Microbial cytosine deaminase is a programmable anticancer prodrug mediating enzyme: antibody, and gene directed enzyme prodrug therapy

Ashraf S.A. El-Sayed a,*, Nabil Z. Mohamed a, Marwa A. Yassin a, Mahmoud M. Amer b, Reyad El-Sharkawy b, Nesma El-Sayed b, Mostafa G. Ali a, b

a Enzymology and Fungal Biotechnology Lab, Botany and Microbiology Department, Faculty of Science, Zagazig University, 44519, Egypt
b Botany and Microbiology Department, Faculty of Science, Benha University, Benha, 13518, Egypt

ARTICLE INFO

Keywords:
Cytosine deaminase
5-fluorocytosine
5-fluorouracil
ADEPT
GDEPT

ABSTRACT

Cytosine deaminase (CDA) is a non-mammalian enzyme with powerful activity in mediating the prodrug 5-fluorocytosine (5-FC) into toxic drug 5-fluorouracil (5-FU), as an alternative directed approach for the traditional chemotherapies and radiotherapies of cancer. This enzyme has been frequently reported and characterized from various microorganisms. The therapeutic strategy of 5-FC-CDA involves the administration of CDA followed by the prodrug 5-FC injection to generate cytotoxic 5-FU. The antiproliferative activity of CDA-5-FC elaborates from the higher activity of uracil pathway in tumor cells than normal ones. The main challenge of the therapeutic drug 5-FU are the short half-life, lack of selectivity and emergence of the drug resistance, consistently to the other chemotherapies. So, mediating the 5-FU to the tumor cells by CDA is one of the most feasible approaches to direct the drug to the tumor cells, reducing its toxic effects and improving their pharmacokinetic properties. Nevertheless, the catalytic efficiency, stability, antigenicity and targetability of CDA-5-FC, are the major challenges that limit the clinical application of this approach. Thus, exploring the biochemical properties of CDA from various microorganisms, as well as the approaches for localizing the system of CDA-5-FC to the tumor cells via the antibody directed enzyme prodrug therapy (ADEPT) and gene directed prodrug therapy (GDEPT) were the objectives of this review. Finally, the perspectives for increasing the therapeutic efficacy, and targetability of the CDA-5-FC system were described.

1. Introduction

Cytosine deaminase (CDA) (E.C 3.5.4.1) is an amidohydrolase that catalyzes the deamination of cytosine into uracil and ammonia (Kilstrup et al., 1989; Danielsen et al., 1992; Mullen, 1994; Mullen et al., 1994). CDA has been distributed in microorganisms with potentiality to convert the non-toxic 5-fluorocytosine (5-FC) into toxic 5-fluorouracil (5-FU) (Polak and Scholar, 1975; Charles et al., 1957). The activity of CDA for deaminating the 5-FC into 5-FU was firstly described by Polak and Scholar (1975). 5-Fluorouracil and its oral prodrug capecitabine are the most efficient chemotherapeutic regimens in cancer therapy (Longley et al., 2003; Miura et al., 2010; Vermorken et al., 2007; Argilés et al., 2020), for treatment of several neoplasms such as head and neck squamous cell carcinoma, gastrointestinal, adenocarcinoma and uterine cervix (Chen et al., 2018; Sakai et al., 2019; Argilés et al., 2020). Recently, 5-FU in combination with vascular endothelial growth factor (VEGF) inhibitors (Ghafouri-Fard et al., 2012) has been recognized as efficient approach for cancer therapy. The activity of 5-FU is basically attributed to the affinity to block the activity of cellular thymidylate synthase (TS), thus, preventing the DNA replication (Peters et al., 1994), in addition to the inhibition of RNA synthesis by integration with the RNA (Silverstein et al., 2011). Nevertheless, the short half-life time, quite lack of selectivity and the drug resistance of 5-FU are the major challenges that limits the clinical applications of this enzyme (Takahashi et al., 2014). Thus, manipulating the toxicity and targetability of 5-FU by the prodrug 5-FC-mediated CDA is one of the most sophisticated anticancer approach. The CDA-5-FC has been recognized as a targeted/directed therapy with little side effects, higher and antiproliferative efficiency, than the traditional anticancer therapies such as chemotherapy and radiotherapy (DeVita et al., 1993; Mullen et al., 1994).

The CDA-5-FC prodrugs enzyme mediated therapy has been developed as one of the most successful cutting-edge technologies for cancer therapy (Karjoo et al., 2013). Recently, the strategy of gene-directed enzyme prodrug therapy (GDEPT) “suicide gene therapy” using CDA

References:

Kilstrup et al., 1989
Danielsen et al., 1992
Mullen, 1994
Mullen et al., 1994
Polak and Scholar, 1975
Charles et al., 1957
Longley et al., 2003
Miura et al., 2010
Vermorken et al., 2007
Argilés et al., 2020
Chen et al., 2018
Sakai et al., 2019
Argilés et al., 2020
Ghafouri-Fard et al., 2012
DeVita et al., 1993
Mullen et al., 1994
Polak and Scholar, 1975
Charles et al., 1957

* Corresponding author.
E-mail address: ash.elsayed@gmail.com (A.S.A. El-Sayed).

https://doi.org/10.1016/j.heliyon.2022.e10660
Received 2 March 2022; Received in revised form 26 March 2022; Accepted 9 September 2022
2405-8440/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
has been established in order to overcome the chemotherapeutic side effects of the traditional approaches (Karjoo et al., 2013). The antiproliferative activity of this prodrug mediated system elaborates from the over activity of uracil pathway in tumor cells compared to normal cells (Heidelberger et al., 1957). Recently, several strategies have been focused on survey of CDA with high turnover and catalytic efficiency, less antigenicity from various microorganisms for activation of the prodrug 5-FC into active 5-FU for selectively targeting the tumor cells with no effect on normal cell (Eastman and Perez, 2006). Bacterial cytosine deaminase (CDA) has received an immense attention over the yeasts enzymes, however, the catalytic efficiency of yeast CDA for deaminating 5-FC have been noticed to be higher than bacterial CDA (Polak and Scholar, 1975). Nevertheless, the conformational/structural and thermal stabilities of CDA is the major challenge that limits their broad spectrum clinical applications (John and Morris, 2002).

The activity of CDA for conversion of cytosine or its analog 5-fluorocytosine was firstly reported by Polak and Scholar (1975) (Figure 1). Also, the potential antifungal activity of 5-FC was attributed to the activity of intrinsic CDA, deaminating the non-toxic 5-FC into toxic 5-FU (Grunberg and Titsworth, 1969). The superior therapeutic efficiency of 5-FC elaborates from its higher solubility in water and smaller molecular size, facilitating its quickly diffusion in the body (Daneshmend and Warnock, 1983). Practically, the 5-FU has a broad bioactivity range against different pathogenic microorganisms including Candida, Cryptococcus, Phialophora, Cladosporium and Aspergillus in addition to the antiprotozoal activity; Leishmania and Acanthamoeba (Polak and Scholar, 1975; Polak et al., 1985; Abdel-Fatah et al., 2021, Ali et al., 2015). CDA is a non-mammalian enzyme, with extraordinary clinical affordability for mediating the conversion of 5-FC into 5-FU upon external infusion (Diasio et al., 1978; Williams et al., 1981). Practically, the intestinal microbes could be the main source for the 5-fluorouracil in human cells (Harris et al., 1986).

Cytosine has been metabolized by pyrimidine-nucleoside phosphorylase (E.C 2.4.2.2) and CDA (E.C 3.5.4.1) into cytidine and uracil (Figure 1). Ribosylpyrimidine nucleosidase (E.C 3.2.2.8) and pyrimidine 5-nucleotide nucleosidase (E.C 3.2.2.10) hydrolyze cytidine and cytidine monophosphate to produce cytosine (Kandeel and Al-Taher, 2020). The antiproliferative activity of 5-FC has been reported to be maximized by combining with many antifungal azole agents such as fluconazole and ketoconazole especially against colorectal carcinoma. Practically, 5-FC displayed an efficient antifungal activity that could be due to the presence of CDA, that being a selective strategy for human fungal pathogen treatment (Vermes et al., 2000). Cytosine and 5-FC are competitive nucleotides for transportation across plasma membrane by cytosine permease, that followed by subsequent hydrolysis by CDA into uracil or 5-FU, respectively (Polak and Grenson, 1973). The conversion of 5-FC to 5-FU is only dependent on the presence of active CDA, some fungi do not possess an active CDA that were described as 5-FU resistant fungi (Polak, 1977). In fungi, after 5-FC uptake and conversion to 5-fluorouracil, two metabolic mechanisms are implemented to fulfill the antifungal activity of 5-fluorouracil (Figure 1). Firstly, uridine phosphoryltransferase (UPRT) converts 5-FU to 5-fluorouridine monophosphate (FUMP), and to 5-fluorouridine diphosphate (FUDP), and finally 5-fluorouridine triphosphate (FUTP) (Waldorf and Polak, 1983; Maamoun et al., 2021). The produced FUTP inhibits the synthesis of cellular proteins by incorporation with fungal RNA replacing the uridylic acid (UTP), hence, altering the acylation of amino acid in tRNA, subsequently disturbing the sequence of amino acid and final block to protein synthesis (Waldorf and Polak, 1983). Secondly, 5-FU is metabolized by uridine 5-monophosphate pyro-phosphorylase to produce 5-fluorodeoxyuridine monophosphate (5-FdUMP) (Waldorf and Polak, 1983), strongly affects DNA biosynthesis by inhibiting thymidylate synthetase (TS) as the main source of thymidine (Dtaszo et al., 1978). Subsequently, 5-fluoro-deoxyuridine monophosphate inhibits the fungal DNA synthesis and in turn their cellular replication (Figure 2) (Polak and Scholar, 1975; Waldorf and Polak, 1983; Vermes et al., 2000; Vandeputte et al., 2012).

Salvage pathways are one of the profound cellular assimilatory pathways in most of microbes for synthesis of cytosine and its derivatives from the non-essential nutrients that usually requires less energy than the de novo pathways (Yao et al., 2005). The de novo pathway for pyrimidine synthesis is an universal pathway for pyrimidine and purine nucleotides synthesis (O’Donovan and Neuhard, 1970). The pyrimidine pathway (Figure 3) was firstly explored in Salmonella typhimurium (Beck et al., 1972), different bacterial (Ovrebo and Kleppe, 1973; Rima and Takashashi, 1977; Jyssum and Jyssum, 1979), and fungal species (Chakrabarty and Loring, 1960; Polak and Scholar, 1975). The pyrimidine salvage pathway was initiated by importing the nucleotides to the cell, followed by metabolic assimilation of these bases (Kern et al., 1990; Yao et al., 2005). The processes of pyrimidine transportation to the cytosol involves; uracil permease (Chevallier, 1982; Jund et al., 1988), 2-
Purine-cytosine transporters that transport cytosine to the cell (Schmidt et al., 1984), and 3- Uridine permease that transports uridine into the cell (Wagner et al., 1998). Unlike the human cells, microorganisms have an active CDA, catalyzing the conversion of 5-FC (non-toxic prodrug) into 5-FU (toxic anticancer drug) (Yao et al., 2005). Thus, expression of CDA in combination with uracil phosphoribosyl-transferase in tumor cells increases the cellular sensitivity to 5-FC and 5-FU (Erbs et al., 2000; El-Kalyoubi et al., 2021, El-Mekawy et al., 2010, El-Sayed et al., 2010, El-Sayed et al., 2011).

1.1. Sources and biochemical properties of CDA

Cytosine deaminase has been widely distributed in various bacterial and fungal isolates (Mullen et al., 1992; Hamaji et al., 2007) as listed on Table 1. Bacterial CDA has received a much attention comparing to the fungal one. Although, the higher conformational stability of bacterial CDA, fungal CDA exhibited a higher catalytic efficiency for converting of 5-FC into 5-FU (toxic anticancer drug) (Yao et al., 2005). Thus, expression of CDA in combination with uracil phosphoribosyl-transferase in tumor cells increases the cellular sensitivity to 5-FC and 5-FU (Erbs et al., 2000; El-Kalyoubi et al., 2021, El-Mekawy et al., 2010, El-Sayed et al., 2010, El-Sayed et al., 2011).

From the kinetics studies of CDA from different sources towards different substrates, CDA from Alcaligenes denitrificans (Kim et al., 1987), A. paraisiticus (Zanna et al., 2012), and E. coli (Porter and Austin, 1993), displayed the highest catalytic affinity for cytosine and 5-FC. Interestingly, the affinity of bacterial (Kim et al., 1987; Mahan et al., 2004) and fungal (Zanna et al., 2012) CDA for deaminating cytosine and 5-fluorocytosine being higher than CDA from yeast’s, as revealed from the Km and Kcat values. For example, the value of Km for Alcaligenes denitrificans CDA was lower than that of baker’s yeast by approximately 31 folds, revealing the higher affinity of bacterial enzymes for cytosine (Ipata and Cerignani, 1978; Katsuragi et al., 1989).

Figure 2. Proposed pathway of 5-FC metabolism and its action mechanism. Uridine phosphoribosyltransferase (UPRT), 5-fluorouridine monophosphate (FUMP), 5-Fluorouridine diphosphate (FUDP), 5-fluorouridine triphosphate (FUTP) and 5-fluorodeoxyuridine monophosphate (FdUMP) as adopted by Vermes et al. (2000).

Figure 3. Salvage pathway of cytosine and cytidine in S. typhimurium and S. cerevisiae. Transporters of the cytosine and cytidine to cell (1), Cytosine deaminase CDA (2), Cytidine deaminase (3), uridine-cytidine kinase (4), uridine phosphorylase (5), uracil phosphoribosyl transferase (6).
CDA from different microorganisms has been characterized by X-ray crystallographic analysis (Ko et al., 2003). The protein structure contains four-stranded β-sheets (β1–β5) with the strand order 2, 1, 3, 4, 5 and with β1 running antiparallel to the other strands. The β-sheets are sandwiched by two α-helices (αb–αd) on the other side (Ko et al., 2003; Yassin et al., 2022). The enzyme forms a tightly packed dimer in the crystal structure without significant differences between the two subunits. The dimer interface constituted by helical layer (αb–αd) and the C-terminal tail. The structural amino acids of CDA from different microbial organisms were resolved, and the conserved regions were determined. Depending on the protein sequence data base, a comprehensive amino acid alignment was conducted to explore the structural and catalytic identities of CDA from different microorganisms using E. coli (WP224491759.1) and S. cerevisiae (AA867713.1) as reference CDA. The sequences of CDA were retrieved from the National Center of Biotechnology Information (NCBI, http://www.ncbi.nih.gov). The alignment and the phylogenetic analysis were performed using ClustalW2 (Myers and Miller, 1988). The amino acid residues of CDA from different bacterial and fungal isolates were aligned. From the alignment profile of the amino acids sequence (Figure 4), the CDA sequences were categorized into clusters clade I, and clade II, representing the fungal and bacterial cluster, respectively. From the alignment profile, the conserved amino acids were D4, A10, E13, G17, E20, G21, G22, G26, D33, K35, G38, H41, N42, V45, Q46, H53, E55, L59, N61, G63, R64, Y70, C82, C85 and W142. From the phylogenetic tree, two clusters of CDA were clearly observed; clade I (Yeast CDA) and clade II (bacterial CDA) (Figure 5). The similarity of amino acid sequence of yeast CDA and bacterial CDA were approximated by about 35%. The amino acid sequence of CDA from A. vadensis (XP025558391.1), A. luchuensis (GAT27366.1), A. niger (XP001388854.1), P. digitatum (XP014537178.1), P. roqueforti (CDEM26386.1), A. nomiae (XP015404871.1), P. aureofaciens (KAB8206987.1), A. oryzae (ET832081.1) displayed a significant proximity with S. cerevisiae CDA (AA867713.1) (clade I) by about 95 %. While, the amino acids sequence of CDA from different bacterial isolates; S. enterica (AVB04088.1), S. laurentii (BAU76665.1) and Nocardia seriolae (APA99657.1) exhibited a similarity ratio 98% with the reference CDA of E. coli (WP224491759.1). Structurally, from the amino acid sequences of CDA and crystal structure, the conserved amino acids have been reported to be localized on the substrate binding active site domains and catalytically active domains of CDA from different microorganisms using E. coli (Katsuragi et al., 1989) and P. aureofaciens (Sakai et al., 1975a,b). The activity of cytosine deaminase from E. coli was strongly inhibited by HgCl2 and CuSO4, suggesting the implementation of SH on the enzyme active sites (Hussein and Al-Baer, 2018) and A. parasiticus (Zanna et al., 2012).

1.2. Amino acids sequences, active sites domains and crystal structure

The crystal structure of yeast CDA has been characterized by X-ray crystallographic analysis (Ko et al., 2003). The protein structure contains four-stranded β-sheets (β1–β5) with the strand order 2, 1, 3, 4, 5 and with β1 running antiparallel to the other strands. The β-sheets are sandwiched by two α-helices (αb–αd) on the other side (Ko et al., 2003; Yassin et al., 2022). The enzyme forms a tightly packed dimer in the crystal structure without significant differences between the two subunits. The dimer interface constituted by helical layer (αb–αd) and the C-terminal tail. The structural amino acids of CDA from different microbial organisms were resolved, and the conserved regions were determined. Depending on the protein sequence data base, a comprehensive amino acid alignment was conducted to explore the structural and catalytic identities of CDA from different microorganisms using E. coli (WP224491759.1) and S. cerevisiae (AA867713.1) as reference CDA. The sequences of CDA were retrieved from the National Center of Biotechnology Information (NCBI, http://www.ncbi.nih.gov). The alignment and the phylogenetic analysis were performed using ClustalW2 (Myers and Miller, 1988). The amino acid residues of CDA from different bacterial and fungal isolates were aligned. From the alignment profile of the amino acids sequence (Figure 4), the CDA sequences were categorized into clusters clade I, and clade II, representing the fungal and bacterial cluster, respectively. From the alignment profile, the conserved amino acids were D4, A10, E13, G17, E20, G21, G22, G26, D33, K35, G38, H41, N42, V45, Q46, H53, E55, L59, N61, G63, R64, Y70, C82, C85 and W142. From the phylogenetic tree, two clusters of CDA were clearly observed; clade I (Yeast CDA) and clade II (bacterial CDA) (Figure 5). The similarity of amino acid sequence of yeast CDA and bacterial CDA were approximated by about 35%. The amino acid sequence of CDA from A. vadensis (XP025558391.1), A. luchuensis (GAT27366.1), A. niger (XP001388854.1), P. digitatum (XP014537178.1), P. roqueforti (CDEM26386.1), A. nomiae (XP015404871.1), P. aureofaciens (KAB8206987.1), A. oryzae (ET832081.1) displayed a significant proximity with S. cerevisiae CDA (AA867713.1) (clade I) by about 95 %. While, the amino acids sequence of CDA from different bacterial isolates; S. enterica (AVB04088.1), S. laurentii (BAU76665.1) and Nocardia seriolae (APA99657.1) exhibited a similarity ratio 98% with the reference CDA of E. coli (WP224491759.1). Structurally, from the amino acid sequences of CDA and crystal structure, the conserved amino acids have been reported to be localized on the substrate binding active site domains and catalytically active domains of CDA from bacterial and fungal sources. The structural stability and catalytic efficiency of CDA are mainly thiols containing amino acids “methionine and cysteine”. The metalloproteinic identity and dependence of CDA on Fe2+ has been reported for the purified enzyme from A. fumigatus (Yu et al., 1991), E. coli (Katsuragi et al., 1989) and yeast (Katsuragi et al., 1989) as revealed from the dialysis against O-phenanthroline. Similar results were reported for CDA from S. marcescens (Sakai et al., 1975a,b) and P. aureofaciens (Sakai et al., 1975a,b). The activity of cytosine deaminase from E. coli was strongly inhibited by HgCl2 and CuSO4, suggesting the implementation of SH on the enzyme active sites (Hussein and Al-Baer, 2018) and A. parasiticus (Zanna et al., 2012).
Table 2. Cytosine deaminase properties from different microorganisms.

| Microorganism            | M. wt. (kDa/subunit) | Optimal pH  | pH stability | Optimal temperature (°C) | Specific activity (Unit/mg of protein) | Enzyme substrate | K<sub>m</sub> (mM) | References                  |
|--------------------------|----------------------|-------------|--------------|--------------------------|----------------------------------------|------------------|----------------|-----------------------|
| S. typhimurium           | 54                   | 7.30–7.50   | 45–50        |                          | Cytosine                              | 0.74             |                 | (West et al., 1982)   |
| Escherichia coli         | 50                   |             |              |                          | Cytosine                              | 0.22             |                 | (Porter and Austin, 1993) |
| Escherichia coli         | 50                   |             |              |                          | Cytosine                              | 0.2              |                 | (Mahan et al., 2004)   |
|                          |                      |             |              |                          | 5-fluorocytosine                      | 3.3              |                 |                       |
| Escherichia coli         | 35 & 46              | 9.0         | 9.0–10.0     | 50                       | Cytosine                              |                 |                 | (Katsuragi et al., 1986) |
| Alcaligenes denitrificans | 37                 | 8.0–9.0     | 12.0         | 4.0                      | Cytosine                              | 0.1              |                 | (Kim et al., 1987)    |
| Chromobacterium violaceum YK 391 | 78     | 7.5         | 40–45        |                          | Cytosine                              | 1.55             |                 | (Kim and Yu, 1998)    |
|                          |                      |             |              |                          | 5-fluorocytosine                      | 5.52             |                 |                       |
|                          |                      |             |              |                          | 5-methylcytosine                      | 10.4             |                 |                       |
|                          |                      |             |              |                          |                                       | 67.2             |                 |                       |
| Escherichia coli         | 48                   | 8.5         | 7.5–9.0      | 45–60                    | 9.0                                    | Cytosine                              |                 | (Husein and Al-Baer, 2018) |
| Pseudomonas aerofaciens  | 45                   | 8.0–9.0     |              |                          | Cytosine                              |                 |                 | (Sakai et al., 1978)  |
| S. marcescens            | 72                   | 8.0         | 7.0–9.0      |                          | Cytosine                              |                 |                 | (Sakai et al., 1978b) |
| Aspergillus parasiticus  | 7.2                  | 40–45       |              |                          | Cytosine                              | 0.19             |                 | (Zanna et al., 2012)  |
| Aspergillus fumigatus IFO 5840 | 32     | 7.0         |              | 35                       | Cytosine                              | 2                |                 | (Yu et al., 1991)     |
|                          |                      |             |              |                          | 5-fluorocytosine                      | 6.5              |                 |                       |
|                          |                      |             |              |                          | 5-methylcytosine                      | 36               |                 |                       |
| Baker's yeast            | 41                   | 7.5         | 7.5          | 30–40                    | Cytosine                              | 3.1              |                 | (Katsuragi et al., 1989) |
|                          |                      |             |              |                          | 5-fluorocytosine                      | 1.2              |                 |                       |
|                          |                      |             |              |                          | 5-methylcytosine                      | 2.5              |                 |                       |
| Baker's yeast            | 34                   | 6.5         | 5.0–9.0      |                          | Cytosine                              | 2.5              |                 | (Ipata and Ceregnani, 1978) |
| Aspergillus fumigatus    | 48                   | 7.0         |              | 37                       | Cytosine                              | 0.08             |                 | (El-Sayed et al., 2021) |

Figure 4. Amino acid alignment of CDA from various prokaryotes and eukaryotes, namely E. coli, S. cerevisiae, A. nitrofafulns, N. seriolsae, S. enterica, Gloecapsa sp. S. laurentii, A. tarcous, A. thermomutatans, A. nomiae, A. parasitica, A. oryzae, A. melleea, A. niger, A. fischer, A. bombycis, A. udagawae, A. vadensis, A. lentulus, P. digitatum, A. pauloenensis, F. pedrosi, A. tamwei, A. luchuensis and P. roqueforti. The conserved residues are shown by colored asterisks. The homologue residues were shown with dots and colons.
stability of the enzymes (Varland et al., 2015). Methionine amino-peptidases is the universal initiator of methionine excision followed by a plethora of predicted modification process especially acetylation, propionylation, palmitoylation and ubiquitylation (Kerwar et al., 1971; Stock et al., 1987; Tooley and Tooley, 2014). The N-terminal domains are mainly related to the in vivo conformational and structural stability of proteins (Hwang et al., 2010; Tasaki et al., 2012; Kim et al., 2014). The fluctuation on the conformational structure and catalytic efficiencies of CDA from both bacterial and fungal sources might be attributed to the variation on the enzyme amino acid constitutions and conformational structures. The bacterial and fungal CDs are distinct from each other and have evolved separately. The 426-residue hexameric CDA had a similarity to adenosine deaminase that belongs to E. coli each other and have evolved separately. The 426-residue hexameric CDA had a similarity to adenosine deaminase that belongs to E. coli and Clade II E. coli CDA.

1.3. Programmability of CDA for mediating the conversion of prodrug 5-FC into drug 5-FU

The development of drug-resistant metabolic criteria to the current chemotherapeutic drugs is one of the main clinical limitations that halt this strategy, thus, the enzyme prodrug mediated therapy is one of the most recent emerged targeted/directed strategies that minimize the off-target effects of the traditional approaches. Cytosine deaminase-5-FC system is one of the remarkable recent approaches for targeting specific metabolic pathways in tumor cells. However, the enzyme catalytic efficiency and localization of the enzyme is the major challenge to the practical application of this system (Kogelberg et al., 2007; Sharma and Bagshawe, 2017).

The non-specificity of cytotoxic agents and their effect on regeneration of normal cells is the major side-effect for most of traditional therapeutic approaches, thus restricting the drug to the tumor cells and decreasing the required dosage is major objective. Thus, generating an active cytotoxic drug from a non-toxic precursor only within or in close proximity to tumor is the main prospective. Localizing the effect of the cytotoxic drugs on sites of tumor cells by prodrug mediated CDA for minimizing the off-target effects of this system is the challenge for biotechnologist. Several strategies have been proposed for directing and localizing the system of CDA-5-FC towards the tumor cells namely; 1-Antibody-directed enzyme prodrug therapy (ADEPT) in which the active enzyme can be delivered to the tumors cells via antibodies specific to tumor cells. 2- Gene-directed enzyme prodrug therapy (GDEPT), and Virus-directed enzyme prodrug therapy (VDEPT), in which the CDA coding genes was delivered to the target tumored tissues, followed by expression of the genes, subsequently the prodrug was provided, that sequentially activated the prodrug into active drug in the target tissues (Portsmouth et al., 2007; Tietze and Schmuck, 2011). There is a promising strategy in gene therapy, in which the combination between gene coding enzyme and a prodrug was occurred, the gene was delivered following by their prodrugs. Additionally, expression of the CDA Suicide gene in vivo was reported in the glioma tumor cell inducing DNA damage after addition of 5-FC, resulted in suppression of glioma cell proliferation (Chang et al., 2020). Implantation of the purified bacterial and fungal CDA in combination with 5-FC for prodrug mediating therapy of various tumor cells were frequently occurred (Michi et al., 2009). CDA is a non-human enzyme (Nishiyama et al., 1985; Katsuragi et al., 1987), however, the human cells possess cytidine deaminase which has the potency to convert 5-FC to the toxic form 5-FU (Hayden et al., 1998), through pyrimidine salvage pathway, that has been considered as the major drawback of this prodrug system. Due to the previous characters, CDA has been in suicide gene therapy (SGT) (Michi et al., 2009). Bystander effect is one of the main practical features of the prodrug mediated therapy, which is an essential factor in gene-prodrug system (Kuriyama et al., 1999) by dissemination to all the surrounding cancer cells. Several in vitro studies using CDA-5-FC, reporting the induction of bystander effect can be achieved by this system in vitro and in vivo (Huber et al., 1994; Kuriyama et al., 1995; Dong et al., 1996). The bystander effect is an important factor in success of suicide gene therapy because by which the cytotoxic effect can diffuse from cells (transduced) to neighboring cells (non-transduced) as shown in Figure 6. Due to the small size of the activated drugs, it can diffuse by
concentration gradient towards the tumor cell, using this phenomena even if less than 10% of cells were transduce, it will be sufficient to influence on the cancer cells (Ardiani et al., 2012; Xiao et al., 2013). So, the spreading of toxic metabolites from cell (transduced) to cells (non-transduced) by active or passive diffusion is the main mechanism for the bystander effect (Karjoo et al., 2016).

1.4. Antibody-directed enzyme prodrug therapy (ADEPT)

Localizing the cytotoxic drug to the site of tumor cells by prodrug-enzyme mediated therapy, implementing the antibody-directed enzyme therapy approach, is the talented technology for minimizing the off-target side effects of the common chemotherapeutic drugs (Kogelberg et al., 2007; Sharma and Bagshawe, 2017). The main objective of ADEPT technology is to restrict the activity of drug to only the tumor sites, utilizing lower dosage of the drug, and avoiding the drug natural clearance, and thus increasing the therapeutic potential of drugs. The inadequate tumor specificity of cytotoxic agents by affecting on the normal cell renewal tissues is the key challenge for various traditional therapeutic approaches, thus restricting the drug to the tumor tissues cells and decreasing the required dosage. This can be attained by generating an active cytotoxic drug from a non-toxic precursor only within or in close proximity to tumor cells, thus guaranteeing that the active drug does not reach normal cell renewal tissues, via the plasma compartment, in dose limiting amounts. During the past 40 years, since the initiation of monoclonal technology, several trials have been proposed to improve the selectivity of cytotoxic drugs by conjugation with antibodies directed to tumor associated antigens (Köhler and Milstein, 1975; Baldwin and Byers, 1986). Similarly, biotoxins like diphtheria toxin and ricin (Moolten and Cooperband, 1970) requires internalization to be cytotoxic that usually directed by the target antibody. The concept of generating an active drug from inactive prodrug necessitate a catalyst, which should be non-human enzyme, completely absent in normal cells. The major challenge of this technology is the spontaneous activation of prodrugs (Paul et al., 1977) (Connors, 1978), in both tumor and normal cells due to the presence of the same enzymes or even isoenzymes with a catalytic efficiency on this prodrug. The concept of the Antibody Directed Enzyme Prodrug Therapy (ADEPT) approach had firstly reported by Philpott et al., (1973), with the main objective to selectively deliver the enzyme to only the cancerous sites without effecting on normal cells. The principle of ADEPT is 1) targeting the enzyme to the tumors by attaching it to antibody directed to a tumor associated antigen, 2) incorporation of the non-toxic prodrug, after clearance of the enzyme from the blood (Figure 6). The targeted enzyme converts the non-toxic prodrug into a potent cell killing drug within tumors to achieve their effective therapy without toxicity to the normal tissue. The principle of ADEPT is the conjugation of enzymes with the monoclonal antibodies that in turn binds to the in vivo tumor associated antigens, followed by subsequent addition of the prodrug. The active generated drug should reach tumor cells in lethal concentration without diffusing out to the normal tissues. To confine the localization of the cytotoxic agent to tumor sites, an effective conjugation of the antibody-enzyme conjugate (Ab-E) with the tumor sites other than normal sites, should be designed, prior administration of the prodrug. The phases of therapeutic efficiency of ADEPT could be categorized as follows: Initial phase in which a high concentration of Ab-E was applied for attaining the maximum binding of antibody with the target tumor sites (Rogers et al., 1986; Jain and Baxter, 1988; Yu et al., 1991). Clearance phase was to ensure the complete clearance of Ab-E, or inactivation of the enzyme in plasma and other normal tissues before the prodrug usage, that is necessary to minimize the prodrug activation at non-tumor sites and consequential toxicity. This involves inactivation and removal of enzyme from the blood and other non-tumor sites prior to prodrug administration, for avoiding the toxicity to normal tissue. To achieve this, numerous novel clearing agents in the form of glycosylated peptidomimetic molecules have been created, with the potency to bind with the enzymes active sites resulting in specific inhibition of the enzyme and subsequent clearance of the complex via the asialo-glycoprotein receptor (Bagshawe et al., 1994). The last phase is the addition of the prodrug. Although, the promising therapeutic potency of the ADEPT approach for minimizing the side effects of drug on the normal cells, the antigenicity of Ab-E, is the major challenge from the practical view.

Overall, the efficiency of the ADEPT depends on the following; 1- target antigen, 2- Choice of enzyme, and 3- Choice of antibody. Firstly, the target antigens should be localized on the surface of tumor cells “plasma membrane” to be more accessible sites for binding with the antibody component of Ab-E (Mason and Williams, 1980). The internalization of the Ab-E on the target cells authenticates the activation of the prodrug only on tumor cells. The target antigens might be a membrane bound or secreted antigens as targets for the antibody based therapy. Membrane bound antigens might be expected to confers longer dwell times for Ab-E than secreted antigens, while, the antigen density might be greater with secreted antigens (Gordon et al., 1982). Secreted antigens are usually present in the plasma, thus interference with the Ab-E might be occurred out of tumor cells (Begent et al., 1980). Thus, selecting of specific antigen for each type of cancer cells might be the proper approach to minimize the off-target effect of antibody. Overexpressed gene products with an external domain on the cell membrane (Park et al., 1992), may be a viable targets, in addition to the growth factor receptors (Perez-Soler et al., 1992). Thus, heterogeneity in the antigen distribution within the population of cancer cells, and the potential to overcome this challenge by specific monoclonal and polyclonal antibodies has been extensively considered (Bagshawe, 1987; Senter et al., 1988).

Secondly, choice of enzyme, the potential enzymes for this approach should be characterized by unique biochemical properties. For example, the optimum enzymatic activity should be close to the pH of tumor extracellular matrix. The catalytic efficiency and affinity of the enzyme...
should be very high to minimize the required enzyme with higher affinity to the target prodrug substrate. Additionally, the enzyme dependence on cofactor and coenzyme should be minimized to reduce the administered components. Several enzymes have been used for mediating the prodrugs by ADEPT technology as listed in Table 3. For example, carboxypeptidase G2 (CPG2) is a non-human enzyme, catalyze of the activation of pro-methotrexate anticancer prodrug (Sherwood et al., 1985), this enzyme has been cloned from Pseudomonas sp, nevertheless, the enzyme antigenicity was the major disadvantage that limits its application. Additionally, presence of isoenzyme homologs in human tissues and plasma, similar to the applied target enzyme, is one of the major tackles that halt the progress on this technology. In terms of specificity, enzymes from non-human sources appear more affordable and their immunogenicity might be controllable via conjugation with natural innate biocompatible polymers such as polyethylene glycol (El-Sayed et al., 2014, 2015, El-Baz et al., 2018, 2019; El-Sayed and El-Sayed, 2020a, El-Sayed and El-Sayed, 2020b). Thirdly, choice of antibody, the first available monoclonal antibodies (W14 and SB 10) to human chorionic gonadotrophin (PHCG) and A5B7 to CEA had been reported (Bagshawe et al., 1994). Several antibodies have been used in ADEPT including human carcinoma associated antigens (Senter et al., 1988), lymphoma (Senter et al., 1988), ovarian carcinoma (Bagshawe et al., 1994), placental alkaline phosphatase (Neuberger et al., 1984) and melanoma (Neuberger et al., 1984). High affinity monoclonal antibodies directed at tumor associated antigens appear to be essential for the ADEPT approach. Bivalent antibodies may be better than univalent in terms of dwell time. The small molecules are usually penetrates tumor cells easily, with desirable rapid blood clearance Jain and Baxter, 1988; Larson (1990); Yu et al., 1991). Conjugates of the enzyme-antibody were prepared by thiolytating the amino groups of antibody fragment with 5-5-acetylthioglycolic acid N-hydroxy-succinimide ester and coupling via physiologically stable thioether bond to the enzyme maleimide groups (Searle et al., 1986; Melton et al., 1993).

### 1.5. Gene-directed enzyme prodrug therapy (GDEPT)

The strategy of gene-directed enzyme prodrug therapy (GDEPT) had been proposed to overcome the challenges and side effects of the traditional chemotherapy (Karjoo et al., 2013; Yuan and Baxter, 1991). The mechanism of selective targeting the uracil pathway in tumor cells than normal cells was firstly explored in 1957 (Heidelberger et al., 1957). In 1970s, a new era of targeted therapy has been emerged with the appearance and conjugation of monoclonal antibodies with cytotoxic compounds (Chabner and Roberts, 2005; DeVita and Chu, 2008, Eastman and Perez, 2006). GDEPT is one of novel strategies in cancer therapy by selective transferring of target gene that catalyzes the production of toxic drug from a nontoxic prodrug (Sprnger and Niculescu-Duvaz, 2000; Portsmouth et al., 2007) (Figure 6). The GDEPT strategy involves two steps, 1-Transduction of gene coding the enzyme into the tumor cells, 2- Mediating the nontoxic prodrug to be converted into toxic metabolites resulted in cell death (Aghi et al., 2000; Hajri et al., 2004; Portsmouth et al., 2007). This approach pledge the efficiency of anticancer drug and decreases its side effects on normal cell that, with potential increase to the therapeutic indexes over the conventional strategies “radiotherapy or chemotherapy” (Karjoo et al., 2013). This strategy provides two unique advantages over the conventional ones, firstly, the suicide gene expression is mainly depends on a specific promoter in the tumor cell, so the expression of this gene can be selectively done in only the tumor cell, but not in normal cells (Saukkonen and Hemminki, 2004; Dorer and Nettelbeck, 2009). This character give the chance to the prodrug to be activated only in the cancerous cell reducing the possibility of off-target toxicity (Yao et al., 2011), leading to a higher concentration of the toxic compound in the target tumor cell over the normal cells (Saukkonen and Hemminki, 2004). Secondly, bystander effect is one of the most potent features that confirms the success of GDEPT over other conventional strategies, allowing the diffusion of cytotoxic effect of drug from the transduced tumor cell to the other

| Enzyme                        | Prodrugs                  | Drugs                  | Action                                                                 | Bystander Effect | References                                      |
|-------------------------------|---------------------------|------------------------|------------------------------------------------------------------------|------------------|-------------------------------------------------|
| Cytosine deaminase            | 5-Fluorocytosine (5-FC)   | 5-Fluorouracil (5-FU)  | Inhibit thymidylate synthetase So, blocks the synthesis of both DNA and RNA, usually affect the dividing cells but at high concentration inhibit both dividing and non-dividing cells. | High             | Hanna et al., 1997                              |
|                               |                           |                        |                                                                        |                  | Johnson et al., 2011                            |
| Purine nucleoside phosphorlylase | 6-Methylpurine deoxyribose | 6-methylpurine         | Inhibits all of DNA, RNA and protein synthesis                        | High             | Lockett et al., 1997                            |
|                               |                           |                        |                                                                        |                  | Martiniello-wilks et al., 1998                   |
|                               |                           |                        |                                                                        |                  | Mohr et al., 2000                               |
| Nitroreductase                | CB1954 and analogs        | 5-(Aziridin 1-yl) 4- 6-nitrobenzamid | Cross linker agent in interstrand of DNA effect on both dividing and non-dividing cells | Very high        | Jabripour et al., 2010                          |
| Herpes simplex virus          | Ganciclovir (GCV)         | Ganciclovir monophosphate (GCV-TP) | Metabolized to triphosphate nucleotide; prevent DNA synthesis by inhibiting DNA polymerase | High             | Moolten, 1986                                   |
| thymidine kinase              |                           |                        |                                                                        |                  | Nicolaus et al., 2003                           |
|                               |                           |                        |                                                                        |                  | Chen et al., 1997                               |
|                               |                           |                        |                                                                        |                  | Chen and Wexman, 2005                           |
| Cytochrome P450                | Oxazaphosphorines:        | 4-Hydroxycyclophosphamide | Cross linker agent in interstrand of DNA                             | Medium           | Chen et al., 1997                               |
|                               | cyclophosphamide          |                        |                                                                        |                  | Chen and Wexman, 2005                           |
|                               |                           |                        |                                                                        |                  | Mohr et al., 2000                               |
|                               |                           |                        |                                                                        |                  | Herz et al., 2007                               |
| Carboxypeptidase G2           | Nitrogen mustard CMDA     | Phenol-bis-ido nitrogen Mustard CMBA | Cross linker agent in interstrand of DNA                             | High             | Herz et al., 2007                               |
|                               |                           |                        |                                                                        |                  | Wierdl et al., 2008                             |
| Carboxylesterase              | Irinotecan (CPT11)        | SN38 (camptothecin)    | Binds to DNA topoisoensemase I so, breaks DNA into single strands.     |                  |                                                 |
neighboring non transduced cells by passive or active diffusion (Karjoo et al., 2013, 2016).

In GDEPT, the enzymes can be classified into two groups: (a) exogenous group; which usually originates from microorganisms as bacteria or viruses and has no counterpart in human, like cytosine deaminase and thymidine kinase, and (b) endogenous group; which can be found in human normal cell like cytochrome P450 (Niculescu-Duvaz and Springer, 2005; Portsmouth et al., 2007). Opposite to the second set, the first set might be likely to be immunogenic with time (Shalev et al., 2000; Karjoo et al., 2013). Despite the second set is less to be immunogenic, it may activate the prodrug in normal cells, producing a lot of side effects (Niculescu-Duvaz and Springer, 2005). The efficiency of suicide gene (transduced gene)/prodrug strategy mainly depends on activity of the transduced gene/enzyme. It should be absent in human, non-toxic to the normal cell, displaying a high catalytic efficiency towards prodrug at low concentration (low $K_m$ and high $K_{cat}$), smaller molecular mass to be more easily expressible in the expression vectors with higher ability to fully activate the prodrug independent on other factors. Prodrug should be capable of penetrating the cancerous cell and diffuse through it, easily to be activated by the enzyme with powerful bystander effect with long half-life time (Lammers et al., 2012). Generally the systems of gene delivery could be microorganisms such as virus, bacteria and yeast, or dendritic or stem cells (Mohit and Rafati, 2013) and synthetic vectors (polymeric and lipid based) (Helen et al., 2010; Karjoo et al., 2016). Several enzyme prodrug systems have been used in GDEPT are summarized in Table 3 (Karjoo et al., 2013, 2016). Vaccinia virus with the prodrug activator gene, might be used as a tool for gene delivery, for augmenting the antitumor efficiency of target prodrug, combined with the effect of vaccinia virus and chemotherapy together (Ding et al., 2020). 5-FC is an orally bioavailable FDA approved antifungal drug with efficiency to crosses the blood-brain barrier (Takahashi et al., 2014). CDA armed Vaccinia virus in conjugation with subsequent 5-FC, provides a direct toxicity killing of tumor cells by local production of 5-FU. Vaccinia virus induced a local and systemic immunotherapeutic response resulting in long-term survival after cessation of 5-FC treatment (Yagiz et al., 2016; Hiraoaka et al., 2017). Yeast CDA gene has been recently cloned and overexpressed in Vaccinia virus VG9, displaying a higher activity in conversion of non-toxic prodrug 5-FC into toxic drug 5-FU (Ding et al., 2020).

1.6. Rationality of CDA/5-fluorocytosine system

The 5-fluorouracil drug had been extensively used in treatment of various types of cancer cells, however, their cytotoxicity to non-cancerous tissues, undesirable effects includes diarrhea, and toxicity to cardiac tissues (Papanastasopoulos and Stebbing, 2014) are the main limiting factors for broad-range application of this drug. The antimetabolite 5-fluorouracil has been produced by the conversion of non-toxic prodrug 5-fluorocytosine by the action of bacterial or fungal CDA, which is a non-human enzyme (Duarte et al., 2012). The drug 5-FU has been characterized by a small size that enables it to diffuse rapidly into/out the neighboring cells resulting in a bystander effect (E. Kievit et al., 2000). The intracellular enzymes convert 5-FU to different metabolites, which finally inhibits thymidylate synthase and causing cell death (Figure 2). Because 5-FC can diffuse through blood brain barrier, this advantageous property can be used in treating tumors such as glioblastoma cancer (Ostertag et al., 2012). 5-FU is a radiosensitizer chemotherapy agent, that can be used in the same time with ionizing radiation in vivo and in vitro which can enhance tumor killing activity (Hanna et al., 1997; Kaliberov and Buchsbaum 2012). However, CDA/5-FC system has a few drawbacks, for example gut normal flora could metabolize 5-fluorocytosine and produce 5-fluorouracil causing some undesirable effects (Karjoo et al., 2016). Bacterial CDA from E. coli displaying an efficient activity towards various tumor cells, however, CDA from yeast displayed a feasible kinetic properties for 5-FC (Johnson et al., 2011). Unfortunately, yeast CDA had a lower thermal stability, thus, a mutant of yeast CDA was created with new features like structural/thermal stability with a higher potency of cancer cells to sense 5-FC treatment comparing to normal yeast CDA (Korkegian et al., 2005; Stolworthy et al., 2008). A new recombinant oncolytic herpes simplex virus type 1 (oHSV-1) armed with E. coli CDA in combination with the prodrug 5-FC was constructed, displayed a strong efficacy against melanoma cell lines (Houtzagers et al., 2020; Liu et al., 2020). Also, combination of CDA and the prodrug 5-FU loaded on chitosan-silver nanoparticle displayed a strong activity in treatment of human breast carcinoma cell line and other solid tumors (Horo et al., 2020). Because of its ability to convert the relatively nontoxic 5-fluorocytosine (5-FC) into 5-FU and its absence in mammalian cells, CD has become an attractive candidate for the reduction of 5-FU toxicity toward normal distal tissues in enzyme-prodrug gene therapy (Greco and Dachs, 2001). Several studies demonstrated that yeast CD significantly had a higher therapeutic efficacy, than E. coli CDA, that might be due to the higher efficiency of the 5-FC into 5-FU by the yeast CD (Kievit et al., 1999; Kievit et al., 2000). Yeast CD, therefore, appears to be a better candidate for gene therapy.

1.7. Protein modification by cross-linking with immunogenically innate polymers

Several approaches have been explored to improve the kinetic properties and stabilizing the catalytic structural orientation of enzymes for in vivo applications. The specificity and selectivity, lower toxicity to normal tissues, solubility formulation, and delivery route to the target tissues, optimum dose and immunogenicity are the common challenges (El-Sayed et al., 2015a; 2015b; 2015c, 2016, 2017, 2020). So, formulation of proteins by cross linking/conjugation to polymers to enhance its activity and pharmacokinetics properties has been recognized as one of the most applicable approach (Pasut, 2014). Several naturally innate polymers such as polyethylene glycol, chitosan, and dextran have been implanted for conjugation of therapeutic enzymes for improving their therapeutic potency such as structural stability, catalytic efficiency, protection from the in vivo proteolytic cleavage, increasing the half-life times of the target drugs (El-Sayed et al., 2014, 2015, 2016, 2017, 2018, 2019). Conjugation of enzymes with polyethylene glycol (PEGylation) strongly improves the enzyme pharmacokinetic properties, reducing antigenicity, and stabilizing the conformational structures of enzymes (Zhang et al., 2015; El-Baz et al., 2011a,b, El-Baz et al., 2018). Dextran has been frequently authenticated as non-immunogenic, biocompatible, biodegradable compound, and stabilizer for the conformational structures of various therapeutic enzymes (El-Sayed et al., 2014, 2016; Badr et al., 2021; Raafat et al., 2021). Dextran is a natural polysaccharide of about 95% glycosidic linkages at position α-1,6 and 5% at 1,3-linkages that has been produced by Leuconostoc mesenteroides and Streptobacterium dextranicum (Varshosaz, 2012). The presence of 95% linear linkages in dextran polymer makes it a water soluble molecule and hence, enzyme conjugation with dextran increase the water hydrophilic identity of the enzyme in addition to shielding the antigenic sites of the enzymes, thus, protecting the enzymes antigenic reactions that in turn increases the half-life of enzymes in vivo (Murakami et al., 2003; Vertommen et al., 2005; El-Baz et al., 2017, El-Ghareeb et al., 2012). The unique chemical features of dextran especially higher hydrophilicity, frequency of glycosidic bonds which increases the higher stability in alkaline and acidic environments, protecting the conjugated enzyme from biodegradation, allow this polymer to be the optimum carrier in drugs delivery systems (Varshosaz, 2012). Several studies confirming the efficiency of dextran as successful carrier in delivery of methotrexate (Dang et al., 1994), 5-fluorouracil (Hao et al., 2006), L-arginine deiminase, L-methionine γ-lyase, peptide γ-L-arginine deiminase, cystathionine γ-lyase, homocysteine γ-lyase and arginase (El-Sayed et al., 2014, 2015a, b, c, d; 2017, 2018, 2019, 2020).
1.8. Future prospective of therapeutic potency of cytosine deaminase

Combinatorial usage of CDA with the prodrug 5-fluorocytosine as an efficient strategy for cancer therapy seems to be the promising approach. However, enzyme antigenicity, targetability, and side effects are the most challenges that limits the auxiliary applications of this technology (Springer and Nicoleuscu-Duvaz, 2000; Khan et al., 2017). Thus, enzymes with higher stability, catalytic affinity to the prodrug, less antigenicity being the most feasible objective for successfulness of this approach (Encell et al., 1999; Patel et al., 2016). Screening for CDA from novel fungal isolates with unique biochemical properties might be one of the most practical ways for improving the efficiency of this approach. Fungi have been considered as a repertoire for novel enzyme with higher human compatibility.

2. Conclusion

Cytosine deaminase (CDA) mediating the conversion of non-toxic prodrug 5-fluorocytosine (5-FC) into toxic drug 5-fluorouracil (5-FU) has been recognized as a powerful cancer-therapeutic approach comparing to the traditional chemotherapies and radiotherapies. 5-FU has been recognized as a powerful cancer-therapeutic approach (Betts et al., 1994). Biotechnol. Rep. 30, e00623. Screening for CDA from novel fungal isolates with unique biochemical properties might be one of the most practical ways for improving the efficiency of this approach. Fungi have been considered as a repertoire for novel enzyme with higher human compatibility.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

The work was supported by the Egyptian Academy of Scientific Research and Technology (ASRT-2022).

Data availability statement

Data included in article supplementary material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abdel-Fatah, S.S., El-Bata, A.I., El-Sherbiny, G.M., Khalaf, M.A., El-Sayed, A.S., 2021. Production, bioprocess optimization and γ-irradiation of Pencillium polonicum, as a new Taxol producing endophyte from Ginkgo biloba. Biotechnol. Rep. 30, e00625.

Aghi, M., Hochberg, F., Breakefield, X.O., 2000. Prodrug activation enzymes in cancer gene therapy. J. Gene Med. 2 (3), 148–164.

Ali, A., Knealian, D., El-Sayed, A.S., 2020. Soluble and volatile metabolites of plant growth promoting rhizobacteria (PGPRs): role and practical applications in inhibiting pathogens and activating induced systemic resistance (ISR). Adv. Bot. Res. 75, 123–152.

Artioli, A., Johnson, A., Ruan, H., Sanchez-Bonilla, M., Serve, K.E., Black, M., 2012. Enzymes to die for: exploiting nucleotide metabolizing enzymes for cancer gene therapy. Curr. Gene Ther. 12 (2), 77–91.

Arlt, G., Tabernero, J., Labianca, R., Hochhausen, D., Salazar, R., Iveson, T., Arnold, D., 2020. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 31 (10), 1291–1305.

Badr, H., El-Baz, A., Mohamed, I., Shetaia, Y., El-Sayed, A.S.A., Sorour, N., 2021. Bioprocess optimization of glutathione production by Saccharomyces boulardii: biochemical characterization of glutathione peroxidase. Arch. Microbiol. 203, 6183–6196.

Bagshawe, K.D., 1987. Antibody directed enzymes revive anti-cancer prodrugs concept. Br. J. Cancer 56 (5), 531–532.

Bagshawe, K.D., Sharma, S.K., Springer, C.J., Rogers, G.T., 1994. Antibody directed prodrug therapy (ADEPT). Ann. Oncol. 5 (10), 879–891.

Batemann, A., Birney, E., Cerruti, L., Durbin, R., Eddy, S.R., Sonnhammer, E.L.E., 2002. The pfam protein families database. Nucleic Acids Res. 30, 276–280.

Beck, C.F., Ingraham, J.L., Neuhard, J., Thomassen, E., 1972. Metabolism of pyrimidines and pyrimidine nucleotides by Salmonella typhimurium. J. Bacteriol. 110, 219–228.

Begroth, R.H.J., Searle, F., Stanway, G., Lewkes, R.F., Jones, B.E., Vernon, P., Bagshawe, K.D., 1980. Radioimmunolocalization of tumors by external scintigraphy after administration of 131I antibody to human chorionic gonadotrophin: preliminary communication. J. R. Soc. Med. 73 (9), 624–630.

Bett, L., Xiang, S., Short, S.A., Carter, C.W., 1994. Cytidine deaminase. J. Microbiol. 203, 6183–6196.

Chabner, B.A., Roberts, T.G., 2005. Chemotherapy and the war on cancer. Nat. Rev. Drug Discov. 4, 65–72.

Chakraborty, K.P., Loring, H.S., 1960. Incorporation of uracil-2-C14 into the nucleic acids of Neurospora crassa. J. Biol. Chem. 235 (7), 2122–2126.

Chang, D.-Y., Jung, J.-H., Kim, A.A., Marastini, S., Lee, Y.-J., Park, S.H., Suk-Kim, H., 2020. Combined effects of mesenchymal stem cells carrying cytosine deaminase gene with 5-fluorocytosine and temozolomide in orthotopic glioma model. American J. Cancer Res 6 (10), 1429–1441.

Charles, H., Heidelfinker, N.K., Chaudhuri, P., Danneberg, D.M., 1957. Fluorinated purine analogues, a new class of tumour-inhibitory compounds. Nature 179, 663–666.

Chen, L., Waxman, D., 2005. Cytochrome P450 gene-directed enzyme prodrug therapy (GDEPT) to cancer. Curr. Pharm. Des. 12 (25), 3825–3840.

Dhaun, Y., Ye, Z., Zhao, Z., Zhou, W., Zhou, J., Wu, C., Zhao, K., 2019. Comparing paclitaxel plus fluorouracil versus cisplatin plus fluorouracil in chemoradiotherapy for locally advanced esophageal squamous cell cancer: a randomized, multicenter, phase III clinical trial. J. Clin. Oncol. 37, 1695–1703.

Chevalier, M.R., 1962. Cloning and transcriptional control of a eucaryotic pericentric gene. Mol. Cell. Biol. 2 (8), 977–984.

Cho, H.-J., Kim, S.-S., 1981. Chemical modification of Soraria macrocera acetolactate synthase with cys, trp, and arg modifying reagents. BMB Reports 28, 40–45.

Choy, W.H., T.A. Biochimie 60 (9), 897–907.

Danesneshmrd, T.K., Warwack, D.W., 1983. Clinical pharmacokinetics of systemic antifungal drugs. Clin. Pharmacokin. 8 (1), 17–42.

Dang, W., Colvin, O.M., Brem, H., Salzman, W.M., 1994. Coupling vectorial methotrexate to dextran enhances the penetration of cytotoxicity into a tissue-like matrix. Cancer Res. 54 (7), 1729–1735.

Daniele, S., Khiar, M., Barbata, K., Jochimsen, B., Neuhard, J., 1992. Characterization of the Escherichia coli codA operon encoding cytosine peroxidase and cytosine deaminase. Mol. Microbiol. 6 (10), 1335–1344.

DeVita, V.T., Chu, E., 2008. A history of cancer chemotherapy. Cancer Res. 68 (21), 8643–8653.

Diato, R.B., Lakings, D.E., Bennett, J.E., 1978. Evidence for conversion of 5-fluorocytosine to 5-fluorouracil in human: possible factor in 5-fluorocytosine clinical toxicity. Antimicrob. Agents Chemother. 14, 903–908.

Ding, Y., Ding, Y., Fan, J., Fan, J., Deng, L., Deng, L., Zhou, B., 2020. Antitumor efficacy of cytosine deaminase–armed vaccinia virus plus 5-fluorocytosine in colorectal cancer. Cancer Cell Int. 20 (1), 1–132.

Dong, Y., Wen, P., Manome, Y., Parr, M., Hirshowit, A., Chen, L., Hirschowitz, E.A., Crystal, R., Weichselbaum, R., Kufe, D.W., Fine, J.K., 1996. In vivo replication-deficient adenovirus vector-mediated transduction of the cytosine deaminase gene sensitizes glioma cells to 5-fluorocytosine. Hum. Gene Ther. 7, 715–720.

Dorer, D.E., Nettelbeck, D.M., 2009. Targeting cancer by transcriptional control in cancer gene therapy and viral oncolysis. Adv. Drug Deliv. Rev. 61, 554–571.

Drazao, R.B., Bennett, J.E., Myers, C.E., 1978. Mode of action of 5-fluorocytosine. Biochem. Pharmacol. 27, 763–770.

Duret, S., Carle, G., Faneca, H., Lima, M. C. P. de, Pierre-Feunteune, C., 2012. Suicide gene therapy in cancer: where do we stand now? Cancer Lett. 324 (2), 160–170.

Eastman, A., Perez, R.P., 2006. New targets and challenges in the molecular therapeutics of cancer. Br. J. Clin. Pharmacol. 62 (1), 5–14.

El Sayed, M.T., El-Sayed, A.S.A., 2020a. Biocidal activity of metal nanoparticles synthesized by Fusarium solani against multidrug-resistant bacteria and mycotoxicogenic fungi. J. Microbiol. Biotechnol. 30, 226–236.

El Sayed, M.T., El-Sayed, A.S.A., 2020b. Tolerance and mycoremediation of silver ions by Punicaria solani. Heliyon 6, e05866.
Kuriyama, S., Kikukawa, M., Matui, K., Okuda, H., Nakatani, T., Sakamoto, T., Tujii, T., 1999. Cytosine deaminase/5-fluorocytosine gene therapy can induce efficient anti-tumor effects and protect immunity in immunocompetent mice but not in athymic nude mice. Int. J. Cancer 81, 592–597.

Larson, S.M., 1990. Improved tumor targeting with radiolabeled, recombinant, single-chain, antigen-binding protein. J. Natl. Cancer Inst. 82, 1173–1174.

Liu, S., Zhang, J., Fang, S., Su, X., Zhang, Q., Zhu, G., Liu, F., 2020. Antimurine efficacy of oncolytic HSV-1 expressing cytosine deaminase is synergistically enhanced by DDP down-regulation and EMF inhibition in uveal melanoma xenograft. Cancer Lett. 497, 123–134.

Lockett, L.J., Molloy, P.L., Russell, P.J.B.G., 1997. Relative efficiency of tumor cell killing in vitro by two enzyme-prodrug systems delivered by identical adenovirus vectors. Clin. Cancer Res. 3 (11), 2075–2080.

Longley, D.B., Harkin, D.P., Johnston, P.G., 2003. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat. Rev. Cancer 3 (5), 330–338.

Maamoun, H.S., Rabie, G.H., Shaker, I., Alaidaroos, B.A., El-Sayed, A.S.A., 2021. New agents for prostatic cancer activated specifically by 5-fluorocytosine. Cancer Res. 81 (2), 1753–1761.

Nicholas, T.W., Read, S.B., Burrow, F.J., Kruse, C.A., 2003. Suicide gene therapy with adenoviral transfer of the endogenous human purine nucleoside phosphorylase gene. Cancer Res. 63 (4), 1571–1577.

Mullen, C.A., Kilstrup, M., Blaese, R.M., 1992. Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine. Nature 356, 661–664.

Mullen, C.A., 1994. Metabolic suicide genes in gene therapy. Pharmacol. Therapeut. 63, 85–107.

Mullen, C.A., 1991. Suicide gene therapy of hepatocellular carcinoma in vitro and in nude mice by adenoviral transfer of the Escherichia coli purine nucleoside phosphorylase gene. Hepatology 13 (5), 85–91.

Mullen, C.A., 1993. Optimization of small-scale coupling of A5B7 monoclonal antibody to 5-fluorocytosine in combination with adenovirus. Cancer Biother. 336 (4), 266–276.

Mullen, C.A., 2004. Random down-regulation and EMT inhibition in uveal melanoma xenograft. Cancer Lett. 495, 113–122.