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Target Therapy in Cancer Treatment: mPGES-1 and PARP

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Abstract

Target therapy is an approach focusing on specific protein or signaling pathways. This therapy is directly aimed to a molecular target such as a receptor, growth factor or enzyme in cancer cells. These targets are used by the tumor cells themselves to obtain uncontrolled proliferation, resistance to traditional therapies and to increase the number of blood vessels in the tissue of origin (neo-angiogenesis). A purpose of target therapy may be to counteract the growth and proliferation of cancer cells through the use of drugs or monoclonal antibodies capable of inhibiting the receptor for the epidermal growth factor (EGFR), that is crucial in the process of neo-angiogenesis, protein kinases (PKs), as regulators of cell growth signals and human epidermal growth factor type 2 (HER2), which is essential in stimulating growth and proliferation of cancer cells. Among anticancer drugs, Bevacizumab, a humanised monoclonal antibody produced by recombinant DNA technique, is used for the first-line treatment of metastatic breast cancer, as it inhibits EGFR and the vascular endothelial cell growth factor (VEGF). Abemaciclib, a protein kinase inhibitor drug, is also used for the treatment of the same cancer. In 20-30% of primary breast tumors, the excessive expression of HER2 is observed; thus, HER2 inhibitors may represent another plausible therapy. A potent HER2 inhibitor is the recombinant humanized igG1 monoclonal antibody Trastuzumab, which was first tested in 1992 and is currently used for the treatment of HER2 positive breast cancer. Unfortunately, despite the numerous advances in finding new therapies, patients treated with these drugs often suffer from severe undesirable side effects. Therefore, the search for new therapeutic targets may be desirable. In this paper we analyse particularly two targets studied quite recently: the microsomal prostaglandin E2 synthase type 1 (mPGES-1) and poly (ADP-ribose) polymerase (PARP) proteins.

Keywords: *mPGES-1, PARP, cancer, tumor, target, mPGES-1 inhibitors, PARP-1 inhibitors.*

Introduction

Several studies have been recently carried out on new therapeutic targets in anticancer therapy, which can be added to the existing ones [1-5]. Different targets were studied in the past for antitumor agents, both synthetic [6-10] and natural [11,12]. Complexes with transition metals [13-16], lanthanides and actinides are widely described in the literature as antitumor agents [17,18]. However, cancer therapies are often responsible of side effects, including cardiac effects, such as cardiotoxicity and arrhythmia; hyperkeratotic skin; gonadotoxicity; nausea; vomiting; diarrhoea; bone marrow suppression [19]. Monoclonal antibody-based immunotherapy is now considered to be a main component of cancer therapy [20,21]. Bevacizumab is a humanised monoclonal antibody produced by recombinant DNA technique in Chinese hamster ovary cells that inhibits epidermal growth factor (EGFR). Initially approved for treatment of metastatic colorectal cancer in combination with chemotherapy, its indications now include metastatic breast cancer, non-small-cell lung cancer, glioblastoma, renal cell carcinoma, ovarian cancer and cervical cancer. Bevacizumab, by binding to vascular endothelial cell growth factor (VEGF), prevents the latter from binding to its receptors on the surface of endothelial cells. The inhibition of VEGF will consequently regress the vascularity of the tumors [22]. A protein kinase inhibitor drug used in the treatment of breast cancer is Abemaciclib. It is used for the treatment of metastatic breast cancer [23]. It is a potent and selective inhibitor of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6). It inhibits retinoblastoma (Rb) protein phosphorylation and blocks cell cycle progression from G1 to S phase, thus suppressing tumor growth. Thymidylate synthase (TS) is a major target for the design of chemotherapeutic agents ad represent a target for the treatment of epithelial-to-mesenchymal transition in non-small cell lung cancer [24,25]. Among protein kinases, targeting tyrosine kinase represent an interesting strategy. Sunitinib is a third-generation tyrosine kinase inhibitor that blocks VEGF receptors. It is used in many cancer diseases, including gastrointestinal stromal tumors, advanced renal cell carcinoma, and progressive, well-differentiated pancreatic neuroendocrine tumors [26]. Finally, excessive expression of HER2 is observed in 20-30% of primary breast tumors. Trastuzumab, a potent HER2 inhibitor, was tested in 1992 and is currently used in the treatment of HER2 positive breast cancer, also during pregnancy. It is a recombinant humanized igG1 monoclonal antibody that binds to HER2 subdomain IV and inhibits the proliferation of tumor cells expressing this growth factor [27]. However, all these drugs are not devoid of toxicity. Thus, searches for new targets are ongoing. One target may be represented by microsomal prostaglandin E2 synthase type 1 (mPGES-1), the main source of prostaglandin E2 (PGE2). The other targets are poly (ADP-ribose) polymerase (PARP), which are proteins that catalyze the reaction of poly (ADP-ribosyl) action activated by DNA strand breaks. PGE2 is known as a key mediator of the inflammatory response. However, a new role of PGE2 has been also demonstrated, *i.e.* it induces the proliferation and growth of cancer cells [28]. mPGES-1 is involved in cancers and inflammatory diseases [29]. Recently, it has been suggested as a potential target of newly identified coronavirus disease 2019 (COVID-19) [30-32]. Understanding the function of polyADP-ribosylation *in vivo* was greatly advanced using *Drosophila melanogaster* [33,34]. Clinical interest in PARP-1 has increased over the past decade with the recognition of its roles in transcription regulation, DNA repair, epigenetic bookmarking, and chromatin restructuring [35].

mPGES-1

Human mPGES-1 was cloned by PCR (polymerase chain reaction) from a placenta cDNA library, while its enzymatic activity was determined by using an HTRF kit. It consists of the net enzymatic conversion of prostaglandin H2 (PGH2) added in potassium phosphate buffer to PGE2. The molecular structure of mPGES-1 consists of a bundle of four helices that packs together to form a homotrimer. mPGES-1 is a very rigid molecule, stabilized by several interhelix hydrogen bonds. It contains an insert of 20 amino acids between the transmembrane helices I and II and forms a small positively charged domain. This domain consists of two structured rings and a short helix called domain C [28]. Helixes II and IV contain pronounced folds determined by the presence of two proline residues (Pro81 and Pro136) that block

the hydrogen bond network of the helix. The helix node II creates a large cytoplasmic cone-shaped cavity in the center of the mPGES-1 trimer. The side chain of Arg73 blocks the connection between the central cavity and the active site. Arg73 residue has two conformations: in the first conformation, it coordinates one of the glutathione (GSH) carboxylate groups and determines a separation of the cavities (Fig. 1A and C). In the alternative orientation, it interacts with the carbonyl of the main Leu69 chain of an adjacent molecule and the solvent structure of the central cavity. The second conformation provides that the pocket of the active site and the central cavity form a continuous surface (Fig. 1B and C). Given the geometric arrangement of the three active sites and the coordination of GSH through the Arg73 residue, an element of cooperativity can be noted. It is noteworthy that Arg73 is only conserved in mPGES-1 and MGST1 from higher vertebrates, so the mechanism related to the conformation of Arg73 will not occur in other members of the MAPEG family. The mPGES-1 requires GSH as an essential cofactor for its activity. The protein was crystallized in the presence of GSH, which binds to the active site of the enzyme defined primarily by TM1 and TM4 for each of the subunits. GSH interacts in a "U-shape" mainly with Arg126, Arg110 and Glu77 of TM4 and His72 of TM1 of another subunit. A model of the open conformation reveals that prostaglandin endoperoxide PGH2 could enter the fissure defined by TM1 and TM4, allowing for the synthesis of PGE2 [36]. GSH is coordinated by hydrogen bonds involving the side chains of helices II and IV (Arg73, Asn74, Glu77, His113, Tyr117, Arg126 and Ser127) and the Arg38 side chain of helix. In addition to the hydrogen bonds, the phenolic group of Tyr130 forms a stacking interaction with the peptide gamma bond between the cysteine and the glutamate side chain of the GSH. Domain does not directly contribute to the interaction with the cofactor. The Asp49 side chain forms a salt bridge with Arg126, thus indirectly contributing to the interaction with GSH. The remaining volume of the GSH binding cavity constitutes a small pocket (site of catalytic activity) [28].

Mechanism of interaction of mPGES-1 and GSH

The conversion of PGH2 to PGE2 (Fig. 2) by mPGES-1 is GSH-dependent and involves the cleavage of the O-O bond of the endoperoxide and the elimination of the hydrogen C-9 as a proton. Deprotonation involves thiolated glutathione that has been suggested to attack C-9 carbon to produce a thioemiketal intermediate, which spontaneously reorganizes into PGE2. An alternative may be the attack of glutathione thiolate (nucleophilic) on oxygen endoperoxide C-9, forming a mixed sulphide. The subsequent deprotonation in C-9 and the cleavage of the O-S bond may lead to the formation of PGE2 and regeneration of glutathione thiolate [37]. New studies carried out on the analysis of the architecture of the active site confirm that α-carboxylate of GSH forms thiolates, through a molecule of crystallographic water firmly bound in the active site. A network of hydrogen bonds is formed from the α-carboxylate fraction of GSH to the thiol group, an ideal position to assist in the deprotonation mechanism during catalysis [38]. The residue of Asp49 may act as a base during the abstraction of the proton, while the primary role of Arg126 is the stabilization of the pK_a for Asp49 and the prevention of the reduction of the intermediate formed [31]. Both the residues are located in the active site and are essential and mutually dependent during catalysis [38].

Key role of mPGES-1 Arg126 residue

The functional role of Arg126 was then examined through mutations of this residue. It was first replaced with a glutamine residue, then with a lysine one, using the site-directed mutagenesis technique. Given that Arg126 and Asp49 participate in an interaction of intermonomeric charge, in the crystalline structure of mPGES-1 the counterpart negatively charged Asp49 was thought to be changed with an asparagine residue. The results obtained lead to understand the key role of the Arg126 residue that totally compromises the functionality of the enzyme. The mutation also causes a reduction of the conversion of PGH2 into PGF2α, rather than in PGE2 [38].

Another new therapeutic target: PARP

In the last decade an important role in oncological treatments has been assumed by PARP: poly (ADP-ribose polymerase) type 1 (PARP-1) and type 2 (PARP-2), respectively [39]. PARP proteins catalyze the reaction of poly (ADP-ribosyl) action which is activated by DNA strand breaks. PARP (in particular PARP-1 and PARP-2) quickly recognize DNA strand breaks generated by genotoxic agents and once activated, use βNAD$^+$ as a substrate, hydrolyze it and release nicotinamide (Nam) and a proton (H^+) [40]. Furthermore, PARPs synthesize and catalyze the transfer of ADP-ribose units on amino acid residues such as glutamate, aspartate, arginine, lysine of acceptor proteins, varying their functioning [41]. PARP proteins are involved in several processes including DNA damage repair, cell death mechanisms (apoptosis and necrosis), transcription and chromatin modification/remodeling and have recently been used as new targets in anticancer therapies [42]. PARP-1 and PARP-2 play a dual role: they act as sensors of DNA damage, while, on the other hand, they act as signal transducers to the downstream effectors [40]. The PARP family of proteins is characterized by the presence of a sequence of 50 amino acids called the PARP "signature" [43] which forms the active site of enzymes [40]. The PARP family includes 17 members, firstly divided into 3 subgroups, but later grouped into 5 subfamilies, as follows: DNA-dependent PARPs: they are active during DNA damage and include PARP-1, PARP-2, PARP-3; Tankyrases: they are responsible for protein-protein interactions and divided into Tankyrase-1 and Tankyrase-2; CCCH PARP: proteins that have a domain related to RNA binding and divided into PARP-7, PARP-12 and PARP-13; Macro-PARP: mediate the migration of proteins towards poly sites (ADP-ribosylation) and include PARP-9, PARP-14, PARP-15; Other PARP including PARP-4, PARP-6, PARP-8, PARP-10, PARP-11 and PARP-16 [43].

PARP-1 and Cancer: Involvement in Tumor Hypoxic Response

The lack of oxygen (hypoxia) in the case of tumors generates phenomena of resistance to therapies as it makes the diffusion of chemotherapy and immunotherapy in poorly vascularized areas more difficult and, at the same time, in the case of radiotherapy, it reduces the synthesis of ROS (Reactive Oxygen Species). The response to this hypoxic condition is important for stabilizing the tumor microenvironment and increasing the volume of the tumor itself. The formation of new vessels supplies the hypoxic tumor with nutrients and oxygen and in this way will favor the growth, proliferation, cellular plasticity, migration and aggressiveness of the neoplastic cells. The hypoxic response occurs following the stabilization of hypoxia inducible factors (HIF). HIF are transcription factors consisting of three chains: HIF1α, HIF2α, HIF3α, stable during hypoxia and able to bind to HRE (hypoxic response elements) [43]. The role of PARP-1 in the interaction with HIF1α and HIF2α in the induction of their activity and stability has been evidenced. In hypoxic conditions, the oxidative stress induced by oxidative phosphorylation involves a hyperactivation of PARP-1 and consequently the stabilization of HIF1α. HIF2α is also capable of interacting with PARP-1. PARP-1 binds to the HIF2α promoter affecting its transcription. HIF2α is important both during tumorigenesis and in promoting tumor aggression and neovascularization. Any inhibition of PARP-1 will cause the reduction of the accumulation of HIF2α in the hypoxic context and consequently all its functions will be compromised [43]. The limitation to the use of PARP inhibitors is related to the presence of resistance phenomena in tumors related to BRCA mutations. It appears that more than 100 genes are involved in the DNA repair pathway, and that BRCA-mutated cells are also HR-defective, due to other alterations. In recent years, PARP inhibitors (PARPi) have emerged as a promising class of chemotherapeutic agents for ovarian cancer associated with defects in homologous recombination DNA repair (HRR) system. Homologous recombination repair deficiency (HRD) is a frequent feature of high-grade serous ovarian, fallopian tube and peritoneal carcinoma (HGSC) and is associated with sensitivity to PARP inhibitor (PARPi) therapy. HRD testing provides an opportunity to optimise PARPi use in HGSC; however, methodologies are diverse and clinical application remains controversial [44]. In the case of cancer, anti-angiogenesis treatments are often used leading to block the formation of new

vessels, limiting the existing ones and sequestering essential elements (nutrients, oxygen and blood) from the tumor itself, thus blocking its growth. The use of angiogenesis inhibition associated with PARP-1 inhibitors has been approved by the FDA for some types of tumors. This association was found to be safe, as it does not generate cross-toxicities. In this context, the use PARP inhibitors at full doses alone may be considered in the future.

mPGES-1 and PARP-1 inhibitors

mPGES-1 is present in low amounts in normal tissues and is induced upon inflammation, but it has been found overexpressed in several human cancers, including prostate, colon, lung, stomach, pancreas, cervix, breast, papillary thyroid carcinoma, head and neck squamous carcinoma, melanoma, and gliomas. mPGES-1 inhibitors have been recently reviewed for their potential activity as anticancer and antinflammatory and cardioprotective [29]. New mPGES-1 inhibitors are under study [45,46]. Sonlicromanol is an oral drug recently discovered as selective mPGES-1 inhibitor that could affect prostate cancer cells-derived spheroid growth. Metabolites are often more active that the parent compound [47-49]. Actually, KH176m, the *in vivo* active metabolite of Sonlicromanol has been studied and suggested as more active that the parent compound thus being a potential novel treatment approach for cancer patients with high mPGES-1 expression [50]. Sorafenib, a multikinase inhibitor, initiated apoptosis by cleavage of caspases 3, 7 and PARP-1 [51-53]. mPGES-1 inhibitors have been also proposed as antifungals. mPGES-1 participated in various pathophysiological states in which both COX-1 and COX-2 are involved, implying that the role of the mPGES-1 enzyme is partially similar to that of COX. Currently, mPGES-1 inhibitors are emerging as the foremost agents in the treatment of inflammatory related diseases, but their antifungal activity is still not clear. Thus, the use of more selective mPGES-1 inhibitors as an alternative pharmacological approach to antifungals front as an alternative may be a wise treatment strategy [54]. Several compounds, including triclosan and many natural products, are considered to be mPGES-1 inhibitors, such as Myrtucommulone from myrtle, Arzanol from Helichrysum, and Curcumin, which have anti-inflammatory and anti-carcinogenic properties [55,56]. There are currently no approved mPGES-1 inhibitors for clinical practice [29]. PARP-1 activation has been associated with many tumors and inflammation-related clinical conditions, including asthma, sepsis, arthritis, atherosclerosis, and neurodegenerative diseases, to name a few [57]. PARP-1 activity has been reported to make more contribution to cells death after ischemic stroke [58]. Recent evidence suggests that PARP-1 inhibitors may exert neuroprotection in the ischemic stroke [59], such as other neuroprotective agents [60,61]. PARP-1 inhibitors have been hypothesized to be involved in Alzheimer's disease (AD). Nicotinamide (vitamin B3) is a key component in the cellular production of Nicotinamide Adenine Dinucleotide (NAD) and an inhibitor of PARP-1. Nicotinamide has been recently proposed as an adjunctive treatment for AD at early stages of the disease not only to increase NAD+ stores but as a PARP-1 inhibitor, raising the hypothesis that other PARP-1 inhibitors might be explored for the treatment of AD [62].

Conclusions

The novel therapeutic targets mPGES-1 and PARP may represent a promising strategy in the treatment of various types of cancer. Through the study of these targets, the mechanisms of proliferation and growth of neoplastic cells was better understood. PARP inhibitors limitation is the appearance of resistance phenomena in tumors related to BRCA mutations. The ideal strategy could be to first measure the complete genomic instability within a tumor by recording the loss of gene heterozygosity, any allelic changes or the presence of somatic mutations. This could allow a more accurate approach capable of detecting sensitivity to PARP inhibitors and thus improving drug treatment for patients. Screening for HRD is able to offer patients the opportunity to be treated with specific PARP inhibitors and further targeted therapies so as to have a greater weapon to counteract tumor progression. Drugs capable of inhibiting mPGES-1 are to be considered a powerful weapon to implement important future strategies for the treatment of cancer.

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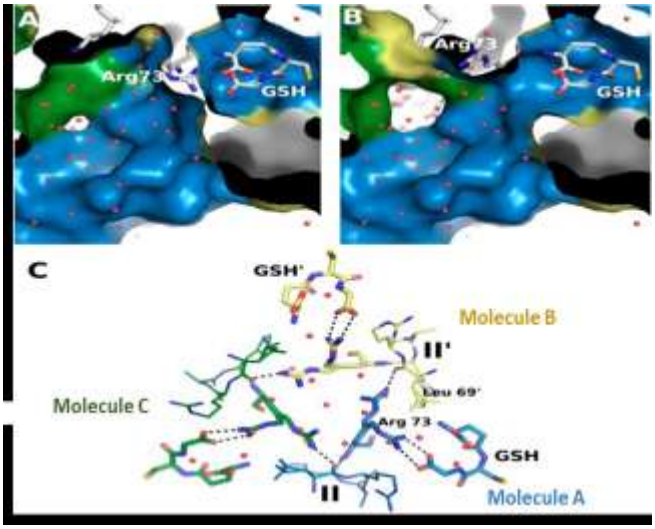

Figure 1. Central cavity and active site in mPGES-1. (**A**) Surface representation of the active site and the central cavity show Arg73 in the GSH coordinating conformation. (**B**) The same as for (A) but with Arg73 in the monomer interaction conformation. (**C**) View of both Arg73 conformations from the luminal side highlighting the potential for cross-talk between the monomers. GSH and a short stretch of helix II is shown for each monomer in blue, yellow, and green, respectively.

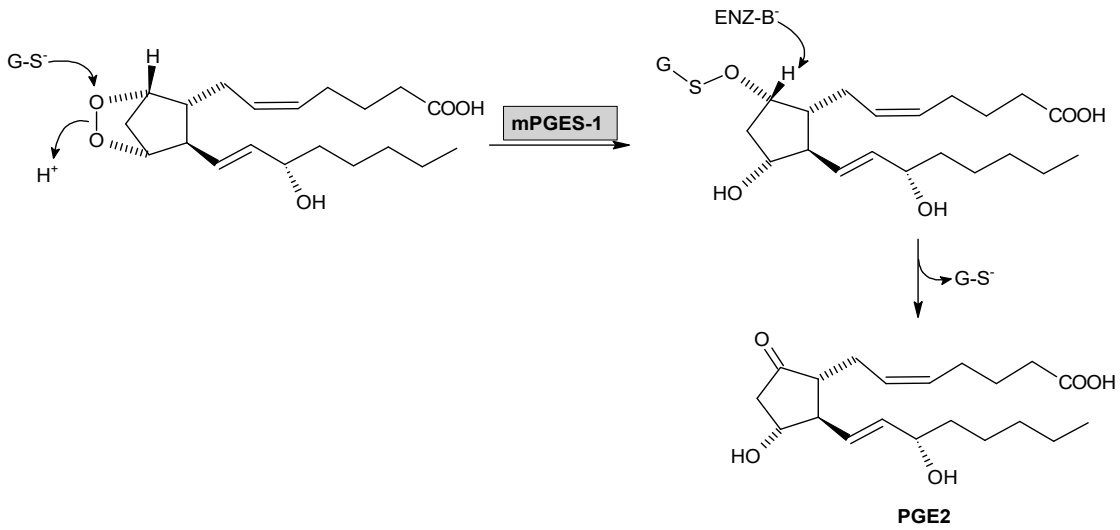

Figure 2. Suggested mechanisms for PGE2 synthase activity of mPGES-1 mutant (GS^- = glutathione thiolate).