Inducing immunogenic cell death in immuno-oncological therapies

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Abstract

Immunotherapy has revolutionized cancer treatment and substantially improved patient outcomes with respect to multiple types of tumors. However, most patients cannot benefit from such therapies, mainly due to the intrinsic low immunogenicity of cancer cells (CCs) that allows them to escape recognition by immune cells of the body. Immunogenic cell death (ICD), which is a form of regulated cell death, engages in a complex dialogue between dying CCs and immune cells in the tumor microenvironment (TME), ultimately evoking the damage-associated molecular pattern (DAMP) signals to activate tumor-specific immunity. The ICD inducers mediate the death of CCs and improve both antigenicity and adjuvanticity. At the same time, they reprogram TME with a “cold-warm-hot” immune status, ultimately amplifying and sustaining dendritic cell- and T cell-dependent innate sensing as well as the antitumor immune responses. In this review, we discuss how to stimulate ICD based upon the biological properties of CCs that have evolved under diverse stress conditions. Additionally, we highlight how this dynamic interaction contributes to priming tumor immunogenicity, thereby boosting antitumor immune responses. We believe that a deep understanding of these ICD processes will provide a framework for evaluating its vital role in cancer immunotherapy.

Keywords: Immunogenic cell death; damage-associated molecular pattern; tumor immunogenicity; antitumor immune responses

Introduction

Cancer immunotherapy has emerged as a powerful therapeutic modality that harnesses the host’s immune system to identify and kill cancer cells (CCs) in primary as well as metastatic lesions. Despite the success of immune checkpoint blockade (ICB) and chimeric antigen receptor T cell (CAR-T) therapy in clinical practice, a sizeable subset of cancer patients possess an inherent/acquired resistance to immunotherapy, and this gives rise to refractory and relapsed cancers (1,2). There is abundant clinical evidence pointing towards the contribution of intrinsic factors of CCs to treatment potency or failure. Due to various challenges of external environment, such as immune surveillance and treatment pressures, CCs generally evolve and develop a low immunogenicity for escaping recognition by immune cells, such as dendritic cells (DCs) and T lymphocytes (3-6). Incidentally, an improved disease-free survival is observed in cases where tumors have low immune evasion capacity and a uniformly
high infiltration of immune cells, or where tumor cells do not present any evidence of DNA immunoediting or disruption to antigen presentation (7-9). Hence, it remains a formidable challenge to compel CC-specific dying through reprogramming intrinsic immunogenicity during cancer immunotherapy.

In the past few years, immunogenic cell death (ICD) has received much attention for boosting anti-neoplastic immune responses. As a form of regulated cell death, ICD can coordinate the complex cell-to-cell communication between dying CCs and immune cells to sequentially trigger antitumor innate immunity and adaptive immunity (10-12). Interestingly, under diseased conditions, like cancer, the cellular apoptosis involved in the continuous cellular turnover is likely to be non-immunogenic or even tolerogenic. Fortunately, ICD inducers can enhance the immunogenicity of dead cells through the production of neoantigens as well as by eliciting damage-associated molecular patterns (DAMPs) and cytokines to recruit and activate antigen-presenting cells (APCs) and effector CD4+ and CD8+ T lymphocytes (13). Through the release of antigenic and adjuvant factors, ICD expresses its immunomodulatory capacities and amends tumor microenvironment (TME) with a “cold-warm-hot” immune status, thereby improving T cell priming and ultimately facilitating T cell-mediated attack of the residual CCs (14,15). Recently, ICD has been recognized as a new and critical pharmacological strategy to improve the effectiveness of cancer treatment and relieve the suffering of cancer patients.

Malignant transformation is initiated by relatively common genetic or epigenetic alterations that result in uncontrolled cell growth, decreased apoptosis, escape from immune surveillance, and ultimately the development of invasive tumors. Zhou et al. reported that intestinal CCs outcompete and eliminate neighboring normal cells by capturing innate immune signaling pathways so that space is available for their own expansion (16). It has been observed that the hypermethylated thymocyte selection-associated high mobility group box (hTOX) promotes the proliferation, migration and invasion of colorectal CCs. Furthermore, activation of the hTOX-driven mammalian target of rapamycin (mTOR) signaling pathway generates an immunosuppressive TME, which is the underlying cause for resistance of CCs to ICB (17). Samur et al. reported that after the initial CAR-T cell infusion in cancer patients, CCs may evolve, leading to the loss of the target antigen; therefore, they develop alternative pathways to survive immune pressure and cancer relapses with resistance to the initial CAR-T cell product (18). These studies demonstrate that the immune system can protect the host from tumor development, but it can also dynamically shape the immunogenicity of developing neoplastic cells. Tumor-immune dynamics lead to continuous changes in the cellular and molecular properties of tumor cells, which can be crucial for patient survival and therapy.

The immune system is composed of specialized cells that protect the body from infections or tumor threats and attempt to maintain homeostasis. Usually, in the continuous battle between CCs and immune cells, the tumors develop a series of immune-excluded features to evade the immune attack of the body; these features include absence of T cell infiltration, reduction of antigen presentation on the cell surface, and recruitment of immunosuppressive regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) (19,20). Interestingly, this tumor immune escape reoccurs, either in part or in its entirety, in response to cancer immunotherapy. Dufva et al. observed that the heterogeneity in the expression of the death receptor gene in various B-cell malignancies can influence T-cell receptor (TCR)-dependent T-cell killing as well as the sensitivity to CAR-T cell cytotoxicity (21). Moreover, the intrinsic loss of the anti-inflammatory protein tumor necrosis factor alpha-induced protein 3 (TNFAIP3) in CCs facilitates immune evasion in a signal transducer and activator of transcription 1 (STAT1)/programmed death-ligand 1 (PD-L1)-dependent manner and impairs the outcome of anti-PD-L1-based therapies (22). Immunologically “hot” tumors often contain a rich immune infiltration, including CD8+ and CD4+ T cells, and this represents the ongoing immune interaction between tumor tissues and the surrounding stroma. Incidentally, non-inflamed (“cold”) tumors, which lack T cells within their immunosuppressive TMEs, are associated with poor prognosis, and their responses to anticancer immunotherapies are weaker than that of the inflamed (“hot”) tumors (23,24). Recent studies have provided evidence that the antitumor effect of the current immunotherapies is generally based on the activation of T-cell immune responses by promoting intratumoral infiltration of cytotoxic T lymphocytes (CTLs) (25,26). Since ICD pathways can engage in a complex dialogue with TME and ultimately evoke DAMP signals to activate tumor-specific immunity and/or overcome resistance to immunotherapy, it is important to determine the mechanisms of stimulating autonomous pathways of CCs that can lead to ICD. This
may represent a novel effective combinatorial strategy for cancer treatment.

**Chief features of ICD**

The immune system has a longitudinal influence on tumor ecosystem, and it establishes its control as an evolutionary bottleneck that is capable of exerting selective pressure on the mutable CCs. At the same time, the evolution of the constituent CCs shapes the phenotypic heterogeneity of the tumors in favor of competing for survival and progression under dynamic immune selection pressures. It has been reported that the oncogenic signals may disrupt the expression of cell cycle and antigen presentation genes of host cells, thereby leading to abnormal proliferation of CCs in the body and a state of unresponsiveness to the defense mechanisms, respectively (27,28). CCs can decrease the downstream signal stimulation of the TCR and lead to dysfunction or apoptosis of T cells through the upregulation of the immune-suppressive molecule PD-L1 (29). Michael Karin and Shabnam Shalapour considered abnormal epigenetic modifications of certain molecular phenotypes, namely death receptors, stress-induced ligands, major histocompatibility complex (MHC)-I molecules, intact antigen processing and presentation machinery (APM), and tumor-associated antigens (TAA), all of which play important roles in the development of CC-intrinsic immune evasion capabilities (30). Hence, these studies already prove that targeting the evolutionary immune tolerogenic features of CCs to induce their “cold-warm-hot” immunogenic reprogramming is a promising approach to improving the efficiency of cancer immunotherapy.

The ICD inducers, such as chemotherapy, radiotherapy (RT), oncolytic virus therapy, and photodynamic therapy (PDT), trigger autonomous molecular signaling pathways in CCs that lead to an immunostimulatory death driven by cellular stressors (31-34). Simultaneously, a large number of tumor antigens, DAMP signals, and inflammatory cytokines are evoked and spatio-temporally exposed or released in concert with systemic tumor specific immune response. DAMP molecules interact with pattern recognition receptors (PRRs) on innate immune cells in a stimulus-dependent manner, and ensure tumor antigens are recognized with priority in a context of “danger” (35). During this process, DCs and macrophages undergo maturation, recruit to tumors, and engulf the dying cells. Subsequently, they emigrate to the draining lymph nodes (dLNs) after adjuvanticity priming of the DAMPs, where they process and cross-present the tumor antigen, thereby activating and promoting T cell-mediated adaptive antitumor responses (Figure 1). Lan et al. have evidenced that localized RT can eradicate immune-cold tumors and increase tumor gene expression of the stimulator of interferon genes (STING) and interferon (IFN) signatures, accompanied by activated proinflammatory responses, augmented leukocyte patrolling and T cell repertoire (36). Hence, this mechanism of CCs dying in an increased immunogenicity fashion is referred to as ICD.

How dying CCs abruptly challenge the immune system and result in downstream inflammatory response is thought to be important. Damaged, endogenously-derived molecules act as “danger” signals and DAMPs that elicit an auto-inflammatory stress response after alterations in organelle homeostasis (37). DAMPs include numerous, intracellular and physiologically active components that exist in the nucleus, mitochondria, or cytoplasm that increase CCs sense to endogenous or external stress (38). For example, reactive oxygen species (ROS) generated by oxidative stress can decorate the chemical structure, properties, and effects of DAMPs proteins to active nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and the interferon regulatory transcription factor 3 (IRF3) by binding the Toll-like receptor 4 (TLR4), which are main effectors of inflammatory cascades (39). When released by dying CCs, immunostimulatory DAMPs transform TME into a pro-inflammatory condition and favor APC and CTL recruitment and activation. In turn, DAMPs amplify and propagate this immunosurveillance processes that ultimately converge to a positive feedback of CCs stress response and participate in an adequate innate and adaptive antitumor immune response (40,41).

The most relevant DAMPs to be considered for elucidating clinical responses in CCs are as follows:

1) The secretion of adenosine triphosphate (ATP) that serves as a “find me” signal. ATP plays an important role in intracellular metabolism pathways. As soon as ATP is located in the intercellular space, it functions as a danger signal and binds to purinergic receptors expressed by neutrophils, monocytes and T cells. This can initiate innate immune responses. Merz et al. found that ATP could guide leukocytes to the sites of inflammation; moreover, the deletion of its receptor almost completely blocked the activity of the macrophages as well as the accumulation of CD4+ T- and B-cells (42). Additionally, ATP from dying CCs can activate the nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome in DCs, thereby
promoting the proteolytic maturation of caspase-1 and the cleavage and release of interleukin (IL)-1β (43). Incidentally, IL-1β is mandatory for the priming of IFN-γ-producing, tumor antigen-specific CD8+ T cells that link the innate and adaptive immune responses against dying tumor cells. Furthermore, ATP release and autocrine feedback regulation contribute to T-cell activation through rapid redistribution of P2X1 and P2X4 receptors to the immune synapse upon TCR stimulation, thus implicating the limited number of TCR molecules that are engaged by APC to fine-tune the efficiency of T-cell responses (44).

2) The pre-apoptotic exposure of chaperones on the cell surface, mainly calreticulin (CALR). CALR is a conserved endoplasmic reticulum (ER) protein that ensures the proper loading of cellular antigens onto MHC-I molecules. In many cancers, the expression of CALR is altered due to transcriptional or protein-coding mutations (45,46). Cancer-related CALR mutants are unable to support the activity of peptide-loading complexes (PLCs), and hence they are associated with reduced antigen presentation on MHC-I molecules, thereby favoring immune evasion upon loss of tumor antigenicity. Various forms of cellular stress induce the secretory trafficking and surface expression of CALR in apoptotic cells. Prior to the post-apoptotic changes in membrane permeability, exposed CALR participates in cell signaling, leading to the assembly and disassembly of focal adhesions; additionally, it serves as a crucial recognition and clearance ligand on apoptotic cells. It appears to mediate the engulfment of apoptotic cells by professional phagocytes via activation of the internalization receptor, LDL-receptor-related protein (LRP) (47). Lin et al. provided evidence that tumor stanniocalcin 1 traps and retains CALR in the mitochondria, reduces membrane levels of CALR, and results in reduced APC phagocytosis (48). Generally, tumor cells inhibit the antigen uptake by APCs and subvert immune recognition through the expression of “do not eat-me” signals, such as CD47 and CD24 (49). Phagocytosis of dead cells is the initial step in the development of immune response against tumors since the APCs capture, process, and present the tumor antigens to T cells. When the “do not eat-me” signals are disrupted, the exposed CALR molecules on the cell surface serve as an important “eat-me” signal, which is crucial to determine the immunogenicity vs. non-immunogenicity of dead tumor cells (50).

3) The post-apoptotic extracellular release of high mobility group box 1 protein (HMGB1). HMGB1 is the most abundant non-histone chromosomal protein in mammalian nuclei, and it is known for its high electro-phoretic motility through polyacrylamide gels. Its chief functions are to bend or distort the DNA double helix and

Figure 1 Schematic representation of ICD reprograms tumor microenvironment with a “cold-warm-hot” immune status and generates DAMPs immunoadjuvant signals to amplify and sustain DC- and T cell-dependent proper innate sensing and antitumor immune responses. ICD, immunogenic cell death; DAMPs, damage-associated molecular patterns; DC, dendritic cell.
increase the affinity of transcription factor-DNA interactions (51). Intracellularly, HMGB1 has a regulatory role in DNA-associated activities, such as replication, repair, transcription and recombination, and it is characterized as a proinflammatory “alarmin” extracellularly (52). Under stress, HMGB1 is actively secreted by immune cells recruited to the site of injury, or it is passively released by necrotic and damaged cells. Tian et al. reported that the formation of HMGB1-DNA complexes resulted in the production of IFN-α and tumor necrosis factors (TNFs) from DCs through the association of the receptor for advanced glycation end-products (RAGE), which is an immunoglobulin “superfamily” member, with the Toll-like receptor 9-myeloid differentiation primary response 88 (TLR9-MyD88) pathway (53). Extracellular HMGB1 binds to the minor groove of DNA and forms multivalent higher-order structures that may be essential for TLR9 crosslinking and modification of the immunostimulatory properties of DNA. As a “danger” signal, HMGB1 polarizes pro-inflammatory microglia through the RAGE-NF-κB pathway, thereby activating the innate immunity (54). After ICD develops in tumor cells, HMGB1 acts on the TLR4 on DCs and promotes the optimal processing of tumor antigens to cross-prime T cells (55). Interestingly, silica nanoparticles activate NLRP3 inflammasome and HMGB1/TLR4/MyD88/NF-κB signaling pathway to induce cytotoxicity on human umbilical vein endothelial cell (HUVEC) through the excessive ROS generation (56). This observation demonstrates how HMGB1 can bolster subsequent inflammatory responses and ICD of target cells.

Therefore, it is only when all these major checkpoints are correctly expressed by dying CCs that DAMPs actually generate immunoadjuvant signals for amplification and sustainability of DC- and T cell-dependent innate sensing and antitumor immune responses.

ICD inducers and associated mechanisms

ER is a crucial cell organelle in which approximately one-third of the polypeptides undergo synthesis, folding and transportation. Interestingly, it can recognize changes in its environment and is extremely sensitive to these changes. Under a deeply hostile TME, CCs hijack the unfolded protein response (UPR) pathway and adapt to the chronic persistence of mild ER stress (mERS), which, in turn, benefits their cellular homeostasis, self-survival and growth (57). Additionally, under mERS, breast CCs can deliver exosomal miR-27a-3p to macrophages, and this inhibits CD8+ T cell cytotoxicity via PD-L1 upregulation (58). When extrinsic pro-transformation homeostasis is disrupted, particularly by pharmacological intervention, severe ERS (sERS) initiates the stress sensor protein kinase R-like endoplasmic reticulum kinase (PERK) pathways for exporting highly protease-resistant DAMPs, mediating CC autophagy, and ultimately inducing apoptosis in normal cells (59).

The ERS response is activated to protect cells from the ERS-induced damage. However, if cells do not recover from the stress situation, this response can lead to cell death. Therefore, some ICD inducers use the evolved biological properties of CCs to overcome the UPR cytotoxicity (60,61). In fact, inhibiting tyrosine kinase ephrin type-B receptor 4 (EPHB4) overexpression in various cancers can induce overwhelming ERS and ICD, which is consistent with the decreased glucose uptake and low intracellular ATP levels (62). The essential effector elements of UPR, namely eukaryotic initiation factor 2α (eIF2α) and inositol-requiring enzyme 1 (IRE1) both serve as sensors of sERS and are increasingly phosphorylated in response to metabolic changes. Indeed, phosphorylation of eIF2α along with the surface exposure of CALR on CCs has been proposed as a pathognomonic marker for ICD (61). Likewise, IRE1 triggers pro-apoptotic cascades via interaction with the adaptor protein TNF receptor associated factor 2 (TRAF2), resulting in activation of the c-Jun N-terminal kinase (JNK) and NF-κB signaling pathways (63). The metallic anticancer drug iridium III complex is an effective ICD inducer that converts dying CCs into therapeutic vaccines and stimulates an antitumor immune response in vivo, following upregulation of the nuclear transcription factor CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) and phosphorylated eIF2α, which is a typical ERS sign (64). Therefore, targeting ERS sensors or their associated UPR pathways might be useful for improving the treatment outcomes in patients with refractory cancer as compared to the current antitumor approaches.

Malignant cells experiencing mERS also modulate the immune-related signals to escape the body’s immune surveillance. In fact, after glucose deprivation in the TME, the mouse EL4 lymphoma cells show decreased expression of surface MHC-I molecules owing to the impairment of optimal peptide synthesis and loading (65). Blunting of the MHC-I-tumor specific antigen (TSA) presentation in neoplastic cells during tumor progression can affect the cues for adaptive immune recognition. The inhibitor of
apoptosis protein (IAP) antagonist ASTX660 has been confirmed to promote the ability of CCs to process and present TSAs through the upregulated expression of MHC-I and other APM components; this, in turn, makes CCs more susceptible to ICD and killed by tumor-infiltrating lymphocytes (TILs) in the absence of APCs (66). Ionizing radiation (IR) boosts the spread of epitopes of stressed or dying CCs and leads to the synthesis of antivaccinity signals by provoking massive DNA double-strand break (DSB) and disrupting the Golgi-ER homeostasis. Lhuillier et al. reported that the number of exposed immunogenic mutated peptides that can be presented by MHC-I and MHC-II molecules increased in the 4T1 breast CCs post-RT, and the killing effect of tumor-specific CD4+ T cells is clearly dependent on patulous MHC-II-neoepitope complexes and the death receptors on the CC surface (67). Hence, RT-induced ICD is a reasonable auxiliary strategy to trigger suboptimal antitumor responses that lead to the secretion of type I IFN chemokines and IL-1β, following the activation of cytosolic nucleic acid-sensing pathways and inflammasomes, and thereby establish an immunologically active micro-environment.

Compared to normal cells, CCs have a higher metabolic rate to meet the aberrant energy demands, organelle integrity, and cellular viability and proliferation in the harsh TME. During this process, they alter their mitochondria-dependent biosynthesis pathways, and reprogram their respiratory metabolism, thereby leading to excessive ROS generation; for instance, this occurs in cases of defective ATP synthesis (68). The subsequent oxidative stress may cause damage to the intracellular active components of CCs, such as DNA, protein and/or lipids, ultimately causing the cells to accumulate ROS beyond the threshold levels, leading to cell death. There is evidence that cytosolic ROS accumulation increases the intracellular release of Ca^{2+} from ER, disrupts the maintenance of ER homeostasis, and activates eIF2α-induced synthesis of apoptosis-related proteins (69). PDT is an effective approach to inducing ICD which depends on the photosensitizer components that produce ROS through the photochemical reactions, thereby leading to the oxidation of a large number of DNA and lipid substances in the tumor cells. PDT causes tumor mitochondrial dysfunction directly via ROS damage and triggers highly stress-induced cell death, thereby provoking a host antitumor response through DC maturation and CTL production (70). Recently, Mishchenko et al. reported that PDT can also induce CCs ferroptosis, an alternative form of iron-dependent cell death, by inhibition of the antioxidant glutathione (GSH) antioxidant (71). Since CCs undergoing early ferroptosis are highly immunogenic in vitro and in vivo, PDT may provide a highly efficient anticancer therapy by circumventing the resistance of CCs to other cell death modalities.

Existing preclinical data indicate that RT and chemotherapy mediate ICD by relying on the DNA damage response of cycling CCs. An important player in the DNA damage-induced apoptosis is the tumor suppressor p53 that mediates morphological changes of ER, facilitates the ER-mitochondrial Ca^{2+} transfer, and stimulates the onset of the cascade (72). Incidentally, DSBs also trigger IRE1 activation and engage the apoptotic program through the induction of unresolved ERS (73). In fact, the elevated amount of energy that RT deposits onto malignant cells can activate another DAMP molecule, namely the second messenger cyclic guanosine monophosphate (cGMP), resulting in the production of IFNα/β driven by STING (74). It is very important that the release of proinflammatory factors, which are crucial components of the immunogenicity of stressed or dying CCs, favors proficient tumor-immune interactions and beneficial antitumor responses. In some cases, the accumulation of cytosolic single-stranded DNA (ssDNA) in CCs is accompanied by the release of T-cell chemoattractants, including C-X-C motif chemokine ligand 10 (CXCL10), as well as the paracrine STING activation in DCs, ultimately upregulating MHC-II molecules and co-stimulatory ligands that support T-cell priming (75).

Furthermore, the selective pharmacological revision of the epigenetic landscape of malignant cells can control the ICD-associated exposure of some DAMPs and increase the tumor immunogenicity so that the adaptive immune responses can work successfully. Under external survival pressures, CCs undergo rapid modifications and convert their phenotypes through transcriptional state changes, epigenetic adaptation and metabolic rewiring. In fact, a frameshift mutation in the histone demethylase gene lysine (K)-specific demethylase 5C (KDM5C) in CCs promotes tumorigenicity by reprogramming the glycogen metabolism and upregulating the pentose phosphate pathway (PPP). A functional KDM5C is required for suppressing the glucose flux as well as the anti-ROS activity of PPP, thereby providing a direct link between epigenetic abnormalities and resistance to ROS-induced cell death (76). Interestingly, Segovia et al. treated bladder cancer
patients by targeting highly active G9a/DNA methyl transferase (DNMT) with the epigenetic inhibitor CM-272 (77). They demonstrated that there was a conversion of non-inflamed “cold” tumors to inflamed “hot” tumors, accompanied by increased apoptosis and autophagy of CCs with CALR expression, HMGB1 secretion, and enhanced IFN immune signaling. The combination of DNMT and histone deacetylase (HDAC) inhibitors also results in the immune priming of myeloma cells that is associated with the altered immune cell constitution of the TME; this, in turn, enhances the activation of DCs as well as CD8+/CD4+ T cells (both naive and memory-like subtypes), leading to antitumor immune responses (78).

**Conclusions**

Currently, the outcomes of the prevalent anticancer immunotherapies are hampered by poor immunogenicity and profoundly immunosuppressive microenvironment in some tumors and lymph nodes. Therefore, it is necessary to determine the means of disrupting the survival signaling pathways of malignant cells and overriding their immune tolerance mechanisms, thereby rendering them as susceptible targets for NK-cell and T-cell killing. The ICD-inducers endow CCs with a proapoptotic suicidal machinery through the activation of diverse stress-damage responses. Furthermore, the dying CCs display enhanced immunogenicity and endogenous adjuvant effects via an inflammatory transcriptional signature. Interestingly, ICD is characterized by tumor antigen release in situ, CTL infiltration in tumor sites, and APC maturation and activation, finally eliciting robust antitumor CTL response at primary and abscopal “infiltrated-inflamed” sites. Hence, ICD can serve as “in situ vaccines”, since its intriguing induction strategy can safely and effectively stimulate an immunogenic “hot” microenviroment to improve outcomes of currently practiced cancer immunotherapies.

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**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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