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REVIEW ARTICLE

Human arginase 1, a Jack of all trades?

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Abstract

Arginine, a conditionally essential amino acid, plays a crucial role in several metabolic and signalling pathways. Arginine metabolism in the body can be significantly increased under stress or during certain pathological conditions. Depletion of circulating arginine by administering arginine-hydrolysing enzyme has been shown to mitigate varied pathophysiological conditions ranging from cancer, inflammatory conditions, and microbial infection. This review provides an overview of such intriguing expanse of potential applications of recombinant human arginase 1 for different pathological conditions and its status of development.

Keywords Arginine · Auxotrophy · Arginase · PEGylation · Fusion protein

Abbreviations

ACE2	Angiotensin-converting enzyme 2
ADEM	Acute disseminated encephalomyelitis
ADI	Arginine deiminase
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
Arg	Arginase
ARG1	Arginase 1
ASL	Argininosuccinate lyase
ASS1	Argininosuccinate synthetase
CNS	Central nervous system
DNA	Deoxyribonucleic acid
Fc	Fragment crystallizable
FcRn	Neonatal Fc receptor
FDA	Food and Drug Administration
EHA	Engineered human arginase
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HFD	High fat diet
HLEP	Half-life extension partners
HSV-1	Herpes simplex virus-1
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IL-17A	Interleukines-17A
IND	Investigational new drug
iNOS	Inducible nitric oxide synthase
IR	Ischaemia–reperfusion
Mcp1	Monocyte chemoattractant protein
M-CSF	Macrophage-colony-stimulating factor
mTOR	Mechanistic target of rapamycin
NFAT c1	Nuclear factor of activated T cells, cytoplasmic 1
NO	Nitric oxide
NOS	Nitric oxide synthase
OTC	Ornithine transcarbamylase
PEG	Polyethylene glycol
PI3K	Phosphoinositide 3-kinase

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PTEN	Phosphatase and Tensin Homolog
RANK	Receptor activator of nuclear factor κ B
RANKL	Receptor activator of nuclear factor kappa-B ligand
rhArg 1	Recombinant human arginase 1
RNA	Ribonucleic acid
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
Th 1/17	T-helper cell 1/17
TRAP	TNF receptor-associated protein

Introduction

Arginine is a semi-essential, cationic amino acid that serves as a precursor for the synthesis of peptides, proteins, polyamines, nitric oxides, and urea, among other things. As a result, it plays a role in a variety of metabolic activities including ammonia detoxification, hormone secretion, and immunomodulation (Morris 2006; Stasyuk et al. 2017). The urea cycle enzymes argininosuccinate synthetase 1 (ASS1) and argininosuccinate lyase (ASL) catalyse conversion of citrulline to arginine, which is an intermediate product of urea cycle. Arginase 1 (Arg 1), ornithine transcarbamylase (OTC), and ASS1 (found in liver cells), on the other hand, catabolise arginine to ornithine and urea, citrulline, and argininosuccinate, respectively (Lam et al. 2018). A few arginine-hydrolysing enzymes are being developed as a therapeutic agent for a variety of diseases and conditions. Arginine deiminase (ADI) converts L-arginine to L-citrulline and ammonia; nitric oxide synthase (NOS) converts L-arginine to L-citrulline and nitric oxide (NO); and arginine decarboxylase converts L-arginine to agmatine and carbon dioxide (Morris 2006; Caldwell et al. 2015). However, arginase 1 has emerged as a most promising candidate, owing to a slew of efficacy and toxicology-related factors.

Human arginase (Arg)

Arg (EC 3.5.3.1) is a metalloenzyme that hydrolyses arginine to ornithine and urea (Fig. 1; Caldwell et al. 2015). It is a homotrimeric enzyme with a molecular weight of 105 kDa and each subunit contains a Mn^{2+} metal ion centre (Khangulov et al. 1998). Mn^{2+} plays a role in catalysis by forming a metal-bound hydroxyl ion (from a water molecule) that serves as a nucleophile, attacking the guanidium carbon of the substrate arginine (Stone et al. 2010; Romero et al. 2012). The two isoforms of the enzyme, Arg 1 and Arg 2, are found mostly in the cytosol of hepatocytes and the mitochondrial compartment of non-hepatic organs, respectively, in humans (Srivastava et al. 2013; Burrage et al. 2015). Both the isoforms have a 60% amino acid sequence similarity,

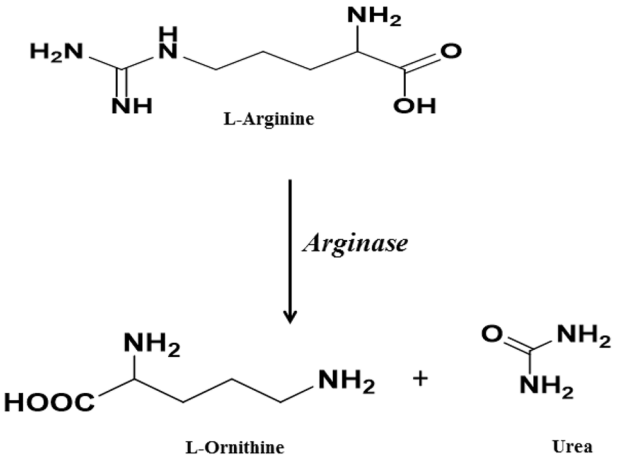

Fig. 1 Arginase catalysed breakdown of arginine

although they differ in gene location and function. Arginase 1 eliminates nitrogen via urea production, and its gene is found on chromosome 6. (6q.23; Ash 2004). Arginase 2, on the other hand, is primarily engaged in cellular development and proliferation, with its gene located on chromosome 14 (14q.24.1; Romero et al. 2012).

The deficiency of arginase 1 leads to a rare hyperargininaemia disorder, which is caused due to a point mutation (R291X) in its gene that results in a truncated protein. This leads to stunted growth and hyperammonia condition in humans (Lavulo et al. 2001). Several types of tumorigenic cells are auxotrophic to arginine due to the lower expression of OTC and/or ASS1 enzymes, and rely on extracellular arginine pool for their growth and survival. Similarly, numerous inflammatory and other disorders have been reported to arise from the imbalance of arginase expression and activity (Clemente et al. 2020). Thus, recombinant human arginase 1 (rhArg 1) is being developed for the treatment of a wide variety of pathophysiological conditions (Fig. 2).

Beneficial effects of recombinant human arginase 1 (rhArg 1) in various disorders/conditions

Arginase 1 deficiency

Arginase 1 deficiency is also known as hyperargininemia or argininemia. It is an autosomal recessive urea cycle disorder. It is a rare and progressive disorder resulting in excessive accumulation of arginine and other guanidine compounds in the blood. Loss of activity of arginase 1 protein due to mutation in ARG1 gene is a main reason for arginase 1 deficiency (Fig. 3; Terheggen et al. 1969; Böger 2007). The patients with arginase 1 deficiency essentially suffer from acute mental issues, neuro-cognitive deficiencies, and loss

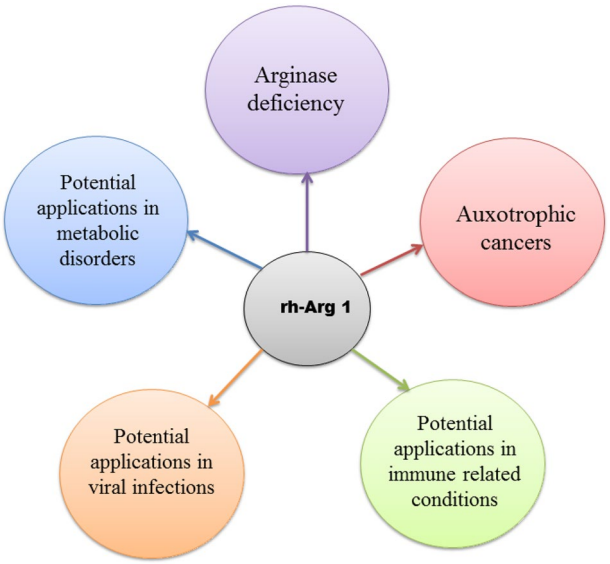

Fig. 2 Pathophysiological implications of rhArg1 (recombinant human Arginase 1)

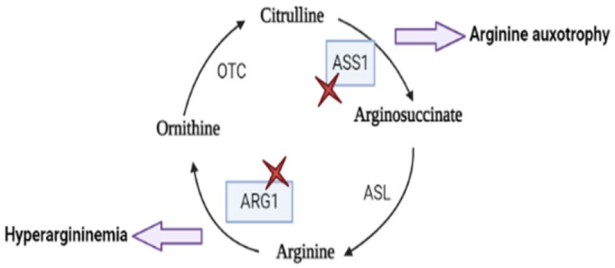

Fig. 3 Hyperargininemia and arginine auxotrophy. Hyperargininemia or Arginase 1 deficiency is a progressive disorder resulting in excessive accumulation of arginine due to mutation in arginase 1 gene (ARG1); whereas, deficiency in synthesis of arginine from citrulline due to silencing of argininosuccinate synthetase 1 (ASS1) gene leads to arginine auxotrophy. *ASL* argininosuccinate lyase, *OTC* ornithine transcarbamylase (Böger 2007; Srivastava et al. 2013)

of ambulation, despite treatment with nitrogen scavenger drugs (Crombez and Cederbaum 2005; Helman et al. 2014; Asrani et al. 2018). In this context, Aeglea Biotherapeutics, a biotechnology company developing next-generation human enzyme to mitigate conditions with unmet medical needs, has achieved US-FDA approval, granting 'Breakthrough Therapy Designation' to Pegzilarginase for the mitigation of arginase 1 deficiency (Rowilson et al. 2019). Pegzilarginase is a PEGylated, cobalt substituted rhArg1 enzyme (Co-rhARG1-PEG) with average molecular weight of ~ 284 kDa and is believed to be superior to native enzyme with increased half-life, stability and catalytic activity. The first open label clinical trial of pegzilarginase was conducted on 16 patients with arginase 1 deficiency and it was found that plasma arginine level of 50% patients was effectively reduced to normal range, with no or less hypersensitivity reaction (Rowilson et al. 2019; Diaz et al. 2021).

Arginine-auxotrophic cancers

Amino acids are known to govern essential cellular processes in both normal and cancer cells, in addition to being involved in the production of peptides and proteins (Andersen et al. 2014). Because of their fast growth and multiplication, cancer cells require more nutrients, particularly amino acids, than typical healthy cells. In addition, certain cancer cells lose the ability to synthesise one or more amino acids (Fernandes et al. 2017). As a result, cancer cells become reliant on exogenous amino acid supplies to maintain their development and metabolism, i.e. cancerous cells become 'auxotrophic' for such amino acids. Thus, amino acid auxotrophy is being explored to develop amino acid deprivation therapy in which the supply of amino acids is inhibited, and this leads to the death of cancer cells (Wang et al. 2021). The use of the L-asparaginase enzyme in the treatment of paediatric acute lymphoblastic leukaemia (ALL) introduced the rationale of amino acid depletion for cancer therapy (Pieters et al. 2011; Phillips et al. 2013). In this regard, it is discovered that more than 30 malignancies are arginine auxotrophic (Jahani et al. 2018). Deficiency in synthesis of arginine from citrulline due to silencing of ASS1 gene or otherwise is a common cause of arginine auxotrophy (Fig. 3; Srivastava et al. 2013). ASS1 may operate as a carcinogenic agent (Tsai et al. 2018) or tumour suppressor (Miyamoto et al. 2017) or as a pro-metastatic factor (Shan et al. 2015) in different carcinomas; hence, its function in tumour biology remains uncertain. It is reported that the direct transactivation of ASS1 by p53 in response to genotoxic stress is the basis for its tumour suppressor function. ASS1 functions as an intrinsic Akt (protein kinase B) repressor by inhibiting the phosphorylation of Akt that is driven by arginine deprivation, though the process by which Akt detects the arginine availability as well as the connection of ASS1 to p53, a major tumour suppressor, is yet to be elucidated (Fig. 4; Miyamoto et al. 2017).

Methylation induced transcriptional silencing has been known to mediate ASS1 loss in some cancer cases (Delage et al. 2012). These include hepatocellular carcinoma (HCC), squamous cell carcinoma, cervical carcinoma, ovarian carcinoma, breast carcinoma, prostate carcinoma, colon carcinoma, lung carcinoma, osteosarcoma, glioblastoma, pre-myelocytic leukaemia and lymphoblastic leukaemia, acute myeloid leukaemia (AML), malignant melanoma, Hodgkin's and non-Hodgkin's lymphoma (Jahani et al. 2018). However, mechanisms like repression of the ASS1 promoter by hypoxia-inducible factor-1 alpha also may play a role in this (Tsai et al. 2009; Datta et al.

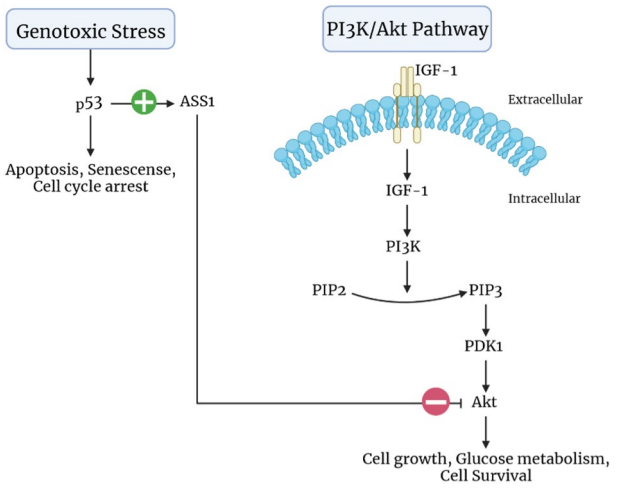

Fig. 4 p53-ASS1 pathway. Insulin-like growth factor-1 (IGF-1) induced phosphoinositide 3-kinase (PI3K) coverts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 is a lipid-derived second messenger that initiates PI3K signal relay by recruiting phosphatidylinositide-dependent protein kinase 1 (PDK1) and Akt (protein kinase B). Akt is a key regulator of several downstream signalling pathways involved in cell survival, proliferation, metabolism and so on. In response to genotoxic stress, p53 directly transactivates argininosuccinate synthetase 1 (ASS1), which act as an intrinsic repressor of Akt (Miyamoto et al. 2017)

2020). Utilisation of this auxotrophy, via the employment of extracellular arginine depleting agents like recombinant human arginase 1, ameliorates such cancerous conditions keeping the normal functional host cells unaffected to a considerable extent (Datta et al. 2020). Even though conventional therapy such as radiotherapy and chemotherapy are available in the market for treatment of numerous cancer types, these treatments fail to get rid of cancer completely and recurrence of cancer is most likely to be seen during the treatment. Thus, there is a dire need to have targeted and efficacious therapy or combination of therapies. Numerous formulations of arginase 1, such as BCT-100, pegzilarginase (AEB1102) and pegtomarginase (PT01) have successfully entered clinical trials while other forms of arginase 1 are under pre-clinical studies (Schaubber et al. 2015; Rowilson et al. 2019; Leung and Shum 2019; Yu et al. 2021). Cheng and Chen disclose a formulation comprising of PEGylated rhArg1 and chemotherapeutic/target therapeutic drug (oxaliplatin, everolimus, paclitaxel or sorafenib) for treating liver or prostate cancer. Also, they have used BCT-100 for the treatment of leukaemia in combination with doxorubicin (Cheng et al. 2014). Further, BCT-100 in combination with chemical drugs such as capecitabine and oxaloplatin, 5-flurouracil or autophagy inhibitors (chloroquine) have shown promising results in clinical trials (Datta et al. 2020).

Viral infection

Several steps in the viral lifecycle of SARS-CoV-2 have been reported to depend on arginine (Grimes et al. 2020). A low arginine environment goes on to affect the production of the nucleocapsid protein (containing 6.9% arginine residues), that plays a significant role in interacting with the negatively charged ribonucleic acid (RNA) strands, enabling efficient virion packing (McBride et al. 2014). In fact, arginine residues on the spike protein are crucial enough to stabilise interaction of the virus with the host cell angiotensin-converting enzyme 2 (ACE2) receptor to facilitate viral entry (Saha et al. 2020). In this regard, studies on other viruses, such as the ribonucleic acid (RNA) influenza virus and the vaccinia virus, also revealed that arginine is essential for both DNA (Deoxyribonucleic acid) synthesis and virion packaging processes of these viruses (Schierhorn et al. 2017; Grimes et al. 2020). Depletion of arginine by utilising pegzilarginase has been reported to inhibit SARS-CoV-2 replication in Vero cells (Grimes et al. 2020). Additionally, arginine deprivation via the employment of arginase 1 has also been found to attenuate pulmonary inflammation, one of the threatening consequences of COVID-19 (Xu et al. 2020). By limiting the availability of arginine for production of nitric oxide (NO) especially via inducible NOS, it might be possible to restrict the hyper-inflammatory response in COVID-19 infection to a considerable extent (Saha et al. 2020). However, such findings are subject to further internal and external grades of validation. In fact, arginine deprivation has been examined as a potential therapeutic strategy against viral infections at the in vitro level of studies, with more success in the context of herpesviridae and adenoviridae families (Becker et al. 1967; Inglis 1968; Sanchez et al. 2016). In herpes simplex virus (HSV)-1-infected cells, treatment with a PEGylated form of native arginase 1 was found to hinder viral replication and restrict production of viral progeny with minimisation of cell-to-cell transmission, thereby blocking the classical cytopathic effects of HSV-1 (Sanchez et al. 2016). It has been reported that the utilisation of PEGylated rhArg1 is effective in acyclovir (a first-line medication for HSV infection) resistant HSV-1 infection (Timothy et al. 2013). In support of the effectiveness and safety of therapeutic arginine depletion as an anti-viral therapy, a clinical trial employing ADI-PEG20 on patients with hepatitis C virus (HCV) serotype 1B showed a viral load decrease of more than 90% (Yang et al. 2010).

Immune-related conditions

Immune system is a highly developed, pervasive system which helps to maintain the integrity of host body by recognising the self and 'foreign' cells. Immune-related diseases are caused by either hypoactive immune system, in which it

fails to defend against the foreign antigens and gives rise to immune-deficient conditions, or hyperactive immune system, where it causes autoimmune disorders due to loss of ability to recognise self-cells (Chaplin et al. 2010). Schabbauer et al. have highlighted the use of PEGylated form of rhArg 1 in treating inflammatory autoimmune diseases (rheumatoid arthritis, multiple sclerosis), supresses the immune response by inhibiting T-cell polarisation, modulate cytokine (IL-6, IFN-γ) release, bone dysfunction (osteoporosis), and organ transplant rejection (Schabbauer et al. 2015). Attention on the role of arginase 1 in immune response increased enormously when it was reported that activated murine macrophages utilise L-arginine with the help of Th2 cytokine induced arginase 1 to form downstream arginine metabolites such as L-ornithine, polyamines and L-proline. These metabolites play a major role in cell proliferation, immune system as well as neuronal regeneration (Munder 2009; Martí and Reith 2021). L-arginine, upon hydrolysis by arginase 1, produces ornithine and urea. Ornithine has essential role in biosynthesis of polyamines. Arginase and ornithine decarboxylase enzymes are rate limiting for production of polyamines (Shosha et al. 2018). Further in vitro and in vivo studies showed that PI3K/PTEN-regulated extracellular arginase 1 in macrophage has regulatory role in immunity and inflammation (Cheng et al. 2008; Sahin et al. 2014). Based on this aspect, BCT-100 was used for immune system modulation studies in cultured cells and mouse models, which showed anti-inflammatory effect by reducing pro-inflammatory cytokines. Pre-incubation of dendritic cells with BCT-100 (30 µg/ml) showed reduced levels of T-helper cells (Th1 and Th17), which induce the secretion of pro-inflammatory cytokines interferon-γ (IFN-γ) and interleukines-17A (IL-17A). This shows the aptness of rhArg 1 to inhibit T-cell polarisation, thereby being an effective solution for T-cell-mediated autoimmune condition like acute disseminated encephalomyelitis (ADEM). Schabbauer et al. (2015) ADEM is an auto-immune condition resulting in inflammation of brain, spinal cord and demyelination of nerves of central nervous system (CNS; Garg 2003). In this context, administration of BCT-100 (10 mg/kg) in experimental autoimmune encephalomyelitis mouse model showed reduced level of IFN-γ and IL-17A cytokines, particularly in lymph nodes, which led to significant reduction in severity and disease progression (Schabbauer et al. 2015).

Multiple sclerosis is an autoimmune chronic disease related to central nervous system where immune cells attack nerve fibre and degrade myelin sheath in CNS, leading to neurodegeneration (Corraliza et al. 1995). This condition can be treated by reducing formation of NO and reactive oxygen species (ROS) which aids in reducing oxidative stress and inflammation (Lange et al. 2004). It was reported that loss of arginase 1 in myeloid cells worsens retinal ischaemia–reperfusion (IR) injury, and macrophages lacking arginase 1 have impaired inflammatory response (Fouda et al. 2018). IR of the retina causes cellular damage in a variety of ocular diseases, such as retinal vascular occlusions, glaucoma, as well as in diabetic retinopathy (Hartsock et al. 2016). Interestingly, administration of PEGylated rhArg 1 in macrophages isolated from mouse retinal IR injury model showed that recombinant human arginase 1 therapy lowered retinal IR injury and suppressed the inflammatory response of macrophages (Qualls et al. 2010; Fouda et al. 2018).

Osteoporosis is a condition with increased risk of fractures, which develops due to the reduction of bone density, that makes bones fragile (Lin and Lane 2004). Bone homeostasis is necessary for bone remodelling, which is accomplished by proper co-ordination between formation of bone by osteoblast and its resorption by osteoclast (Matsuoka et al. 2014). One of the major causes of osteoporosis is the imbalance of osteoclast–osteoblast interaction during chronic inflammation, that results in increased rate of osteoclast differentiation through receptor activator of nuclear factor kappa-B ligand (RANKL) signalling, which is produced by immune cells such as dendritic cells, neutrophils and T lymphocytes (Pietschmann et al. 2016). Bone marrow cells from mice were treated with RANKL and Macrophage colony-stimulating factor (M-CSF) were subjected to dose-dependent administration of BCT-100 ranging from 1 to 1000 ng/ml. It was found that a high dosage of recombinant arginase 1 can inhibits osteoclastogenesis and reduce the expression of RANK, TRAP and NFAT c1 genes which are necessary for osteoclast formation. This pre-clinical study using BCT-100 also demonstrated the therapeutic potential of recombinant arginase 1 in osteoporosis (Schabbauer et al. 2015). Rheumatoid arthritis is an autoimmune chronic inflammatory disease mainly affecting joints, bones, and causing pain, inflammation and swelling of affected organs. Collagen induced mice were used to see the effect of recombinant human arginase 1. It was observed that blood level of inflammatory cytokines such as IL-17A, ILP2/23 and IL-6 decreased drastically. These studies manifest the importance of arginase 1 in inflammatory conditions (Leung et al. 2010).

Metabolic disorders

Obesity is a disorder resulting from the accumulation of excessive amount of body fat, which leads to many metabolic disorders such as insulin resistance, dyslipidaemia, hypertension, type-2 diabetes mellitus, cardiovascular diseases and cancer which are considered as some of the major global health challenges of this century (Engin 2017). It was reported that intermittent fasting is an effective method against obesity and related metabolic disorders, as it can induce autophagic flux in cells. Autophagy is a catabolic process which gets activated due to amino acid starvation during intermittent fasting with the help of mechanistic

target of rapamycin (mTOR) complex 1, which senses amino acid availability in cells (Fig. 5; Goberdhan et al. 2016; Zachari et al. 2017). Hong Kong Polytechnic University disclosed an 'arginase 1-albumin binding domain fusion protein' (N-ABD094-rhArg) which is capable of mimicking intermittent fasting and inducing autophagic flux. Their studies showed anti-obesity effect of arginase 1-albumin binding domain fusion protein (600U) in C57BL/6J mouse strain. Within 6–7 weeks itself effective weight loss was observed and the animals showed improvement in insulin responsiveness as well as glucose tolerance, which highlighted its therapeutic potential in insulin-resistance diseases. They have also reported that fusion (cobalt substituted rhArg-N-ABD094-rhArg-Co^{2+}) and PEGylated form (PEG-His-rhArg) of arginase 1 showed reverse adiposity and reduction in fat mass in high fat diet (HFD) fed mouse model (Leung et al. 2021). Furthermore, it was observed that HFD induced obesity mouse model administered with N-ABD094-rhArg (500U) can reduce lipogenesis, thereby decreasing triglyceride content and lipotoxicity in organs such as liver, heart and pancreas. This demonstrates the possibility of using rhArg 1 for the treatment of steatosis, an abnormal accumulation of lipids inside the cells. The pre-clinical study in mouse model also showed that N-ABD094-rhArg can downregulate the expression of pro-inflammatory adipokine monocyte chemoattractant protein 1 (Mcp1) in white adipose tissues, which is responsible for obesity-induced inflammation (Leung et al. 2020). All these results indicate the significance of modified/fusion recombinant human arginase 1 as a therapeutic drug in obese-related disorders.

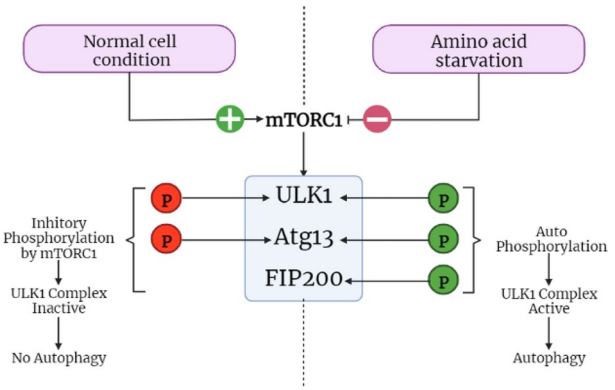

Fig. 5 Amino acid starvation-induced autophagy. Mammalian target of rapamycin complex 1 (mTORC1) phosphorylates unc-51-like autophagy activating kinase 1 (ULK1) and autophagy-related protein 13 (Atg13) and renders them inactive under normal cellular conditions with enough amino acids and nutrients. When mTORC1 is inhibited during amino acid deprivation, the inhibitory phosphorylation is alleviated by phosphatases, and the ULK1 complex becomes active through autophosphorylation of ULK1, a serine/threonine protein kinase, and other components including Atg13 and FIP200 (focal adhesion kinase family interacting protein of 200 kDa). This initiates the downstream signalling pathways for the autophagy process (Zachari et al. 2017)

Development of rhArg 1 for therapeutic use

Native hArg 1 has an optimal pH of 9.6, making it less effective at physiological pH. Furthermore, arginase 1 shows K_m value of 10.5 mM for arginine substrate, implying that a substantial amount of the enzyme is required to achieve the desired results. Also, arginase 1 has circulation half-life of ~ 30 min resulting in inferior pharmacokinetic and pharmacodynamic features. This aspect limits the applicability of the native form of arginase 1, necessitating the urgent development of modifications to overcome these constraints (Patil et al. 2016; Datta et al. 2020). Two of these modified enzymes are discussed below.

PEGylated rhArg 1

The Hong Kong-based Bio-cancer Treatment International Limited (BCT) has been working on the development of rhArg 1 for therapeutic usage (Hsueh et al. 2012; Cheng et al. 2014). BCT has announced that microbial expression systems, such as *Bacillus subtilis* and *Escherichia coli*, can be used to make recombinant human arginase 1. The rhArg 1 is linked to PEG-5000 using a succinamide propionic acid linker (BCT-100) to increase its circulatory half-life (Cheng et al. 2007). BCT-100 was shown to be functionally active, with a half-life of 3 days in vivo, and to be efficacious against a variety of arginine auxotrophic malignancies, including melanoma, prostate cancer, acute myeloid leukaemia, and HCC (Xiong et al. 2016). Apart from it, Aeglea BioTherapeutics at Austin in the United States has been developing rhArg 1 for therapeutic applications. They discovered that replacing two Mn^{2+} ions in the active region of the native enzyme with Co^{2+} results in a significant increase in the enzyme affinity for arginine (Georgiou and Stone 2014; Wang et al. 2016; Iyengar et al. 2019). At physiological pH, the combination of these effects is reported to result in a ten-fold increase in the enzyme's total catalytic activity. The addition of Co^{2+} enhances the serum stability of the enzyme, resulting in a 12–15 times reduction in the IC_{50} value for killing HCC and melanoma cell lines (Stone et al. 2010). US-FDA has approved the investigational new drug (IND) application of pegtomarginase, which is a site-specific linear PEGylated rhArg1, for the treatment of patients with advance stage carcinomas (Datta et al. 2020).

Regardless of the efficacy of PEGylation in the modification of therapeutic proteins, clinical use of PEGylated proteins is recognised to have a few numbers of drawbacks, prompting bio-therapeutic companies to find more cost-effective and safer ways for the modification of therapeutic

proteins. PEG toxicity, immunogenicity, and hypersensitivity to PEG are all aspects to consider, as are challenges emerging from the heterogeneity of PEGylated proteins and the cost of generating PEGylated proteins (Langenheim and Chen 2009; Fee and Alstine 2011; Li et al. 2013; Haeckel et al. 2016).

Fusion rhArg 1

The fused protein undergoes either neonatal fragment crystallizable receptor (FcRn)-mediated recycling and/or reduced glomerular clearance when rhArg 1 is genetically fused to the IgG Fc domain (Sockolosky et al. 2012). IgG1, IgG2, and IgG4 have been found to have nominal circulatory half-lives of ~ 480 h, whereas IgG3 has a half-life of ~ 144 h (Strohl 2015). It is reported that other properties of fusion proteins, such as solubility and stability are also improved by Fc fusion (Levin et al. 2015). Furthermore, anti-cancer activity of IgG1 Fc fused to rhArg 1 (rhArg 1-Fc), with a circulatory half-life of 4 days, has been investigated and found that it can be used as a therapeutic candidate for malignancies (Li et al. 2013). According to in vitro mutagenesis and related binding studies, it is observed that the conserved H166 residue of the human FcRn heavy chain, positioned opposite the FcRn-IgG contact site, plays a key role in the pH-dependent FcRn–albumin interaction (Zhao et al. 2013). A patent also describes engineering and characterisation of rhArg 1 albumin binding domain fusion protein, with a claimed circulatory half-life of 7 days (Leung et al. 2019). Using protein engineering approach, we have also engineered fusion variants of hArg1 in which the hArg1 enzyme is fused to a variety of half-life extension partners (HLEPs) via peptide linker. The lead engineered human arginase 1 (EHA) variant exhibited not only enhanced in vivo circulatory half-life but also desirable anti-cancer activity in a mouse xenograft model of hepatic cancer (data not published).

Conclusion

Arginine is a versatile amino acid that is essential to many physiological and pathological processes. Moreover, arginine plays a crucial hub role in the viral lifecycle of several viruses, including SARS-CoV-2, herpesviridae, and adenoviridae families. Arginine requirement in the body can be substantially increased during conditions such as rapid growth, stress or pathological circumstances. Thus, the import of arginine from the extracellular fluid is necessary for cells lacking the enzyme(s) necessary for its biosynthesis. Also, many malignant cells depend on the body pool of arginine for growth and maintenance because they lack or produce inadequate levels of the enzyme(s) required for arginine synthesis. Hence, depletion of arginine by administering arginine-hydrolysing enzyme has emerged as a powerful approach to selectively target and destroy such pathophysiological conditions. rhArg 1 is a potential candidate, not only for the treatment of a variety of cancers but also for a vast expanse of other pathological conditions including viral infections, multiple sclerosis, rheumatoid arthritis, autoimmune diseases, congenital hyperargininaemia, inflammation, obesity, and metabolic disorders and related complications and comorbidities (either alone or in combination with other drugs). However, issues concerning its pharmacokinetic profile do threaten to compromise its therapeutic functionality. The successful development of fusion variant(s) of rhArg 1 with increased circulatory half-life, therefore, is an important leap towards developing a safer and longer acting arginase 1 molecule for clinical use.

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Author contributions AJ and PSK contributed equally. AJ, PSK, SD and SSJ involved in conceptualization, data curation and writing the manuscript. UCB involved in reviewing and editing of the manuscript. AHP involved in conceptualization, visualisation, investigation, supervision, reviewing and editing. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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