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REVIEW ARTICLE OPEN ACCESS

Functions of glutaminyl cyclase and its isoform in diseases

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Abstract – Glutaminyl cyclase (QC; isoform: isoQC) is a zinc-dependent enzyme that catalyzes the intramolecular cyclization of N-terminal glutamine and glutamic acid residues into a pyroglutamate residue (pGlu). This conversion is a type of posttranslational modification called pyroglutamylation. The expression of QC/isoQC is regulated by epigenetics, cell homeostasis, and its substrates. Pyroglutamylation is an important maturation process during the synthesis and secretion of hormones, functioning in different diseases, such as Alzheimer's disease, tumors, and other kinds of chronic diseases mediated by inflammation. IsoQC has been identified as a key regulator of the CD47-SIRPα checkpoint and is critical for the pyroglutamylation of CD47 at its SIRPα binding site, thus helping cancer cells evade immune surveillance. Inhibition of isoQC blocks the interaction between CD47 and SIRPα, leading to constrained tumor growth, indicating that isoQC is a novel target for immunotherapy. Targeting isoQC overcomes the side effects of targeting CD47 because isoQC is Golgi resident and is not expressed on erythrocytes. Small molecules and antibodies have been developed to target isoQC, and some of them have been tested in preclinical or clinical studies. Here, we briefly review the discovery history of QC/isoQC and then discuss its regulation and function in different diseases, emphasizing the unique role of isoQC in immunotherapy. Finally, we summarize the development of inhibitors and their progress in clinical trials with the hope of providing useful insights for future investigation of QC/isoQC and targeting it in various diseases.

Key words: Glutaminyl cyclase, *QPCTL*, Pyroglutamylation, Phagocytosis, CD47, isoQC.

Introduction

Glutaminyl cyclase (QC) is encoded by the glutaminyl-peptide cyclotransferase (*QPCT*) gene, and the isoform of QC (isoQC) is encoded by the glutaminyl-peptide cyclotransferase-like (*QPCTL*) gene [1]. QC was first isolated from the latex of the tropical plant *Carica papaya* in 1963 [2, 3] and later from animals, plants, and bacteria [4], while the first isoQC of humans was isolated in 2008 [1]. QC is expressed in the mammalian pituitary, hypothalamus, other parts of the brain, adrenal medulla, and B lymphocytes [4, 5], with the highest expression in the striatum and hippocampus [6].

QC/isoQC catalyzes the formation of N-terminal glutamine and glutamic acid residues on target proteins into an N-terminal pyroglutamate residue (pGlu) [1]. The catalytic function of QC was first determined in plants and later in mammals [3, 4, 7]. The cyclization of the N-terminal glutamine occurs spontaneously and slowly under physiological conditions. IsoQC is highly expressed in brain tissue in Alzheimer's disease (AD) and some chronic diseases caused by inflammation [8]. It mainly protects target proteins from degradation and plays an important role in the initiation of neurovegetative diseases and monocyte migration.

Pyroglutamate formation of the cluster of differentiation 47 (CD47) catalyzed by isoQC is critical for the binding between CD47 and the signal-regulatory protein alpha (SIRPα) at their binding site, thus contributing to cancer cell immune surveillance [9, 10]. CD47 creates a "don't eat me" signal by binding with SIRPα on myeloid cells with high specificity. Tumor cells highly express CD47 to evade immune surveillance by the CD47-SIRPα axis [11]. The CD47-SIRPα axis has been considered a tumor phagocytosis checkpoint. Targeting CD47 or interrupting the CD47-SIRPα axis exerts efficacy in solid tumors and hematological malignancies while causing adverse effects such as anemia since CD47 is also highly expressed on red blood cells (RBCs). Inhibition of isoQC enhances the phagocytosis of myeloid cells [9]. IsoQC resides in the Golgi complex, which is absent in RBCs [12–14]; therefore, targeting isoQC overcomes the adverse side effects (anemia) of targeting CD47 [15].

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In this review, we briefly summarize the structure, regulation, and function of QC/isoQC. Then, we highlight the function of the critical enzyme isoQC in the pyroglutamylation of CD47 in the CD47-SIRPα pathway and the targeting of isoQC as a novel therapy. We believe that this short review will further our understanding of the importance of QC/isoQC in various diseases and will help researchers design a better anti-CD47 strategy in tumor immunotherapy.

Introduction and regulation of QC/isoQC

Introduction of QC/isoQC

Mammalian *QPCT* cDNA was isolated from the bovine pituitary in 1991 [6]. The gene encodes a glycoprotein QC containing sulfhydryl groups [4], a zinc-binding site [16, 17], and a mixed α-helix and β-sheet structure functioning in the polarization of the γ-amide group of the substrate and the interaction with competitive inhibitors of QC [18].

QCs exhibit overlapping but distinct homologies, structures, and characteristics among different species [6, 19–22]. The murine *QPCT* gene is located on chromosome 17, and the human *QPCT* is on chromosome 2; the murine *QPCTL* is located on chromosome 7, while the human *QPCTL* is on chromosome 19. Both *QPCT* and its isoform *QPCTL* are ubiquitously expressed in mice; *QPCT* expression is higher in neuronal tissue, while there is no significant difference in *QPCTL* expression in tissues [1]. Human *QPCTL* and murine *QPCTL* display broad substrate specificity and a preference for hydrophobic substrates [23].

As a metalloenzyme, both human QC and isoQC contain a zinc-binding domain, which reveals the conservation of the zinc-binding motif [1]. Human isoQC carries an N-terminal signal anchor that is different from that in QC [1]. Human isoQC exhibits 10-fold lower activity than human QC, indicating that isoQC has an overall lower enzymatic activity [1]. Human QC colocalizes with the Golgi apparatus, endoplasmic reticulum, and secretory granules, where it is thought to play an important role in the maturation of different proteins [1, 24], while its isoform is exclusively localized within the Golgi complex and is obviously retained by the N-terminus [1]. IsoQC shows 46% sequence identity to QC, but they have almost identical substrate specificity *in vitro* [1].

Regulation of QC/isoQC expression

The expression of QC/isoQC is tightly regulated at multiple levels, including epigenetic mechanisms, cell homeostasis, and its substrates.

Histone methylation activates QC maternally

QPCTL has been predicted to be a potential placenta-specific imprinted gene expressed maternally. Genomic imprinting is an epigenetic process by which the male and female germ lines confer specific marks (imprints) onto certain gene regions, leading to one allele with the imprinted gene being active and the other one being silenced [25–27]. QC could be a target for the treatment of diseases related to the development of the placenta and embryo. The DNA methylation level does not affect the imprinting level of QC, but histone methylation of H3Kme3 results in maternal activation of QC [28]. Whether isoQC is also regulated by H3Kme3 has not been explored and needs further investigation.

Ca^{2+} homeostasis enhances QC expression and its enzyme activity

Ca^{2+} homeostasis enhances QC mRNA expression and enzyme activity. The QC promoter contains a putative binding site for the calcium-dependent transcription factors *c-fos* and *c-jun,* which are induced by Ca^{2+}-related stimuli, and their upregulation enhances QC expression. In neuronal cells, the selective upregulation of QC via Ca^{2+}-dependent transcription factors is the major consequence of unsettled Ca^{2+} homeostasis [29]. Whether Ca^{2+} homeostasis regulates the expression of isoQC is not clear.

Proinflammatory cytokines upregulate QC/isoQC under inflammation

Chemokines play a pivotal role in different inflammatory disorders, such as atherosclerosis fibrosis, AD, and tumor progression [30–32]. The expression of QC/isoQC is upregulated by proinflammatory cytokines such as CCL2 and CX3C chemokine ligand 1 (CX3CL1), which act as its substrates.

CCL2 is a chemokine known to recruit monocytes to sites of inflammation, playing important roles in cancer progression [33]. CX3CL1 is a multifunctional inflammatory chemokine whose function depends on several factors, including its structures [34]. Both CCL2 and CX3CL1 are typical cytokines that mediate cell adhesion and migration in inflammatory processes [35], and both are substrates of QC/isoQC. The N-terminus of both chemokines has a glutamine in the first position and can be post-translationally modified by QC/isoQC to form a pGlu residue [35]. Under inflammatory conditions, the release of chemokines such as CCL2 and CX3CL1 increases the expression of QC/isoQC at the mRNA level, thus ensuring that all these chemokines are released in their fully pyroglutamylated forms [35]. Furthermore, the expression of QC/isoQC correlates with CCL2 expression at the mRNA level in an NF-κB-dependent manner [1, 33].

The function of QC/isoQC

QC/isoQC is a zinc-dependent enzyme that catalyzes the intramolecular cyclization of N-terminal glutamine and glutamic acid residues into pGlu [36]. This conversion is a type of posttranslational modification named pyroglutamylation. Pyroglutamylation occurs at the N-terminus of a number of peptide hormones and secretory proteins, such as gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), neurotensin and fibronectin [37, 38].

IsoQC possesses almost the same substrates as QC but differs in subcellular localization [1, 23, 39]. The functions of QC and isoQC probably complement each other, suggesting

a pivotal role of pyroglutamylation in protein and peptide maturation [23].

QC/isoQC function in protein stabilization

The formation of the N-terminal pGlu is an important maturation step during the synthesis and secretion of hormones [40, 41]. The pyroglutamylation modification allows regulatory peptides to adopt a proper conformation for binding to their receptors and renders the protein more resistant to protease degradation [4, 7] and more susceptible to hydrophobic interactions, aggregation, and neurotoxicity [7, 42, 43]. In snakes, QC and isoQC also contribute to the stabilization of peptide toxins [44].

QC/isoQC protects proteins in cerebrospinal fluid from degradation

Proteins and peptides in human cerebrospinal fluid (CSF) are potential substrates of QC/isoQC, whose activity is a characteristic feature of CSF [43]. The pyroglutamylation of proteins in CSF stabilizes the peptide from degradation by aminopeptidases [45]. This may play an important role in neurological disorders, which are characterized by the inappropriate expression of QCs or their substrates [46].

QC/isoQC function in chemokine activity and stability

The pyroglutamylation of CCL2 catalyzed by both QC and isoQC plays critical roles in the synthesis, secretion, maturation, and function of CCL2 [1, 39, 47] and protects CCL2 from proteolytic degradation during these processes *in vivo* [39, 48]. The activity and stability of CCL2 also depend on its pyroglutamylation [1, 39, 47].

QC/isoQC also catalyzes the N-terminal pyroglutamylation of CX3CL1, which is important for its stability and its interaction with the CX3CL1 receptor and provides new insights into the function of QC in inflammation [35]. Loss of pyroglutamylation results in increased accessibility for N-terminal proteolytic degradation or directly affects ligand-receptor interaction (Fig. 1a).

QC/isoQC function in monocyte migration

QC-catalyzed pyroglutamylation of monocyte chemoattractant proteins is required for monocyte migration in the inflammatory process; thus, QC may be a potential target for some inflammatory disorders [48].

QC/isoQC plays essential roles in monocyte homeostasis. It supports mouse monocyte migration and sustains monocyte infiltration and tumor growth. Pharmacological isoQC inhibition prevents monocyte accumulation in established tumors and promotes the remodeling of macrophage compartments. Suppressing isoQC also improves $CD8^+$ T-cell responses upon programmed cell death-ligand 1 (PD-L1) blockade and enhances antitumor activity. Moreover, isoQC inhibition constitutes an effective approach for myeloid cell-targeted cancer immunotherapy, and disruption of isoQC can be used in combination with checkpoint inhibitors to enhance the antibody-mediated phagocytosis of tumor cells [49].

The function of isoQC in cancer immunotherapy

Brief introduction of CD47

CD47 is a glycolyzed 5-transmembrane protein expressed on many types of cells. It is composed of a glycosylated N-terminal extracellular domain (ECD), a 5-transmembrane-spanning domain, and a short C-terminal domain (CTD) [50–53]. The ECD contains a V-set immunoglobulin superfamily (IgSF) domain and is a cell surface marker of self that binds to SIRPα.

The expression of CD47 is regulated at the transcriptional level, translational level, and posttranslational level. Transcription factors such as Myc [54, 55], hypoxia-inducible factor-1 (HIF-1)α [56–58], The nuclear factor κB (NF-Kb) [59, 60], and α-Pal/NRF-1 [61, 62] regulate the expression of CD47; various cytokines such as tumor necrosis factor (TNF)-α [59, 63, 64], interferon-γ [65, 66], and interleukins [67, 68] potentiate CD47 expression. Posttranscriptional modification of CD47, including glycosylation and pyroglutamylation, regulates CD47 function [69, 70]. The glycosylation of CD47 is essential for its binding to thrombospondin 1 (TSP-1) [71, 72], and the pyroglutamylation of CD47 is essential for the CD47-SIRPα axis to function in immunotherapy [9, 10].

CD47 plays important roles not only in biological processes such as proliferation, autophagy, and phagocytosis but also in various types of cancer. It has been regarded as a key antiphagocytic molecule that renders tumor cells resistant to immune surveillance.

The CD47-SIRPα pathway

CD47 regulates a cascade of signaling pathways by binding to its receptors, such as SIRPα and TSP-1. SIRPα is a transmembrane glycoprotein that contains three Ig-like domains, one transmembrane domain, and four tyrosine phosphorylation sites with two immune receptor tyrosine-based inhibitor motifs (ITIMs) in the cytoplasmic tail [73–75]. The NH2-terminal domain of SIRPα binds to the lgV domain of CD47, and the interaction between CD47 and SIRPα leads to the phosphorylation of ITIMs in SIRPα. Phosphorylated ITIMs recruit and activate the inhibitory molecules Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2. The recruitment of SHP-1 and SHP-2 enables the phosphorylation of myosin IIA and then suppresses the function of non-muscle myosin IIA, which modulates phagolysosomal biogenesis in macrophages and plays important roles in phagocytosis [76]. Finally, the interaction between CD47 and SIPRα inhibits phagocytosis and cytotoxicity [76–78]. When the binding between CD47 and SIPRα is blocked, the latter's ITIMs are not phosphorylated, and the lack of SHP-1 and SHP-2 recruitment enables the activation of prophagocytic receptors to trigger phagocytosis.

CD47 creates a "don't eat me" signal by binding SIRPα on myeloid cells with high specificity. The CD47-SIRPα axis inhibits phagocytosis and protects RBCs from

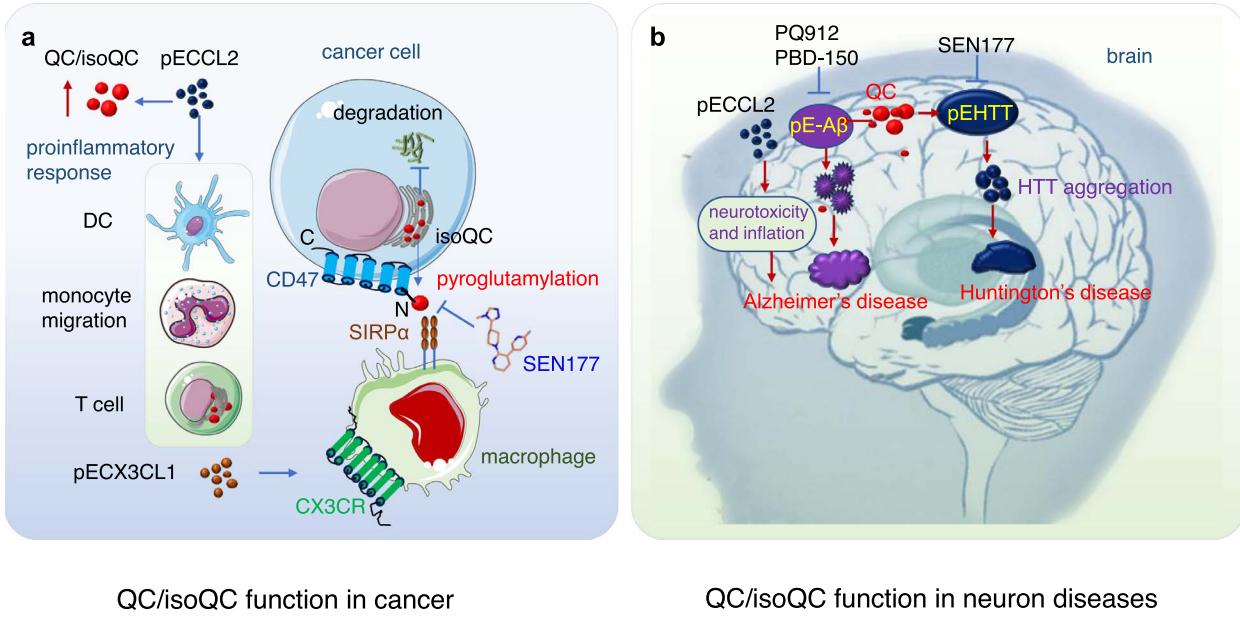


Figure 1. The function of QC/isoQC in diseases. (a) *Function of QC/isoQC in cancer*: IsoQC catalyzes the pyroglutamylation of CD47 and contributes to the interaction with the CD47-SIRPα axis and finally leads to the cancer cell immune surveillance evasion. Targeting isoQC by SEN177 or other molecules inhibits the pyroglutamylation of CD47, thus CD47 cannot interact with SIRPα, and cancer cells are phagocyted by the macrophages. The pyroglutamylation of CCL2 upregulates CCL2 expression and recruits more immune cells such as DCs, monocytes, and T cells into the tumor microenvironment. The pyroglutamylation of CX3CL1 is critical for its binding to CX3CR and blocks the CX3CL pyroglutamylation leading to the degradation of the protein and affecting its binding to receptors. The basic function of QC/isoQC is to catalyze the target protein N terminal pyroglutamine and protect it from degradation. (b) *Function of QC in neuro diseases*: QC is primarily and highly expressed in the brain including the hippocampus and cortex and other peripheral tissues. It converts beta-amyloid (Aβ) N-terminal peptides at position 3 or 11 into pGlu. Abnormally upregulation of QC in AD functions as a key inducer in the initiation of AD by catalyzing the generation of different mediators such as pyroglutamylation of Aβ and CCL2, which are the main causes of neurotoxicity and inflammation. Small molecules such as PBD-150 and PQ-912 inhibit QC activity and reduce the syndrome of AD, PQ-912 is in a Phase II clinical trial now. In Huntington's disease, the pyroglutamylation catalyzed by QCs leads to the aggregation of huntingtin (HTT) protein and finally leads to Huntington's disease. A small molecule such as SEN177 targeting QC/isoQC rescues Huntington's disease phenotypes efficiently and reduce HTT protein aggregation.

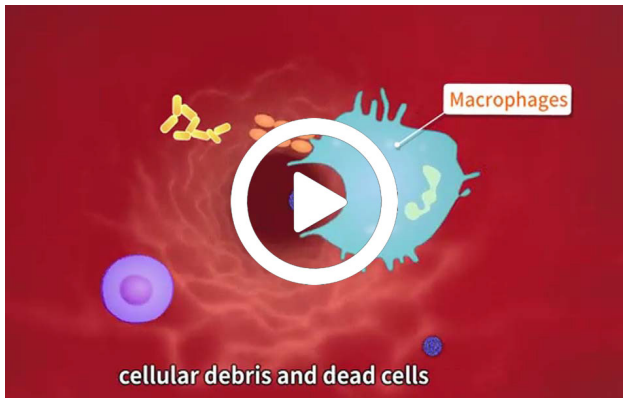
erythrophagocytosis [79]. Tumor cells utilize this mechanism to evade immune surveillance by highly expressing CD47 [11, 80–85]. Therefore, the CD47-SIRPα axis has been considered a tumor phagocytosis checkpoint [83–86]. Targeting CD47 or disrupting the CD47-SIRPα axis has shown efficacy against solid tumors and hematological malignancies while causing adverse effects such as anemia since CD47 is also highly expressed on RBCs. Therefore, it is important to gain a deeper understanding of the CD47-SIRPα axis with the hope of identifying modulators that could be targeted for myeloid cell checkpoint blockade.

The pyroglutamylation of CD47 contributes to the CD47-SIRPα pathway

Crystal structures of the CD47-SIRPα interaction have revealed the involvement of pyroglutamate at the N-terminus of CD47 based on its hydrogen bonding [87]. The N-terminal Q19 of CD47 is modified to a pGlu residue by isoQC [10]. This process involves the loss of an NH_3 from N-terminal glutamine, leading to the formation of a cyclic amide structure, thus generating the pyroglutamate derivative [43]. IsoQC-mediated CD47 modification occurs very early in the protein life cycle [9]. This pyroglutamate is assumed to form spontaneously upon cleavage of the CD47 signal sequence [88]. The role of isoQC as a modifier of CD47 is observed in many cells, including malignant melanoma and other types of cancer cells [9].

The N-terminal pyroglutamylation of CD47 is an important posttranslational modification of CD47, which can be specifically recognized by SIRPα and contributes to the interaction between CD47 and SIRPα [9, 87]. CD47-SIRPα binding critically depends on a CD47 posttranslational modification of its N-terminal glutamine to a pyroglutamate [9]. isoQC was discovered to be involved in CD47-SIRPα by two different screens in two labs. Logtenberg et al. used a fluorescence-activated cell sorting (FACS)-based haploid genetic screen with an antibody against human CD47 and found that isoQC is a typical CD47 regulator [9]. Our lab used a FACS-based whole-genome screen in the colon cancer cell line HCT116 and obtained similar results [10]. By coculturing tumor cells and macrophages, we found that isoQC inhibits the phagocytosis of tumor cells by macrophages.

The interaction between CD47 and SIRPα is druggable by compounds such as QC/isoQC inhibitors, which have been verified by two independent studies [9, 10]. This indicates that pyroglutamylation serves as an immunotherapeutic target by



Video 1. https://vcm.edpsciences.org/10.1051/vcm/2022008#V1.

abolishing immune checkpoint signaling and that isoQC is a potential therapeutic target for the treatment of various cancers (Video 1).

QC/isoQC function in diseases

QC and isoQC play important roles in cancers such as melanoma [89], papillary thyroid carcinoma [90, 91], and renal cell carcinoma [28, 92]. Abnormal upregulation of isoQC in AD functions as a key inducer in the initiation of AD, catalyzing the generation of different mediators, such as pyroglutamyl-Aβs (pE-Aβs) and pE-CCL2, which are the main causes of neurotoxicity and inflammation [93–99]. More details on QC/isoQC function in diseases will be addressed in the next section.

Targeting QC/isoQC in cancers and other diseases

Targeting QC/isoQC in cancers

QC/isoQC correlates with survival and drug resistance

QC/isoQC is highly expressed in melanoma [100, 101], papillary thyroid carcinoma [90], glioma, cholangiocarcinoma, and colon cancer. The relationship of QC/isoQC expression with patient survival is inconsistent. In most cancers, the high expression of QC/isoQC is associated with a lower survival rate, while in cholangiocarcinoma and colon adenocarcinoma, the high expression of QC/isoQC indicates a higher survival rate [102]. In sunitinib-nonresponsive renal cell carcinoma (RCC), the methylation level in QC is decreased, suggesting QC may function in tumor progression and drug resistance [103]. QC colocalizes with HRAS in the cytoplasm and binds HRAS to promote its stability and reduce its ubiquitin-mediated degradation, finally leading to drug resistance [103]. Therefore, the expression of QC may be related to sensitivity to drugs in RCC.

Targeting isoQC in the CD47-SIRPα pathway in cancers

CD47-SIRPα is the key pathway through which tumor cells escape phagocytosis by macrophages, and pyroglutamylation of CD47 is the key modification for CD47 to bind to SIRPα [104]. IsoQC is the central enzyme catalyzing the pyroglutamylation of CD47. Pyroglutamylation contributes to the macrophage-mediated phagocytosis of tumor cells [9, 10]. Genetic and pharmacological interference with isoQC activity enhances antibody-dependent cellular phagocytosis and increases the cellular cytotoxicity of tumor cells and the neutrophil-mediated killing of tumor cells *in vivo* [9, 10]. Knockout of isoQC induces the accumulation of macrophages expressing phagocytic genes, indicating that this modality of antitumor immunity may be enhanced by two isoQC-mediated mechanisms. Targeting isoQC significantly reduces the binding ability of CD47 to SIRPα to regulate tumor immunity [10, 105, 106]. Unlike CD47-targeted monoclonal antibodies, isoQC inhibitors avoid the side effects on RBCs. In addition, a recent article reported that isoQC depletion also results in impaired monocyte recruitment, affected monocyte homeostasis, and improved $CD8^+$ T-cell responses [49] (Fig. 2).

The facilitating function of isoQC in the CD47-SIRPα pathway and in monocyte migration suggests that targeting isoQC is a means of engaging and enhancing multiple antitumor mechanisms, including macrophage-mediated phagocytosis and adaptive T-cell responses.

Targeting QC/isoQC in other diseases

QC/isoQC plays important roles in the pathology of several diseases, such as rheumatoid arthritis, osteoporosis [107], AD [107, 108], and septic arthritis [109]. IsoQC is significantly upregulated and closely related to decreased patient survival [10].

QC/isoQC in inflammatory diseases

Acute and chronic inflammatory disorders are characterized by detrimental cytokine and chemokine expression. The chemotactic activity of cytokines depends on a modified N-terminus of the polypeptide. For example, CCL2 is modified by pyroglutamylation to protect it against degradation *in vivo*. QC/isoQC inhibitors attenuate atherosclerotic pathology in a model of accelerated atherosclerosis. A combinatorial approach of QC/isoQC knockout as well as the pharmacological inhibition of QC/isoQC represents an alternative therapeutic strategy to treat CCL2-driven disorders such as atherosclerosis/restenosis and fibrosis [39]. Targeting QC/isoQC activity leads to the degradation of CCL2 and the reduction of kidney inflammation [110].

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of hepatic pathology, and inflammation is an integral part of NAFLD. The chemokine CCL2 and its primary receptor CCR2 are key regulators of inflammation. The pyroglutamylation of CCL2 protects it from degradation by aminopeptidases, and this truncated form reduces its potential to attract immune cells in the inflamed liver [111]. Therefore, inhibition of QC/isoQC is a promising therapy for NAFLD.

QC/isoQC in neuron diseases

(a) IsoQC in Alzheimer's disease

AD is a common chronic and progressive neurodegenerative disease [112]. Deposition of amyloid-β (Aβ) remains a

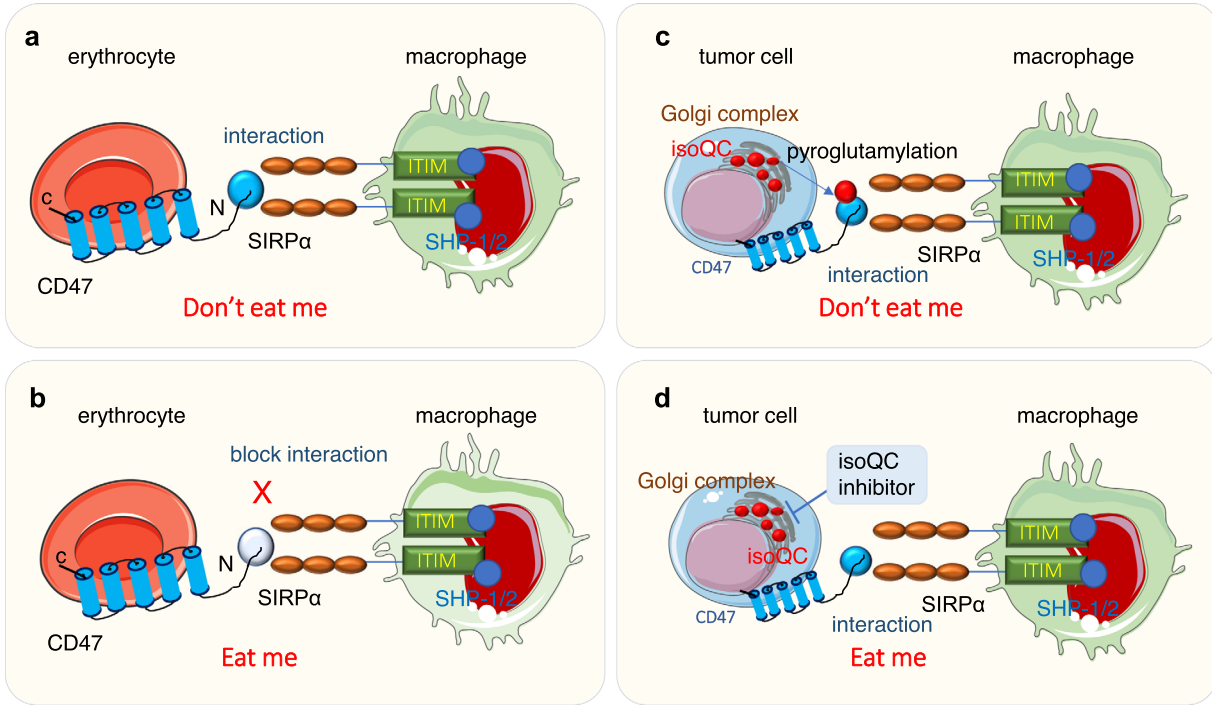


Figure 2. Targeting isoQC in cancer immunotherapy. The interaction between CD47 on erythrocyte and SIRPα on macrophages triggers a "don't eat me" signal, thus the erythrocyte is not engulfed by macrophages. (b) Interrupting the CD47-SIRPα pathway either by blocking CD47 or SIRPα lead to the phagocytosis of erythrocytes by macrophages. (c) The interaction between CD47 on tumor cells and SIRPα on macrophages triggers a "don't eat me" signal, thus the tumor cells are not engulfed by macrophages, by this mechanism, tumor cells realize their immune surveillance evasion. The pyroglutamylation of CD47 is critical for the binding between CD47 and SIRPα. (d) Blocking the CD47-SIRPα pathway leads to the phagocytosis of tumor cells by macrophages but meanwhile brings the adverse effect that the erythrocytes are phagocytized by the macrophages. Targeting isoQC by SEN177 or other inhibitors inhibits the pyroglutamylation of CD47 and thus blocks the interaction between CD47 and SIRPα. IsoQC is a Golgi-resident enzyme that is not expressed on erythrocytes, thus the erythrocytes would not be affected by targeting isoQC.

hallmark feature of AD, yet the precise mechanisms by which this peptide induces neurotoxicity remain unknown. Research on this point has grown exponentially over the past few decades. The contribution of pyroglutamylation of Aβ to the neurodegenerative process in AD has been widely studied over the last few years [113, 114].

QC is primarily and highly expressed in the brain, including the hippocampus and cortex, as well as in peripheral tissues. It is highly involved in the posttranslational modification of Aβ peptides by converting its N-terminal peptide at position 3 or 11 into pGlu. Abnormal upregulation of QC in AD functions as a key inducer in the initiation of AD by catalyzing the generation of different mediators, such as pyroglutamylation of Aβ and CCL2, which are the main causes of neurotoxicity and inflammation [93–99] (Fig. 1b).

Pyroglutamylated Aβ peptides (AβpEs) are found in self-aggregated Aβ peptides and act as initiators of Aβ accumulation, favoring plaque seeding [115, 116]. AβpE is only found in AD brains and constitutes approximately 50% of the total Aβ [117], so the level of AβpE has been treated as a potential biomarker of AD [118]. AβpE is upstream of the neurotoxic amyloid cascade, triggering neurodegeneration and enhancing AD pathology [119–121].

AβpE has been treated as a drug target in AD, and drugs such as donanemab (a humanized IgG1 monoclonal antibody to the pyroglutamylated form of Aβ) are in phase II clinical trials [122–126]. Many Aβ peptides possess a pGlu residue catalyzed by QC [127], and the pyroglutamylation of Aβ is highly abundant in the brains of AD patients, protecting it from protease degradation and making it more hydrophobic and prone to aggregation, which might increase its neurotoxicity [24, 101, 128, 129]. QC catalyzes the pyroglutamylation of Aβ, and QC inhibition lowers the amount of pyroglutamylated Aβ and lessens the AD-like pathology. Oral application of a QC inhibitor resulted in reduced Aβ(3pE-42) burden in two different transgenic mouse models of AD and in a new Drosophila model [101].

In conclusion, QC/isoQC is a potential target in drug development due to its pronounced stability, neurotoxicity, and aggregation propensity, and various inhibitors have been synthesized targeting pyroglutamylation in AD [130].

(b) QC in Huntington's disease

Huntington's disease (HD) is an incurable neurodegenerative disease caused by an abnormally expanded polyglutamine tract in the huntingtin protein (HTT) [131]. In a cell-based siRNA screen, QC had one of the strongest effects on mutant HTT-induced toxicity and aggregation, and it rescued these phenotypes in Drosophila. Targeting QC/isoQC with new inhibitors using in silico methods followed by *in vitro* screening

rescued the phenotype and disrupted HTT aggregation [131] (Fig. 1b).

QC/isoQC in osteoporosis

Osteoporosis is a disease of low bone mass, high bone fragility, and high morbidity [132]. Hormone levels are involved in the occurrence and progression of osteoporosis [133]. QC/isoQC is an essential modifier of pituitary peptide hormones, including GnRH, which is associated with osteoporosis. Genetic variations in QC are important factors affecting the bone mineral density (BMD) of adult women and thereby contribute to susceptibility to osteoporosis [107].

The common polymorphisms in QC are closely associated with BMD in both the Chinese and Japanese populations [107, 134]. The *QPCT* gene lies on chromosome 2p22.3, within a region influencing forearm BMD, suggesting bone mass differences between individuals may be influenced by the nucleotide variations of the *QPCT* gene. By investigating 13 known polymorphisms, it was found that a single-nucleotide polymorphism (SNP) variation at isoQC contributes to BMD in adult women [107].

In conclusion, the expression and function of QC/isoQC in many types of diseases are under research, and advanced research is focused on neuron-related diseases. Its function and working mechanisms in cancer require more investigation. Due to its importance in various diseases, targeting QC/isoQC by small molecules is developing fast.

Small molecules targeting QC/isoQC

Since QC and isoQC have 46% conserved sequences, including sites with catalytic activity, many QC substrates can also be catalyzed by isoQC to form pyroglutamide, suggesting that they have similar substrate selectivity [1]. Theoretically, inhibitors of QC would also inhibit isoQC, as demonstrated by Holger et al. [1]. IsoQC, as an isoenzyme, is an important target to reduce the risk of cross-reactivity and side effects.

QC inhibitors developed from 2004 to 2021 have been reviewed elsewhere [135, 136]. Many inhibitors of QC have been prepared and screened *in vitro* [16, 18, 101, 137–145]. Since the catalytic activity of QC is zinc-dependent, some types of QC inhibitors were designed based on its zinc domain, such as imidazole, triazole, and benzimidazole [146], and they all contain, in general, the following distinct structural features: a zinc-binding group, a hydrogen-bonding donor and an aromatic group to interact with Phe325 in the pocket as a key amino acid for potent binding. They mimic two or three amino acids of the sequence of *N*-truncated Aβ, NH2–Glu–Phe–Arg [18, 137–139, 147].

Imidazole and its derivatives were identified as competitive inhibitors of QC. They interact with accessible zinc atoms in the active site of carboxypeptidase A to form stable complexes with active-site residues of serine proteases in the presence of zinc ions [148–150]. The first effective inhibitors for human QC were described in 2006 [17, 18]. Most of the inhibitors of QC/isoQC were designed based on the imidazole/methylimidazole ring as a zinc-binding motif (motif 1). Later, more compounds presented extended motifs, consisting of an H-bonding donor (motif 2) and an aromatic moiety (motif 3) or even a fourth motif mimicking an arginine guanidinium moiety [18, 137, 140–142]. The minimal requirements to obtain a high-affinity inhibitor are a Zn (II)-binding moiety, either imidazole or triazole and a hydrophobic aromatic ring [151]. Some compounds are predicted to be able to permeate the blood-brain barrier (BBB), and they can be used as oral drugs [152]. Here, we summarize a couple of QC and isoQC inhibitors.

PBD-150

Researchers have further modified imidazole to improve its inhibition of QC enzyme activity [23] and found that thiourea derivative modification on the nitrogen atom of imidazole No. 1 can greatly improve its inhibition of QC [39].

PBD-150 is a human QC Y115E–Y117E variant inhibitor with a high affinity for QC (inhibition constant K_i = 490 nM for human QC and K_i = 173 nM for murine QC) [18, 153]. PBD-150 thiourea is a representative compound. Its inhibition of QC is more potent than that of isoQC (isoQC, K_i = 7.3 μM; QC, K_i = 60 nM) [128, 154]. PBD-150 is the prototype QC inhibitor with imidazole as a zinc-binding group, and it was discovered by Probiodrug AG through fragment-based screening [18].

PBD-150 can reduce the deposition and aggregation of pyroglutamate-modified amyloid-β peptides in the brains of transgenic mouse models of AD, leading to a significant improvement in learning and memory in those transgenic animals [18, 101, 155]. Furthermore, PBD150 plays important role in monocyte migration simulated by monocyte chemoattractant proteins; it reduces N-terminal uncyclized-MCP-stimulated monocyte migration without affecting pGlu-containing MCP-induced cell migration [48].

Even PBD-150 has been demonstrated *in vitro* and *in vivo* to decrease the formation of pGlu-Aβ, but the preclinical evaluation of PBD-150 demonstrates that it cannot cross the BBB [154] due to the polarity of this compound [156], suggesting that the therapeutic effect of QC inhibition may be associated with peripheral mechanisms [156]. Future works in the development of this therapeutic agent may focus on the improvement of BBB permeability, which would let it serve as a companion diagnostic when developed as a PET radioligand.

SEN177

The SEN177-binding mode to human QC differs from that of the known human QC inhibitors. SEN177 is designed based on a triazine ring as the human QC-binding motif and brings different extension motifs [151]. Crystal studies of the SEN177–human QC complex show two interactions between the enzyme and the inhibitor in terms of inhibitor orientation: the pyridine ring with Trp207 and the fluorine atom of 2-fluoropyridine with the hydrogen atom of His330 [152]. SEN177 exhibits comparable or better potency compared to other inhibitors, such as PBD-150 and PQ912 (K_i = 24.7 nM) [151]. Its K_i to human QC is 20 nM, and its half-maximum inhibitory concentration is only 0.013 μM for isoQC, so it is one of the best inhibitors of isoQC.

SEN177 is the first reported small-molecule antagonist of the SIRPα-CD47 interaction. Treatment with the isoQC inhibitor SEN177 reduces human SIRPα-Fc staining in many cancer cell lines, thus leading to the reduced binding of CD47-SIRPα even though the total CD47 levels are not significantly affected [9]. Knockout of isoQC reduces the binding of the CD47 antibody CC2C6, which has the same recognition site (pGlu) as SIRPα [9]. The SIRPα-CD47 interaction is also quantitatively measured in live and fixed tumor cells via a new assay relying on laser scanning cytometry (LSC) [4]. Furthermore, blocking CD47-SIRPα by SEN177 enhances tumor cell killing by polymorphonuclear neutrophilic granulocytes (PMNs) with different EGFR antibody isotypes [157].

In addition, SEN177 can efficiently rescue HD phenotypes and reduce HTT protein aggregation in mammalian cell lines, mouse neurons, Drosophila eyes, and zebrafish [131].

PQ529

PQ529 is a newly reported isoform-nonspecific QC/isoQC inhibitor [39]; its full name is 1-(1H-benzo[d]imidazol-5-yl)-5-(4-propoxyphenyl) imidazolidine-2,4-dione, and it possesses benzimidazole as a warhead. The K_i-values of PQ529 at pH 8 are 38 nM for human QC, 4 nM for human isoQC, 27 nM for murine QC, and 2 nM for murine isoQC [109]. Treatment with PQ529 results in a significant delay in the development of arthritis [109]. PQ529 significantly inhibits CCL2 and amyloidβ *in vivo* and *in vitro*. It has potential therapeutic effects on atherosclerosis and AD [39, 158].

PQ912

PQ912 (drug name: Varoglutamstat) is an inhibitor of QC that plays a critical role in the formation of synaptotoxic pyroglutamate-A-beta oligomers (AβOs) and has no significant toxicities when used in neurodegenerative diseases. The model of action of PQ912 is as follows: AβOs, which are synaptotoxic and lead to cognitive decline, play important roles in the pathophysiology of AD [159–161]. pE-Aβs initiate and sustain the formation of synaptotoxic AβOs. PQ912 is a QC inhibitor that can decrease the formation of pE-Aβ [162]. Therefore, inhibition of QC with PQ912 leads to a decrease in pE-Aβs and consequentially a reduction in AβOs [115]. Treatment with PQ912 in a mouse model of AD improves cognition and attenuates the pathology of AD [144, 163]. PQ912 prevents the formation of pGlu3-Aβ in different compartments of cells, and the combination of PQ912 and a pGlu3-Aβ-specific antibody (m6) in transgenic mice exert additive effects on brain Aβ pathology [164].

PQ912 is a first-in-class QC inhibitor in clinical development, and it has undergone rigorous preclinical and clinical investigations, including *in vitro* and *in vivo* animal and phase 1 clinical studies [144, 162]. A phase 2a study aims to investigate the highest dose of PQ912 that was used in a phase I study of the same compound to evaluate its safety and tolerability in patients with mild cognitive impairment due to AD. The results have shown that PQ912 is safe and highly effective [162, 165]. It is currently under phase 2b study, and the results are expected in early 2023 [166].

In addition to all the above inhibitors, new heterocyclic and peptidomimetic derivatives to inhibit QC and isoQC are still under investigation. In Dec. 2021, the cyclopentylmethyl derivative (214) exhibited the most potent in vitro activity (IC50 = 0.1 nM), which is 290-fold higher than that of PQ912, and benzimidazole (227) showed the most promising *in vivo* efficacy [123].

Natural products targeting QC/isoQC

In addition to small molecules, natural products and traditional Chinese medicines are widely used for various diseases [167]. A myriad of isolated natural products, such as natural phenol compounds, marine products, and flavonoid derivatives, have revealed new active scaffolds with isoQC-inhibitory properties, and some of them have exhibited promising and exciting results [130, 140].

Flavonoids exhibit many desired pharmacological effects, including antioxidation and anti-inflammation [168, 169]. Luteolin, a natural flavonoid compound, has potent anticancer effects *in vitro* and *in vivo* due to its natural antioxidant ability [170, 171]. Luteolin has been verified as a novel inhibitor of isoQC by our lab; it directly interacts with isoQC protein, reduces pGlu modification of CD47 and its surface binding to SIRPα, and finally promotes macrophage-mediated phagocytosis [172]. Other natural compounds from microalgae have been synthesized to target QC or isoQC [173].

Conclusion and prospects

QC/isoQC is a critical enzyme that functions in both biological and pathological conditions via the pyroglutamylation of proteins. Most of the research focuses on its function in AD or other neurodegenerative diseases. We explored and summarized its function in immunotherapy due to its unique role in the CD47-SIRPα checkpoint.

Cancer immunotherapy is now a pillar of cancer treatment that continues to develop. Cancer immunotherapies, including checkpoint inhibitors and adoptive cell therapy, manipulate the immune system to recognize and attack cancer cells [174]. Cancer cells escape immune surveillance by hijacking inhibitory pathways through the overexpression of checkpoint genes; thus, the downregulation of checkpoint gene expression can be a potential weapon in immunotherapy. Besides T cells, macrophages and neutrophils are also targets of immunotherapy [9, 175]. IsoQC blocking is a novel immunotherapy approach that relies on innate myeloid checkpoint (CD47-SIRPα) blockade [176, 177]. The physiological role of isoQC is probably to stabilize bioactive peptides, and the unique role of isoQC in macrophage-mediated phagocytosis gives it a role in cancer immunotherapy. In CD47-highly-expressing tumors, targeting CD47 suppresses tumor growth but also has side effects, such as limited penetration of tumor tissues associated with their structure and large size, which may impede its clinical application. Targeting isoQC provides new opportunities to increase the therapeutic efficacy of tumor-directed antibodies by interfering with the CD47-SIRPα axis. Targeting isoQC not only avoids the anemia problem but also leads to better tumor penetration, fewer infusion reactions, and higher patient compliance [178].

Knowledge of QC/isoQC has increased considerably, and the therapeutic potential of QC/isoQC inhibitors has been explored. Difficulties in obtaining large quantities of pure protein may limit the use of crystallographic screening for drug development on this target, and the complicated metabolism *in vivo* requires more experiments [179, 180]. QC enzymes are pharmacologically interesting targets to be used as an AD-modifying therapy [136]. Many inhibitors have been developed and optimized, but only PQ-912 has been applied in clinical trials and has completed a phase 2a trial in AD [165]. Regarding the application of isoQC targeting in cancer immunotherapy, no inhibitors are on the market until now. In Feb. 2022, Insilico Medicine nominated the preclinical candidate compound ISM004-1057D, which targets isoQC in the CD47-SIRPα pathway, for innovative tumor immunotherapy. ISM004-1057D is in the investigational new drug (IND) stage. Once the clinical trial application is approved, the cooperating company Fosun Pharma will conduct clinical trials immediately.

In the future, innate myeloid checkpoint blockade will become another important therapeutic option [176, 177], and isoQC as a potential target deserves more investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

P.W. designed the study. Y.L. contributed to the literature search, designed the video, and wrote the paper. J.Z gave suggestions in revision. Y.S. and P.W. revised the manuscript based on their rich research experience. All authors read and approved the final manuscript.

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