



Review

The role of choline in prostate cancer

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ABSTRACT

Choline is an essential nutrient that is necessary for cell membrane synthesis and phospholipid metabolism and functions as an important methyl donor. Multiple roles for choline in cancer development have been suggested. Choline can affect DNA methylation and lead to a disruption of DNA repair. It can also modify cell signaling that is mediated by intermediary phospholipid metabolites, and it can support the synthesis of cell membranes and thus support cell proliferation. A higher intake or status of choline in plasma and tissues has been related to higher cancer risks. Prostate cancer shows elevated levels of choline uptake and levels of certain choline metabolites. Choline metabolites can be used as potential prognostic biomarkers for the management of prostate cancer patients. Targeting certain enzymes, which are related to choline metabolism, provides promising therapeutic opportunities for tumor growth arrest. This review summarizes the potential role of choline metabolism in cancer, especially in prostate cancer.

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Introduction

Choline is an essential nutrient [1]. The main dietary sources of choline are beef and chicken liver, eggs, wheat germ, and dried soybeans [2]. The recommended daily requirements for choline have

been set to 550 mg/day for men and 425 mg/day for non-pregnant women [1]. Choline is necessary for the synthesis of acetylcholine, membrane and signaling phospholipids, and as a source of methyl groups [3].

Dietary intake is not the only source of choline, since a considerable amount of this compound can be produced de novo from phosphatidylethanolamine via phosphatidylcholine (PtdCho). Pathological changes in the liver and muscles were observed in 77% of men on a diet that was poor in choline [4]. These observations suggest that the endogenous synthesis of choline is not sufficient to meet the daily requirements. Genetic variations of choline dehydrogenase and phosphatidylethanolamine *N*-methyltransferase can influence the dietary requirements for choline [5,6].

Choline metabolism has been linked to malignant transformation characterized by a higher proliferation rate and increased phosphocholine (PCho) and other choline-containing compounds [7,8]. Inducing the proliferation of normal cells by growth hormones was not

Abbreviations: CCT, CTP-phosphocholine cytidyltransferase; CDP-Cho, cytidine diphosphate choline; CHK, choline kinase; CHTs, high-affinity choline transporters; CPT1, diacylglycerol cholinephosphotransferase 1; CTLs, choline transporter-like proteins; CTP, cytidine triphosphate; DAG, diacylglycerol; Eth, ethanolamine; GPC, glycerophosphocholine; GPC-PDE, glycerophosphocholine phosphodiesterase; GPE, glycerophosphoethanolamine; Lyso PLA1, lyso-phospholipase A1; OCTs, organic cation transporters; OCTNs, organic cation/carnitine transporters; PEth, phosphoethanolamine; PCA, prostate cancer; PCho, phosphocholine; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PtdCho, phosphatidylcholine.

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associated with an increase in PCho and total choline levels in one study [8]. Therefore, the role of choline in cancer goes beyond the expected higher requirements caused by cell division and the increased synthesis rate of new cell membranes. Cancer cells exhibit an abnormal choline phospholipid metabolism [8–10]. The extensive alterations in choline metabolism in malignant transformation have been shown to be related to the expression of enzymes involved in this pathway [7]. Moreover, aberrant choline metabolism might be related to malignant transformation via genetic and epigenetic dysregulations [11,12].

Few studies observed a positive association between the dietary choline intake or plasma concentration of choline and the risk of some types of cancer [13,14], including PCA [15]. Other studies observed no significant relationship [16,17]. Prostate cancer (PCA) accounts for 14% of all newly diagnosed cancer cases worldwide in 2008 [18]. The rising incidence rate of PCA reflects in part the widespread screening for prostate-specific antigen (PSA). Risk factors such as age, ethnicity, family history, lifestyle, and androgens are also discussed in relation to the PCA risk [19,20]. Beyond enhanced lipid biosynthesis, altered choline phospholipid metabolism is one of the characteristic features of PCA [21,22]. Dietary choline, as a major source of choline, phospholipids, and methyl groups might be a potential modifiable risk factor or risk marker for PCA.

The role of choline phospholipid metabolism in cell function and signaling makes it one important target candidate for tumor treatment or prevention. This review summarizes the current knowledge regarding the implications of choline in carcinogenesis and discusses the potential usage of choline and choline phospholipid metabolites as prognostic biomarkers in patients with PCA.

The role of choline as a methyl donor

The proportional distribution of dietary or endogenous choline between phospholipids and the methylation pathways has not been studied much. Choline is oxidized to betaine via two-step irreversible reactions mediated by choline dehydrogenase and betaine aldehyde dehydrogenase. Betaine homocysteine methyl transferase mediates the transfer of the methyl group from betaine to homocysteine to produce methionine that is in turn converted into S-adenosylmethionine (SAM), the universal methyl donor (Fig. 1). Choline is utilized to generate the methyl group required for phospholipid metabolism. The SAM-dependent phosphatidylethanolamine *N*-methyltransferase catalyzes the methylation of phosphatidylethanolamine (PtdEth) to PtdCho, an important source for the de novo synthesis of choline [3,23].

Epigenetic mechanisms are important in prostate carcinogenesis [19,24]. SAM is required for DNA methylation. Altered DNA methylation and disruption of DNA repair were reported in cancer patients [25], including those with PCA [19]. For example, the glutathione S-transferase 1 can detoxify reactive chemical species through conjugation with reduced glutathione thus preventing or attenuating the

development of cancer upon exposure to carcinogens [26]. The lower expression of the π -class glutathione S-transferase 1 was related to hypermethylation of CpG island of the promoter region of π -class glutathione S-transferase 1 in more than 90% of PCA cases [24]. Nakayama et al. observed hypermethylation of the promoter region of the glutathione S-transferase 1 gene in the majority of areas of carcinoma and high grade prostatic intraepithelial neoplasia lesions, but not in the epithelium and hyperplastic epithelium [27]. Therefore, the role of choline in carcinogenesis may be related to the extent of its utilization as a methyl group donor. Since epigenetic mechanisms precede the development of the tumor, follow-up studies can provide information about a potential predictive value for choline levels in cancer.

The role of choline in phospholipid metabolism

Choline is utilized for the de novo synthesis of PtdCho via the Kennedy pathway (also called CDP-choline pathway) (Fig. 2) [28]. The Kennedy pathway involves three reactions. In the first step, choline kinase (CHK) catalyzes the phosphorylation of choline into PCho. This reaction can be a rate-limiting step for PtdCho biosynthesis [29]. CHK has three isoforms (CHK α 1, CHK α 2, and CHK β) all of them have choline kinase activity [30]. The second reaction in the Kennedy pathway is catalyzed by the CTP-phosphocholine cytidyltransferase (CCT) that yields cytidine diphosphate choline (CDP-Cho) from PCho and cytidine triphosphate (CTP). CCT mediates the rate-limiting step in the Kennedy pathway and its activity depends on the association with membrane structures, the phosphorylation state, and some transcription factors [28,31]. CCT α encodes one isoform and CCT β encodes three isoforms of the enzyme (β 1, β 2, and β 3). CCT α is found in all tissues, while CCT β is expressed in certain tissues [32]. In the final step, the CDP-Cho and diacylglycerol (DAG) are converted into PtdCho by 1,2-diacylglycerol cholinephosphotransferase (CPT1). CPT1 seems not to be a rate-limiting step in PtdCho biosynthesis. Choline phosphotransferase and ethanolamine phosphotransferase-1 genes encode the two CPT isoforms, *cpt-1* and *cept-1*, respectively [30,33].

Cancer pathogenesis is discussed in relation to enzymes involved in the synthesis or the degradation of phospholipids. The enzyme CHK seems to enhance the malignant transformation of cancer cells [34]. For example, CHK is overexpressed in PCA thus causing higher PCho and supporting malignant transformation [21]. This in turn enhances choline uptake and membrane phospholipid synthesis in malignant cells [21,35]. Furthermore, PCho might be involved as a second messenger in the growth-signaling cascade [30]. In line with this evidence, the inhibitors of CHK show antitumor activity and reduces tumor growth [30,35].

Moreover, the endogenous synthesis of choline seems to be upregulated in cancer cells. The higher requirements of choline in cancer cells are supported by the enhanced choline transport activities and increased degradation of PtdCho by phospholipase D (PLD). Choline can be synthesized from PtdCho by means of phospholipase A (PLA) and PLD, while phospholipase C (PLC) converts PtdCho into PCho. There are three reactions that produce choline from PtdCho. First, phospholipase A2 (PLA2) catalyzes the hydrolysis of PtdCho yielding 1-acylglycerophosphocholine, which is converted to glycerophosphocholine (GPC) by the enzyme lyso-phospholipase A1 (Lyso-PLA1). The enzyme GPC phosphodiesterase (GPC-PDE) then converts GPC into choline [7]. Recent data demonstrated that GPC-PDE constitutes a source of choline from GPC for the Kennedy pathway [36,37]. Moreover, GPC-PDE has been demonstrated to play a critical regulatory role in cell migration in vitro [37]. Second, choline and phosphatidic acid are produced from PtdCho via two isoforms PLD1 and PLD2 [38]. The activity of the enzyme PLD that synthesized choline from PtdCho is increased in tumor cells [38,39]. Several lines of evidence indicated that PLD might be implicated in cell proliferation, survival signaling,

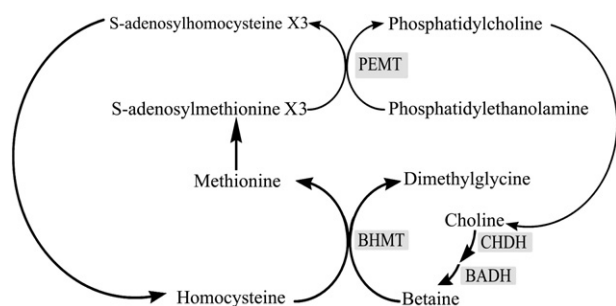


Fig. 1. One-carbon metabolism, the choline cycle. BADH, betaine aldehyde dehydrogenase; BHMT, betaine homocysteine methyltransferase; CHDH, choline dehydrogenase; PEMT, phosphatidylethanolamine *N*-methyltransferase.

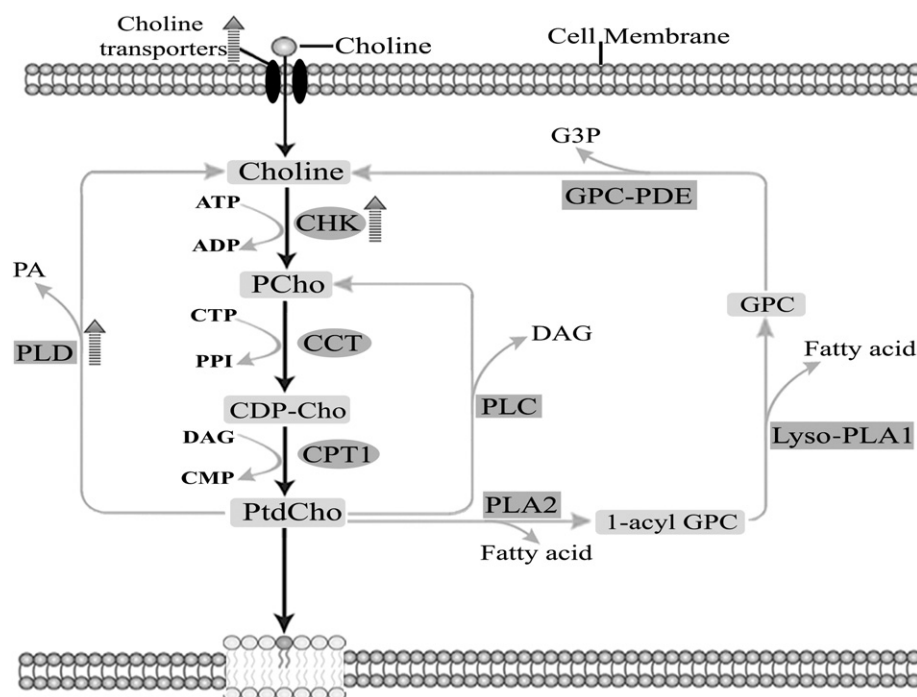


Fig. 2. The enzymes involved in choline phospholipid metabolism in the cell. Black arrows represent the biosynthetic pathway. Gray arrows represent the catabolic pathway. Dashed arrows indicate increased enzyme activity in prostate cancer. 1-acyl GPC, 1-acylglycerophosphocholine; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CCT, CTP-phosphocholine cytidyltransferase; CDP-Cho, cytidine diphosphate choline; CHK, choline kinase; CMP, cytidine monophosphate; CPT1, diacylglycerol cholinephosphotransferase 1; CTP, cytidine triphosphate; DAG, diacylglycerol; GPC, glycerophosphocholine; GPC-PDE, glycerophosphocholine phosphodiesterase; G3P, glycerol-3 phosphate; Lyso-PLA1, lyso-phospholipase A1; PA, phosphatidic acid; PCho, phosphocholine; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PPI, diphosphate; PtdCho, phosphatidylcholine.

malignant transformation, tumor progression and metastatic process [7,38].

Recent studies pointed to a probable interaction between CHK, PLD, and GPC-PDE. A high co-expression of these enzymes in the tumor resulted in higher PCho and lower GPC and led to a characteristically switched PC/GPC ratio [36,37]. The glycerophosphodiester phosphodiesterase domain isoform 5 (GDPD5) is a transmembrane protein that is ubiquitously expressed in human tissues. It is thought to confer GPC-PDE activity [40], since the overexpression of GDPD5 contributed to GPC reduction. GDPD5 is most probably involved in membrane PtdCho metabolism and at least partially responsible for the malignant choline metabolite profile in breast cancer [36].

PtdCho can be hydrolyzed by PLC to yield PCho as well as DAG, a critical second messenger that activates protein kinase C, an important player in several signal transduction cascades [41]. Inhibition of PLC activity in PC3LN3 human prostate carcinoma cells showed a decrease in cell migration, suggesting a possible role for PLC in cell motility and invasion process [42].

Taken together, higher CHK expression in PCA supports tumor development via PCho formation. A higher uptake of choline by tumor cells might support the requirements of the cells.

Choline uptake in normal and PCA cells

The plasma concentrations of free choline are around 10 $\mu\text{mol/L}$ [1,3]. These concentrations are maintained via dietary choline and endogenous synthesis. Dietary choline is absorbed in the intestine [43]. The quaternary amine choline is a charged hydrophilic cation, which needs specific transporters to pass the membrane lipid barrier [44]. Choline transporters mediate the cellular uptake and the transport is the rate limiting step for the synthesis of PCho [7]. Four choline transporters have been described showing different affinity for choline, specificity, and Na^+ -dependency [7].

1. *High-affinity choline transporters (CHTs)* have been attributed to CHT1 (also known as SLC5A7), which has a K_m of 0.5–5 μM for choline. CHT1 is mainly expressed in cholinergic neurons and it is choline-specific and Na^+ -dependent [44,45].
2. *Choline transporter-like proteins (CTLs)* are characterized by intermediate-affinity (K_m 20–200 μM) and they are choline-specific and Na^+ -independent. CTL1 is ubiquitously expressed in most human tissues and provides choline for phospholipid synthesis [7,44]. The CTL proteins have been identified in human, mouse, and rat [44]. The CTL family comprises at least six genes where all gene products undergo complex alternative splicing [7].
3. *Organic cation transporters (OCTs)* transfer organic cations in a Na^+ -independent and reversible manner. Since 1994, several members of the OCTs family have been cloned from different tissues (OCT1, OCT2, and OCT3). They are part of the SLC22 gene family and carry out a low-affinity choline transport in addition to the transport of other organic cations. OCTs facilitate polyspecific cationic transport, whereas their exact role in choline transport is unclear [44].
4. *Organic cation/carnitine transporters (OCTNs)* are part of the SLC22 gene family and exist as two isoforms. The OCTN1 (also known as SLC22A4) is pH-sensitive and functions in a Na^+ -independent manner. The OCTN2 (also known as SLC22A5) can function as a Na^+ -dependent co-transporter for certain zwitterions, as well as a Na^+ -independent transporter of organic cations. OCTN1 and OCTN2 are structurally related. However, the exact role of OCTNs in choline transport is not known [46,47].

An increase in the expression of choline transporters and the transport rate have been documented in PCA compared with normal prostate tissues [48]. The transporters involved in increased uptake of choline in human PCA and the mechanism of this enhanced uptake are not completely clear. CTL1 is expressed in PCA cells and the kinetic properties of this transporter are similar to that of human CTL1.

However, one study has shown that choline transporters in the PCA cell line PC-3 have intermediate affinity, but are Na⁺-dependent [7,48]. Thus, combined transport systems (Na⁺-dependent and Na⁺-independent) might be involved. Therefore, a potential role for choline transporters in PCA deserves further investigations.

Choline metabolism in relation to oncogenic signaling, hormone therapy, and hypoxia

Molecules derived by the breakdown of choline-containing phospholipids such as DAG and PCho can act as second messengers in mitogenic signal transduction pathways. Other molecules like lyso-phosphatidic acid can activate enzymes involved in choline metabolism such as PLD [38,39,41]. Moreover, phosphatidic acid, the precursor of lyso-phosphatidic acid and DAG, has regulatory properties that are implicated in oncogenic signaling and activation of enzymes involved in choline metabolism [7]. The interactions between oncogenic signaling pathways and the choline metabolic pathway are summarized in Table 1.

PCA hormone therapy such as androgen deprivation therapy might influence choline metabolism or choline uptake, but the evidence is not consistent [49–51]. The influence of androgen deprivation therapy on the uptake of radiolabelled choline (¹¹C-choline) was tested by using Imaging Positron Emission Tomography (PET/CT). Androgen deprivation therapy lowered choline uptake in prostate tissues in two studies that tested patients before and after androgen deprivation therapy treatment [49,50]. A third study reported enhanced choline uptake in patients treated with androgen deprivation therapy compared to those not treated with this type of therapy [51]. Experimental studies showed that androgens increased choline uptake (2.0-fold) and caused accumulation (2.5 fold) of phospholipids in androgen-dependent human prostate cells LNCaP [52,53]. In contrast, choline uptake in androgen-independent PC-3 cells was similar both in the presence and absence of androgens [53]. These findings suggest a potential influence of androgens on choline metabolism, but this effect might differ with each cancer type.

Hypoxia might occur because of the decrease in the vascular support when PCA cells grow. The subsequent activation of hypoxia-inducible factor 1 [54] might cause resistance to hormones, radio- and chemo-therapy [53,54]. However, the hypoxia-inducible factor 1 has been shown to increase PCho levels in PCA cells [53,55] and to induce the expression of CHK in PC-3 cells, resulting in raised levels of total choline and PCho [55]. Furthermore, hypoxia might decrease the uptake of choline in PCA cells, while the choline uptake is enhanced under aerobic conditions [53]. Therefore, the relationship between

hypoxia and choline metabolism in PCA seems to affect both choline uptake and utilization.

Choline phospholipid metabolites as prognostic markers in PCA

Accurate clinical staging of PCA is very important for the management of the disease and optimization of the therapy regimen. Total choline levels in prostate tissue have been shown to be positively related to the Gleason score and tumor aggressiveness in patients with PCA [56]. Several PET/CT techniques that depend on studying the uptake of radiolabelled choline (¹¹C-choline) in tumor cells have shown that ¹¹C-choline might be helpful in monitoring the recurrence of PCA, but not for initial staging of the tumor [57,58]. Magnetic resonance spectroscopy imaging (MRSI) that depends on measuring the levels of choline phospholipid metabolites in prostate tissues showed that total choline and PCho correlate with the stage of PCA. Therefore, MRSI might be used for diagnosing and monitoring the progression of PCA [58,59]. The low sensitivity and low accuracy of PET/CT in comparison with MRSI might explain the differences in the diagnostic utility of both methods.

The relationship between choline and ethanolamine (Eth) metabolite levels and PCA stage was recently investigated in benign and prostate tissues by using MRSI. In addition to Eth, four choline- and Eth-containing metabolites (PCho, GPC, and their Eth counterparts phosphoethanolamine (PEth) and glycerophosphoethanolamine (GPE)), were tested [56,60]. One study indicated that PCho levels were significantly higher, while Eth levels were lower in cancer versus benign prostate tissues [60]. The other metabolites, PEth, GPE and GPC, showed no significant differences [60]. Furthermore, PCho/PEth ratio provided the best discrimination between benign and PCA tissues. Moreover, PCho/PEth, PCho/GPC, PEth/Eth, and GPE/Eth ratios were also increased significantly in cancer prostate tissues compared with benign prostate tissues [60]. Another study confirmed lower levels of Eth and higher PCho levels in PCA versus benign prostate tissues [56]. The levels of PEth, GPE, and GPC were higher in PCA compared with benign prostate tissues in this study [56]. Additionally, the levels of the choline-containing metabolites (PCho and GPC) were significantly elevated in high-grade PCA versus benign prostate tissues whereas the Eth-containing metabolites (Eth, PEth, and GPE) were significantly higher in low-grade PCA versus benign prostate tissues [56]. Therefore, it seems that the Eth-containing metabolites (PEth + GPE) discriminate between individual benign and low-grade PCA tissues. Moreover, the choline-containing metabolites (PCho + GPC) seem to discriminate between high- and low-grade PCA tissues [56].

Table 1

The interaction between oncogenic signaling and the choline phospholipids metabolism.

Oncogenic signaling	Choline transporter	CHK	CCT	PLD	PLC	PLA2
RAS GTPase		↑ CHK activity [61]		↑ PLD activity [62]	↑ PLC activity [63]	PLA2 mediate downstream of IRAS [64]
RAF kinases MAPK/ERK PI3K	PI3K inhibition reduce the choline uptake [67]	↑ CHK activity [61]	↓ CCT activity [65]	↑ PLD activity ^a [38]		↑ PLA2 activity [66]
RALGDS JNK Hypoxia-inducible factor 1 Activator protein 1		↑ CHK activity [61] ↑ CHK transcription [55] ↑ CHK transcription [70]	↓ CCT activity [69]	↑ PLD activity ^a [68]		↑ PLA2 activity [66]
SREBP Specificity protein 1	↑ hCTL1 transcription [72]		↑ CCT activity [71] ↑ CCT transcription [71,73]	↑ PLD transcription [74]		

^a Negative feedback pathway. CCT, CTP-phosphocholine cytidyltransferase; CHK, choline kinase; ERK, extracellular signal-regulated kinase; hCTL1, human choline transporter-like protein 1; JNK, JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; RAF, rapidly accelerated fibrosarcoma; RALGDS, RAL GTPase guanine nucleotide dissociation stimulator; RAS, rat sarcoma; SREBP, sterol regulatory element binding protein.

Conclusion

Choline might affect PCA risk via several mechanisms, including DNA methylation or phospholipid metabolism. Alternatively, choline metabolism in PCA might be dysregulated in order to meet the tumor requirements for phospholipids and methyl groups. Common polymorphisms in genes involved in choline metabolism such as phosphatidylethanolamine *N*-methyltransferase and choline dehydrogenase might interact with choline metabolism and affect its requirements. Tissues might vary in terms of choline uptake, susceptibility to choline depletion as well as the extent of the choline utilization as a methyl donor. Despite the controversy over the use of choline phospholipid metabolites in staging and monitoring PCA, the usefulness of total choline and PCho as prognostic markers in PCA has been established. The diagnostic usefulness of other phospholipid metabolites for better PCA management has to be tested in future studies.

Conflict of interest

The authors have no conflict of interests regarding the content of this article.

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