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**The emerging role of cytidine deaminase in human diseases: a new opportunity for
therapy?**

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ABSTRACT

The recycling activity of cytidine deaminase within the pyrimidine salvage pathway is essential to DNA and RNA synthesis. As such, CDA deficiency can lead to replicative stress, notably in Bloom syndrome. On the other hand, CDA also can deaminate cytidine and deoxycytidine analogue-based therapies, such as gemcitabine. Thus, CDA over-expression is often associated with lower systemic, chemotherapy-related, adverse effects but also with resistance to treatment. Considering the increasing interest of CDA in cancer chemoresistance, the aims of this review are to describe CDA structure, regulation of expression and activity, and to report the therapeutic strategies based on CDA expression that recently emerged for tumor treatment.

INTRODUCTION

Cytidine deaminase (CDA) is a ubiquitous enzyme, whose major role is to participate in the recycling of free pyrimidines. The pyrimidine salvage pathway appears to have two different aims: one is the recycling of pyrimidines for the synthesis of other nucleotides that will be integrated into DNA and RNA, the other is the degradation of pyrimidines to ensure a constant source of carbon and nitrogen to the cell. Interestingly, CDA has a pivotal role in both pathways. Over the past decades, cancer researchers also took interest in this enzyme for its additional role on chemoresistance. Accordingly, this review aims to gather known data on CDA, from structure to activity regulation, and the first evidence of CDA relevance to cancer treatment.

CDA GENE ORGANIZATION

CDA (EC 3.5.4.5) can also be referred to as cytidine aminohydrolase, CD or CDD and is responsible for cytidine (and deoxycytidine) transformation to uridine (and deoxyuridine), respectively. This enzyme belongs to the cytidine and deoxycytidylate deaminase family that rely on zinc-binding ¹. *CDA* gene is located on chromosome 1 (1p36.12) ^{2,3}, spans on a 29.961 base pair (bp) region but the open reading frame (divided in 4 exons) is encoded only by 441 bp ^{4,5}. *CDA* gene and protein sequences are well conserved among species. Orthologous *CDA* genes are found in many species with a high percentage of sequence homology with human *CDA* (*hCDA*). For instance *hCDA* gene is 83.79% identical to the mouse *CDA* gene, and 99.77%, 68.99% and 51.88% identical to chimpanzee, zebrafish and *Saccharomyces cerevisiae* *CDA* genes, respectively ³. The protein sequence of *hCDA* is also 39% identical to *Escherichia Coli* *CDA* protein sequence ¹.

CDA PROTEIN STRUCTURE

CDA is composed of a CMP (cytidine monophosphate) /dCMP-type deaminase domain that consists of a central β -sheet with one or more α helix on each side ⁶. This feature is shared with all APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) proteins. However, unlike its counterpart AI-CDA (for activation-induced cytidine deaminase, extensively described by R. Kumar et al. ⁷), CDA does not belong to the APOBEC family and thus, processes only free pyrimidines. The CDA gene codes for a 146 amino acid protein (15kDa) that associates with a zinc ion (Zn^{2+}) ⁸ and is organized into a 52kDa homotetramer to ensure its enzymatic function ^{9,10}. The enzyme isoelectric point is 4.5, and the pH range in which it is active is between 3.5 and 10.5 ¹⁰. As the crystallization of the CDA revealed a second order axis of symmetry, it is assumed that the enzyme is actually a dimer of dimers ¹¹. S. Vincenzetti et al. elucidated the roles of different amino acids in CDA using targeted mutagenesis. They found that each monomer binds to a zinc atom in coordination with the cysteines C99 and C102 while C65 is essential to the correct orientation of zinc ion ¹². E67 has a central role in the initiation of catalysis since it is the glutamate carboxylic moiety that authorizes the deprotonation/protonation of the water molecule/amine group of the substrate respectively, which initiates the nucleophilic attack of the hydroxide on the C4 of the pyrimidic ring ¹³. The reaction intermediate is a tetrahedral interaction between the pyrimidic ring of the substrate, the hydroxyl group formed by the water molecule, the glutamate carboxylate moiety and the zinc ion (Figure 1) ¹⁴. Each catalytic site is formed by the interaction of three monomers, which explains why it needs to be tetrameric to be active ¹¹. Among others, F137, Y33 and R68 are important for interaction with the substrate since they participate in the formation of the catalytic site

and Y60 in the recognition of the substrate ^{15,16}. Interestingly, all the amino acids of interest mentioned in this section are conserved in the mouse CDA protein sequence ¹⁷.

CDA FUNCTION

The molecular landmark of CDA is its deaminase function, that catalyzes the transformation of cytidines and deoxycytidines into uridines and deoxyuridines, respectively, ¹⁸ by hydrolyzing the amine moiety into ketone with ammonia release (Figure 2) ¹¹. The deamination of cytidine to uridine contributes, on the one hand, to maintaining nucleotide pool balance for DNA and RNA synthesis. On the other hand, such conversion is also necessary for the catabolism of the pyrimidine cycle, a source of carbon and nitrogen, which results in the formation of beta-alanine (Figure 2) ^{19,20}. Therefore, CDA has a complementary role within the pyrimidine salvage pathway to the *de novo* pyrimidine synthesis pathway (Figure 3). Large scale interactome studies revealed many CDA potential partners (Figure 4A, Table 1), for which gene ontology analysis is provided (Figure 4B) ²¹. While this could lead to the discovery of new functions and new roles for this enzyme. However, none of these potential molecular interactions have been studied further.

SUBCELLULAR LOCALIZATION OF CDA

Databases describe CDA as a cytoplasmic protein ²², which is in agreement with recycling free pyrimidines from the cytoplasm. Pyrimidines are then passively transported into the nucleus or actively transported into the mitochondria by the family of adenine nucleotide carriers ANT or SLC25A19 where they are integrated into DNA or RNA ^{23,24}. It is therefore commonly accepted that CDA is present mainly in the

cytoplasmic fraction of cells. However, A. Somasekaram et al. reported that CDA might also be nuclear, due to a bipartite NLS sequence (a specific nuclear localization signal composed of two clusters of basic amino acids, separated by 10 amino acids) recognized by the importin receptor ²⁵. CDA can also be found in the serum, which demonstrates release from the cell ²⁶. CDA serum activity has been monitored in several cancers and it is correlated with chemotherapy response or chemotherapy-related toxicities ²⁷⁻³⁰. Moreover, CDA serum activity is measured to predict abnormal pregnancy with complications ³¹, or during acute inflammation in rheumatoid arthritis ³². Indeed, as CDA leaks out into serum when the membrane of PMN (polymorphonuclear neutrophils) is damaged ³³, one can speculate that CDA serum activity increase during may reflect higher numbers of damaged cells in tissues expressing high levels of this enzyme. However, addressing CDA activity in blood is not (yet) part of routine clinical practice.

REGULATION OF CDA EXPRESSION

CDA expression is heterogenous between tissues. CDA is most commonly expressed in bone marrow and liver, and more moderately or even undetectably in other tissues ³⁴. In white blood cells, CDA activity is 279 nmol/h/mg protein in peripheral lymphocytes, and 2443 nmol/h/mg protein in granulocytes.³⁵ More generally, the activity of CDA is higher in mature hematopoietic cells than in immature cells ³⁶. In cancer cells, *CDA* gene is not lost, nor amplified, meaning that regulation of *CDA* expression is mainly at the transcriptional level ³⁷. Several binding sites to transcription factors have been described in the literature in *CDA* promoter or enhancer regions (cEBPs, SP1, AP1, Myc/Max, HFH2, Lyf1, ARP1 and GATA1 ^{5,38}).

As several natural mutations of the *CDA* gene are found in the population, and in particular within the promoter region ³⁹, this is likely to modify transcription factors binding sites and to alter the level of transcription of *CDA*, as exemplified by R. E. Ferrell's group ³⁹. More recently, M. Amor-Gueret's team showed that the level of expression of *CDA* in tumors is negatively regulated by DNA methylation of the *CDA* promoter, as epigenetic drugs increase *CDA* expression ⁴⁰. S. Watanabe and T. Uchida showed that the injection of vitamin D3 induces an increase in the level of expression of *CDA* mRNA in tumors ³⁴. S. S. Shord and R. P. Patel showed that the administration of paclitaxel (antineoplastic anticancer agent) induces a decrease in *CDA* mRNA level in cancer cell lines, while not having a significant effect on protein, but nonetheless resulted in *CDA* increased activity ⁴¹. In another study, K. K. Frese et al. showed that albumin-conjugated paclitaxel (nab-paclitaxel) reduces *CDA* protein due to reactive oxygen species (ROS) production, and that *CDA* levels can be restored when cells are treated with N-acetylcystein (NAC, a ROS scavenger molecule) ⁴². This study demonstrates at least in part why cells treated by taxol are more sensitive to gemcitabine.

CDA expression seems to be finely tuned by microRNAs, a class of short, non-coding RNAs experts in gene silencing. Along this line, Z.-M. Shao's team negatively correlated miR-484 and *CDA* expression in breast cancer cells; they found that miR-484 directly inhibits *CDA* translation by targeting *CDA* 3' UTR, and relieves tumor cells' chemoresistance ⁴³. In another study, A. Rajabpour et al. correlated the decrease of miR-608 and the up-regulation of *CDA* protein in pancreatic cancer cells resisting to chemotherapy ⁴⁴. Finally, Z. Gil's team showed that tumor-associated macrophages induce *CDA* expression in response to treatment with gemcitabine ^{45,46}, *via* miR-365 microRNA ⁴⁷.

REGULATION OF CDA PROTEIN ACTIVITY

Many polymorphisms of the *CDA* gene have been identified in the population. The work of K. Kühn et al.⁴⁸ and J. Laliberté and R. L. Momparler⁴⁹, supplemented by the numerous studies of Vincenzetti et al.⁵⁰, have highlighted the existence of two major variants of CDA mRNA that differ by codon 27 (79A>C) that alternatively gives a glutamine or a lysine. These two proteins called CDA1 and CDA2, respectively, are capable of forming the different combinations of tetramers, *i.e.* 5 enzymatic isoforms that have essentially the same physico-chemical and enzymatic properties as the amino acid is not located in the active site of the enzyme⁵⁰. According to these authors, the only difference would be in the net charge of each monomer, giving them a different capacity for migration under electrophoresis, with CDA1 being revealed slightly lower than CDA2 on polyacrylamide gel (PAGE)⁵⁰. These polymorphisms are found in the population with variable frequencies according to ethnicity. The CDA2 variant is the one most commonly found in the Caucasian population (65%)⁵¹. S. Koizumi's team also highlighted another variant in codon 70 (208G>A) which consists of a threonine instead of an alanine, but found in only 4.3% of the Japanese population studied⁵². Other studies showed that this variant appears to be mostly found in African and Asian populations^{53,54}. The simple nucleotide substitutions (SNP) 79A>C and 208G>A have been the two most studied polymorphisms.

These works have been supplemented by numerous clinical trials aimed at demonstrating a possible correlation between the numerous variants of CDA and the activity of the enzyme. Indeed, genetic variations can affect the structure of the enzyme, albeit only a few of them have been correlated to a variation in the catalytic activity of the enzyme. In humans, serum CDA activity was measured in a cohort of 150 cancer

patients, ranging from 0.6 to more than 18 U/mg, which shows a high degree of inter-individual variability ²⁷.

Despite some inconclusive studies and even contradictory results on the correlation between the enzymatic activity of CDA and these SNPs, studies showed that SNP 208G>A (A70T) results in a lower activity of CDA ^{30,52} while 79A>C (K27Q) mutation favors cytidine and deoxycytidine deamination ^{55,56}. In addition, S. Vincenzetti's group identified an activating haplotype of CDA (-451T/-92G/-31Del/79C/435C) ⁵⁷. These polymorphisms also have repercussions on drug metabolism with a 68% decrease in activity of A70T CDA mutants against Ara-C (cytarabine) ⁵². The picture is less clear when it comes to K27Q mutation. One group showed that recombinant CDA27Q has a 23% to 70% decrease in activity for Ara-C ⁵⁸ and a 34% decrease in activity for gemcitabine ⁵⁹, while another study showed an increase in activity for Ara-C but a decrease for decitabine for the exact same mutant ⁵⁶. Such discrepancies between CDA activity and SNPs presence strongly suggests that gene polymorphisms cannot fully explain inter-patient heterogeneity in serum CDA activity.

Unfortunately, data about CDA activity modulation mechanisms are rare. Along this line, database mining reveals one potential site of phosphorylation on CDA protein on Y79 residue, documented by seven different large scale studies ⁶⁰. However, no functional studies were further carried out to better understand the consequences of CDA post-translational modification nor the mechanism by which CDA is phosphorylated. To date, CDA activity regulation by the nucleotide pool remains to be documented. Although CDA products such as uridine and its derivatives, or CMP have the ability to inhibit the enzyme by competing with substrates, their affinity for CDA is very moderate and their concentration in the environment is far too low to exert significant inhibition of the enzyme ⁶¹. However, regulation of CDA expression or

activity by the nucleotide pool could be further considered, as this was reported for related salvage enzymes, such as cytosine deaminase (COD) ⁶² or adenosine deaminase (ADA) which is regulated by the presence of inosine ⁶³.

CDA INHIBITORS

Current known pharmacological inhibitors of CDA are based on the same mechanism of action. The conversion of cytidine to uridine by CDA goes through a reaction intermediate resulting from the nucleophilic attack of the water/zinc complex on the C4 of the pyrimidine cycle, following which ammonia is released. By replacing the NH₃ moiety with a proton for example (as for zebularin, Figure 5), the reaction intermediate is then blocked. The molecule therefore behaves as a powerful competitive inhibitor of the enzyme. Although other CDA inhibitors such as 3,4,5,5,6-tetrahydrouridine (THU) or 1,3-diazepinone riboside (DR) bear more distant structures (Figure 5), their mechanism of action is also based on blocking the enzyme in an unresolved reaction intermediate state. S. Vincenzetti's group conducted a screening of CDA inhibitors, that were sorted by inhibitory constants (K_i), with best candidates (THU, zebularin and its fluorinated derivative) with K_i less than or equal to the *Michaelis* constant (K_m) of CDA for cytidine (*i.e.* <3.9) ⁶¹. In greater details, THU was first identified and purified in 1967 following affinity capture with CDA as a bait ⁶⁴, and studied by R. Cohen and R. Wolfenden in 1971 in *Escherichia coli* to understand its mechanism of action and pharmacokinetics profile ⁶⁵. THU mechanism of action is based on its C4 hydroxyl moiety in the pyrimidic ring which binds to zinc instead of the water molecule ⁶⁶. However, Y. Manome's team recently put at stakes the specificity of THU for CDA, as they showed that THU is cytostatic regardless of CDA expression in target cells, strongly suggesting a mechanism of action distinct from CDA inhibition, as THU can

impact cell cycle distribution by inhibiting E2F1 transcription factor ⁶⁷. Since the bioavailability of THU is weak (~20% ⁶⁸), T. Tsukamoto's team synthesized new fluorinated versions of the drug, (namely (4R)-2'-Deoxy-2',2'-difluoro-3,4,5,6-tetrahydrouridine), with better oral bioavailability. The IC₅₀ of this new compound is comparable to that of THU (0.4μM versus 0.34μM for THU), and has a better pharmacokinetic profile for CDA inhibition due to its stability ⁶⁹. This team therefore suggests using this compound as an oral complement should improve Ara-C efficacy, yet no clinical trial has been registered to date.

The CDA inhibitor Zebularin was first described by J.S. Driscoll's group in 1980 ⁷⁰. Zebularin blocks CDA in a tetrahedral intermediate thanks to its proton in C4 ¹⁴. The efficacy of this drug was also studied in *Escherichia coli* to understand its precise mechanism of action and the reaction kinetics ⁷¹. However, zebularine is far from being specific to CDA only, as this drug interacts with cytosine-[C5]-DNA methyltransferases (C5 MTases), to regulate DNA methylation on a large scale ⁷². Diazepinone riboside (DR) was discovered in 1981 ⁷³, but its mechanism of action was only elucidated much later. Due to its structure, DR cannot interact with CDA through coordination with the water/zinc complex. Consequently, its mechanism of action is therefore slightly different from the first two above mentioned inhibitors. S. J. Chung et al. demonstrated that DR inhibitory activity on CDA rather results from the electrostatic interaction (π - π type) between the double bond of the DR ring and the aromatic ring of the amino acid Phe137 of the catalytic site of the enzyme ¹¹. However, to date, DR has never been tested in cell cultures. Several groups recently investigated for more stable inhibitors of CDA, such as pseudoisocytidine ⁶¹, often at the expense of specificity or inhibitory potential.

ROLE OF CDA IN PHYSIOLOGY AND PHYSIOPATHOLOGY

Animal models for CDA knockout are still cruelly lacking, meaning that the role of CDA during development is unknown to date. Historically, CDA deficiency has been long associated with Bloom's syndrome ⁷⁴, due to the pioneering work of Pr M. Amor-Guérêt. Bloom syndrome is a rare autosomal disease caused by mutation of the Bloom (*BLM*) gene on both alleles that results in high genetic instability ⁷⁵. *BLM* encodes for a DNA helicase, which main function is to maintain genomic integrity ⁷⁶. *BLM* is relocated to DNA breaks in a complex that also contains RAD51, which reports DNA damages and thus contributes to their management by repair mechanisms. Generally, mutations found in *BLM* are deletions or base insertions that result in decreased gene transcription or termination of translation due to a nonsense mutation of mRNA ⁷⁶. Therefore, defect or alteration of this protein induces a malfunction of the homologous recombinant repair system. Spontaneous mutations of somatic cells are thus much more frequent than in WT *BLM* genetic background. A systematic feature of genetic instability associated with Bloom syndrome is the presence of segment exchanges between sister chromatids of a chromosome, which has long been a method of diagnosing this disease, now confirmed by sequencing ⁷⁷. As a result, patients with this condition develop congestive redness of the skin, called telangiectatic erythema, as well as growth retardation and other malformations ⁷⁸. These patients are also more predisposed to cancer, in particular hematologic cancers and carcinomas of diverse organs, which considerably reduces their life expectancy ⁷⁴. Pr Amor-Guérêt's group nicely showed that loss of CDA expression is concomitant to *BLM* mutation. They found that CDA has an important role in maintaining the nucleotide pool, to prevent from replicative stress that is harmful to the cell ⁷⁹. Among the many chromatid

segregation defects, chromatin bridge formation (consequences of poor sister chromatid segregation) is directly due to the absence of BLM, while the presence of ultra-fine bridges (UFB) (which follow incomplete DNA replication when cells enter mitosis) results from an imbalance in the pyrimidine pool due to CDA deregulation⁸⁰. The mechanism explaining this phenomenon is the accumulation of dCTP due to the absence of CDA, which induces a decrease in the activity of PARP-1, a protein involved in the response to DNA damage⁸⁰. C.P. Witte's team supplemented this hypothesis by showing that the accumulation of intermediate species of pyrimidine catabolism due to CDA invalidation is toxic¹⁹.

As stated before, a significant functional aspect of CDA is the ability to deaminate synthetic or natural cytidine analogues. This is the reason why numerous studies have been focusing on CDA expression as a marker of cancer chemoresistance since the 1960s, with the emergence of the first anti-metabolites anticancer analogs of cytidine and deoxycytidine such as cytarabine (or Ara-C)⁸¹. Indeed, CDA is recognized as a key chemo-resistance factor to cytidine and deoxycytidine analogues such as Ara-C, Gemcitabine (dFdC), or Decitabine (5-Aza-dC)⁸² *in vitro* (Figure 5), since it catalyzes their transformation into inactive metabolites^{81,83}. Indeed, CDA proceeds synthetic metabolites and natural substrates with similar affinities ($K_m=11\mu\text{M}$ for Ara-C, $K_m=3.9\mu\text{M}$ for cytidine)^{61,84}. In mice, CDA largely participate in gemcitabine inactivation and clearance²⁷. In humans, 80% of intravenous Ara-C bolus is eliminated in the urine, 90% of which in its uracil form, thus strongly suggesting direct and active "detoxification" by CDA⁸⁵. Further, a study conducted on 40 patients with pancreatic cancers treated with gemcitabine correlated high activity of CDA (>6 U/mg) with progressive disease and concluded that patient with high CDA activity are five times more likely to progress following gemcitabine-based therapy⁸⁶. Very recently,

commensal CDA was identified as a key player of chemoresistance originating from the tumor microbiome. L. T Geller et al. identified that 76% of patients with pancreatic cancer are positive for γ proteobacteria, which possesses an orthologous CDA capable of deaminating gemcitabine and so to participate in resistance to treatment ⁸⁷. Quite the contrary, capecitabine, a synthetic deoxycytidine analogue, has to be deaminated to be active. Enforced CDA expression consequently improves capecitabine chemotherapy efficacy, *in vitro* ⁸⁸.

Therefore, CDA has an important therapeutic role since its modulation would make it possible to defeat the chemoresistance of some cancers. On the other hand, CDA levels may also predict toxicities associated with chemotherapies; indeed, patients with lower CDA activity than normal treated with chemotherapy (Gemcitabine or Ara-C) have serious side effects due to exacerbated toxicity of the treatment ^{27,89,90}. Along the same line, J. Ciccolini et al. showed that CDA-deficient patients (*i.e* with a CDA activity <1.3 U/mg) develop serious adverse effects (grade 3-4) when treated with Gemcitabine ²⁷. Thus, CDA may have a role in genetic diseases and in response to treatment in cancer patients. CDA might be an appealing molecular target, particularly in patients treated with chemotherapy.

THERAPEUTIC STRATEGIES BASED ON CDA EXPRESSION

Given the high heterogeneity of expression of CDA, different therapeutic strategies can be considered depending on the pathological context. Most cancers under-express CDA compared to healthy tissues ^{91,40}. Prof. Amor-Gueret's team focused on breast tumors that poorly express CDA due to DNA methylation. They showed that tumors with low CDA expression are more sensitive to aminoflavone derivative AFP464 ⁴⁰. Tissues that

express higher levels of CDA are, on the other hand, resistant to these drugs, which would place CDA as a predictive biological marker of response. High-CDA expressing tumors should be in theory more resistant to cytidine-based therapies. In line with this assumption, several studies combining various chemotherapy and CDA inhibitors have been conducted so far ^{68,92-95}. However, only few clinical trials resulted from these studies. Phase I clinical trials studied the effects of 5-fluoro-2'-deoxycytidine with THU (NCT00359606, NCT01041443, available at ClinicalTrial.gov) and two Phase II clinical trials were completed recently combining 5-fluoro-2'-deoxycytidine with THU and another combining THU and decitabine with nivolumab (NCT00978250, NCT02664181, available on ClinicalTrials.gov) but none of them shared the results yet. Another interesting hypothesis made by Z. Gil's group could be to target macrophages in order to reduce CDA levels and thus chemo-sensitize the tumor to gemcitabine ⁴⁵. M. Zauri's group recently performed an elegant study, focusing on tissues that express high levels of CDA, including pancreatic cancer, in which CDA appears to be expressed mainly in epithelial tumor cells and not in the stroma ⁹⁶. They took advantage of CDA-overexpression to metabolize synthetic deoxycytidine analogues to be inserted into DNA ⁹¹. In greater details, overexpression of CDA provokes the deamination of deoxycytidine analogues 2'-Deoxy-5-(hydroxymethyl)cytidine (5hmdC) and 5-formyl-2'deoxycytidine (5fdC) into 5hmdU and 5fdU respectively, which are then phosphorylated and incorporated into DNA, triggering cell cycle arrest and cell death ⁹¹, due to activation of surveillance factors such as uracil glycosylase, which causes multiple DNA breaks ⁹⁷. In normal cells, these nucleotides are not integrated into DNA due to the presence of cytidine monophosphate kinase 1 (CMPK1), involved in nucleoside recycling and metabolism pathways, that acts as barrier to protect the

genome. These observations clearly provide a therapeutic opportunity to treat cancers that overexpress CDA.

Another interesting issue is that CDA, through induction of chemoresistance, also protects tissues from the toxic effect of such intravenous drugs throughout the body, as severe toxicity is observed in rapidly proliferating tissues with low levels of CDA^{27,89,90}. A therapeutic strategy developed by T. Moritz consists in overexpressing CDA in these tissues using gene transfer, to protect them from side effects^{98,99}. This group demonstrated the interest of such an approach in acute leukaemia treated with Ara-C post-transplantation to protect the healthy marrow.

Lastly, stepping back from cancer management, CDA can also be also considered as a target *per se*. Indeed, CDA, as well as other enzymes from the pyrimidine salvage pathway, may represent interesting targets for killing pathogens such as trypanosomiasis (flagellated protozoa causing, for example, sleeping sickness or leishmaniasis) that do not produce some enzymes in the *de novo* pathway and are therefore dependent on CDA for their nucleotide synthesis¹⁰⁰.

CONCLUSION

CDA is historically known to be involved in the salvage of pyrimidines by recycling free cytidines and deoxycytidines into uridines and deoxyuridines, respectively, for DNA and RNA synthesis. This enzyme is well conserved among species, highlighting the importance of this pathway during evolution. In Bloom syndrome, CDA defects induces replicative stress. In cancer, CDA detoxifies cytidine and deoxycytidine analogue-based therapies such as gemcitabine and Ara-C and can be considered as a key player in tumor resistance to treatment. Taking advantage or modulating CDA activity

in patients is at reach of hand and might constitute a promising strategy in cancer therapy. On the other, CDA can be heterogeneously expressed in tumors, regardless of chemotherapeutic treatment. This advocates for a better understanding of the molecular mechanisms involved in CDA regulation of expression, but also suggest a new and undiscovered role of CDA in oncogenesis. Thus, better understanding the role and function of CDA may not only help overcome chemo-resistance and improve the management of patients with cancer, notably pancreatic cancer, and may also reveal the unforeseen role of CDA during carcinogenesis to help develop new therapeutic strategies.

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AUTHOR CONTRIBUTIONS

A.F. and P.C. conducted the bibliographic search and wrote the paper.

DISCLOSURE/CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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FIGURE LEGENDS

Figure 1 : Tetrahedral reaction intermediate of cytidine following deamination to uridine by cytidine deaminase.

Figure 2 : Cytidine deaminase reaction equation from cytidine to uridine and catabolism of uridine to β -alanine.

Figure 3 : *de novo* pyrimidine synthesis, pyrimidine salvage pathways and metabolism of pyrimidine nucleosides to nucleotides.

Figure 4. A. Theoretical interactome network of CDA using BioGRID (www.thebiogrid.org). Physical edges are shown in yellow.**B.** Gene ontology analysis of CDA potential partners. Pie charts were generated with PANTHER14.1 from the 2018_04 release.

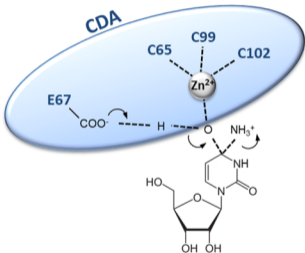
Figure 5: Schematic representation of the natural substrates, substrates analogues used as chemotherapies and inhibitors of CDA.

TABLES

Acronym	Full name
AAR2	AAR2 splicing factor homolog (<i>S. cerevisiae</i>)
APBA3	amyloid beta (A4) precursor protein-binding, family A, member 3
C20ORF195	chromosome 20 open reading frame 195
CDKN2C	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)
CLIC4	chloride intracellular channel 4
CPNE1	copine I
CPNE3	copine III
DPH2	DPH2 homolog (<i>S. cerevisiae</i>)
DPYSL2	dihydropyrimidinase-like 2
EVPL	envoplakin
G6PD	glucose-6-phosphate dehydrogenase
GBE1	glucan (1,4-alpha-), branching enzyme 1
LIN7A	lin-7 homolog A (<i>C. elegans</i>)
LNX1	ligand of numb-protein X 1, E3 ubiquitin protein ligase
LYPLA1	lysophospholipase I
MCM2	minichromosome maintenance complex component 2
MPP2	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)
MPP6	membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)
MTMR6	myotubularin related protein 6
MTMR9	myotubularin related protein 9
PFKP	phosphofructokinase, platelet
PLEKHB2	pleckstrin homology domain containing, family B (evectins) member 2
PSMA1	proteasome (prosome, macropain) subunit, alpha type, 1
PTGER3	prostaglandin E receptor 3 (subtype EP3)
RC3H1	ring finger and CCCH-type domains 1
RNF181	ring finger protein 181
SDCBP	syndecan binding protein (syntenin)
SH3KBP1	SH3-domain kinase binding protein 1
TIMM8A	translocase of inner mitochondrial membrane 8 homolog A (yeast)
TRIM25	tripartite motif containing 25
TTC38	tetratricopeptide repeat domain 38
WARS	tryptophanyl-tRNA synthetase
WDYHV1	WDYHV motif containing 1
WIBG	within bgcn homolog (<i>Drosophila</i>)
XPO1	exportin 1
ZBTB24	zinc finger and BTB domain containing 24
ZMYM6	zinc finger, MYM-type 6

Table 1: abbreviated and full name of CDA potential interactants.

FIGURE 1



Tetrahedral reaction intermediate

FIGURE 2

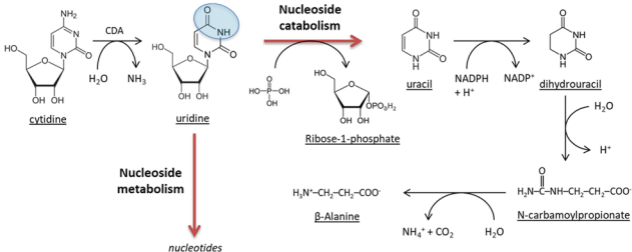


FIGURE 3

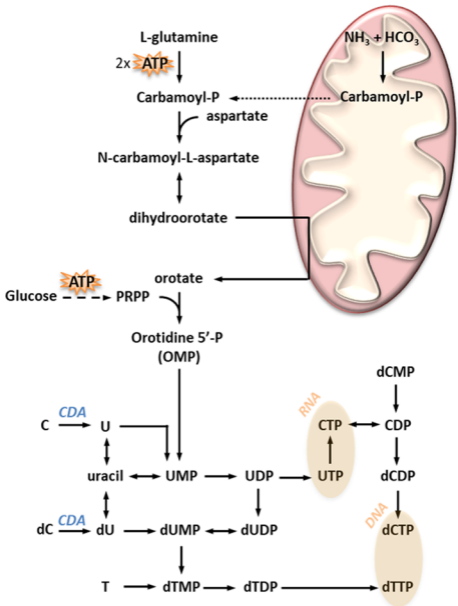
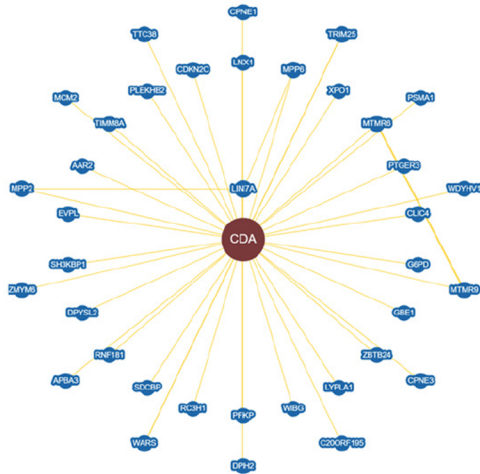


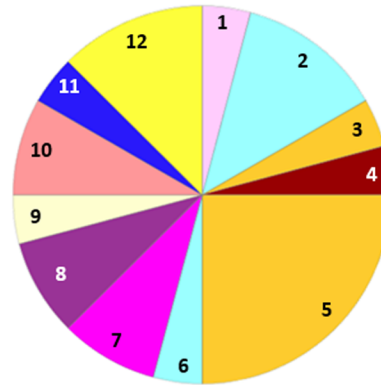
FIGURE 4

A



B

Protein Class



- | | |
|----|--------------------------|
| 1 | Cell adhesion molecule |
| 2 | Cell junction protein |
| 3 | Cytoskeletal protein |
| 4 | Enzyme modulator |
| 5 | Hydrolase |
| 6 | Ligase |
| 7 | Membrane traffic protein |
| 8 | Nucleic acid binding |
| 9 | Oxidoreductase |
| 10 | Receptor |
| 11 | Transcription factor |
| 12 | Transferase |

Pathway



- | | |
|---|---|
| 1 | Alzheimer disease-amyloid secretase pathway |
| 2 | Axon guidance mediated by semaphorins |
| 3 | Gonadotropin-releasing hormone receptor pathway |
| 4 | Notch signaling pathway PI3K pathway |
| 5 | PI3K pathway |
| 6 | Parkinson disease |
| 7 | Pyrimidine metabolism |

FIGURE 5

Natural substrates



Substrates analogues



Inhibitors

