DNA [17–19]. It is therefore of great interest to restore the natural immune function within the tumor micro-environment to trigger a powerful antitumor response and in this regard, CDNs have already shown potent and promising *in vivo* antitumor effects in melanoma [5–7], breast [4], colon [5] and oral cancer [20].

CDNs as anticancer drugs

CDNs face obvious practical problems and limitations as drug candidates: their net charge and polar profile strongly limit their membrane passage and cellular uptake; and phosphodiester linkages are prone to enzymatic hydrolysis. The modification of the phosphodiester groups has been the core strategy for the drug candidate ADU-S100, which is currently in a Phase I clinical trial for patients with advanced/metastatic solid tumors or lymphomas. However, the quest for potent small molecule agonists of hSTING remains ongoing and is a promising field of exploration. The availability of high resolution crystal structures of STING bound CDNs allows for the rational design of such compounds and medicinal chemists will surely soon identify new chemical entities with *in vitro* and *in vivo* efficacy.

5,6-dimethylxanthenone-4-acetic acid, a proof of concept

An interesting point is the case of 5,6-dimethylxanthenone-4-acetic acid (DMXAA): this compound is a potent non-nucleotidic agonist of the murine STING protein. Human and mouse STING exhibit 68% amino acid identity and 81% similarity [13]. Not only does DMXAA treatment lead to strong type I IFN induction in mice, but its use also results in powerful and promising anticancer effects *in vivo*. Indeed, STING activation by DMXAA stimulates $CD8^+$ T-cell responses in leukemia models and enhances *in vivo* survival through adaptative immunity [3]. In wild-type B16 melanoma-inoculated mice, a single intratumoral dose of 500 μg DMXAA induced potent tumor regression and complete tumor rejection in the majority of the animals, while STING knockdown mice showed no response to this treatment [8].

Therefore, the anticancer ability of a non-nucleotidic STING agonist that is both very efficient *in vitro* and *in vivo*, constitutes a proof of concept for the design of novel non-nucleotidic agonists for the hSTING protein, and it is unlikely that the human form would not be accessible to such small molecules.

Conclusion: hope for the next-gen agonists

We have briefly highlighted herein the recent basis for the development of novel STING agonists for cancer immunotherapy. Early results, showing *in vivo* promise, combined with the availability of crystal structures of the human protein, warrant the development of this new class of immunotherapeutic compounds for cancer treatment.

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