Explaining deuterium-depleted water as a cancer therapy: a narrative review

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Deuterium is a natural heavy isotope of hydrogen, containing a neutron and a proton. This gives it distinct biophysical and biochemical properties, compared with hydrogen. Deuterium alters enzymatic activity in significant ways. Human metabolic processes minimize the amount of deuterium in mitochondrial water, because it causes a dysfunction in mitochondrial ATPase pumps, leading to excessive reactive oxygen species (ROS) and loss of ATP production. Mitochondrial dysfunction is a characteristic feature of cancer and many other diseases. Lactate plays an important role in cancer progression, and a central role holds also for vacuolar ATPases (V-ATPases). In the presence of excess deuterium, cancer cells show a remarkably altered metabolic policy, enabling invasion and proliferation. Cancer cells protect their mitochondria from excessive ROS by minimizing the use of ATPase to synthesize ATP. Instead, they rely on glycolysis to supply ATP and support the massive synthesis of lactate, which is excreted into the microenvironment. They also use V-ATPases in an unusual way at the plasma membrane to pump deuterium-depleted protons out of the cell, enriching cytoplasmic deuterium. These complex processes suggest that cancer cells are able to sense deuterium levels in the medium and commit apoptosis when deuterium levels are low or proliferate when they are high. Tumorigenesis involves a metabolic switch that supports increased cellular deuterium levels, decreasing the deuterium burden overall in the organism. Strong clinical evidence supports deuterium-depleted water (DDW) as an anticancer treatment. More investigations on cancer autophagic behavior are needed to guide DDW clinical use. *European Journal of Cancer Prevention* **XXX: XXXX–XXXX Copyright © 2025 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Deuterium (heavy hydrogen) plays a critical role in mitochondrial disease. It interferes with the synthesis of ATP by mitochondrial F1F0-ATP synthase (F-ATPase) and induces the release of high amounts of reactive oxygen species (ROS) (Olgun, 2007). Nicotinamide adenine dinucleotide (NADH) is a critical player in the maintenance of low deuterium in mitochondrial water. Since NADH dehydrogenase directly supplies protons to F-ATPase in mitochondrial respiratory complex I. The proton supplied by NADH is unlikely to be a deuteron, as explained later in this article. Maintenance of the NAD pool in the mitochondria is essential for mitochondrial health, and the ratio of NAD^+ to NADH is tightly regulated (Stein & Imai, 2012; Wikström & Hummer, 2012).

Cancer is a metabolic disease with a drastically altered metabolic policy, supporting rampant proliferation. Through the Warburg effect (increased energy supply obtained through glycolysis), cancer cells suppress oxidative phosphorylation in their mitochondria, while producing large amounts of lactate derived from glycolysis and releasing it into the external environment (Warburg *et al.*, 1927; Potter *et al.*, 2016). By reducing the role of ATPase in energy supply, however, they underutilize their mitochondria for energy supply, protecting them from DNA damage (through increased production of ROS).

During growth, cancer cells induce well above normal activity of the glycolysis pathway. This produces increased amounts of lactate that is excreted into the tumor microenvironment, which usually becomes acidic. Cancer cells need to survive and proliferate under extracellular hypoxic conditions and often under glucose deprivation, and they have developed special adaptations for this (for review see Hu *et al.*, 2017). In order to maintain their basic intracellular pH and survive during hypoxic conditions, however, cancer cells need to intensify proton extrusion (Zheng *et al.*, 2020) and increase their alkaline [e.g. bicarbonate (HCO_3^-)] intake, which they achieve by altered and increased ion channel/transporter operation, for example, SLC4A4, found in most cancers. Altered ion channels under hypoxic conditions have been shown

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experimentally to promote the progression of gastric cancer (Chen *et al*., 2021).

Cancer cell growth is suppressed in a medium containing half the normal deuterium concentration, whereas it is accelerated with double the amount, opposite to the effect of deuterium on normal cells (Zhang *et al*., 2020). This suggests that cancer cells may be able to sense the amount of deuterium in the medium, and, when it is elevated, they are triggered to proliferate through complex signaling mechanisms. An experiment involving nasopharyngeal carcinoma (NPC) cells demonstrated that deuterium-depleted water (DDW) with deuterium concentrations at 100, 75, and 50 ppm suppressed the growth and invasiveness of tumor cells and caused cell cycle arrest. By contrast, DDW promoted the growth of normal control cells (Wang *et al*., 2012). DDW extends the lifespan of cancer patients in multiple studies (Somlyai *et al*., 2016, 2023; Yaglova *et al*., 2023).

Immune cells swarm to the tumor microenvironment, but signaling processes often suppress their ability to attack the tumor (Tie *et al*., 2022). 'Tumor immune escape' is a well-established phenomenon, mediated by programmed death ligand 1 (PD-L1)/PD-1 signaling (Jiang *et al*., 2019). An optimal T-cell response, a hallmark of successful cancer immunotherapies, is essential for efficiently killing cancer cells (Waldman *et al*., 2020). Kovács *et al*. (2022) exposed mice to carcinogens, and then the animals were supplied with DDW at 30 ppm deuterium. DDW treatment prevented tumor development compared with control mice. It is conceivable that immune cells regain mitochondrial health due to DDW, which then enables their antitumor effectiveness.

Here, we investigate the effects of DDW on the metabolic policy of cancer cells. DDW launches a cascade in cancer cells that results in suppressed autophagy and commitment to apoptosis. The effects of DDW treatment on cancer cell cycle arrest are explained through metabolic dependence of cancer on autophagy to discard damaged organelles and continue proliferation. Autophagy is a critical internal recycling process that cancer cells use to survive (Alvarez-Meythaler *et al*., 2020).

Lactate and isomerases

Isomerases are enzymes that convert two isomers back and forth from one to the other, a seemingly futile reaction, but, importantly, this achieves deuterium scrubbing from the isomers. Phosphoglucose isomerase in spinach chloroplasts has a very strong deuterium kinetic isotope effect (KIE), which results in essentially stripping deuterium from C2 of glucose (Maloney *et al*., 2024). The first pathway in glucose metabolism in a human cell is anaerobic glycolysis, which takes place in the cytoplasm and yields pyruvate as the product. Glucose is first converted into two isomeric triose phosphates, and a triosephosphate isomerase converts them back and forth from one to the other while depleting deuterium on the affected carbon atom (Leadlay *et al*., 1976). The enzyme has a high deuterium KIE in both directions and any deuterium that was initially present in glyceraldehyde-3-phosphate (G3P) exchanges with hydrogen in the medium as an intermediary step in the reaction. The repeated cyclic activity of the isomerase eventually nearly completely scrubs deuterium from the carbon atom in G3P that ultimately delivers a proton to NAD^+ via a dehydrogenase (glyceraldehyde-3-phosphate dehydrogenase), in the next step in the pathway. Thus, the proton in the NADH molecule is highly unlikely to be deuterium.

Lactate is produced from pyruvate by reverse activity of the enzyme lactate dehydrogenase (LDH), which transfers a deuterium-depleted (deupleted) proton from NADH to pyruvate. Hence, lactate released from cancer cells carries a low-deuterium proton that becomes very valuable to the recipient cell, which can convert lactate back to pyruvate, supplying a deupleted proton to mitochondrial NAD^+, and, ultimately, to the ATPase pumps via NADH dehydrogenase.

Vacuolar ATPase and microenvironment proton deupletion

Vacuolar ATPase (V-ATPase) plays a critical role in cancer pathology, and it is considered an attractive therapeutic target in cancer (Whitton *et al*., 2018). The two main types of ATPases in biological organisms are F-ATPase and V-ATPase. F-ATPase in the mitochondria synthesizes ATP from ADP while reducing oxygen to metabolic water. V-ATPases have an opposite function. Protons are pumped across a membrane from a proton-sparse medium to a proton-dense medium, and this requires energy, which is supplied by converting ATP to ADP. When localized to the plasma membrane, they pump protons into the extracellular space; whereas, when at the membrane of the endosome/lysosome organelle, they can acidify the endosome to convert it to a lysosome (Perzov *et al*., 2001).

A study evaluating the deuterium KIE of yeast V-ATPase acting at the plasma membrane discovered that the extrusion of H^+ ions was reduced by as much as 90% when the cells were suspended in heavy water. The authors wrote: 'It thus appears that the binding site [in V-ATPase] for protons (or hydronium ions) to be transported does not accept deuterons (or deuteronium ions) with equal ease *or perhaps not at all*' (Kotyk *et al*., 1990). Thus, yeast V-ATPase has an extremely high deuterium KIE. Human breast cancer cells with high metastatic potential overexpress V-ATPase at the plasma membrane, and they use it presumably to transfer deupleted protons into the extracellular space, thus enriching deuterium levels in the cell's cytoplasm and decreasing deuterium levels extracellularly (Sennoune *et al*., 2004).

Vacuolar ATPase, acidification, lactate, and M2 macrophages

Most tumors have a slightly elevated internal pH (7.3–7.7) compared with a low external pH (5.6–7.0), where the polarity is opposite to that of normal cells. Lactate concentrations in the tumor microenvironment can be as high as 40 mmol. The low pH in the tumor microenvironment is caused predominantly not by lactate but by the activities of V-ATPase pumps. The abnormal overexpression and unusual subcellular locations of V-ATPases in tumor cells are associated with a poor prognosis in many types of cancer. Plasma-membrane V-ATPase promotes tumor cell invasion, metastasis, and drug resistance (Chen *et al*., 2022). The release of lactate and acidification of the neighboring microenvironment by tumor cells accompanies metastasis, angiogenesis, and cancer progression (Pérez-Tomás & Pérez-Guillén, 2020).

Lactate is a valuable nutrient to fuel the tricarboxylic acid (TCA) cycle in regulatory T cells and bone-marrow-derived macrophages. LDH expression was found to be essential for $CD8^+$ T cells to promote their cytotoxic activity (Apostolova & Pearce, 2022). LDH results in the synthesis of NADH to support deupleted protons for the ATPase pumps. The lactate anion is well tolerated by $CD8^+$ T cells in pH-neutral conditions (Barbieri *et al*., 2023). When $CD8^+$ T cells were exposed in culture to a pH-neutral environment along with high lactate availability during the T-cell activation phase, the lactate promoted rapid expansion and an enhanced effector profile, increasing their cytotoxic potential (Barbieri *et al*., 2023). Subcutaneous administration of sodium lactate solution at pH 7.4 to tumor-bearing mice leads to improved immune function of $CD8^+$ T cells, by inhibiting histone deacetylases and boosting stem-like properties, resulting in significant suppression of tumor growth. Therefore, it is the low pH of the tumor microenvironment that promotes tumor progression rather than the lactate itself (Feng *et al*., 2022).

Excess lactic acid in the tumor microenvironment, however, indicates immunosuppression (Caslin *et al*., 2021). Lactate facilitates the recruitment of immunosuppressive cells, including tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells. TAMs, the most abundant immune cells in the tumor microenvironment, play a powerful role in reprogramming T cells toward an immunosuppressive state, as well as promoting tumor initiation and progression (Hayes *et al*., 2021). Macrophages can be characterized as being in either an M1 (proinflammatory) or M2 (anti-inflammatory) state. Lactic acid skews macrophages toward an M2 state, particularly under low external pH conditions (Noe *et al*., 2021).

Lactate can induce marked changes in gene expression, in part through histone lactylation (Zhang D *et al*., 2019). In response to a bacterial infection, M0 macrophages differentiate into proinflammatory M1 macrophages, with an associated increase in glycolysis. As time passes, lactate accumulates in the cell, and this leads to histone lactylation, which then induces a transformation into an M2 phenotype, in preparation for wound healing and resolution of the inflammatory state. In TAMs isolated from lung tumors and melanoma cultures, the overexpression of *Arg1*, an M2-associated gene, was linked to lactylated but not acetylated histones. Exogenous lactate (e.g. released by cancer cells) also increased histone lactylation levels in immune cells and induced *Arg1* expression, along with other M2-associated genes (Zhang D *et al*., 2019).

M2 macrophages facilitate tumor metastasis and augment drug resistance (Wang *et al*., 2024). Acidic pH, independent from lactate, can promote the polarization of macrophages toward an M2 state in prostate cancer cells. This promotes tumor survival, including enhanced tumor cell proliferation, loss of macrophage cytotoxicity, and release of angiogenic factors (El-Kenawi *et al*., 2019). Tumor relapse after chemotherapy is a major clinical problem, often involving inoperable metastasis. M2 macrophages accumulate around blood vessels after chemotherapy, promoting tumor revascularization and relapse (Hughes *et al*., 2015). In glioblastoma cells, extracellular vesicles containing lactate dehydrogenase A are released by macrophages recruited to the tumor microenvironment, to promote tumor cell glycolysis, proliferation, and survival (Khan *et al*., 2024).

Both LDH and monocarboxylate transporter 1 (MCT1), the transporter that imports lactate into the cell, show deuterium KIEs that would further support low deuterium levels in the mitochondria (Grimshaw and Cleland, 1980; Geistlinger *et al*., 2023). MCT1 has a significant deuterium KIE, with the uptake of lactate (and protons/deuterons) slowed significantly in D_2O at p^2H 6.8 compared with H_2O at pH 6.8 (Geistlinger *et al*., 2023). A study on the effects of deuterium on the activity of human LDH found a solvent isotope effect of 2.75 for D_2O and a kinetic isotope of 2.74 for NAD^2H instead of NADH, at pH 4.3 (Wang *et al*., 2016).

Deuterium-depleted water therapy as a treatment for cancer

DDW exerts strong antitumor activity (Wang *et al*., 2012; Zhang *et al*., 2020). Determining exactly how DDW suppresses cancer growth, however, has been a challenging task. Human clinical studies identify a potent anticancer activity obtained by DDW treatment (Somlyai *et al*., 2016, 2023; Boros *et al*., 2021; Yaglova *et al*., 2023). Glioblastoma multiforme (GBM) is an aggressive brain tumor with a relatively short median survival time (MST), especially at later onsets of disease (Wu *et al*., 2021). Patients who had sustained surgical excision of a brain tumor consumed wholly DDW as a daily fluid, to reduce deuterium levels

Table 1 A summary of indicative in-vitro and in-vivo deuterium-depleted water treatment effects that hold promise for its use as anticancer treatment

DDW treatment	Form and duration of DDW treatment	Clinical laboratory results	Effect on life span
Cell lines: Nasopharyngeal carcinoma (NPC) cells and normal preosteoblast MC3T3-E1 cells Wang *et al.* (2012) Wang *et al.* (2013)	RPMI1640 cell media contained a range of deuterium concentrations [50 (DDW)[a]-150 ppm].	DDW: cell cycle arrest of NPC cells at G1/S phase. Significantly increased expression NQO1[b], and decreased expression of PCNA and MMP9 in NPC cells.	DDW: inhibition of NPC cell proliferation, colony formation, migration, and invasion. Promotion of growth of normal MC3T3-E1 cells.
Double-blind, randomized phase II placebo control human study in prostate cancer (PC) patients Kovács *et al.* (2011)	Prospective study: 22 PC patients replaced daily water intake with DDW for 4 months and 22 PC patients received normal water. Both groups received conventional therapy for PC. Retrospective study: 91 DDW PC patients were evaluated.	Prospective study: progression of the disease was diagnosed in 4 DDW PC patients and 8 non-DDW PC patients. Significant decrease in prostate volume and reduction of prostate volume increase in the DDW PC group. Similar marked reduction of prostate serum antigen in the DDW group (>50%). Cease in urination complaints. Retrospective study: DDW extended the patient medium survival time (MST).	Prospective study: reduction of death after 3 years with a ratio of 8 DDW/12 non-DDW. Retrospective study: MST was extended to 11.02 years. Decrease of deaths in nonmetastasis patients.
Human clinical study in 129 patients with small cell and non small cell lung cancer (SCLC and NSCLC). Supplementary expression analysis in mouse lungs. Gyöngyi *et al.* (2013)	Humans: patients voluntarily consumed DDW for an extended period of time in addition to conventional chemotherapy and radiotherapy. Animals: DDW group (25 ppm of DDW) instead of tap water for 8 days before sacrifice.	Expansion of MST. Animal study: DDW attenuates the expression of DMBA, Bcl-2, Kras, and myc proto-oncogenes in DDW mouse lung cells.	Relative extension of survival time by consuming DDW, especially in patients with brain metastasis. Extension of 5-year survival in DDW SCLC group (from 6–12% to 25%). Especially for females, the survival expands to 50%. For NSCLC patients, the MST expands to 33.7 months for males and 74.1 months for females (greater than normally observed 15–20 months, respectively)
36 male and 50 female pancreatic adenocarcinoma patients consumed DDW compared with 30 control patients who consumed natural water. Supplementary MIA-PaCa-2 pancreatic cancer cell line DDW treatment analysis. Boros *et al.* (2021)	Humans: adenocarcinoma patients consumed 85 ppm DDW that was gradually decreased to 65–45 ppm DDW for 1–3 months. Control patients consumed natural water. All patients received conventional chemotherapy apart from one. MIA-Paca-2 cells: cancer cells were grown in 50–150 ppm deuterium concentrations in growth medium.	Elongation of MST in DDW-treated pancreatic cancer patients. MIA-PaCa-2 showed an in-vitro growth inhibition either with DDW alone or in combination with cisplatin.	Humans: DDW consuming group had increased MST to 16.9 months compared with 6.36 months of control group that consumed natural water. Cancer cells: decrease in proliferation rate of adenocarcinoma cell line grown in DDW medium.

DMBA, 7,12-dimethylbenz(a)anthracene; MMP9, matrix metalloproteinase 9; NADPH, nicotinamide adenine dinucleotide phosphate; NQO1, NADPH:quinone oxidoreductase-1; PCNA, proliferating cell nuclear antigen.
[a]DDW is equal to or less than 0.0050% or 50 ppm.

in their body. DDW intake was given as an adjuvant therapy to chemotherapy and radiation. DDW extended their MST from 14.6 months to 30 months. DDW was especially effective for female patients, whose MST was increased to 42 months. Moreover, patients starting DDW concurrently with radiation lived for 47 months on average (Somlyai *et al*., 2023).

Pancreatic cancer is an aggressive and invasive cancer (Zhao and Liu, 2020). DDW in combination with conventional chemotherapy in pancreatic cancer patients remarkably increased their MST from 6.36 months to 19.6 months. These authors also conducted *in vitro* experiments where pancreatic cancer cells were grown in DDW. The lower the deuterium concentration the more the pancreatic cancer cell proliferation was inhibited (Boros *et al*., 2021). Deuterium depletion also delayed progression and prolonged MST in prostate cancer patients. DDW intake amount correlated inversely with prostate serum antigen levels. Moreover, DDW treatment significantly prolonged the MST of patients who suffered from distant metastasis (Kovács *et al*., 2011). DDW treatment is a promising anticancer approach as an adjuvant to either chemotherapy and/or radiation therapy, since cancer cells are sensitized to die upon deuterium depletion. DDW is a nontoxic anticancer strategy that has proved its efficacy in animal and human clinical trials (Lu & Chen, 2024). A number of indicative subclinical and clinical findings for DDW use as anticancer treatment are summarized in Table 1.

The role of cell cycle arrest

Deuteration (the exchange of hydrogen with deuterium) of active biomolecule structures involved in

mitochondrial metabolism has long been shown to affect the normal mechanisms of hydrolysis of ATP (Dorgan & Schuster, 1981). In the case of cancer, one of the hallmarks of antitumor activity is to increase the rate of autophagy and/or apoptosis of malignant cells at certain stages of oncogenesis (Mulcahy Levy & Thorburn, 2020).

Autophagy is a catabolic process that sequesters damaged organelles into compartments known as autophagosomes, where they are degraded and removed. Autophagy plays a complex role in cancer cells. The Warburg effect is sustained by autophagy activation. The response to activation of autophagy depends on the stage and microenvironmental conditions. Autophagy often promotes cancer progression and metastasis, and it can facilitate multiple drug resistance (Alvarez-Meythaler *et al*., 2020). Stimulation of autophagy can reduce the toxic effects of deuterium through the turnover of defective mitochondria and heavily deuterated macromolecules. Autophagic mechanisms must be working efficiently to discard defective highly deuterated organelles (Olgun, 2007). Thus, the effects of deuteration can be delayed or prevented by decreasing the intake of deuterium and/or increasing the turnover of organelles and macromolecules by stimulating autophagy.

The application of DDW promotes the senescence of breast cancer cells (Lajos *et al*., 2018). Moreover, experiments on colorectal cancer cells treated with DDW showed similar senescent phenomena, but also revealed a proapoptotic effect, suggesting DDW-related promotion of autophagic and cancer cell death mechanisms (Chira *et al*., 2018). Further experiments on breast cancer cells showed that DDW treatment had a direct growth inhibitory effect, due to a G1/S cell cycle arrest, enhancing the anticancer activity of fluorouracil (5FU) (Yavari & Kooshesh, 2019). Recent experiments showed that DDW induces a similar G1/S phase cell cycle arrest that causes the death of colorectal cancer cells (Haseli *et al*., 2023). Moreover, DDW inhibits the proliferation, migration, and invasion of NPC cells. Strikingly, by contrast, the growth of normal preosteoblast cells (mesenchymal progenitors of osteoblasts) was promoted in the presence of DDW (Wang *et al*., 2013).

Understanding the mechanism of tumor suppression by deuterium-depleted water

Zhang X *et al*. (2019) used a proteomics approach to reveal a disbalance between ROS production and ROS neutralization brought on by DDW exposure to cancer cells, leading to suppression of activity of several proteins that maintain tumor growth. Tumor cells grown in culture and exposed to DDW experienced 32% inhibition of proliferation. The authors sought to understand exactly how

Table 2 Proteins shown to be downregulated in tumor cells in response to exposure to DDW

Enzyme	Significance
ALDH4A1	Mitochondrial NAD-dependent dehydrogenase; essential role in pathway converting proline to glutamate
FDXR	Mitochondrial flavoprotein that converts $NADP^+$ to NADPH
H6PD	A dehydrogenase that converts $NADP^+$ to NADPH
DHFR	Regenerates THF by reducing NADPH to $NADP^+$
GPX4	Oxidizes glutathione, an important antioxidant enzyme in mitochondria

ALDH4A1, aldehyde dehydrogenase 4 A1; DHFR, dihydrofolate reductase; FDXR, ferredoxin reductase; GPX4, glutathione peroxidase 4; H6PD, hexose-6-phosphate dehydrogenase; THF, tetrahydrofolate.

DDW arrests tumor growth, causes oxidative stress, and induces apoptosis in tumor cells. The regulatory protein p62, overexpressed in cancer cells, was highly oxidized in response to the treatment. Oxidation of p62, an autophagy receptor protein, promotes its oligomerization which in turn leads to increased autophagy, including autophagy (and clearance) of p62 itself (Carroll *et al*., 2018; Islam *et al*., 2018). Decreasing its level lowers proliferation rates (Zhang *et al*., 2023).

This proteomics analysis revealed several specific enzymes whose expression was significantly downregulated in response to DDW treatment. The most downregulated enzymes are listed in Table 2. Most of these enzymes carry out reactions that involve either NADH or NADPH. Downregulation of aldehyde dehydrogenase 4 A1 would impair the ability of the tumor cell to convert proline to glutamate, decreasing its supply of glutamate, which is an important alternative fuel in the TCA cycle in cancer cells (Yi *et al*., 2019). Glutamate deprivation in prostate cancer cells decreased proliferation, migration, and invasion, leading to apoptotic cell death (Koochekpour *et al*., 2012). Both ferredoxin reductase and hexose-6-phosphate dehydrogenase replenish NADPH, which is needed to maintain glutathione in its reduced state. In parallel, glutathione peroxidase downregulation suppresses the ability to detoxify H_2O_2 in the mitochondria via glutathione oxidation. Dihydrofolate reductase (DHFR) regenerates tetrahydrofolate (THF) from dihydrofolate (DHF), so its suppression leads to a reduced supply of THF, an essential coenzyme for *de novo* synthesis of both pyrimidine and purine nucleotides, needed for DNA synthesis in proliferating cells. DHFR inhibitors such as methotrexate are common antifolate drugs used in cancer therapy (Taylor, 1993).

Zhang X *et al*. (2019) reported that the most highly oxidized enzyme was hydroxymethylglutaryl CoA synthase 1 (HMGCS1), the first step in the mevalonate pathway. This pathway leads to the synthesis of cholesterol, which is essential for assembling the plasma membranes of proliferating cells. Its suppression lowers mitochondrial membrane potential and increases the release of

proapoptotic factors (Tricarico *et al.*, 2015). Depletion of isoprenoids, other mevalonate pathway products, leads to endoplasmic reticulum (ER) stress and apoptosis in liver cells (De Giorgi *et al.*, 2020).

DDW may not be toxic to the tumor cells per se, but rather cancer cells are programmed to respond to low deuterium by suppressing proliferation and inducing apoptosis. DDW induces complex metabolic changes in tumor cells that interfere with proliferation and promote autophagy and apoptosis. Dietary choices can also influence mitochondrial function. A high-fat (ketogenic) diet is a low-deuterium diet (Somlyai *et al.*, 2022). Pesticide exposure can induce mitochondrial dysfunction and increase oxidative stress (Sule *et al.*, 2022). Riboflavin deficiency can interfere with the supply of NAD(P)H (Schramm *et al.*, 2014). These ideas are summarized in Fig. 1.

Discussion

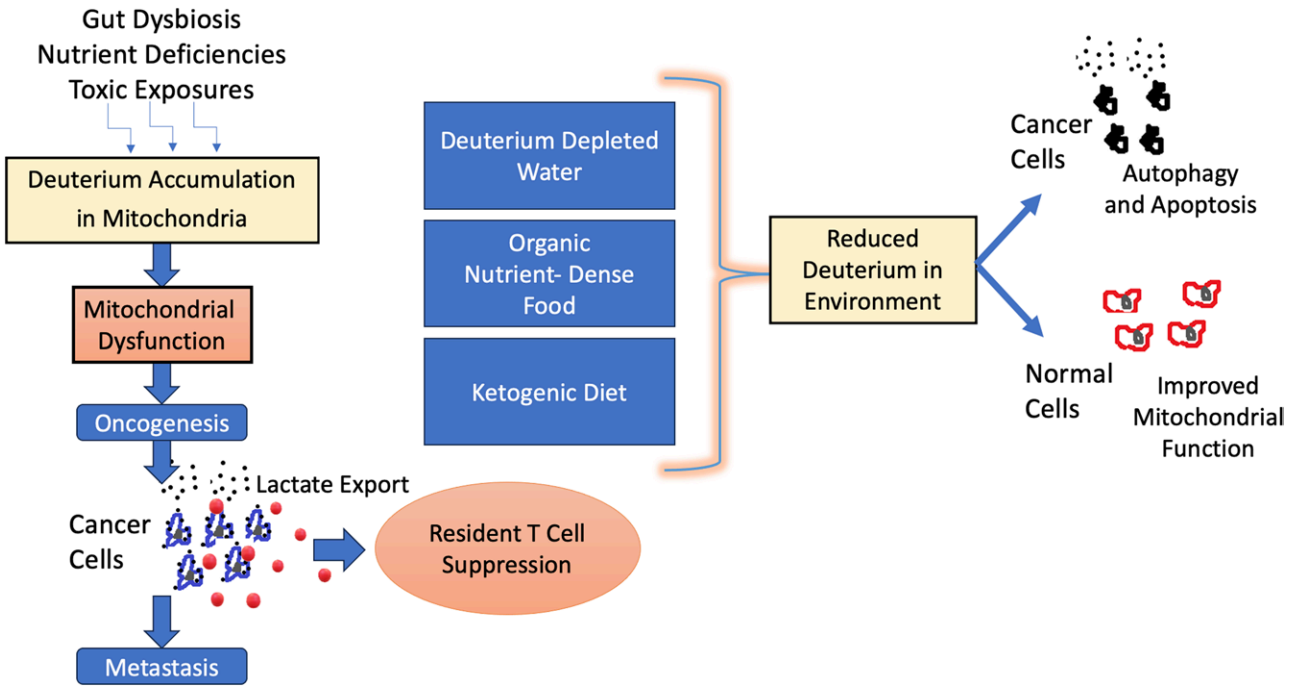
Many of the unusual aspects of cancer metabolic policies can be explained as a strategy to produce and release deupleted nutrients and deupleted protons into the external milieu while concentrating deuterium internally. By severely restricting the activity of the F-ATPase pumps, tumor cells protect their defective mitochondria from the primary damaging effects of deuterium. M2-like TAMs infiltrating the tumor microenvironment promote tumor growth, angiogenesis, and metastasis and suppress T-cell activation (Wang *et al.*, 2024). Nutrients supplied by the tumor, however, can support mitochondrial and lysosomal healing of the immune cells, eventually enabling them to become activated and destroy the tumor.

Deuterium-depleted water, autophagy, and apoptosis

DDW has been found in many studies to be a promising therapy to treat cancer, with minimal side effects (Yavari & Kooshesh, 2019; Zhang X *et al.*, 2019; Lu & Chen, 2024). It appears that cancer cells can sense deuterium levels and respond through signaling pathways to launch an apoptotic program. DDW may restore oxidative phosphorylation and result in an accumulation of ROS in cancer cells, which results in accelerated aging and shrinkage of the tumor, due perhaps to deficient antioxidant defenses and impaired ability to repair damaged DNA (Zhang X *et al.*, 2019).

The mechanisms generating an autophagic response in cancer cells are tightly linked with apoptosis (Mulcahy Levy & Thorburn, 2020). Targeting autophagy to induce apoptosis in tumor cells, however, is complicated. The response depends on which kind of tumor is being targeted, and at which stage of oncogenesis (e.g. stages where a benign tumor turns malignant) autophagy is inhibited (Bhutia *et al.*, 2013). Generally, at the initial

Fig. 1



Schematic of the processes by which excess deuterium in mitochondria can lead to a change in metabolic policy toward oncogenesis, proliferation and metastasis, and how reduced deuterium intake can reverse these processes, leading to recovery from cancer.

stages of oncogenesis, cancer cells need autophagic recycled molecules to maintain their excessive proliferative metabolism. When autophagy is inhibited at this stage, a provoked cancer cell death by apoptosis can be achieved (Yun and Lee, 2018).

The inhibition of autophagy to produce an anticancer effect, however, may be reversed for certain cancers under certain conditions. For example, in contrast, in lung cancer, restoring the autophagy response during early oncogenesis can have an antitumor effect. But autophagy at later stages of oncogenesis of lung cancer promotes tumor progression via a dysregulated T regulatory cell response and an impaired ability of mutant p53 activity to safeguard the genome (Rao *et al*., 2014). In simple words, restoration of autophagy in early oncogenesis of lung cancer has an antitumor effect, whereas at late stages of oncogenesis, the inhibition of autophagy is needed to have an antitumor effect. Therefore, the practice of using DDW to promote autophagy and apoptosis in cancer cells needs further clinical research evaluations to define at which stage of oncogenesis and in which types of cancer this can be applied to optimize anticancer effects.

The crosstalk between senescence, apoptosis, and cell cycle arrest induced by deuterium-depleted water treatment in cancer

Under normal conditions, a cell induces cell cycle arrest via the transcriptional activation of *p21* by tumor suppressor protein p53. p53 forms a complex with p21, initiating a molecular cascade to inhibit cell cycle progression to mitosis (Engeland, 2022). Autophagy, on the other hand, is inhibited as the cell progresses to mitotic division (Mathiassen *et al*., 2017). Initiation of autophagy and cell cycle arrest share common cellular pathways, where, in both cases, the activity of p53 is catalytic. In most cancers, either p53 is mutated or the cell suffers from biological inactivation of its related pathways that otherwise safeguard the genome (Hernández Borrero & El-Deiry, 2021). In cancer cells, the mutant isoforms of p53 do not induce cell cycle arrest but instead promote the continuation of cellular proliferation and oncogenesis (Ozaki & Nakagawara, 2011).

The activity of *Tp53* and other oncogenes, namely *c-myc*, and *Ha-ras*, is inhibited by deuterium depletion (Gyöngyi & Somlyai, 2000). Zhang X *et al*. (2019) found that the p53 signaling pathway was the most downregulated signaling pathway of DDW-treated lung cancer cells. A possible explanation for how DDW might induce cell cycle arrest in cancer cells is the reduction of intracellular content of deuterium, which then blocks the expression of cancer-related genes (Kovács *et al*., 2022). DDW, by delaying the expression of *Tp53*, also reduces the concentration of mutant p53 that induces cellular proliferation and oncogenesis. This will prevent or even stop cancer proliferation and development. Moreover, DDW's lowering of the D to H ratio in the cytosol in malignant cells initiates apoptosis, autophagy, and senescence, phenomena that are coupled with cell cycle arrest (Chira *et al*., 2018; Lajos *et al*., 2018; Yaglova *et al*., 2023).

Senescence is a permanent phenomenon of cell cycle arrest in which the aging cells are unresponsive to mechanisms that induce the repair of DNA damage events (Kumari & Jat, 2021). Incubation of lung cancer cells in DDW shows substantial ROS accumulation. In cancer cells, the normal mitochondrial function for depleting deuterium is expected to be disrupted, and therefore deuterium accumulates. Possibly, when the antioxidant defenses (e.g. glutathione) are insufficient (Zhang X *et al*., 2019), ROS accumulate, and cancer cells finally suffer from accelerated aging and apoptosis (Lu & Chen, 2024).

Deuterium-depleted water and vacuolar ATPase

One way in which DDW might affect cancer cell proliferation is by decreasing the activity of V-ATPase pumps. These pumps reverse the polarity of the membrane by pumping protons out, and they have a very high deuterium KIE (Kotyk *et al*., 1990). The acidic environment created by this activity promotes tumor growth. There exist organic chromophores that can distinguish D_2O from H_2O based on their slight difference in acidity. This feature has been exploited in the design of D_2O optical sensors to quantitatively detect levels of D_2O in water (Luo *et al*., 2019). It is conceivable that biological organisms possess deuterium sensors that could have signaling capabilities to suppress the membrane-bound ATPase pumps under low-deuterium conditions. These, however, have not yet been identified. V-ATPase pumps increase multidrug resistance and promote proliferation, migration, and invasion (Stransky *et al*., 2016). V-ATPase inhibitors cause slowed growth and increased cancer cell apoptosis, simply by normalizing membrane polarity (De Milito *et al*., 2010). More research is needed to better characterize the role that deuterium may play in regulating V-ATPase pumps in cancer.

Strongholds and limitations on how deuterium-depleted water treatment works against cancer

A promising view, as deduced from results of DDW use in human clinical trials in prostate, lung, and pancreatic cancer studies, is that (1) deuterium can be regarded as a nutritional agent, and (2) its depletion from the organism extends the MST of cancer patients. DDW can be regarded as a nontoxic and easily noninvasive supplementation approach in these studies. The problem of cost in introducing the DDW approach, however, remains to be evaluated. The Boros *et al*. (2021) study describes an easy manufacturing laboratory process to produce DDW for patients. In this study, however, the optimum D content in DDW to provide the highest anticancer effect remains to be evaluated in future studies. Furthermore, the relatedness of first appearing cancer and its complications

(metastasis) and the relation of DDW to oncogenesis stages of cancer remains to be elucidated further.

We had hoped in this article to be able to determine exactly how deuterium depletion works to attenuate essential factors that enforce cancer cell growth, but the ultimate answer still eludes us. It is plausible, however, that cancer cells, upon DDW treatment, suffer from levels of oxidative stress that cannot be handled. It has been observed experimentally that DDW induces apoptosis in cancer cells whereas it supports survival in normal cells (Wang *et al*., 2012). Oxidation of HMGCS1, as reported by Zhang X *et al*. (2019), likely leads to apoptosis following ER stress, due to depletion of isoprenoids. HMGGCS1 suppression also depletes the supply of cholesterol needed to populate the membranes of cell clones. The list of downregulated proteins in cancer cells in response to DDW provided in Table 1 predicts a deficiency in glutamate synthesis and in the regeneration of NADPH from $NADP^+$. NADPH is urgently needed for antioxidant support when ROS production is high. Therefore, suppression of these critical enzymes may lead to sufficient mitochondrial damage to provoke mitochondrial outer membrane permeabilization, the release of cytochrome C into the cytoplasm, caspase activation, and a programmed apoptotic response (Lopez & Tait, 2015). We still do not know exactly how DDW induces these observed effects on protein expression in cancer cells, but our conjecture is that it is through an ability for cancer cells to sense deuterium levels in the medium and react to a reduction in deuterium via a signaling response that leads to these reductions in expression of critical proteins that support cancer survival and proliferation.

Finally, we want to highlight a remarkable new study conducted by a group of Russian scientists who showed that DDW fed to male Wistar rats delayed or even reversed thymic involution, a hallmark of immune cell aging. This study showed that, after a short time period of suppression, thymopoiesis not only becomes restored, but the rate of producing differentiated T cells exceeds the values of the animals that were not fed DDW (Yaglova *et al*., 2023). The activation of T-cell proliferation and the increase in the numbers of progenitor cells suggest that DDW counteracts and reverses thymic involution. Thymic involution is responsible for cancer emergence, relapse, and decreased immune anticancer activity (Wang *et al*., 2020). Therefore, the possible enhancement of thymic function in patients consuming DDW can be another important factor for the anticancer effects and prolongation of survival, a topic that urgently needs further research.

Much more research is needed to identify immune cell functions that are enhanced by DDW treatment. The clinical applications of DDW are relatively easily applied in medical settings and can be considered to have an impact above and beyond the already ongoing conventional therapeutic regimes.

Conclusion

A causal factor in oncogenesis is excess deuterium in mitochondria. Cells whose mitochondria are sufficiently damaged by deuterium may transform into tumor cells, and this transformation is associated with a readjustment of metabolism to reduce the need for mitochondria-derived energy. Simultaneously, they engage in a program that results in further enrichment of cytoplasmic deuterium through the export of deupleted protons and the overproduction of lactate, a carrier of a deupleted proton derived from NADH, exported to the medium. Cancer cells support deuterium sequestration and release deupleted nutrients, primarily lactate, to support neighboring immune cells and distant organs. The lactate and acidic pH produced by cancer cells lead to cancer progression and immunosuppression of resident immune cells.

DDW has promising therapeutic value in treating cancer. DDW appears to suppress autophagy and interfere with the clearance of damaged mitochondria, and this may then lead to cell cycle arrest. The substantial accumulation of ROS in cancer cells during DDW treatment causes senescence that leads to apoptosis. Clinical evaluation is needed to discover DDW's influence on autophagic-related death by apoptosis at different stages of oncogenesis in different cancer types.

A future challenge would be to identify (or, ideally, reduce) the cost of DDW application in clinical settings providing anticancer treatment. Moreover, although many hydrogen/deuterium exchange studies prognose for the intracellular effects; these are primarily on a theoretical level. Apart from the phenomenal reduction of proliferation rate and induction of cell cycle arrest, which macromolecules are being affected during depletion of deuterium remains to be identified. This will enlighten more clinical anticancer approaches in the future.

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Conflicts of interest

There are no conflicts of interest.

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