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REVIEW

Zeta-crystallin: a moonlighting player in cancer

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Abstract

Crystallins were firstly found as structural proteins of the eye lens. To this family belong proteins, such as ζ-crystallin, expressed ubiquitously, and endowed with enzyme activity. ζ-crystallin is a moonlighting protein endowed with two main different functions: (1) mRNA binding with stabilizing activity; (2) NADPH:quinone oxidoreductase. ζ-crystallin has been clearly demonstrated to stabilize mRNAs encoding proteins involved in renal glutamine catabolism during metabolic acidosis resulting in ammoniagenesis and bicarbonate ion production that concur to compensate such condition. ζ-crystallin binds also mRNAs encoding for antiapoptotic proteins, such as Bcl-2 in leukemia cells. On the other hand, the physiological role of its enzymatic activity is still elusive. Gathering research evidences and data mined from public databases, we provide a framework where all the known ζ-crystallin properties are called into question, making it a hypothetical pivotal player in cancer, allowing cells to hijack or subjugate the acidity response mechanism to increase their ability to resist oxidative stress and apoptosis, while fueling their glutamine addicted metabolism.

Keywords RNA metabolism · Metabolic acidosis · Apoptosis · Cancer metabolism · Ammoniagenesis in cancer

Introduction

Crystallins are the main structural proteins in eye lens. They were discovered in 1894 by Morner [1] and are separated into two classes: taxon specific and ubiquitous. The former class is constituted by ζ (zeta), λ (lambda), ρ (rho), η (eta), τ (tau), μ (mu), ω (omega), ι (iota), υ (ypsilon), ε (epsilon) and δ (delta) crystallins, also called phylogenetically restricted crystallins, which are endowed with enzyme activity or that have lost such an activity [2]. The latter class is constituted by α (alpha), β (beta), and γ (gamma) crystallins, classified by decreasing molecular weight of native proteins. Due to their high similarity in protein sequence, topology and structure, β and γ crystallins are grouped in the superfamily βγ-crystallins. α and βγ-crystallins constitute the major structural proteins of vertebrate eye lens, contributing to maintain the transparency and refractive index. From a pathophysiologic point of view, according to their distribution, crystallins are often involved in cataract. Moreover, in recent years many crystallins have been identified also outside the lens, and characterization of their functions revealed roles in other pathologies (Table 1).

α-Crystallins form large protein complexes in the lens, constituted by the subunits referred to as αA- and αB-crystallins, which are encoded by *Cryaa* and *Cryab* genes. αA and αB-crystallins belong to the family of small heat-shock proteins, which are a group of molecular chaperone proteins that are ubiquitous in all organisms; their function is to prevent aggregation of other proteins under stress conditions. These proteins are characterized by a central region called α-crystallin domain. The main function of α-crystallins in the lens is to act as chaperone, protecting the lens against stress conditions, although subsequent studies suggest that they are also involved in cytoskeletal protection and apoptosis inhibition (by binding to pro-apoptotic Bcl-2 associated X (Bax) and Bcl-2 like 1 X_s (Bcl-x_s) proteins) [3, 4].

βγ-Crystallins are characterized by antiparallel β-sheet secondary structure. Due to the similarity of this motif to paintings on ancient Greek pottery, it is called "Greek key

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Table 1 Crystallins and related pathologies

Crystallins	Related pathologies	References
αA-crystallin	Cataract	[98]
	Diabetes	[99]
	Diabetic retinopathy	[100]
	Retinoblastoma	[101]
αB-crystallin	Cataract	[102]
	Desmin-related myopathy	[103]
	Multiple sclerosis	[104]
	Dilated cardiomyopathy	[105]
	Alexander's disease	[106]
	Parkinson's disease	[107]
	Alzheimer's disease	[108]
	Creutzfeldt–Jakob disease	[109]
	Solid tumors	[110]
	Infantile myofibrillar myopathy	[111]
	Huntington's disease	[107]
	Skeletal muscle myopathy	[108]
	Tauopathies	[112]
	Down syndrome	[113]
	Renal cell carcinoma	[114]
	Ischemia	[108]
	Pulmonary fibrosis	[115]
	Diabetes	[116]
	Inflammatory disorders	[117]
	Retinoblastoma	[101]
	Breast carcinoma	[118]
	Glioblastoma	[101]
	Diabetic retinopathy	[119]
	Glaucoma	[120]
	Osteosarcoma	[121]
	Colorectal cancer	[122]
β-Crystallin	Cataract	[123]
	Glaucoma	[123]
	Schizophrenia	[124]
γ-Crystallin	Cataract	[125]
	Auditory hindbrain	[126]
μ-Crystallin	Hearing loss	[5]
	Facioscapulohumeral muscular dystrophy	[127]
	Amyotrophic lateral sclerosis	[128]
	Obesity	[129]
ζ-Crystallin	Cataract	[8]
	Leukemia	[16]

motif", and it recurred four times in all the members of βγ-crystallins [3]. These motifs allow a dense packaging of the proteins to minimize light scattering, guaranteeing optimal transparency to the lens.

The class of enzyme of phylogenetically restricted crystallins comprises μ-crystallin and ζ-crystallin. These crystallins are variably expressed inside and outside the lens and are related to different pathologies comprising cataract, hearing loss, and muscular dystrophy according to their distribution (Table 1). μ-Crystallin is a structural protein in the lens, but is also endowed with enzymatic activity. According to Oshima et al., μ-crystallin may be involved in the potassium ion recycling system together with Na,K-ATPase being responsible for deafness when mutated [5].

ζ-Crystallin (CryZ) was originally found in the lens of guinea pig, where it constitutes about 10% of the total soluble lens proteins [6]. Genes encoding CryZ homologs are distributed from bacteria to higher plants and animals. Contrary to guinea pigs, in mouse and human lens CryZ is not expressed at high level [3, 7], but rather it can be found at enzymatic levels practically ubiquitously in the various organs and tissues. In Fig. 1, *CryZ* mRNA expression levels can be seen in different human tissues outside the lenses. The only mutation in *CryZ* gene functionally characterized to date, resulting in a shortening of the protein, leads to an autosomal-dominant cataract in the 13N mutant line of guinea pigs (Table 1) [8]. Like many crystallins, CryZ is a moonlighting protein, i.e., a protein performing very different functions with often cryptic or no relationships. A prototypic moonlighting protein is cytochrome C, involved in energy metabolism and apoptosis effector phase, respectively [9, 10]. CryZ is endowed with enzyme and nucleic acid-binding activities. With respect to its enzyme activity, CryZ protein is structurally related to alcohol dehydrogenase with no such activity [11], but with NADPH:quinone oxidoreductase (QOR) activity, the relevance of which for the cells is still to be elucidated [12]. However, CryZ protein was firstly discovered for its ability to bind DNA in cell-free settings [13, 14]. Unfortunately, after the appearance in the literature of some evidences suggesting the possibility that CryZ could be involved in transcriptional regulation, no in vitro/in vivo data have been ever provided. Later, Curthoys' group provided strong evidence that CryZ is an mRNA-binding protein. In a renal cell model, CryZ stabilizes rat *glutaminase* (*GLS*) mRNA [15] in the context of an adaptive response of the cell to acidosis. Independently, our group identified CryZ as a *Bcl-2*- and *Bcl-x*$_L$-binding protein [16, 17] involved in leukemia cell resistance to apoptosis. The group of Bazzi, looking for CryZ inhibitors, identified analgesics such as paracetamol, ibuprofen, acetylsalicylic acid (ASA) and salicylic acid (SA) as inhibitors of the enzymatic function of this protein [18, 19], and our group demonstrated that ASA and SA can also inhibit CryZ-mRNA binding [17]. Such findings suggest mechanisms by which these drugs are considered for anticancer therapy [20]. The implications in cancer of the many functions of CryZ are discussed.

ζ-Crystallin as a nucleic acid-binding protein

ζ-Crystallin as a DNA-binding protein

CryZ has been first discovered as a bovine DNA-binding protein by DNA–cellulose affinity chromatography [21]. Subsequently, in an effort to characterize the promoter region of the gene *alcohol oxidase-1* (*AOX1*) of *Pichia*

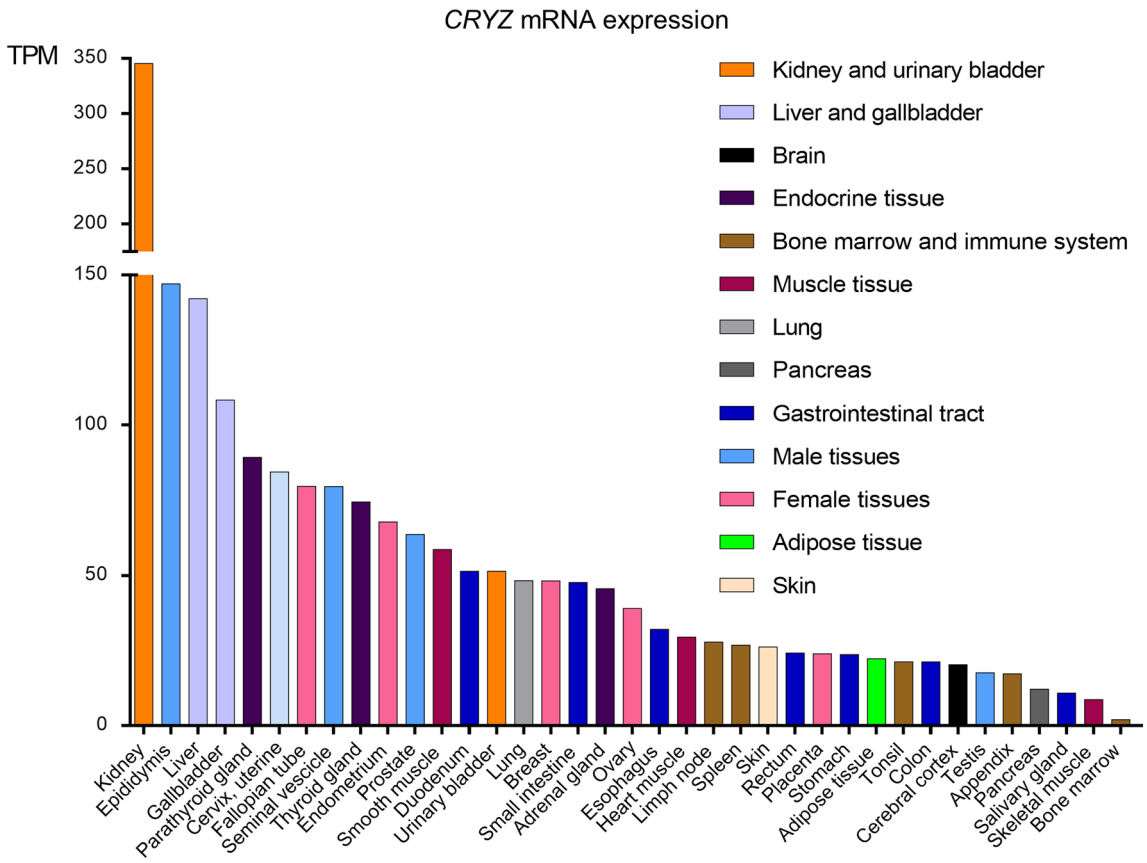

Fig. 1 Overview of CryZ expression at RNA level in various human tissues [96]. Color coding is based on tissue groups, each consisting of tissues with functional features in common. Data are relative to *CryZ* mRNA expression levels in different human normal tissues and arise from Human Protein Atlas (HPA) dataset [97]. Data are reported as mean transcript per million (TPM), corresponding to mean values of the different individual samples from each tissue. Specimens were collected with the consent from patients and all samples were anonymized in accordance with approval from the local ethics committee (ref #2011/473) and Swedish rules and legislation. All tissues (for a total of 172 tissue samples) were collected from the Uppsala Biobank and RNA samples were extracted from frozen tissue sections

pastoris, CryZ, was shown to be a single-stranded (ss)-DNA-binding protein whose bond can be reversed by its enzyme cofactor NADPH [13]. It has been demonstrated that in cell-free transcription experiments, CryZ increased six- to tenfold the transcription rate of the *αA2-crystallin* gene [22]. Furthermore, bovine CryZ was seen to strongly bind to double-stranded (ds)-Z-DNA and ss-DNA, and to a lesser extent to ds-B-DNA. Bovine CryZ was also demonstrated to possess DNA unwinding/protecting and transactivating properties [13]. These observations led to the hypothesis that CryZ can act as a transcriptional enhancer for lens crystallin and non-crystallin genes [14], albeit no in vitro/in vivo evidences have been provided so far. The DNA-binding ability of CryZ will be discussed later, in the light of its other better characterized functions.

ζ-Crystallin as a RNA-binding protein

ζ-Crystallin in glutaminolysis

Glutaminolysis represents a series of biochemical reactions catabolizing glutamine to downstream metabolites, such as glutamate and α-ketoglutarate, in which two key enzymes such as GLS and glutamate dehydrogenase (GDH) are involved [23]. Acidosis is an acid–base disorder due to different mechanisms resulting in acidemia. In particular, the acute onset of metabolic acidosis triggers a reprogramming in the glutamine metabolism involving a variation of inter-organ flux of this amino acid. Indeed, liver switches from a glutamine utilizing configuration to a producing one and more glutamine is provided by the liver and the muscle in the systemic circulation. At the same time, kidneys increase

glutamine extraction from blood and glutamine reabsorption from filtrate, thus leading to an increased glutamine availability for renal glutaminolysis. In the chronic phase of metabolic acidosis, this adaptive response is partially compensated with a concomitant increased expression of genes involved in glutamine catabolism [24], such as *GLS* and *GDH*. Bicarbonate generated by activated glutaminolysis in the proximal tubular cells is transported into the blood to buffer pH, while NH3, $NH4^+$ and H^+ are secreted in the proximal tubular lumen. In the thick ascending limb (TAL) of Henle loop, $NH4^+$ is then reabsorbed mainly into the peritubular space. This mechanism of secretion/reabsorption generates an interstitial axial ammonium gradient, increasing from the cortex to the papillary end, which allows intercalated cells of the inner medullary collecting duct (IMCD) to transport NH3 and $NH4^+$ from the interstitial space to the lumen. Maintaining this gradient involves induction of a number of transporters, such as BSC1/NKCC2 (mainly responsible for ammonium reabsorption from TAL to interstice), molecules such as sulfatides [25] and enzymes such as GLS and GDH [26, 27]. In this context, Curthoys' group characterized a novel mechanism of post-transcriptional regulation of genes involved in glutaminolysis during chronic metabolic acidosis in a proximal renal cell line [28]. The authors identified CryZ as a *GLS* mRNA-stabilizing protein able to bind a pH response element (pH-RE). pH-RE belongs to the adenine-uracil-rich element (ARE) family and is sufficient to impart a pH-responsive stabilization to mRNA that harbor it [15]. In addition, Schroeder et al. found that CryZ also stabilizes rat *GDH* mRNA by binding to peculiar motifs in its 3′-untranslated region (UTR) that are homologous to the *GLS* mRNA pH-RE [29]. Moreover, Fernandez et al. reported the strength and the specificity of human CryZ binding to the ARE consensus sequence $A(UUA)_{5–6}$, demonstrating that this protein can be classified as an ARE-binding protein (AUBP) [30]. In a physiological acid–base balance, the pH-RE in the 3′-UTR of the *GLS* mRNA recruits an isoform of the AU-rich element rna-binding protein 1 (AUF-1), which has been hypothesized to promote the observed *GLS* mRNA de-adenylation and thus degradation; instead, the presence of metabolic acidosis causes an increase of the binding affinity of CryZ for the pH-RE, which, in turn, confers increased protection to the *GLS* mRNA from deadenylation and results in an increased stabilization of the *GLS* mRNA [31]. This mechanism of *GLS* post-transcriptional regulation has been regarded as a "paradigm for determining the mechanism by which mRNAs are stabilized in response to metabolic acidosis" [28]. In support of the paradigm, CryZ mediates also the acid pH-induced increase of $Na^+/K^+/2Cl^-$ cotransporter BSC1 by increasing its mRNA stability [32], thus contributing to reabsorbing ammonium from the lumen into thick ascending limb cells of the loop of Henle [33]. In addition, it has been shown that CryZ expression levels increase following pH lowering in the same cells [32].

The enhanced binding of CryZ to its mRNA targets, including the *GLS* and *GDH* mRNAs, may be mediated by its phosphorylation status. CryZ is a phosphoprotein [34] whose activity could be regulated by a kinase of the p38 mitogen-activated protein kinase (MAPK) signaling pathway [35]. Recently, the role of MAPK-activated protein kinase 2 (MAPKAPK2 or MK2), a downstream substrate of p38 MAPK, has been highlighted as a master regulator of RNA-binding proteins [36]. Indeed, a decrease in intracellular pH leads to p38 MAPK activation in renal cells, which, in turn, determines the activation of ATF2 transcription factor inducing transcription of specific genes involved in adaptation to metabolic acidosis [35]. On the whole, this hypothetic pathway provides a mechanistic explanation about the molecular events that allow renal cells to sense changes in pH and mediate a response leading to increased transcription of specific genes and the selective stabilization of certain mRNAs.

An increasing number of studies are highlighting the pivotal role of glutamine metabolism in cancer onset and progression [37]. Glutamine is a major anaplerotic substrate promoting cancer cell growth. Cancer cells are heavily sensitive to glutamine deprivation, while glutamine addiction results in enhanced production of by-products necessary for proliferation and drug resistance [38]. Chronic acidification of microenvironment and hypoxia are hallmarks of neoplasia [39]. The release of lactate/H^+ by hypoxic cells, the hydration of CO_2 to bicarbonate plus H^+, and the large amount of lactate/H^+ released by tumor cells exhibiting an intense aerobic glycolysis contribute to the acidification of the tumor extracellular microenvironment, with pH values as low as 6.4. It has been reported that extracellular pH of different tumor tissues is 0.3–0.7 pH units lower than that of the corresponding normal tissues [40–42]. Remarkably, acidification of the microenvironment occurs early in cancer, during the avascular phase of progression [43]. This causes cancer cell evolution versus phenotypes with increased resistance to acid-induced toxicity, which accelerates malignant progression and adaptation to therapeutic strategies [39, 44]. Short and long exposure of cancer cells to acidosis induces a metabolic reprogramming toward glutaminolysis, as the main fulfillment of their cellular bioenergetics [38, 45]. In addition to the anaplerotic role of glutamine, its metabolism leads to ammonia production, not only in renal cells, but also in cancer cells where it has been proposed to increase protective autophagy in a paracrine way by diffusing to the more stressed cells in the nutrient-poor areas of the tumor microenvironment [46, 47]. Also, Spinelli et al. [48] provided a robust demonstration that ammonia can be recycled by breast tumor cells to produce amino acids in a sort of nitrogen fixation reminiscent of that in plants, bacteria and yeasts [49]. More recently, it has been demonstrated that

even cancer-associated fibroblasts (CAFs) participate in extracellular ammonia clearance and to ammonia-dependent cancer biomass increment through the conversion of inorganic ammonia derived from cancer cell metabolism into several secreted amino acids (including glutamine and glutamate) [37, 50]. To date, a mechanistic link between acidification of cancer microenvironment and induction of glutaminolysis has not been clearly demonstrated. Considering the impact of CryZ on renal glutamine catabolism, it can be supposed that among the deregulated mechanisms leading to glutaminolysis induction in cancer cells, a CryZ-dependent mechanism could be enrolled by cancer cells, contributing to upregulating the levels of enzymes (such as GLS and GDH) critically contributing to this induction. As an evidence supporting this hypothesis, we revealed the induction of the expression of CryZ in different cancer cell lines following medium acidification [17].

ζ-Crystallin in apoptosis

In addition to its role in glutaminolysis, as *GLS* and *GDH* post-transcriptional regulator, CryZ plays a key role also in apoptosis by the regulation of antiapoptotic factors such as Bcl-2. Our group firstly explained the relationship between CryZ and Bcl-2, confirming the hypothesis that CryZ controls the expression of *Bcl-2* at the post-transcriptional level, thus affecting the stability of its mRNA [16]. We demonstrated that an ARE in the 3′-UTR of *Bcl-2* mRNA modulated *Bcl-2* expression in physiological conditions by interacting with specific AUBPs [51, 52]. One of the *Bcl-2* AUBPs was CryZ, which is responsible, following overproduction and overactivation in leukemia cells, for a greater stability of the *Bcl-2* mRNA [16]. A link between the apoptosis-related *tp53* gene and *CryZ* has been also demonstrated. p53 is an important oncosuppressor protein that transregulates different apoptosis-related genes following DNA damage sensing [53]. Other than to transactivate genes of the *Bcl-2* family, three mechanisms have been identified through which p53 performs a repressive function: competition with transcription activators for DNA binding; sequestration of transcription activators or recruitment of corepressor/chromatin-modifying factors. Relative to this latter mechanism, p53 represses target genes via recruitment of chromatin remodeling of the Sin3 transcription regulator/histone deacetylase (HDAC) 1/2 complex. Sin3 proteins (Sin3A and Sin3B) regulate gene expression at chromatin level by serving as an anchor onto which the core Sin3/HDAC complex is assembled [54]. The theme of the work of the group of Bansal was to establish the role of Sin3B in p53-mediated repression [55]; they demonstrate a direct protein–protein interaction between human N-terminal region of p53 and the amino acids 1-199 of Sin3B. Genotoxic stress induced by adriamycin led to increase of Sin3B levels that is recruited to the promoters of p53-target genes. *CryZ* is one of the p53 repressed genes at transcriptional level. The observed transrepression by an important pro-apoptotic gene, together with the mRNA stabilization of the antiapoptotic Bcl-2 protein [16], suggests a strong, direct, and positive impact of CryZ on cell survival.

ζ-Crystallin as an enzyme

As previously reported, CryZ retains a role as a NADPH:QOR [56]. This ability it is not fully understood. As suggested by Malik et al. [57], this activity can be regarded as a part of a detoxification mechanism. The enzyme may bind to NADPH to preserve the reducing environment in the lens and protect it from oxidative damage [58]; alternatively, the oxidoreductase activity may function as a defense against quinones and different oxidizing agents [59]. Rao's group examined various quinones demonstrating that CryZ showed enzymatic activity on orthoquinones, such as 1,2-naphthoquinone and 9,10-phenanthrenequinone, while non-quinoidal compounds, such as naloxone and nitrofurans, were not utilized as substrates [12]. The authors concluded that quinones are the only biological compounds identified as substrates of CryZ. In recent years, a new protective role exerted by CryZ against lipid peroxidation products has been described in humans [60]. CryZ homologs from plants have also been shown to be induced by oxidative stress [61]. A possible suggestion about the role of CryZ as enzyme in cancer cells comes from NAD(P)H quinone dehydrogenase 1 (NQO1), another QOR, which is upregulated in many cancers [62] and which is also endowed with mRNA-binding ability [63]. NQO1 activity is strictly related to cholangiocarcinoma cell migration, proliferation [64] and chemoresistance [65]. Other than in cholangiocarcinoma, NQO1 is frequently upregulated in human liver cancer, and its expression correlates with tumor grade and low survival rate [62]. *CryZ* mRNA expression levels in 17 different cancer types retrieved from The Cancer Genome Atlas (TCGA) database are shown in Fig. 2 [66, 67]. Among the different cancer types present in the TGCA database, *CryZ* is significantly overexpressed in lymphoid neoplasm diffuse large B cell lymphoma, testicular germ cell tumor, thymoma and glioblastoma multiforme with respect to normal tissues [68, 69]. Very interestingly, higher expression of *CryZ* in astrocytoma, the most common gliomas, strongly correlates with a worse prognosis (in term of overall survival) [68].

Despite that CryZ and NQO1 use a different mechanism of catalysis (1 versus 2 electrons, respectively), it can be inferred that CryZ could provide protection to cancer cells against oxidative stress to maintain the optimal DNA complement [70]. In fact, despite cancer being highly aneuploid and mutated, once established it still needs protection against oxidative stress [71], probably with other endogenous factors

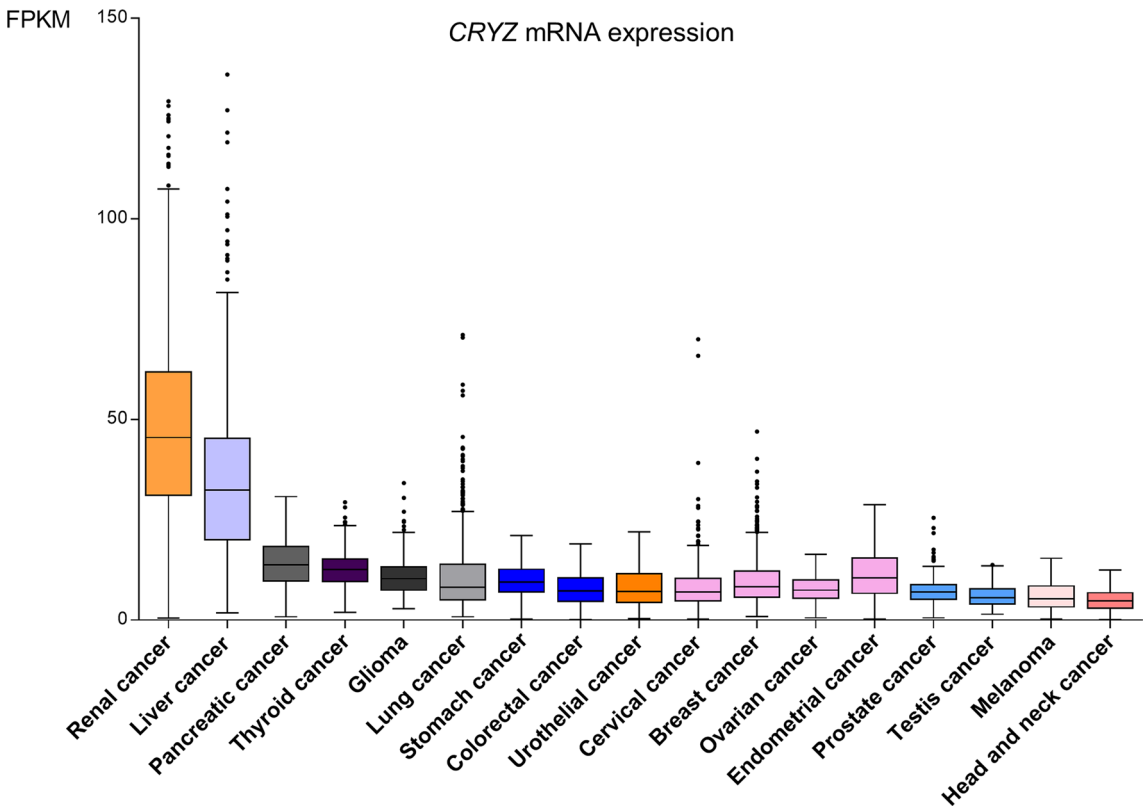

Fig. 2 Overview of *CryZ* mRNA expression in 17 human cancer types. Color coding is based on the normal organ (the same as in Fig. 1) the cancer originates from. Data arising from RNA-seq analyses are reported as median FPKM (number of Fragments Per Kilobases of exon per Million reads) and are generated by The Cancer Genome Atlas (TCGA) [97]. Normal distribution across each dataset is visualized with box and whiskers plots: box is shown as median and 25th and 75th percentiles; whiskers are plotted following calculation by Tukey method

such as aneuploidy [72], anastasis [73], chromothripsis [74] and neosis [75] devoted to drive the higher plasticity of cancer cell with respect to the normal somatic cell. This putative important function of CryZ in cancer helps to explain why there is only a report of *CryZ* mutation that is related to cataractogenesis in Guinea pig implicating its enzymatic activity and/or structural changes [8].

Emerging perspectives

Curthoys' group demonstrated that CryZ is involved in the fate of at least one mRNA (*GLS*) either in physiological or pathological (acidosis) conditions. They hypothesized that the machinery for *GLS* mRNA degradation/stabilization involves also AUF1 and ELAV-like RNA-binding protein 1 (HuR), two well-characterized proteins involved in mRNA metabolism [28]. Many authors outlined the possibility that instead of specific AUBPs, each specifically targeted to a particular mRNA, a pool of common AUBPs orchestrates the turnover of a set of mRNAs, pretty as much as a transcriptional operon does [76]. Our group also collected a number of evidences that CryZ, AUF1 or HuR is able to bind the ARE of *Bcl-2* mRNA affecting its half-life [16, 77, 78]. It is therefore highly probable that cancer cells hijack the acidity responsive mechanism to increase their ability to resist oxidative stress and apoptosis while fueling their glutamine-addicted metabolism. It can be speculated that these results could be realized in different ways: (1) through qualitative mutations in *CryZ* or genes involved in CryZ pathways, favoring the stabilizing activity of the protein; (2) increasing the level of CryZ protein as an effect of mutation or aneuploidy; (3) increasing the level of CryZ as an effect of simple adaptation to environmental conditions, such as acidity; (4) increasing the nucleic acid binding and/or enzyme activity of CryZ as an effect of adaptation to environmental conditions. Until now, the only report of *CryZ* mutation has been related to protein inactivation and cataract [8], while the only report of constitutive CryZ upregulation has been represented by our previous paper about leukemia cells [16]. Also, mutations in downstream transacting elements of CryZ such as CryZ-binding sequences on mRNAs have been rarely reported. In particular, mutations in the UTR of mRNAs causing diseases are very rare, especially those

in the 3′-UTR [79]. Thus, we speculate that the adaptive mechanisms are the most relevant for cancer to thrive.

In the light of the different physiological roles of CryZ, a mechanism where the protein is pivotal in sustaining cancer metabolism and survival can be hypothesized (summarized in Fig. 3). On one hand, CryZ can fuel cancer requirement for energy by glutaminolysis activation; from the other hand, its increased expression and/or activation following acidification of the tumor microenvironment can contribute to cancer cell survival and drug resistance by increasing antiapoptotic gene expression, especially in those cells in which *tp53* is mutated and could not contribute to a CryZ transrepression. Furthermore, its putative detoxifying enzyme role could contribute to protect cancer cells from excess quinones-derived oxidative stress. We speculate that CryZ-promoted glutaminolysis, activated in renal cells following blood acidosis, can be a mechanism opportunistically exploited by cancer cells to provide fuels for its OXPHOS energetic metabolism. Microenvironment acidification, promoted by the Warburg effect, has been demonstrated to be a special hallmark of moderately hypoxic cancer microenvironment [80]. In such conditions, outer low pH could be sensed by tumor cells and translated into CryZ upregulation and/or overactivation that in turn contributes to replenish TCA cycle with glutamine derivatives, and to detoxification activity, respectively. As a by-product of glutaminolysis, ammonia can provide substrate for cancer biomass and help protect cells from stressing conditions by triggering autophagy [46–50]. At the same time, CryZ upregulation/overactivation could contribute to stabilize ARE-containing mRNAs encoding pro-survival factors such as Bcl-2 and other antiapoptotic Bcl-2 family members. The increase in these factors could account for a general increase in apoptotic threshold paralleled by drug resistance, placing CryZ at a pivotal position in cancer survival and progression. Lastly, CryZ was first discovered as a DNA-binding protein, whose binding to DNA is competed by NADPH [13, 56]. It is not surprising that RNA-binding proteins are also endowed with DNA-binding ability in that many examples are reported in the literature [81]. An estimate of the DNA/RNA-binding proteins (DRBPs) is an impressive 2% of the human proteome [81]. This double activity can accomplish many purposes in different ways. In T-cell activation, the nuclear factor 90 (NF90) and its partner NF45 regulate transcription, mRNA turnover, translation and miRNA processing by binding DNA, mRNA and miRNA, respectively [82–84]. In most cases, the ability to bind DNA and RNA are functionally related but can be accomplished in same or different spatio/temporal frames. In some cases, simultaneous DNA/RNA-binding serves the need to gather regulative DNA sequences to long noncoding RNAs (lncRNAs), affecting

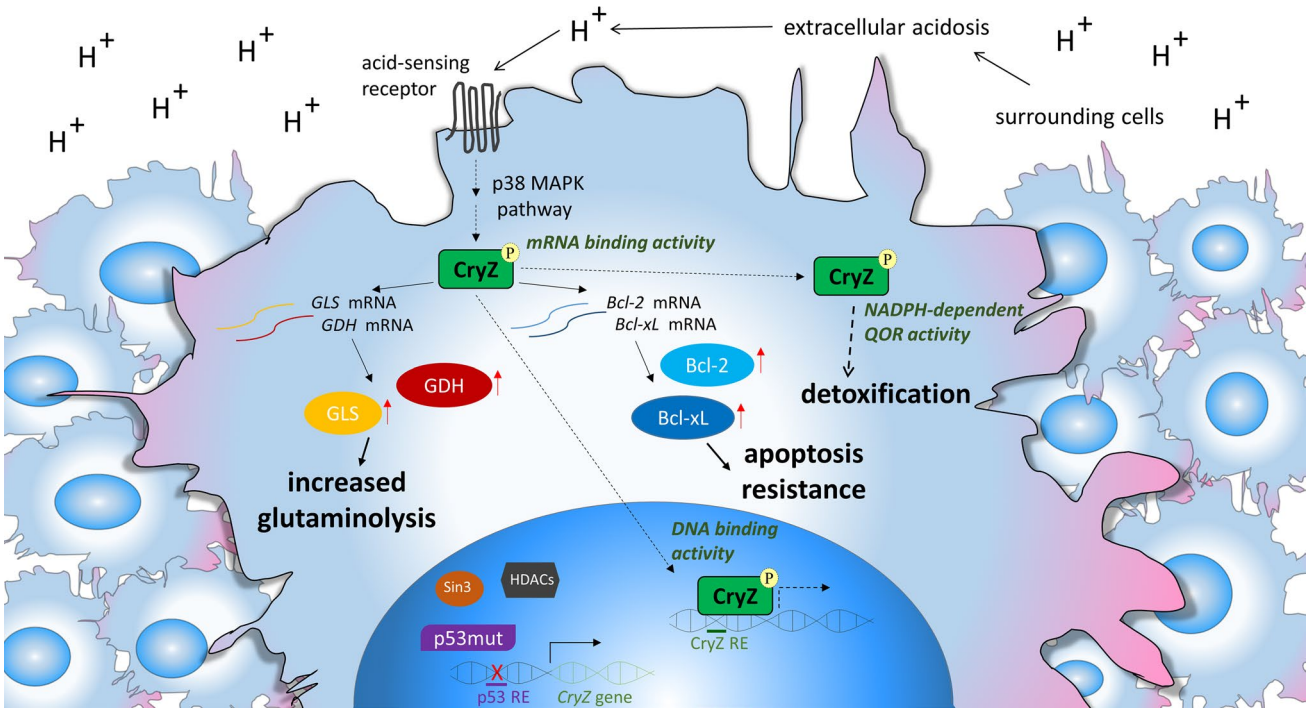

Fig. 3 The proposed CryZ integrated circuitry in cancer cells. Extracellular acidosis is a hallmark of cancer metabolism. Extracellular pH can be sensed by specific membrane receptors. The receptor-coupled intracellular signaling is translated into CryZ activity modifications impacting on apoptosis resistance and glutaminolysis increase. Gene transactivation and quinone detoxification could be also triggered. Inactivating mutations of p53 avoids *CryZ* gene transrepression, thus possibly strengthening CryZ activities by protein accumulation

gene expression [85]. For example, transcriptional modulation of nuclear receptors such as steroidogenic factor 1 (SF1) is realized by simultaneous binding of downstream gene promoters and the lncRNA co-activator *steroid receptor RNA activator* (*SRA*) [86]. Also, simultaneous DNA/RNA binding is required in RNA-guided DNA methylation as an example of epigenetic regulation driven by DRBPs. In other cases, DNA/RNA binding is not simultaneous but competitive, as for a transcription factor (e.g., the glucocorticoid receptor, or GCR) which DNA-binding ability is prevented by binding to a decoy lncRNA [81]. However, the present knowledge about CryZ in this respect is too scarce and does not allow to address the in vivo functions related to its putative DNA-binding activity. To date, protein localization studies revealed that CryZ mainly localizes in the cytoplasm, where it accomplishes the control of RNA turnover. Further studies in different cell types and pathophysiological conditions (including cancer) are needed to unveil its putative nuclear localization and a clear role in DNA-binding-mediated gene expression control.

Whether CryZ actually represents a crossroad between apoptosis and cancer metabolism, the development of CryZ inhibitors could be regarded as a potent tool that interferes with cancer growth, interfering with the specific attitude of cancer cells to live in an acidity adapted status. Bazzi's group identified aspirin-like analgesic as potent inhibitors of CryZ enzymatic activity [18, 19]. Epidemiological studies in man have shown that these analgesics protect against or delay various types of cataracts [87], which may be caused by mutations in CryZ. Bazzi et al. evaluated the inhibition of CryZ enzymatic activity by means of the various analgesics tested and noted that the inhibition was almost instantaneous after the addition of the inhibitor, was time-dependent, and they observed that aspirin was the most potent uncompetitive (with respect to NADPH) inhibitor; ibuprofen was less effective than aspirin; and paracetamol was the least effective as it only marginally inhibited CryZ [18, 19]. At the same time, the binding of CryZ to target mRNAs has been shown to be competitively prevented by NADPH, suggesting that the cofactor-binding site is involved in RNA binding [56]. We also demonstrated, prompted by the above evidences, that aspirin and salicylic acid are able to prevent CryZ binding to *Bcl-2* and *Bcl-x*$_L$ mRNAs in melanoma cells [17]. Several epidemiologic studies showed that aspirin exerts a chemopreventive effect and decreases the incidence and/or mortality or could be a candidate for anticancer therapy of melanoma, prostate, hepatocellular carcinoma, glioblastoma, colorectal cancer, and breast cancer [88–93]. Although aspirin is a well-known cyclooxygenase-2 (COX-2) inhibitor, the anticancer molecular mechanisms are still elusive. Indeed, the wide range of antiproliferative effects of aspirin in tumor cells do not correlate exclusively with its COX-2 inhibitory activity and deserve further investigation.

Conclusion

Similarly to the much more studied hypoxia response, further research will be necessary to elucidate the acidity response mechanism, either in cancer or normal cells, but it is now clear that it plays a main role in cancer thriving [80]. In the light of the reported evidences, CryZ could represent a highly hierarchical player in this response, probably initiated by membrane H^+ sensing receptors, such as acid-sensing ion channels (ASICs) or proton-sensing G-protein-coupled receptors (GPCRs) [94, 95]. Based on the competitive effect of NADPH on CryZ bond to nucleic acids, we are tempted to speculate, as have been already done by others for the NQO1 enzyme [63], that CryZ could behave as a sensor of the redox state of the intracellular environment, able to translate an increase in $NADP^+$/NADPH ratio into a tighter binding to nucleic acids and a consequent upregulation of a number of genes both at transcriptional (as suggested by a few weak biochemical experimental data) and post-transcriptional level (as strongly supported by in vitro/in vivo experimental data), respectively.

It can be easily imagined that cancer cells living in an acidic microenvironment are more sensitive to a CryZ pathway-targeted therapy with respect to normal cells, which only occasionally or in specialized functions such as the reported renal ones are challenged by low pH.

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