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Choline metabolism-based molecular diagnosis of cancer: an update

Kristine Glunde[*,1,2], **Marie-France Penet**[1,2], **Lu Jiang**[1], **Michael A Jacobs**[1,2], and **Zaver M Bhujwalla**[*,1,2]

[1]The Russell H. Morgan Department of Radiology and Radiological Science, Division of Cancer Imaging Research, Baltimore, MD, USA

[2]Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Abnormal choline metabolism continues to be identified in multiple cancers. Molecular causes of abnormal choline metabolism are changes in choline kinase-α, ethanolamine kinase-α, phosphatidylcholine-specific phospholipase C and -D and glycerophosphocholine phosphodiesterases, as well as several choline transporters. The net outcome of these enzymatic changes is an increase in phosphocholine and total choline (tCho) and, in some cancers, a relative decrease of glycerophosphocholine. The increased tCho signal detected by ^{1}H magnetic resonance spectroscopy is being evaluated as a diagnostic marker in multiple cancers. Increased expression and activity of choline transporters and choline kinase-α have spurred the development of radiolabeled choline analogs as PET imaging tracers. Both tCho ^{1}H magnetic resonance spectroscopy and choline PET are being investigated to detect response to treatment. Enzymes mediating the abnormal choline metabolism are being explored as targets for cancer therapy. This review highlights recent molecular, therapeutic and clinical advances in choline metabolism in cancer.

Keywords

cancer; choline kinase-α; choline metabolism; choline transporters; GDPD5; GDPD6; phospholipase C; phospholipase D; targeting; treatment strategy

Over the past two decades, abnormal choline metabolism has emerged as one of the most consistent hallmarks of cancer [1]. The molecular causes underlying abnormal choline metabolism are being gradually unraveled and are providing potential novel targets in the treatment of cancer [1]. Since our first review in 2006 [2], there have been several advances and new insights into choline metabolism in cancer. The field of choline in cancer is stimulating increasing interest from fundamental and translational investigators as is evident

[*]Authors for correspondence: kglunde@mri.jhu.edu; zaver@mri.jhu.edu.

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from the trend in annual publications. In 2000, there were 111 annual publications in this field that doubled to 220 by 2010 and have increased to 256 in 2013. PET choline imaging [3,4] has eclipsed ^{1}H magnetic resonance spectroscopy (MRS) in human oncological applications, including the detection of response to treatment [5,6], although ^{1}H MRS is still the technique of choice in preclinical studies. The past 5 years have witnessed significant insights into the molecular causes underlying abnormal choline metabolism in cancer, novel directions in targeting choline metabolism for therapy and a clearer understanding of the role of choline metabolism in oncological transformation and oncological pathways.

Molecular causes & potential targets of abnormal choline metabolism

The most well-established molecular causes for the increased phosphocholine (PC) and total choline (tCho) levels in cancer cells and tumors are an increase in choline kinase (Chk)-α expression [7–9] and activity [10–13], a higher rate of choline transport [14,15], and increased phosphatidylcholine (PtdCho)-specific phospholipase C (PC-PLC) [11–13] and D (PC-PLD) activities [12,16]. More recently discovered enzymes that alter the choline metabolite profile are the glycerophosphocholine phosphodiesterases (GPC-PDEs) GDPD5 and GDPD6. These enzymes, among others, constitute the complex network of biosynthetic and breakdown pathways of the major membrane phospholipid PtdCho, with one or more enzymes acting per pathway as shown in Figure 1 [1].

The role of Chk-α in malignant transformation and progression in multiple cancer types such as breast [10], colorectal [7], prostate [7], ovarian [12], lung [7,9] and more recently endometrial [17] and pancreatic [18] cancers is well documented. Chk catalyzes the phosphorylation of free intracellular choline (Cho) to PC. In mammalian cells, two separate genes *Chk-α* and *Chk-β* encode the three known isoforms of Chk, Chk-α1, Chk-α2 and Chk-β. Chk-α1 and Chk-α2 are formed as the result of alternative splicing of the Chk-α transcript [19–21]. The enzymes are active as homo- or heterodimers [19]. Despite being members of the same family, Chk-α and Chk-β behave differently when overexpressed in cells [21]. Chk-α expression and activity are important in oncogenesis, tumor progression and metastasis of many cancers [1,22]. Increased levels and activity of Chk-α have been observed in human breast [10], colorectal [7], lung [7,9], prostate [7], ovarian [12] cancer and most recently in endometrial [17] and pancreatic [18] cancer. Chk-α expression was also associated with negative estrogen receptor (ER^{-}) status in breast cancer [10] and with worse clinical outcome in non-small-cell lung cancer [9]. Increased Chk-α expression in human breast cancer cells was found to increase invasiveness [23]. Chk-α inhibition and siRNA-based downregulation decreased the phosphorylation of ERK1/2 to p-ERK1/2 on T202/Y204, and the phosphorylation of AKT to p-AKT on S473, highlighting its role in the regulation of MAPK and PI3K/AKT signaling [24,25]. Chk-α is phosphorylated by c-Src and was found to form a complex with EGFR that regulates cell proliferation and tumorigenesis [26]. These studies suggest that enzyme stability rather than activity is critical for oncogenesis. A noncatalytic role of Chk-α was also observed where inhibition of the choline kinase catalytic activity alone was not sufficient to kill cancer cells [27]. Chk-α inhibition also resulted in prolonged endoplasmic reticulum stress, partially mediated by the transcription factor CHOP [28]. Increased invasiveness and drug resistance have also been observed with Chk-α overexpression in breast cancer cells [23]. Chk-α silencing in ovarian cancer cells resulted in

reduced migration and invasion, as well as increased sensitivity to platinum, paclitaxel and doxorubicin [29]. Combined treatment with 5-fluorouracil and siRNA silencing [30] or Chk-α inhibition [31] demonstrated synergistic effects of both treatments in breast and colorectal cancer models, respectively. Advances have also been made in characterizing the structure of Chk-α through the identification of a new binding site that may result in the design of more effective compounds [32,33].

PC-PLC and PC-PLD also play a role in modifying choline metabolism in cancer cells. PC-PLC activity was found to be significantly increased in ovarian cancer cells compared with nonmalignant immortalized ovarian cells [12,13]. However, the gene for mammalian PC-PLC enzyme has not as yet been identified. Nevertheless, PC-PLC has been implicated in cell signaling through MAPK and oncogene-activated protein kinase pathways, in programmed cell death, activation of immune cells and stem cell differentiation [34–37]. PC-PLC accumulation has been observed to be localized to the plasma membrane of ovarian cancer cells [38], human EGFR2-overexpressing breast cancer cells [39], mitogen-stimulated fibroblasts [34] and cytokine-activated human natural killer cells [40–42].

PC-PLD is a ubiquitous enzyme involved in the hydrolysis of PtdCho to phosphatidic acid (PA) and Cho [43]. PA is known to activate the mTOR signaling pathway by binding directly to mTOR [44]. PA is further converted either to diacylglycerol or lysophosphatidic acid by PA, phosphohydrolase and phospholipase A2 [43]. Two mammalian genes, *PLD1* and *PLD2*, each with splice variants, have been identified [45–47]. PLD1 and PLD2 can accelerate EGFR endocytosis by interacting with Dynamin, a critical mediator of membrane fission [48]. G proteins, such as ARF, Rho and Rac, activate PLD1 [49]. PLD1 is overexpressed in uterine [50] and endometrial carcinoma [51], and may be a critical downstream mediator of H-Ras-induced tumors [52]. Overexpression of either PLD1 or PLD2 in transformed fibroblasts conferred anchorage-independent growth [53]. Elevated PLD1 protein expression has been found to generate rapamycin resistance in breast cancer cells [54,55]. Recently, a correlation between the expression of Chk-α and PLD1 was reported with breast cancer malignancy, as shown in Figure 2 [56]. An association between ER status and Chk-α and PLD1 expression was also observed [56]. In addition, downregulation of Chk-α with siRNA increased PLD1 expression, and downregulation of PLD1 increased Chk-α expression, demonstrating that these two enzymes were interactive [56].

In addition to Chk-α, PC-PLC, PLD1 and PLD2, an elevation of choline uptake by choline transporters, followed by phosphorylation by Chk-α can also increase endogenous PC [57]. Increased expression of the high-affinity choline transporter CHT1 with a Km of ∼2 μM, also called solute carrier family 5 member 7 (SLC5A7) [58], has been observed in breast cancer cells [15]. Another choline transporter, choline transporter-like protein 1 with a Km of ∼68 μM, was overexpressed in human lung and colon carcinoma cells [59,60]. Increased CHT1 and choline transporter-like protein 1 expression was recently reported in human pancreatic cancer xenografts [18].

More recently, GDPD6 (alternatively named EDI3 or GPCPD1) was identified as an enzyme that cleaves GPC to form glycerol-3-phosphate and choline, and positively regulates cancer

cell migration via protein kinase C-α signaling [61]. A negative association between high GDPD6 expression and relapse-free survival time was observed in clinical endometrial and ovarian cancers in a screen that compared mRNA levels in metastasizing versus nonmetastasizing cancers [61]. GDPD6 was also described as overexpressed in endometrial cancer, along with overexpressed Chk-α and lysophospholipases A1 and A2, resulting in a 70% increase of PC levels [17]. Recent studies have also shown that the GPC-PDE encoded by GDPD5 is associated with breast cancer malignancy in highly malignant ER^- breast cancer in cells and tumors originating from patients [62]. ER^- breast tumors with high GDPD5 expression also displayed high levels of PC, tCho and PC/GPC [62]. GDPD5, Chk-α and PLD1 were significantly overexpressed in a concerted manner in highly malignant ER^- tumors in this patient cohort [62]. Orthotopic breast cancer xenograft models in which GDPD5 was stably silenced with GDPD5-specific shRNA contained increased levels of GPC and phosphoethanolamine (PE) compared with control tumors [63]. Cell-based studies showed that GDPD6 positively regulates cell migration via disruption of the protein kinase C-α signaling pathway [61], and its association with integrin expression, cell adhesion and spreading in breast and ovarian cancer cells [64].

Enzymes in the choline phospholipid metabolism provide attractive potential targets for anticancer therapy, especially for cancers that lack specific receptors or targeting pathways. Chk-α [8,10,33,65–67], PC-PLD [13,68] and PC-PLC [12,13] have already been targeted by gene silencing or enzyme inhibition in studies of MRS-monitored, targeted anticancer therapies. As the high PC levels in tumors are, in large part, caused by an increased expression and activity of Chk-α, this enzyme presents an attractive molecular target for ^{31}P or ^{1}H MRS-monitored anticancer therapy [8,10,22,31,65–67,69,70] and is currently being tested in clinical trials [71].

Although significant progress has been made in characterizing the molecular causes of abnormal choline metabolism in cancer, several enzymes remain to be investigated. Increased PE has been observed in tumors almost as consistently as increased PC [72], but understanding the role of PE in cancer is relatively unexplored. An increased signal from PE is, however, only observed in tumors but not in culture because, whereas mammalian plasma contains both choline (~10–40 μM) and ethanolamine (~10 μM), most culture media only contain choline (~1–20 μM). Although Chk-α has a dual choline/ethanolamine kinase activity [21], ethanolamine kinase (Etnk)-1 and 2 are the two enzymes that convert ethanolamine to PE [73–76]. Interestingly, the *ETNK1* gene contains somatic missense mutations in systemic mastocytosis with eosinophilia and chronic myelomonocytic leukemia [77]. ETNK in cancer cells and tumors should be a focus of future studies and be investigated as a potential therapeutic strategy in cancer.

The power of analytical methods that generate big data, such as mass spectrometry imaging (MSI), is also providing unique insights into the abnormal choline metabolism in cancer at the metabolite [78], lipid [79] and protein [80] levels. As shown in Figure 3, matrix-assisted laser desorption ionization MSI performed on tumor sections detected the spatial distributions of individual PC and Cho, among many others [78]. PC and Cho were increased in viable compared with necrotic regions of invasive and metastatic triple-negative MDA-MB-231 tumors but were relatively homogeneously distributed in nonmetastatic ER^+

MCF-7 tumors [78]. The molecular distributions of PC and other compounds detected by MSI distinguished several subregions within viable tumor regions, which were not apparent with hematoxylin and eosin staining [78]. PC was a major principal component that allowed identification of subregions within MDA-MB-231 tumors, but not MCF-7 tumors, likely due to low PC concentrations in MCF-7 compared with MDA-MB-231 tumors [78].

Cancer detection & monitoring response to treatment

^{1}H and ^{31}P MRS were the initial techniques to detect increased tCho (^{1}H MRS) and phosphomonoester (^{31}P MRS) in human tumors [81]. These observations have led to evaluations of ^{1}H and ^{31}P MRS for detecting cancers. Increased tCho, detected with ^{1}H MRS, is consistently observed in cancer cells but not in non-malignant cells [12,82,83]. As a result, tCho detected by ^{1}H MRS is being evaluated as a diagnostic and prognostic biomarker in multiple human cancers [1]. Although most of these human studies have confirmed the increase of PC and tCho in brain [84–86], breast [87–91], prostate [92], ovarian [93], endometrial [17] and cervical cancers [93], there were cases where increases in tCho were noted in normal tissue [88]. Other factors that hamper the widespread use of MRS are the variability in data due to differences in field strength, coil design and sequences used across different centers, and the inability to standardize data acquisition. These factors have proven to be major barriers for these techniques to consistently influence outcome and achieve approval by Centers for Medicare and Medicaid Services for routine diagnostic testing [87]. In addition, because of the relatively poor sensitivity of MRS, spatial resolution is a limiting factor, as is the resulting longer patient scanning time [87]. Continuing increases in field strength, improvement of coil design, novel sequences that minimize motion artifacts and increase the rapidity of scan time [87], and efforts to achieve standardization, are providing solutions to these barriers. A major advantage of MRS is the ability to detect intrinsic metabolic contrast that does not require the delivery of an extrinsic marker for detection. As a result, confounding effects due to poor delivery of contrast agents and probes are minimized.

The tCho signal has been incorporated as one relevant parameter in combination with multiparametric MR readouts of magnetic resonance spectroscopic imaging (MRSI)-based metabolites, together with MRI-based parameters such as the apparent diffusion coefficient (ADC) and diffusion tensor imaging obtained from diffusion-weighted MRI, and blood volume from contrast-enhanced perfusion-weighted MRI, to successfully discriminate between recurrent glioma and radiation injury [94], and to grade brain gliomas [95]. The predictor set with tCho from MRSI, ADC from diffusion-weighted MRI, and the relative cerebral blood volume from perfusion-weighted MRI maximized the discrimination between recurrent glioma and radiation injury [94]. The use of multiparametric MRI in locally advanced breast cancer for the prediction of partial clinical response has been shown to be highly predictive [96]. Multimodal multiparametric studies combining [^{18}F]-fluorodeoxyglucose PET and MRI approaches that incorporate the MRSI-detected tCho signal and rely on multiparametric machine-learning approaches demonstrated a significant improvement in the detection of glioma progression [97]. A novel application for MRSI is in the intraoperative MR suite for tissue characterization and optimization of tumor resection in glioma patients [98]. Intraoperative MRS at the resection margin correctly diagnosed tissue

signal increases in tCho/*N*-acetylaspartate and tCho/total creatine ratios and assisted in achieving total tumor resection in conjunction with intraoperative conventional MRI [98]. Novel clinical correlations such as a positive correlation between ^{1}H MRS-detected tCho and immunohistochemistry-detected calcium-sensing receptor in clinical breast cancers have recently been revealed [99].

Innovative specialized ^{31}P MRS approaches based on ^{1}H to ^{31}P polarization transfer are currently emerging to quantify *in vivo* levels of PE, PC, GPE and GPC noninvasively on small animal and clinical MR scanners [100–103]. Unlike the tCho signal detected by ^{1}H MRS that has overlapping signals from PC, GPC and cho, in ^{31}P MR spectra signals from PC, PE, GPE and GPC metabolites do not overlap, and are of significant interest because the PC/GPC ratio can track increasing aggressiveness of breast and ovarian cancer cells [12,82]. 31P MRS can detect these individual signals, but has relatively low signal-to-noise ratio due to the intrinsically low sensitivity of the ^{31}P nucleus [104,105]. The detection of individual PE, PC, GPE and GPC is demanding even at higher field strength, especially in heterogeneous cancer tissues where the homogeneity of the magnetic field is poor [106]. Polarization transfer techniques transfer the polarization of excited ^{1}H spins through J-coupling to the ^{31}P spins, increasing the signal-to-noise ratio and eliminating resonances without ^{1}H-^{31}P coupling, thereby flattening the baseline and significantly increasing the signal-to-noise ratio of PE, PC, GPE and GPC at 1.5T [107], 3T [100] and 7T [106]. Using specialized hardware to adapt high-field ^{31}P MRS at 7T, it was possible to detect ^{31}P MR spectra from a small 3-mm diameter lymph node in the axilla [108]. These recent advances in ^{31}P MRS coil and pulse sequence design will enable the detection of individual PE, PC, GPE and GPC in cancers in the near future.

[^{11}C]-choline, [^{18}F]-fluoromethylcholine and [^{18}F]-fluoroethylcholine are the main tracers used in PET imaging to visualize choline uptake and metabolism. The short half-life of 20 min of ^{11}C restricts its use to centers with cyclotrons, whereas the longer half-life of 110 min allows the use of ^{18}F-choline in centers without an onsite cyclotron [109]. A metabolically more stable analog of [^{18}F] -fluoromethylcholine, which is deuterated on the ethyl backbone, was recently developed and its biodistribution and radiation dosimetry were evaluated in healthy volunteers and resulted in no adverse events and dosimetry profiles comparable to other common [^{18}F]-PET tracers [110]. Choline PET/computed tomography (CT) in local disease evaluation and staging of prostate cancer surpasses conventional [^{18}F]-fluorodeoxyglucose PET imaging for nodal staging of prostate cancer and for patients with suspected metastases [109]. The US FDA approved [^{11}C]-choline PET in clinical applications for prostate cancer in 2012 [111].

Both ^{1}H MRS [5] and [^{11}C]-choline-PET [6] imaging are finding important roles in monitoring response to radiation treatment in patients with glioblastoma multiforme [5] and in detecting recurrence in patients with prostate cancer [6]. Increasingly, radiation oncologists are incorporating these imaging modalities in restaging and assessment of response in these patients [5,6]. A recent study demonstrated that [^{11}C]-choline-PET/CT positive lung tumors also contained high expression levels of choline acetyltransferase and Chk-α [112]. Figure 4 shows examples of combined [^{11}C]-choline PET images, a T_2-weighted image, and MRSI maps acquired from a glioma-bearing mouse (Figure 4A–C)

with corresponding tumor and brain ^{1}H MR spectra (Figure 4D–E) [113]. In this preclinical study, negative correlations were observed between tCho measured with MRS and [^{11}C]-choline PET tumor-to-brain ratio, and between MRS-measured tCho and [^{11}C]-choline PET tumor-to-cerebellum ratio [113]. Positive correlations were observed between tCho tumor-to-brain ratio and the percentage injected dose per milliliter, tCho tumor-to-brain ratio and the [^{11}C]-choline PET tumor-to-brain ratio [113]. These findings suggest that while MRSI highlights the areas of high tCho concentrations that are localized to the tumor rim, [^{11}C]-choline PET identifies regions of high choline turnover in mouse models [113]. These results suggest that ^{1}H MRS and [^{11}C]-choline-PET imaging could have complimentary roles. 1H MRS could provide qualitative and quantitative tumor assessment, early indication of tumor spread, gliosis and inflammation, and [^{11}C]-choline PET could be used as a biomarker for proliferation, treatment planning and therapy monitoring as shown in early preclinical models [113]. The tCho signal shows promise as a surrogate marker for assessing tumor response in novel targeted treatments. For example, ^{1}H MRSI metabolic maps of tCho/*N*-acetylaspartate showed superior performance, when compared with contrast-enhanced MRI, in assessing the response to antiangiogenic treatment with bevacizumab or cabozantinib in preclinical studies [114].

The availability of whole-body PET/MR scanners that provide multimodality data is likely to increase both specificity and sensitivity of tumor detection [115]. PET/MRI permits simultaneous monitoring of morphologic, functional and metabolic tumor properties for diagnosis and therapeutic response assessment [116]. Benefits of combining either [^{18}F]-fluoroethylcholine or [^{11}C]-choline PET with MR have been shown in pediatric astrocytic brain tumors and prostate cancer, respectively [117,118]. As shown in Figure 5, anatomical referencing can also be improved by combining [^{11}C]-choline PET with T_1-weighted or T_2-weighted sequences compared with CT [118]. Combining PET with MR provided a morphological correlate for the majority of the intraprostatic [^{11}C]-choline foci [118]. Simultaneous measurement of [^{18}F]-fluoroethylcholine PET and ADC by MRI was evaluated in a pilot study in children with astrocytic tumors for diagnosis, and response assessment showing that baseline [^{18}F]-fluoroethylcholine uptake matched areas of contrast enhancement and restricted diffusion [117]. A negative correlation trend between maximal standard uptake value and mean ADC, and a positive correlation trend between maximal standard uptake value and tumor size, were observed in this study, highlighting the use of simultaneous [^{18}F]-fluoroethylcholine PET/MRI in monitoring morphological and metabolic response and changes during therapy [117].

Expert commentary

The use of ^{1}H and ^{31}P MRS and MRSI to help diagnose a variety of cancers and to monitor the efficacy of cancer treatment is not yet broadly applied in the clinic, which is mostly due to the still developmental stages of these techniques. As ^{1}H and ^{31}P MRS benefit from higher magnetic fields, the introduction of human 7T MR scanners for research, although not FDA approved for routine clinical imaging, is currently giving an additional boost to the clinical development of ^{1}H and ^{31}P MRS techniques. Clinical multicenter trials of relatively simple single-voxel ^{1}H MRS measurement of tCho have revealed a large variation in the acquired data. This variability arises from lack of standardized data acquisition across

different centers due, in part, to differences in field strength, coil design and sequences used across different centers, as well as the absence of quantitative image analysis. The field of MRS in cancer requires careful evaluation of those newly developed techniques that merit large multicenter clinical trials, and careful identification of the particular cancers and the time points in clinical care at which imaging should be performed. For monitoring treatment, it is of paramount importance to carefully evaluate the time point at which MRS or MRSI should be performed after a particular therapy has been given. Once a clinical MRS protocol for multicenter testing has been agreed on, personnel running the studies should be sufficiently trained to eliminate poor standardization and quantification.

The use of [^{18}F]-fluoroethylcholine or [^{11}C]-choline PET is restricted to specialized centers and is not as yet widely available in the clinic. This is not only partially due to the high cost and the need for a cyclotron on site but also mostly due to the early developmental stages of these techniques. Additional clinical studies to identify the appropriate applications for cancers that are otherwise difficult to diagnose, or to monitor their treatment response are required, followed by larger multicenter trials for the identified niche applications. PET/MRI applications with choline-based PET tracers are in their infancy, which is due to the short time frame during which PET/MRI scanners have been available, and the need for the technology to mature and develop clinical imaging protocols with clear indications that can be tested in large clinical multicenter trials.

Pathology-based clinical applications that measure choline metabolites with the use of MSI from thin tissue sections [78,119] or intraoperatively [120,121] are currently emerging but are at an even earlier stage of clinical development than the above-mentioned MRS and PET imaging applications. The clinical use of these techniques is still being evaluated, and the first multicenter studies are just being conducted for MSI proteomics in breast cancer [119], whereas MSI metabolomics and lipidomics applications that could make use of the abnormal choline metabolism in cancer are still being developed [78,120,121].

Molecular studies of enzymes involved in choline metabolism of cancer have accelerated over the past two decades. The regulation and role of Chk-α, as well as PC-PLD and PC-PLC, in cancer have been extensively studied. However, additional studies are necessary even for these three well-studied enzymes, as gaps still exist regarding their promoter regions such as hypoxia-regulation of Chk-α, signaling pathways driving PC-PLD and the cloning of PC-PLC. Other enzymes in choline metabolism such as the GPC-PDEs need to be studied more extensively. The parallel metabolic pathways of ethanolamine metabolism, which display a significant amount of cross talk and use the same enzymes as in choline metabolism, need to be investigated in cancer as discussed in detail above. Although an enzyme-focus is important in molecular characterization, it is also important to develop systems biology approaches where all choline and ethanolamine pathways are considered as a whole to identify critical nodes and regulators. As cancer cells are supported by stromal and immune cells in their growth, the evaluation of choline and ethanolamine metabolism in cells such as tumor-associated fibroblasts and macrophages would represent important steps forward in this field.

Translational therapeutic strategies to target choline metabolism in cancer have mainly focused on small molecule inhibitors of Chk-α, as their translation is the most feasible clinically. The Chk-α inhibitor RSM-932A inhibited proliferation of several cancer cell lines *in vitro*, and reduced tumor growth in animal models *in vivo* with no toxicity at therapeutic doses [122]. It was therefore selected for further clinical testing [71]. The success or failure of these ongoing studies should be rapidly communicated to allow follow-up clinical studies or improvement of the currently available small molecule inhibitors of Chk-α. Strategies targeting other enzymes in choline or ethanolamine metabolism have so far been limited either because these enzymes are currently still being evaluated as potential targets or because suitable small molecule inhibitors are not available. As a first step, molecular and systems biology studies should clarify which enzymes in choline and ethanolamine metabolism are worth targeting. This should be followed by the design and development of suitable small molecule inhibitors or molecular reagents such as siRNA for these enzymes.

Five-year view

Increased tCho has been frequently observed in inflammatory conditions such as prostatitis and studies have identified an association between COX-2, Chk-α and PC [123]. A recent study has demonstrated the effect of Chk-α inhibition on decreasing the migration of fibroblast-like synoviocytes that play an important role in synovial inflammation in inflammatory arthritis [124]. These studies suggest that enzymes in choline metabolism may provide targets to reduce inflammation and will likely provide new avenues of investigation in the next 5 years. Interestingly, the presence of PC on proteins has been found to provide an escape from immune surveillance [125] and has been postulated as one reason why cancers have high PC. The role of PC in escaping immune surveillance by cancer cells is another area that merits investigation and will be pursued within the next 5 years.

A recent study investigating associations between plasma choline metabolism and colorectal cancer risk showed that plasma betaine/choline ratio may be a potential indicator of colorectal cancer risk and could be used for colorectal cancer screening [126]. Measurement of alterations in choline metabolism in the plasma could prove to be a useful tool in early tumor detection. Although MRS and PET imaging have been applied to solid tumors, their applications in the detection and management of liquid tumors is relatively unexplored and may provide a new frontier for this field within the next 5 years [127].

The role of the CDP-ethanolamine pathway in malignant transformation merits further investigation. The apparent positive feedback mediated by enzymes in the choline pathways as well as the interaction and compensatory mechanisms between enzymes in the choline and ethanolamine pathways that may allow cancer cells to adapt and survive downregulation of individual enzymes are other areas that require focus in the future. The use of radiolabeled compounds based on ethanolamine for PET imaging is relatively unexplored and may provide improved pharmacokinetics and biodistribution for PET imaging of cancers. *In vitro* studies have shown a two- to sevenfold higher uptake of ^{14}C-ethanolamine and ^{14}C-N-N′-dimethyl ethanolamine compared with ^{14}C-choline uptake in a variety of tumor cell lines [128], supporting the potential use for ethanolamine-based PET imaging that needs to be further explored within the next 5 years.

Further development of specialized ^{31}P MRSI sequences and their clinical testing will enable the detection of individual PE, PC, GPE and GPC in the clinical setting in a variety of solid tumors. The tCho signal as one parameter in multiparametric MRI and multimodal PET/MRI approaches, in conjunction with multiparametric machine-learning models, has so far been used in limited studies [94,95,97] and should be further explored and expanded. The use of tCho MRSI in the intraoperative MR suite for optimizing resected tumor margins has shown promise during brain tumor surgeries [98] and requires further evaluation in larger clinical studies. PET/MRI permits simultaneous monitoring of morphologic and metabolic tumor properties for diagnosis and therapeutic response assessment and has shown promise in pediatric astrocytic brain tumors and prostate cancer for combining either [^{18}F]-fluoroethylcholine or [^{11}C]-choline PET with MR [117,118]. Clinical MSI fingerprinting applications are currently being developed to detect choline metabolites and lipids that will help identify distinct tumor regions with diagnostic and therapeutic relevance [78], as well as tumor margins intraoperatively [120,121]. These MSI advances will mature within the next 5 years and provide novel clinical applications.

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Key issues

- Standardization of acquisitions and normalization to achieve quantitative data is of paramount importance when normalizing MR metabolite signals to water to achieve accurate total choline quantification.
- Comprehensively understanding the mechanisms underlying abnormal choline metabolism in cancer, including the interactions between cancer cells and stromal cells, and the transcription factors regulating enzymes and transporters in the choline pathways is critical for effective targeting of choline metabolism.
- New methodologies such as MSI and hyperpolarized ^{13}C MRSI in combination with molecular biology techniques should be applied to perform a genomic to proteomic characterization of cancer choline metabolism.
- The role of the CDP-ethanolamine pathway in malignant transformation needs to be investigated in relation to choline phospholipid metabolism.
- As the connectivity, networks and feedback mechanisms in choline metabolism become more evident, a systems biology approach will be necessary to identify critical nodes to target in this pathway.
- The sequencing of all genes in the choline cycle will be necessary to allow a better understanding of the deregulated choline metabolism in cancers.
- Targeting enzymes involved in choline metabolism may prove to be highly effective against cancer cells, and could be detected noninvasively by MRSI and/or positron emission tomography in vivo for image-guided therapy.
- Image-guided siRNA delivery targeting enzymes will allow downregulation of multiple enzymes in choline metabolism.

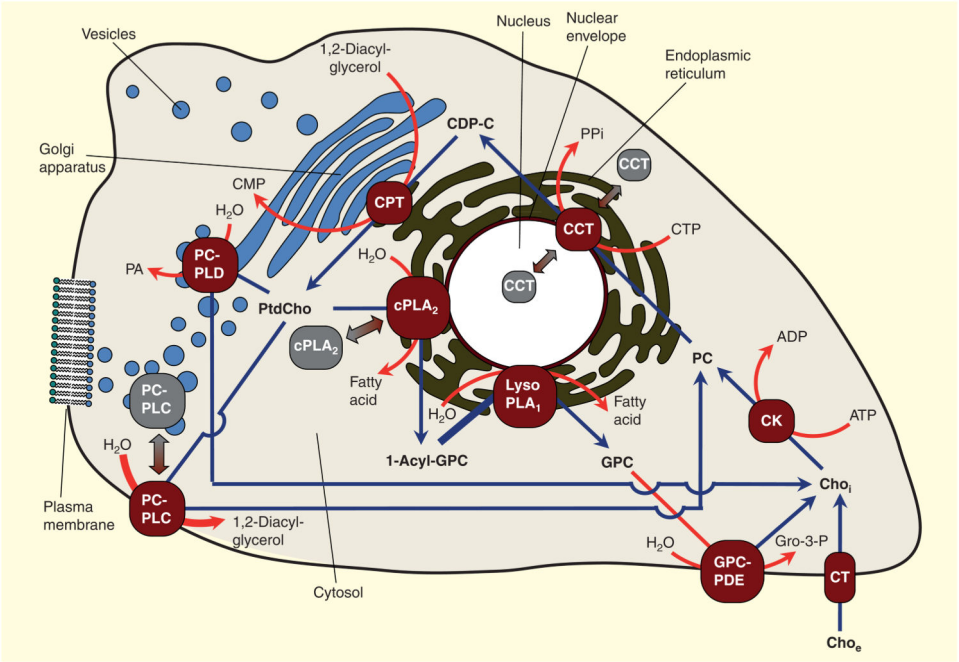

Figure 1. Major enzymes involved in choline phospholipid metabolism in the cell

Enzymes shown in red indicate active choline cycle enzymes, which are shown in the organelle in which they are active. Enzymes shown in gray indicate the location of choline cycle enzymes that are deactivated by translocation to a different organelle. Blue arrows represent choline metabolism pathways, proteins in red catalyze the reaction that is depicted by the corresponding orange arrow and choline cycle metabolites are shown in bold. Red–gray arrows show translocation to different subcellular locations, which can deactivate (gray) or activate (red) the enzyme.

CCT, CTP: Phosphocholine cytidylyltransferase; CDP-Cho: Cytidine diphosphate-choline; CHKα: Choline kinase-α; Cho_e: Extracellular free choline; Cho_i: Intracellular free choline; CHPT1: Diacylglycerol cholinephosphotransferase 1; CMP: Cytidine monophosphate; CTP: Cytidine triphosphate; FA: Fatty acid; GPC: Glycerophosphocholine; GPC-PDE: Glycerophosphocholine phosphodiesterase; Gro-3-P: Glycerol-3-phosphate; Lyso-PLA1: Lyso-phospholipase A1; PCho: Phosphocholine; PC-PLC: Phosphatidylcholine-specific phospholipase C; PC-PLD: Phosphatidylcholine-specific phospholipase D; PLA2: Cytoplasmic phosphatidylcholine-specific phospholipase A2; PP: Diphosphate.

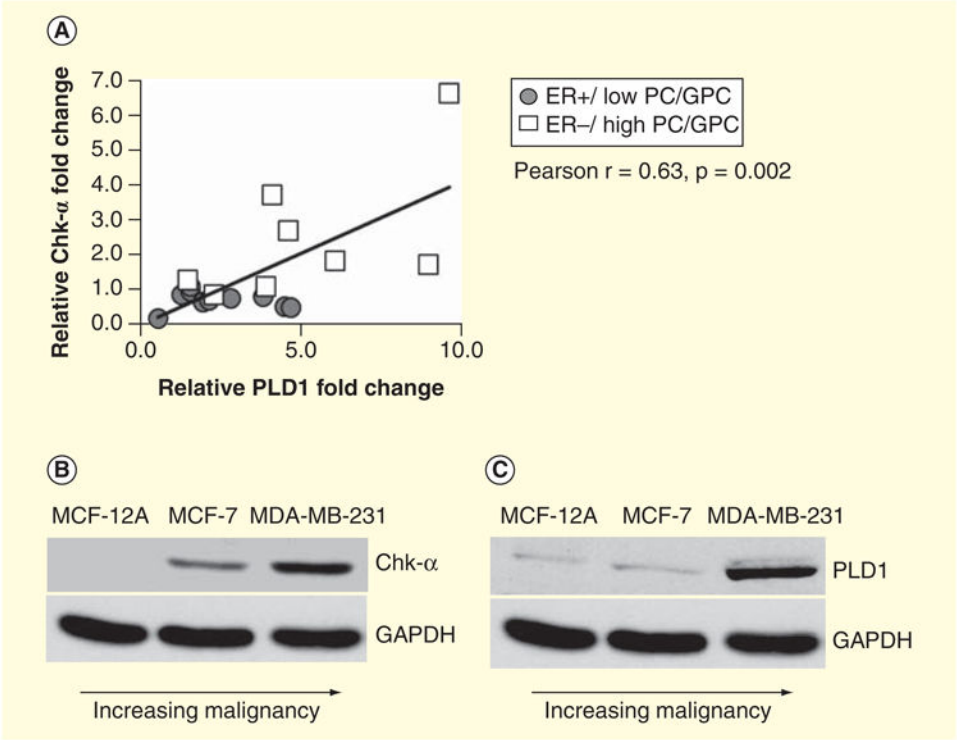

Figure 2. Chk-α and PLD1 expression increases with increasing malignancy in breast cancer

(A) Relative fold change in PLD1 mRNA and Chk-α mRNA in patient-derived tumor samples that are either ER^+ (n = 11) or ER^- (n = 8). **(B)** Immunoblots representing the protein expression of Chk-α in nonmalignant MCF-12A, nonmetastatic ER^+ MCF-7 cells, and highly metastatic ER^- MDA-MB-231 cells. **(C)** Immunoblots representing the protein expression of PLD1 in MCF-12A, MCF-7 and MDA-MB-231 cell lines. GAPDH was used as a loading control.

ER: Estrogen receptor; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase. Adapted by permission of the publisher (Taylor & Francis Ltd., http://www.tandfonline.com) [56].

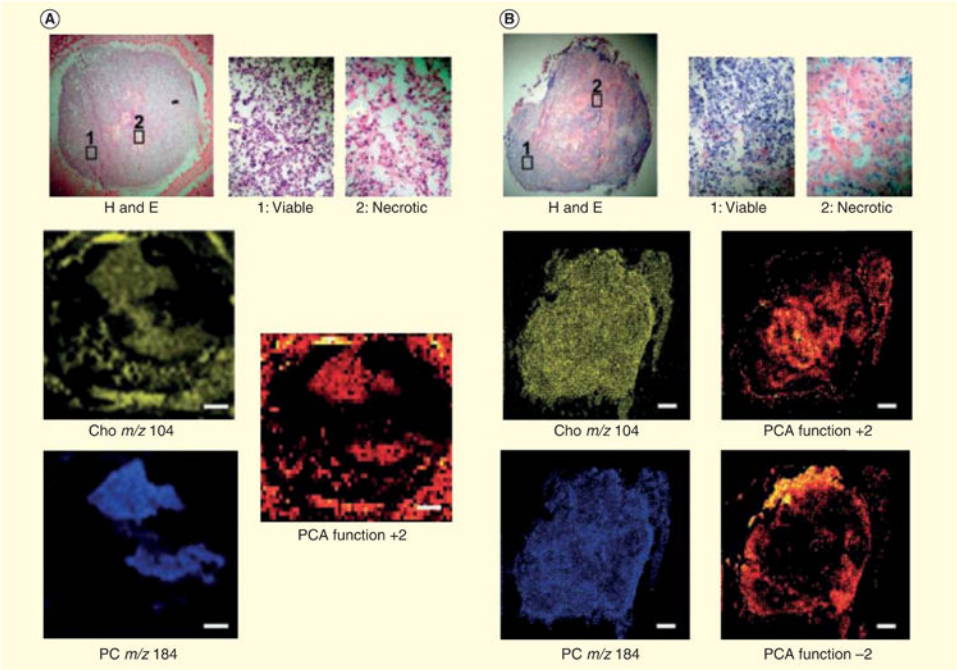

Figure 3. MSI of choline metabolites in breast tumor models

(A) Matrix-assisted laser desorption ionization-MS microscope mode data set of a representative MDA-MB-231 tumor showing the hematoxylin and eosin image, including magnified areas of (1) viable and (2) necrotic tumor regions, the corresponding MS imaging of Cho and PC, and the PCA image of the function +2. **(B)** Secondary ion mass spectrometry microprobe data set of a representative MCF-7 tumor showing the H&E image, the corresponding MS imaging of Cho and PC, and the PCA images of the functions +2 and −2 providing inverse images, in which tumor regions are defined by masses other than Cho and PC. Scale bar, 1 mm.

Cho: Choline PC: Phosphocholine; PCA: Principal component analysis.

Adapted with permission from [78].

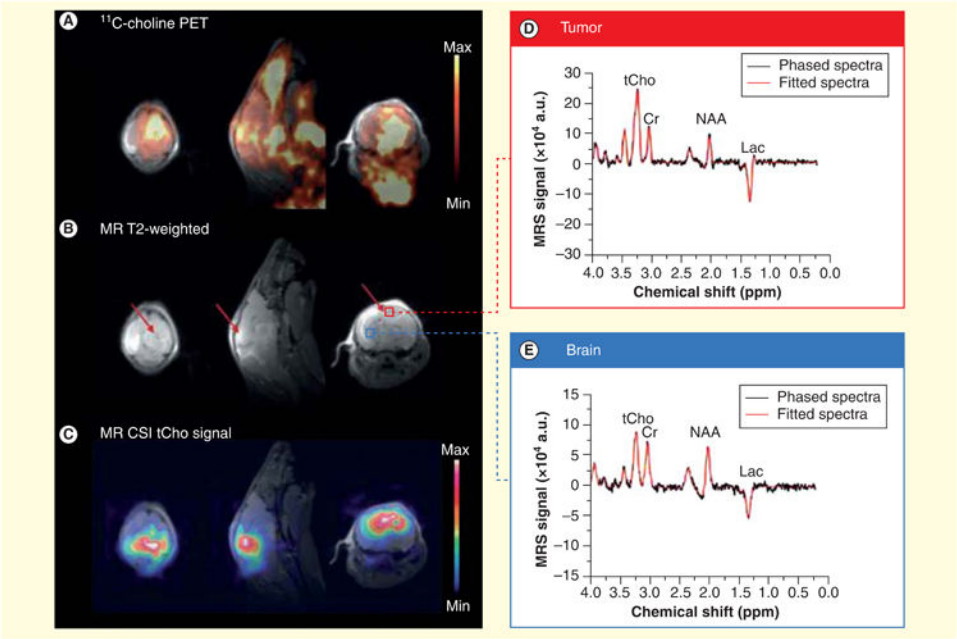

Figure 4. [^{11}C]-choline PET combined with tCho MRSI maps in a glioma mouse model

(A) [^{11}C]-choline PET showing the total uptake of [^{11}C]-choline including its metabolites, **(B)** T_2-weighted MR images as anatomical reference, **(C)** chemical shift imaging (CSI) map showing the total choline (tCho) distribution, **(D)** tumor, and **(E)** brain spectra of a murine glioma 18 days post-implantation. Transverse, sagittal, and coronal planes of a representative mouse head are shown from left to right. The tumor is pointed out with a red arrow.

Cr: Creatine; NAA: *N*-acetylaspartate; tCho: Total choline.

Adapted from [113], with permission from AACR.

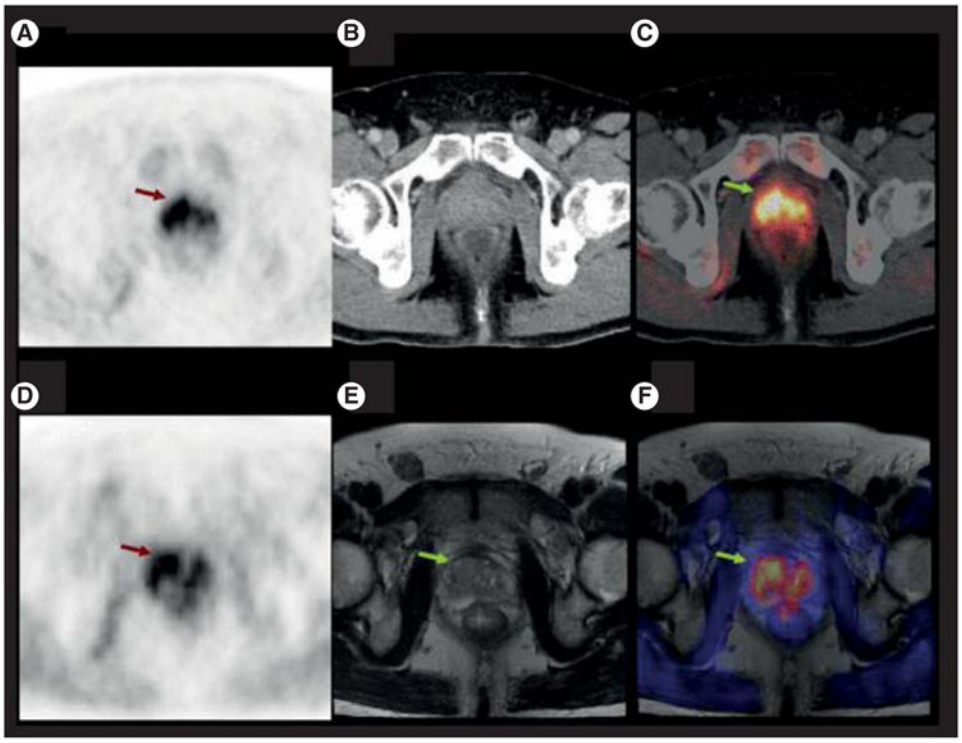

Figure 5. PET/CT and MRI of a 73-year-old man diagnosed with prostate cancer

(A–C) PET/CT imaging. **(A)** Axial PET image showing intense [^{11}C]-choline focal uptake in the right prostate central zone (arrow). **(B)** CT image demonstrates no differences in the Hounsfield units or density of the prostate parenchyma. **(C)** Fused PET/CT image showing increased [^{11}C]-choline uptake in the prostate (arrow). **(D–F)** PET/MR imaging. **(D)** Axial PET image showing intense [^{11}C]-choline focal uptake in the right prostate central zone (arrow) corresponding to the uptake observed in PET images. **(E)** Axial T_2-weighted MR image and **(F)** fused PET image showing a hypointense nodular formation in the central zone of the right prostate as a morphological correlate of the uptake observed in the PET image (arrow).

CT: Computed tomography.

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