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Choline metabolism in malignant transformation

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Abstract

Abnormal choline metabolism is emerging as a metabolic hallmark that is associated with oncogenesis and tumour progression. Following transformation, the modulation of enzymes that control anabolic and catabolic pathways causes increased levels of choline-containing precursors and breakdown products of membrane phospholipids. These increased levels are associated with proliferation, and recent studies emphasize the complex reciprocal interactions between oncogenic signalling and choline metabolism. Because choline-containing compounds are detected by non-invasive magnetic resonance spectroscopy (MRS), increased levels of these compounds provide a non-invasive biomarker of transformation, staging and response to therapy. Furthermore, enzymes of choline metabolism, such as choline kinase, present novel targets for image-guided cancer therapy.

The complexity and adaptability of cancer cells and their ability to recruit and modulate host stromal cells to create a tumour microenvironment that enables survival has led to the disease continuing to confound conventional treatments and molecular targeting. Because of their genomic and phenotypic plasticity, cancer cells have a multitude of redundant pathways and networks, as well as a genomic and proteomic diversity that highlights the

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Competing interests statement
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DATABASES
National Cancer Institute Drug Dictionary:
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Kristine Glunde's homepage: http://www.hopkinsradiology.org/Radiology%20Faculty/Research%20Faculty%20Bios/Glunde
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need for personalized medicine in this era of 'omics' and disease fingerprinting. A handful of common hallmarks of cancer[1] have emerged that cut through this variation. Activated choline metabolism, which is characterized by increased phosphocholine (PCho) and total choline-containing compounds (tCho) and which we refer to as the cholinic phenotype, is a fairly new metabolic hallmark that was discovered mostly owing to the introduction of magnetic resonance spectroscopy (MRS) studies of tumours in the 1980s[2,3] (TIMELINE). Initially, the increased PCho levels in cancer cells were interpreted as a requirement for the high rate of cell proliferation[4]. In subsequent studies, non-malignant breast or prostate epithelial cells that were induced to proliferate as rapidly as cancer cells using growth hormones still exhibited significantly lower levels of PCho and tCho levels, identifying malignant transformation rather than just cell proliferation as the cause of abnormal choline metabolism in cancers[5]. The tumour microenvironment also influences choline metabolism. Tumours have abnormal physiological environments such as hypoxia and acidic extracellular pH[6]. In studies with perfused mammalian cells, an acidic extracellular pH was found to significantly increase glycerophosphocholine (GPC) levels and to decrease PCho levels[7]. Hypoxia was also found to increase tCho and PCho levels in a human prostate cancer xenograft model that was genetically engineered to express green fluorescent protein under the control of a hypoxia response element[8]. A close correlation was observed between regions of high tCho levels and hypoxia *in vivo*[8], which was subsequently identified to be due to the regulation of choline kinase-α (CHKα) expression by the transcription factor hypoxia-inducible factor 1 (HIF1)[8]. The conditioned growth medium from cultured cancer cells was also found to increase PCho levels when used to culture human vascular endothelial cells[9], suggesting that cancer cells can influence stromal cells in the tumour microenvironment.

Because of the ability of non-invasive clinically available imaging techniques such as MRS and positron emission tomography (PET) (BOX 1; FIG. 1) to detect these changes in choline metabolism, the cholinic phenotype is being exploited for radiological diagnosis, prognosis, monitoring response and the development of novel treatments. In fact, early ^{31}P MRS studies showed a close association between phosphomonoesters (PMEs) and phosphodiesters (PDEs) with therapeutic response[2,3], and paved the way for later studies with ^{1}H MRS to study choline metabolism. The search for biomarkers to detect cancer and to non-invasively monitor the response to treatment has led to several clinical studies evaluating the non-invasive detection of tCho for these objectives. Similar to studies in cancer cells[10], human tumour xenograft models[10] and excised tumour tissue[11,12], clinical studies have confirmed the activation of choline metabolism in human tumours *in vivo*[10,13].

Phosphocholine is both a precursor and a breakdown product of phosphatidylcholine (PtdCho), which, together with other phospholipids such as phosphatidylethanolamine (PtdEtn) and neutral lipids, forms the characteristic bilayer structure of cellular membranes and regulates membrane integrity[14]. The synthesis of PtdCho and PtdEtn, which are the most abundant phospholipids in the cell membrane, was first described by Kennedy and Weiss in 1956 (REF. 15) and this *de novo* biosynthesis of PtdCho and PtdEtn is termed the Kennedy pathway. The high-energy intermediates cytidine diphosphate (CDP)-choline and CDP-ethanolamine are required to synthesize PtdCho and PtdEtn. These two mirroring

pathways, one that uses choline and the other that uses ethanolamine, are called the CDP-choline and CDP-ethanolamine pathways, respectively. The biosynthesis and hydrolysis of PtdCho mediates mitogenic signal transduction events in cells, and products of choline phospholipid metabolism, such as PCho[16], diacylglycerol (DAG)[17,18] and arachidonic acid metabolites[17], may function as second messengers that are essential for this mitogenic activity. Growth factor stimulation[18], cytokines[19], oncogenes[5] or requirements for eicosanoid production[17] also regulate choline phospholipid metabolism (FIGS 1,2). Underlying these phenotypic alterations in choline metabolism, is a network of transporter systems and enzymes involved in choline phospholipid metabolism that are deregulated in cancer cells.

This Review highlights the causes and consequences of the cholinic phenotype within the context of radiological applications, the molecular mechanisms and the interconnected networks underlying this phenotype, and the possibility of targeting this metabolic hallmark of cancer.

Key enzymes in choline metabolism in cancer

The increased PCho and tCho levels that have been detected in human cancers are caused by interplay between multiple enzymes, which are at the core of choline metabolism (FIG. 3) and which constitute the biosynthetic and catabolic pathways of PtdCho. Here, we review the different enzymes that are involved in choline metabolism. Reported enzyme expression levels and activities in human cancers are compared with those in normal tissue of the same origin and are summarized in TABLE 1.

Choline transporters

Choline is an essential nutrient that is derived from the diet, with plasma concentrations of about 10 μM. The enhanced transport of free extracellular choline into cancer cells (FIG. 3) has been identified as a dominant cause for the cholinic phenotype, as it can be a rate-limiting step in PCho formation under some circumstances[20]. Four different types of choline-transporting transmembrane systems have been implicated in cancer. These are high-affinity choline transporters (CHTs), choline transporter-like proteins (CTLs), organic cation transporters (OCTs) and organic cation/carnitine transporters (OCTNs). Subtypes of each transporter have increased expression levels in cancer cells (TABLE 1).

High-affinity choline transport has been attributed to CHT1 (also known as SLC5A7)[21], which has a K_m (Michaelis constant) for choline of less than 10 μM. CTLs are responsible for intermediate-affinity, sodium-independent choline transport[22]. The CTL family consists of at least six genes, and all gene products undergo complex alternative splicing[23]. *SLC44A1* (which encodes CTL1)[23] exists as two splice variants. It is ubiquitously expressed in human tissues[24] and supplies choline for phospholipid biosynthesis[22,24]. Both OCTs and OCTNs are part of the SLC22 family and carry out low-affinity choline transport in addition to the transportation of other organic cations[25]. Human OCTs exist as three subtypes and translocate organic cations in an electrogenic, sodium-independent and reversible manner[25]. There are two human OCTN isoforms[25]. OCTN1 (also known as SLC22A4) functions in a sodium-independent manner[25]; whereas OCTN2 (also known as SLC22A5) functions as a

sodium-dependent co-transporter for certain zwitterions, and as a sodium-independent transporter of organic cations[25].

CTL1 was shown by microarray analysis to be expressed in cancers of the central nervous system (CNS), ovary, breast and prostate, as well as in leukaemia, and to be highly expressed in melanoma, and renal and colon cancer[24]. Human pulmonary adenocarcinoma tissues highly overexpress CTL1 and somewhat overexpress OCT3 (also known as SLC22A3) compared with matched normal tissues, as shown by immunohistochemistry[26]. In lung adenocarcinoma cell lines, enhanced choline uptake mostly depends on CTL1, and only partially on OCT3, OCTN1 and OCTN2 (REF. 26). However, in human HT-29 colon carcinoma cells, enhanced choline transport is predominantly mediated by CTL1, and to a lesser extent by CTL2 (also known as SLC44A2) and CTL4 (also known as SLC44A4)[27]. Increased choline transport[20] and elevated mRNA expression of *SLC5A7* (which encodes CHT1) and *SLC22A2* (which encodes OCT2)[28] was observed in breast cancer cells compared with mammary epithelial cells. Choline transport in PC-3 prostate cancer cells had a facilitative component that was characterized by sodium dependence and intermediate affinity, as well as non-facilitative components, although the transporters involved were not identified[29].

More molecular characterization is required to decipher the exact roles of the different transporters and how they lead to the enhanced choline transport that is observed in cancer cells. As a common theme so far, CTL1 is overexpressed in multiple types of cancer. However, it is also possible that different types of cancer use different choline transport mechanisms depending on their tissue of origin.

Choline kinase

CHK was first described in 1953 by Wittenberg and Kornberg[30], and first cloned in 1992 by Hosaka *et al*.[31]. It catalyses the phosphorylation of free choline using ATP as a phosphate donor, thereby producing PCho, and CHK can take on a rate-limiting, regulatory role in PtdCho biosynthesis under some circumstances[32]. CHK is primarily located in the cytoplasm of cells from various tissues, and its enzymatic properties have been extensively studied (reviewed in REF. 32). At least three isoforms of CHK exist in mammalian cells, and these are encoded by two genes: choline kinase-α (*CHKA*) and choline kinase-β (*CHKB*)[32]. The two functional isoforms, CHKα1 and CHKα2, are derived from *CHKA* by alternative splicing[32]. Homodimeric or heterodimeric forms of CHK are enzymatically active[32]. Both CHKα1 and CHKα2 homodimers display a dual choline and ethanolamine kinase activity, with a lower K_m for choline, whereas the CHKβ homodimer predominantly has ethanolamine kinase activity, and the CHKα–CHKβ heterodimer has an intermediate substrate specificity[33,34]. The proportions of different homodimer and heterodimer populations are tissue-specific[33], and studies with knockout mice demonstrate that *Chka* loss, but not *Chkb* loss, is embryonically lethal[35,36]. Taken together, knockout studies suggest that CHKβ cannot compensate for the loss of CHKα, which is necessary to sustain PtdCho biosynthesis[35,36]. Although the activity of yeast choline kinase (CKI) can be upregulated by protein kinase A (PKA)-dependent phosphorylation at both Ser30 and Ser85, there has been only one report indicating that PKA-mediated phosphorylation partially

regulates human CHK[37]. Thus, the upregulation of CHK activity in cancer probably results from an increase in CHKα expression, which would lead to a higher proportion of CHKα–CHKα dimers in cancer cells and in turn a higher CHK activity level than CHKα–CHKβ heterodimers or CHKβ–CHKβ homodimers.

Overexpression of CHKα, but not of CHKβ, has been reported in several human tumour-derived cell lines of multiple origins, as well as in biopsy samples of lung, colon and prostate carcinomas, among others, which were compared with matched normal tissue from the same patient[34,38,39] (TABLE 1). These findings, combined with the studies in knockout mice, indicate that CHKα, but not CHKβ, is essential for PtdCho biosynthesis, which is required for the uncontrolled growth of cancer cells. In addition, the activity of CHKα was shown to increase in human colon cancers[40] and in human breast carcinomas compared with normal breast tissue[41]. Increased enzymatic activity and overexpression of CHKα correlated with advanced histological tumour grade and negative oestrogen receptor status in breast carcinomas[41], which is consistent with the increased levels of PCho and tCho that are observed in breast cancers[42]. By contrast, no significant correlation was found with age, tumour size, progesterone receptor status, vascular invasion and histological tumour type, or with expression of p53, ERBB2 or Ki-67, in these breast tumours[41]. These clinical findings, combined with a large body of work in cancer cells, suggest that CHKα expression and activity is directly associated with increased cancer cell proliferation and malignancy, making it a potential prognostic marker of some cancers, such as non-small-cell lung cancer[39]. CHKα expression and activity levels have not yet been investigated in metastases.

CTP:phosphocholine cytidylyltransferase

CTP:phosphocholine cytidylyltransferase (CCT) catalyses the synthesis of CDP-choline and PP_i from PCho and cytidine triphosphate (CTP) (FIG. 3). CDP-choline is the most highly activated Cho intermediate of the Kennedy pathway, and it is directly used by diacylglycerol cholinephosphotransferase 1 (CHPT1) to form the membrane lipid PtdCho. First described in 1957 by Kennedy *et al*.[43], CCT catalyses the ratelimiting step in PtdCho synthesis in normal cells, and it catalyses the main rate-limiting and regulatory step in PtdCho biosynthesis in cancer cells[44], but CHK[32], and choline transporters[20], can take on an important regulatory role in some cases. CCT exists as an inactive soluble form and as an active lipid-bound form in the nuclear membrane[45]. Four highly homologous isoforms of CCT exist in mammalian cells: CCTα (also known as PCYT1A), which is ubiquitously expressed and active as a homodimer[45]; and CCTβ1, CCTβ2 and CCTβ3, which are splice variants of *PCYT1B* that exhibit tissue-specific expression[46]. Much of the work on CCT was carried out before the different isoforms were identified; nonetheless, it is clear that the control of CCT activity is complex and that it involves multiple factors that modulate membrane localization, phosphorylation and transcription of CCTs[47]. All of these factors have been linked to oncogenic signalling.

Hepatocarcinogenesis was demonstrated to be associated with increased CCT activity and mRNA expression, whereas phosphatidylethanolamine *N*-methyltransferase (PEMT) activity and mRNA expression were decreased, suggesting a reciprocal relationship between these two enzymes[48]. PEMT converts PtdEtn to PtdCho and can therefore replenish cellular

PtdCho levels at the expense of PtdEtn[48]. An increase in CCT activity would therefore result in the depletion of cellular PCho levels. However, ^{31}P MRS has frequently demonstrated that liver tumours contain significantly increased cellular PCho levels compared with normal liver[49]. Because several enzymes, other than CCT, such as CHK and PtdCho-specific phospholipase C (PC-PLC), can increase intracellular PCho levels, these enzymes may counteract a possible depletion of cellular PCho levels by increased CCT activity in liver tumorigenesis (TABLE 1). CCT expression and activity were also increased in colon cancer, which resulted in elevated PtdCho levels in colon cancer[50]. Taken together, CTL1, CHKα and CCT expression and activity are increased in colon cancer, which results in elevated PCho and PtdCho levels, thereby facilitating enhanced cell growth and proliferation (TABLE 1).

PtdCho-specific phospholipase D

PtdCho-specific phospholipase D (PC-PLD) was discovered in plants in 1947 (REF. 51), but it only attracted widespread attention when experiments in mammalian cell cultures revealed that PLD1 and PLD2 were rapidly activated in response to extracellular stimuli (reviewed in REF. 52). PLD1 and PLD2 hydrolyse PtdCho to phosphatidic acid and Cho, which is one of the breakdown pathways in PtdCho metabolism that directly produces intracellular free Cho (FIG. 3). PLD1 and PLD2 occur as three splice variants and share 50% sequence homology[53,54]. PLD1 is predominantly localized to Golgi membranes, whereas PLD2 is associated with both Golgi membranes and the plasma membrane[55]. Both PLD enzymes were also found in the cytoplasm and a variety of other organelles, and their location may depend on the cell type and physiological state of the cell[56]. PLD1 and PLD2 are crucial in cell proliferation, survival signalling, cell transformation and tumour progression[57].

Elevated activity of PC-PLD has been observed in gastric[58] and ovarian cancers[59], as well as in epithelial ovarian cancer cell lines[59]. PLD1 activity and expression were increased in breast cancers[60,61], as well as several human breast cancer[62] and melanoma[63] cell lines. PLD2 activity and expression were elevated in colorectal[64,65] and renal[66] cancers. A C1814T polymorphism occurs in *PLD2*, resulting in T577I substitution, which is associated with colorectal cancer but which does not affect PLD2 activity[67]. PLD1 or PLD2 expression were able to transform cells that overexpressed a tyrosine kinase such as epidermal growth factor receptor (EGFR) or SRC, suggesting a contribution of PLD1 or PLD2 to malignant progression in cells with elevated tyrosine kinase activity[68] (TABLE 1).

The overexpression of PLD1 and PLD2 in mouse fibroblasts caused neoplastic transformation, resulting in upregulated matrix metalloproteinase 9 (MMP9) activity, anchorage-independent growth in soft agar and the formation of undifferentiated sarcoma in nude mice[69]. Multidrug-resistant colon and breast cancer cells exhibited a higher level of PLD2 activity and expression than the parental drug-sensitive cells[70]. A correlation between increased PC-PLD activity and loss of oestrogen receptor expression in breast cancer cells has been observed, suggesting that PC-PLD could provide a survival signal that is normally provided by oestrogen in a developing tumour[71]. PC-PLD activity has also been implicated in tumour invasion and may play an important part in the metastasis of cancer cells[72,73]. High levels of PC-PLD activity correlate with high invasive potential in human breast

cancer cells[71], and elevated PC-PLD activity was shown to correlate with increased protease secretion, which is commonly observed in invasive cancer cells[72–75].

PtdCho-specific phospholipase C

PC-PLC catalyses the hydrolysis of PtdCho, thereby producing PCho and DAG (FIG. 3). DAG is a second messenger that induces protein kinase C (PKC) activation, which phosphorylates multiple target proteins and which is involved in several signal transduction cascades. PtdCho hydrolysis produces a longer and more sustained action of DAG than phosphatidylinositol cleavage by PC-PLC[76]. To date, no mammalian PC-PLC isoforms have been sequenced or cloned, and studies investigating the putative mammalian PC-PLC enzyme have relied on detecting PC-PLC activity and/or protein expression with rabbit polyclonal antibodies against bacterial PC-PLC from *Bacillus cereus*. PC-PLC translocates from a perinuclear cytosolic area to the plasma membrane following activation by growth factors, insulin or phorbol esters[77] (FIG. 3).

A function for PC-PLC has been demonstrated in hepatocarcinogenesis[78], in which the activity of calcium-dependent PC-PLC, but not of calcium-independent PC-PLC, increased significantly in a rat model of *N*-nitrosodiethylamine-induced hepatocarcinoma[78]. Stable transfection of NIH3T3 fibroblasts with bacterial PC-PLC led to chronically elevated levels of DAG and PCho, and a transformed phenotype in these cells that was mediated by RAF1 and PKCζ[79]. In ovarian cancer cells, the elevated PCho levels, which are linked to ovarian carcinogenesis, are partially caused by PC-PLC activation[80]. In breast cancer cells, PC-PLC accumulates on the plasma membrane of HER2 (also known as ERBB2)-overexpressing cells and associates with HER2 in lipid raft domains[81].

Other choline-related enzymes

Additional enzymes, such as PtdCho-specific phospholipase A2 (PC-PLA2), lysophospholipase, GPC phosphodiesterase (GPC-PDE; also known as GPCPD1) and CHPT1, may also be important in establishing the cholinic phenotype by altering the metabolite levels of PCho, GPC and Cho (FIG. 3). PC-PLA2 exists as 19 isoforms, complicating its investigation. Ovarian cancer lines contained unaltered or decreased expression of PC-PLA2 isoforms, which was accompanied by decreased overall PC-PLA2 activity[59]. The expression of cytosolic phospholipase A2 (PLA2; also known as PLA2G4A) was decreased in breast cancer cells[82], and may modulate the cellular MRS-detected GPC levels[83]. Sphingomyelinases, which catalyse the cleavage of the phosphodiester bond in sphingomyelin to form ceramide and PCho, are involved in cancer pathogenesis[84], and may potentially contribute to elevated PCho levels that constitute the cholinic phenotype. Inhibition of CHKα has been reported to increase ceramide levels, and therefore sphingomyelinases may have a role in the action of CHKα inhibitors as antitumour drugs[85].

Oncogenic regulation of choline metabolism

The first indication that choline metabolism could be regulated by oncogenic signalling came from early work showing that PCho levels increased following growth factor stimulation of NIH3T3 fibroblasts[86]. Subsequent studies (discussed below) have

demonstrated the complex and often reciprocal interactions (FIG. 2) between oncogenic signalling pathways and the enzymes that are involved in choline metabolism.

The RAS pathway

Each of the enzymes involved in choline metabolism is affected by the RAS pathway (FIG. 2). Activation of CHK and elevated PCho levels were initially reported in serum-stimulated, KRAS- or HRAS-transformed NIH3T3 fibroblasts[87–89]. Detailed investigations indicate that CHK activation downstream of oncogenic HRAS occurs via combined RAL GTPase guanine nucleotide dissociation stimulator (RALGDS) and PI3K signalling[90]. Activation of CHK could also be mediated by the small GTPase RHOA and its effector RHO-associated coiled-coil-containing protein kinases (ROCKs), which are associated with transformation and metastasis, and which are overexpressed in various cancers[91].

Oncogenic HRAS controls the transcription of CCT through the activation of the MAPK pathways[92]. MAPK signalling also modulates the expression of sterol regulatory element binding proteins (SREBPs). SREBPs, in turn, can induce CCT expression, but are more likely to lead to increased CCT activity by enhancing fatty acid synthesis, which promotes membrane translocation and thus the activation of CCT[93]. By contrast, other studies indicate that RAS signalling can result in the inhibition of CCT. Phosphorylation, and thus inhibition, of CCT is mediated by ERK[94] and JUN N-terminal kinase (JNK)[95]. Accordingly, oncogenic signalling can reduce CCT activity and can increase PCho levels[92,94,96]. Elevated CCT expression could lead to enhanced PtdCho synthesis, enabling rapid cell proliferation and tumour growth. However, the importance of reduced CCT activity in cell transformation remains unclear.

The activation of PC-PLD was first reported in serum-stimulated HRAS- or KRAS-transformed NIH3T3 cells[97]. Recent work has demonstrated the complex oncogenic regulation of PC-PLD with two positive regulatory pathways that are mediated by RALGDS and PI3K, and two negative feedback mechanisms that are mediated by RAF and RALGDS[71,98]. In turn, PC-PLD was identified as a regulator of cell transformation and tumour progression[71]. This effect could be mediated by phosphatidic acid, which couples EGFR stimulation to RAS activation through SOS[99], or it could be mediated through a positive feedback loop via WNT[57,100].

The activation of PC-PLC in cells that express oncogenic HRAS was hypothesized in early studies as an explanation for the presence of elevated PCho levels in these[18]. Subsequently, increased PC-PLC activity was observed downstream of serum stimulation, or platelet-derived growth factor (PDGF) stimulation via RAF1, and was associated with cell transformation[79,80,101]. However, an alternative mechanism for the elevation of PCho levels downstream of PDGF has been proposed and involves increased PC-PLD expression combined with increased CHK activity[102]. Unfortunately, the lack of molecular biological information regarding PC-PLC has hampered more detailed studies into its role in cancer.

The expression of PLA2 is mediated through JNK and ERK signalling [103], and its activity is further induced by ERK through phosphorylation on Ser505 (REF. 104). In turn, PLA2 has been reported to mediate cell transformation downstream of HRAS[105].

The PI3K–AKT pathway

Inhibiting the PI3K–AKT signalling pathway blocked choline uptake in lung adenocarcinoma cell lines, suggesting that this pathway might participate in the regulation of choline transport[26]. As mentioned above, PI3K–AKT signalling also affects CHK activation, which occurs via combined RALGDS and PI3K signalling[90] (FIG. 2). Recent studies point to a reciprocal interaction between the enzyme and oncogenic pathways. CHKα overexpression resulted in the differential expression of multiple genes that are associated with cell cycle progression, whereas partial inhibition of CHKα resulted in cell cycle arrest or apoptosis[106]. Knockdown of CHKα using small interfering RNA (siRNA) led to the simultaneous attenuation of MAPK and PI3K–AKT signalling and was associated with the inhibition of cell proliferation[107]. A separate study indicated that knockdown of CHKα or CHKβ inhibited AKT-S473 phosphorylation independently of PI3K, and this was associated with the inhibition of cell proliferation[108]. Consistent with this observation, treatment with the CHKα inhibitor Mn58b inhibited tumour growth *in vivo* [108]. Most recently, a role for CHKα in mediating the synergistic actions of EGFR and SRC in breast cancer development has also been shown[109]. These observations reinforce the proposed role of *CHKA* as an oncogene, with its overexpression contributing, through a positive feedback loop, to increased MAPK and PI3K signalling.

Transcription factors

Transcriptional control of the regulators of choline metabolism by factors associated with cancer has been most clearly demonstrated for CHK. In the liver, the binding of JUN to a distal activator protein 1 (AP1) element in the promoter region of *CHKA* mediates its expression, indicating a possible link between the role of AP1 in cell proliferation and transformation and CHK[110] (FIG. 2). Two additional transcription factors, which are important in oncogenesis and which are likely to be involved in the control of CHK expression, are MYC and HIF1. A possible role for the proto-oncogene *MYC* was indicated in studies that showed elevated levels of CHK and PCho in $Myc^{+/+}$ compared with $Myc^{-/-}$ rat fibroblasts[111]. In the case of HIF1, a recent study clearly identified putative hypoxia response elements within the promoter of *CHKA* and showed that hypoxia and HIF1 stabilization increase PCho levels[8] (FIG. 2). How this effect could enhance cancer cell survival under hypoxic conditions is unclear, but this observation indicates that cells that survive in a hypoxic environment are likely to demonstrate elevated PCho levels.

With regard to other transcription factors that are associated with choline metabolism, SP1, which is involved in cell growth and which is upregulated in a variety of cancers, controls the expression of CTL1 (REF. 24), CCTα[112] and PLD1 (REF. 113). Collectively, these enzymes could therefore enhance PtdCho synthesis, facilitating cell growth and proliferation.

Clinical implications

Several clinical implications arise from the deregulated choline metabolism of cancers. The most evident of these is non-invasively detecting cancer with MRS, and indeed many centres throughout the world are evaluating the use of spectroscopic imaging to assist in the

diagnosis and the staging of cancer. Two additional, equally valuable, areas of application are in detecting therapeutic response and exploiting choline phospholipid metabolism to identify new targets for cancer treatment (FIG. 3).

Diagnosis and staging of cancer

Several ongoing multi-centre trials evaluating ^{1}H MRS imaging (MRSI) of breast[114], brain[115] and prostate[116,117] cancer, have reported an elevation of tCho levels in tumours. Increased tCho levels have been associated with aggressiveness in breast cancer[118]. The elevation of tCho expression is a specific biomarker of prostate cancer and correlates with Gleason score and aggressiveness[117]. In breast and ovarian cancers, the low PCho and high GPC levels that are observed in non-malignant cells change to high PCho and low GPC levels with malignant transformation[5,59]. PCho, GPC and tCho concentrations in human cancers versus normal tissue of the same origin are summarized in TABLE 1. The elevated tCho level in brain tumours has been used for detection[119] and grading[120], radiation therapy treatment planning[121], and determining recurrence from radiation-induced necrosis and reactive astrocytosis[122].

Although the spatial resolution of MRS is orders of magnitude lower than histological evaluation and staging from biopsies or excised tumours, MRS has the advantage of non-invasively detecting tumour metabolism, and can be combined with clinically approved magnetic resonance imaging (MRI) methods in the same setting. Unlike the histological evaluation of biopsies, MRS also provides information about the entire tumour.

MRS applications in the clinic are technically challenging and, unfortunately, are currently not reimbursed by healthcare providers in the United States[123]. This prevents patients from receiving the potential benefits of MRS, and slows down future progress in clinical MRS techniques, as, without reimbursement, there is no incentive for manufacturers to support MRS development or for radiologists to use MRS[123]. Nonetheless, several large multi-centre clinical trials are currently evaluating the use of MRS for the detection of tCho and other metabolites in diagnosis, staging and therapeutic response monitoring. This reflects the fairly early stages of MRS technology in terms of clinical translation[111,112,114,115]. However, the value of MRS in identifying tumours and predicting survival has been demonstrated[111,112,114,115]. Furthermore, the introduction of higher field clinical MR scanners, such as 7 Tesla and higher, although to date technically more demanding, should allow for better spatial and spectral resolution of non-invasive MRS in clinical applications, probably achieving resolution of the tCho signal into GPC, PC and Cho and further enhancing the information obtained by ^{1}H MRS.

Recent advances in the process of dynamic nuclear polarization (DNP) and the development of commercial polarizers provide a novel method to dramatically increase the MRS signal that arises from exogenous hyperpolarized compounds and their metabolites. The use of hyperpolarized ^{13}C MRS is now in clinical trials at the University of California in San Francisco (UCSF), USA, with very promising results. Whereas monitoring of hyperpolarized PCho synthesis from hyperpolarized choline has not yet been demonstrated in cells or tumours, some studies indicate that this approach might be feasible in the future. Synthesis of PCho from hyperpolarized ^{15}N-labelled choline was observed *in vitro* using

purified CHK[124], and the presence of ^{15}N choline, al though not its metabolism, was reported *in vivo* in rat brain[125]. Metabolism of hyperpolarized ^{13}C-labelled choline to acetylcholine was also observed *in vitro* [126]. In addition, ^{11}C- and ^{18}F-labelled choline and choline derivatives are being evaluated for non-invasive PET radiological diagnosis of metastatic or recurrent cancers[127].

Detecting therapeutic response

Treatment with most conventional chemotherapeutic agents results in a decrease of tCho levels in responding tumours in preclinical models[128] and in human tumours[129,130]. Studies investigating the molecular basis underlying these changes are urgently needed. These studies will provide much needed insight into how choline metabolism in cancer cells is affected by chemotherapy, and whether treatment outcome would be improved by combining conventional chemotherapy with the downregulation of enzymes in this pathway.

Because the enzymes that control choline metabolism are modulated by oncogenic signalling pathways (FIG. 2), the inhibition of this signalling results in altered choline-containing metabolite levels of Cho, PCho and GPC. Accordingly, these metabolites could provide downstream metabolic readouts of effective inhibition of these pathways. Several studies have used MRS and have demonstrated the value of this approach for the non-invasive detection of drug molecular action. The inhibition of oncogenic signalling in mutant HRAS-transfected NIH3T3 cells resulted in reduced levels of PCho, whereas PCho levels remained unchanged in the parent line[131]. Inhibition of PI3K or MAPK signalling also resulted in reduced PCho and tCho levels, with recent studies indicating that this effect is primarily mediated by CHKα inhibition as a result of its reduced expression downstream of HIF1 activation[8,132–135]. Consistent with these findings, and with the fact that p53 can regulate HRAS[136], loss of p53 function also led to an increase in PCho levels[137]. In addition, the inhibition of HIF1α[138] resulted in reduced PCho levels, and tumours deficient in HIF1β (also known as ARNT), which HIFα heterodimerizes with to form the HIF transcription factor, showed lower levels of PCho than controls[139].

Other metabolic imaging studies highlight the need for further investigation. The inhibition of heat shock protein 90 (HSP90), which results in reduced signalling through both the MAPK and the PI3K pathways, leads in most cases to an unexpected increase in PCho and GPC levels[140,141] and to increased uptake of the PET tracer [^{11}C]-choline[142]. These effects could be mediated by the increased expression of the choline transporter CTL1 (REF. 143), but this varies across cancer types, which possibly reflects differences in genetic background[144]. Drug-induced inhibition of fatty acid synthesis resulted in reduced PCho levels and lower CHK activity, potentially identifying fatty acids as post-translational modulators of CHK activity, which is similar to their role in the control of CCT activity, but such an effect remains to be confirmed[145]. The inhibition of cyclooxygenase resulted in reduced levels of PCho and an increase in GPC levels with no concomitant changes in the expression of the enzymes that are associated with choline metabolism, but this study identified several candidate genes that could be involved in altering choline metabolism through secondary, as yet undetermined, effects[146]. Finally, phenylbutyrate induced increases in GPC levels, which was consistent with an as yet undetermined mechanism

whereby peroxisome proliferator-activated receptor-γ (PPARγ) is responsible for increased PLA2 activity[147].

Therapeutic opportunities

The increased expression and activity of CHKα make it an attractive molecular target for anticancer therapy[148–151]. Lacal and colleagues first suggested CHKα inhibition as a potential antitumour therapy[152], and developed novel chemical compounds for this purpose[152]. The most promising of these compounds exhibited significant antiproliferative activity, induced the reduction of tumour growth and was specific for CHKα inhibition[152,153]. Overall, these novel pharmacological inhibitors targeting CHKα[151] resulted in tumour growth arrest and apoptosis through the induction of a cytotoxic increase in ceramide levels[85,149]. A CHKα inhibitor (TCD-717) is currently undergoing Phase I clinical trials for toxicity testing in cancer patients.

Targeting CHKα results in decreased PCho and tCho levels, which can be detected non-invasively with ^{31}P or ^{1}H MRS[149,150]. Silencing of CHKα in human breast cancer cells by RNA interference (RNAi) has been demonstrated to decrease cellular ^{1}H MRS-detectable PCho levels, while leaving human mammary epithelial cells unaffected[148,150,154]. A significant decrease of cell proliferation and induction of differentiation in highly invasive and metastatic human breast cancer cells and tumour xenografts was observed with CHKα downregulation[148]. Non-invasive ^{31}P MRS detected a reduction in PCho levels following gene therapy with an intravenously injected lentiviral vector that delivered short hairpin RNA targeting CHKα in a breast cancer model[150]. Combining CHKα silencing with chemotherapeutic 5-fluorouracil treatment resulted in a more pronounced anti-proliferative and growth inhibitory effect in breast cancer cells, but not in non-malignant breast epithelial cells[154]. Radiolabelled choline or choline analogues can also be used as pharmacodynamic markers to monitor CHKα-targeted therapies using PET[155].

Hemicholinium-3 (HC-3), which inhibits low-affinity sodium-independent choline transport[156], has been proposed as a potential therapeutic anticancer agent[152]. However, HC-3 is not specific to choline transport and also inhibits CHK[152]. Radiolabelled HC-3 was evaluated as a molecular imaging agent in a preclinical study, to potentially detect prostate cancer by PET[29].

Hexadecylphosphocholine (HePC; also known as miltefosine) is a synthetic alkylphosphocholine that is currently being investigated for antineoplastic therapy and it has been approved for the topical treatment of cutaneous metastases of mammary carcinomas[157]. Its mechanism of action involves the inhibition of CCT activity by preventing CCT translocation to the membrane[158,159]. This leads to elevated cellular PCho levels, and decreasing cellular PtdCho levels, and identifies a potential approach for monitoring the effects of treatment[158,159].

Because PC-PLD is involved in several aspects of cell proliferation and oncogenic signalling, it could prove to be valuable as a target for therapeutic intervention in cancer. HePC was also shown to modulate PC-PLD activity[160]. Chronic exposure of different non-tumorigenic mammalian cell lines to HePC strongly inhibits PC-PLD activity, which may be

related to its antitumour activity[160]. The selective oestrogen receptor modulators tamoxifen and raloxifene, which are commonly used for the chemotherapy of oestrogen receptor-positive breast cancer, were reported to differentially affect PC-PLD activity[161]. Multidrug resistance in human cancer cells was accompanied by increased PLD2 activity in detergent-insoluble glycolipidrich and caveolar membranes[70]. High levels of PC-PLD activity also conferred resistance to rapamycin, an inhibitor of mTOR, in human breast cancer cells[162].

The anti-oestrogen drug tamoxifen, which is widely used for the treatment of breast cancer[163], as well as some other cancers[164,165], activates cellular PC-PLD and PC-PLC, thereby causing increased levels of DAG, PCho and phosphoethanolamine (PEtn), and sustained translocation and activation of PKC, which may ultimately lead to changes in cell growth[166]. PC-PLC inhibition reduced HER2 expression on the plasma membrane, thereby inducing antiproliferative effects, which suggests that it may provide a valuable strategy to counteract the tumorigenic effects of HER2 and thus complement HER2-targeting therapies[81]. The antiviral, potentially antitumoural xanthate D609 was shown to be a potent inhibitor of PC-PLC[167], and significantly reduced PCho levels along with cell proliferation in ovarian cancer cells[59]. D609 can also inhibit sphingomyelin synthase[168]. Although it has shown promising results in cancer cell lines and transformed cells *in vitro*[169], D609 exhibited little antitumour activity in *in vivo* animal studies[170]. The dual-specificity phosphatase inhibitor SC-ααδ93,4-(benzyl-(2-[(2,5-diphenyloxazole-4-carbonyl)amino] ethyl)carba-moyl)-2-decanoylaminobutyric acid, which exhibits antitumour activity *in vivo*, was also shown to be a potent inhibitor of PC-PLC[171].

Conclusions

As a disease, cancer continues to confound the technological advances that are available in the twenty-first century. Common pathways are a rarity in this disease, but these provide the vision and hope for finding effective treatments (FIG. 3). Over the past decade, aberrant choline metabolism is one common pathway that has been consistently revealed by bench-to-bedside MRS studies evaluating tCho levels as a biomarker for detection and monitoring response to treatment (FIG. 1). Elevated levels of PCho and tCho have been observed in almost every cancer type studied. Although substantial advances have been made in understanding the molecular mechanisms that drive these differences, the challenges for the future are to comprehensively understand the mechanisms underlying this elevation, interactions between cancer cells and stromal cells, and the transcription factors that regulate enzymes and transporters in the choline pathway. New methodologies such as mass spectrometric imaging[172] in combination with molecular biology techniques such as PCR carried out on tissue sections should allow a genomic- to proteomic-scale characterization of deregulated choline metabolism. Although ^{31}P MRS studies have identified increased PEtn levels in tumours[13], the role of the CDP-ethanolamine pathway in malignant transformation and progression is almost completely unexplored and merits further investigation. The apparent positive feedback that is mediated by enzymes in the choline cycle, as well as the interaction and compensatory mechanisms between enzymes in the choline cycle and the ethanolamine cycle that may allow cancer cells to adapt and survive the downregulation of individual enzymes, are other areas that require focus in the future. As the connectivity, networks and feedback mechanisms in choline metabolism become more evident, a systems

biology approach will be necessary to identify the crucial nodes that should be targeted in this pathway. Other challenges are the sequencing of all genes in the choline cycle to allow a better understanding of deregulated choline metabolism in cancers. Targeting the enzymes involved in choline metabolism may prove to be highly effective against cancer cells, and MRSI would lend itself most easily to clinical applications to detect the effect of targeting choline metabolism non-invasively *in vivo* for image-guided therapy. The development of image-guided siRNA delivery to target enzymes[173] could allow the downregulation of multiple enzymes in the choline cycle, and the clinical translation of these technologies provides hope for the future.

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Glossary

Total choline-containing compounds	(tCho). The sum of choline (Cho),phosphocholine (PCho) and glycerophosphocholine (GPC),this term was coined because these three metabolite signals appear as one overlapping signal in non-invasive *in vivo* ^{1}H magnetic resonance spectra owing to limited spectral resolution
Magnetic resonance spectroscopy	(MRS). A technique that measures the nuclear magnetic resonance of ^{1}H,^{31}P and other magnetic resonance-active nuclei to determine their physical and chemical properties either non-invasively in live organisms or at high spectral resolution in *ex vivo* samples
Phosphomonoesters	(PMEs). Phospholipid metabolism intermediates, such as phosphocholine (PCho) and phosphoethanolamine (PEtn), which contain one ester bond between the phosphate group and the specific phospholipid head group alcohol
Phosphodiesters	(PDEs). Phospholipid metabolism intermediates, such as glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE),which contain two ester bonds at the phosphate group,one to the specific phospholipid head group alcohol and a second one to glycerol
Magic angle spinning	(MAS). A specialized magnetic resonance spectroscopy technique that avoids tissue extraction to detect high-resolution spectra by spinning solid tissue samples at the angle of 54.74°with respect to the magnetic field. These tissue samples can be used for subsequent histological,biochemical and genetic analyses

Phorbol esters	A class of compounds originally derived from esterification of the plant compound phorbol,which promote tumorigenesis through the activation of protein kinase C
MRS imaging	(MRSI). Applies magnetic resonance spectroscopy (MRS) in a spatially resolved manner, thereby providing metabolite concentrations in tissue in two or three spatial dimensions and allowing for the display of metabolic maps throughout the tissue

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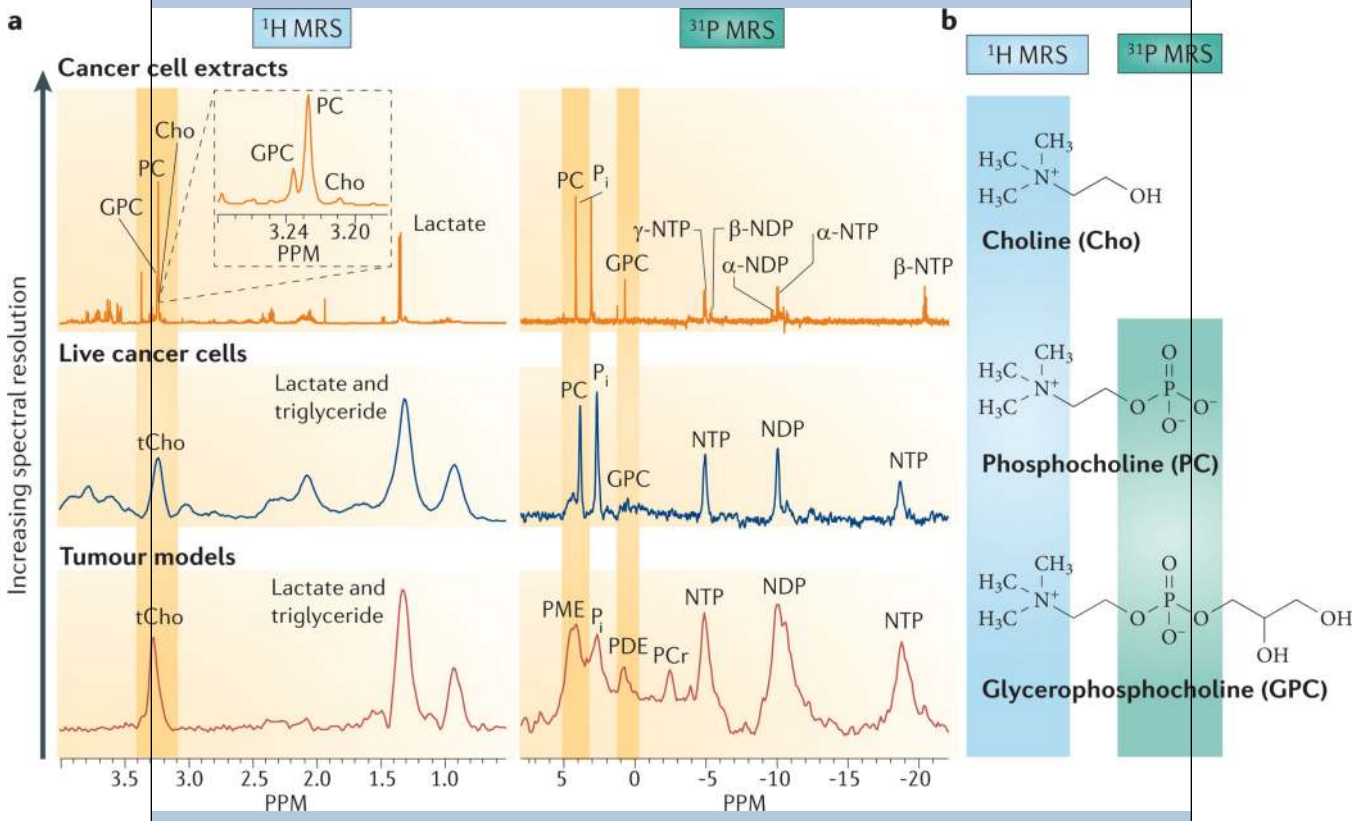
Box 1

Detecting deregulated cancer choline metabolism with MRS



Choline metabolism in cells and tumour xenografts has been extensively investigated with ^{1}H, ^{13}C and ^{31}P magnetic resonance spectroscopy (MRS). In 1H MR spectra obtained *in vivo*, the total choline-containing compounds (tCho) signal in tumours is detected as a single peak observed between 3.2 ppm and 3.3 ppm (see part **a** of the figure). This peak consists of choline (Cho), phosphocholine (PCho) and glycerophosphocholine (GPC), which can be resolved in high-resolution 1H MR spectra of tissue and cell extracts, or in high-resolution magic angle spinning (MAS) ^{1}H MR spectra of tissue biopsy samples (see part **a** of the figure). These peaks arise from the nine chemically equivalent protons in the highlighted choline-$N(CH_3)3$ groups of Cho, PCho and GPC (see part **b** of the figure).

In vivo ^{31}P MR spectra contain signals from phosphomonoesters(PMEs)that consist of the overlapping peaks from membrane precursors PCho and phosphoethanolamine (PEtn), and from phosphodiesters (PDEs) that consist of the overlapping peaks from membrane breakdown products GPC and glycerophosphoethanolamine (GPE) (see part **a** of the figure). PCho and PEtn can also be formed during the breakdown of phosphatidylcholine (PtdCho) and phosphatidylethanol-amine (PtdEtn). Individual PCho, PEtn, GPC and GPE peaks are detected in high-resolution ^{31}P MR spectra (see part **a** of the figure). These PCho and GPC peaks arise from the ^{31}P nuclei in the highlighted phosphate groups (see part **b** of the figure). Increased levels of PMEs were evident in the very first MR

spectrum of a human sarcoma, which was acquired in 1983 (REF. 3). Because of its low sensitivity, ^{31}P MRS has limited application in human studies.

^{13}C MRS studies of choline metabolism are carried out to track the metabolism of exogenously supplied ^{13}C-labelled choline in cells[143] or *in vivo*[174]. The incorporation of ^{13}C-labelled choline into different metabolites in the choline phospholipid metabolic pathways can be used to calculate flux rates from the rate of enrichment of the metabolite pool. Future studies might demonstrate the value of hyperpolarized Cho for monitoring Cho uptake and phosphorylation with greater sensitivity.

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At a glance

- Some of the levels of metabolic intermediates that are generated in choline phospholipid metabolism, particularly phosphocholine (PCho) and total choline (tCho), are elevated in cancer, and can be used for non-invasive detection in cancer diagnosis and staging by using magnetic resonance spectroscopy (MRS) or positron emission tomography (PET).
- Several enzymes in choline metabolism, such as choline transporter-like protein 1 (CTL1), choline kinase-α (CHKα), CTP:phosphocholine cytidylyltransferase (CCT), phosphatidylcholine-specific phospholipase D (PC-PLD) and PC-PLC, are overexpressed and/or activated in cancer and can potentially be used as prognostic markers.
- Overexpression and activation of choline cycle enzymes are mediated by oncogenic signalling via pathways such as the RAS and PI3K–AKT pathways, and by transcription factors associated with oncogenesis such as hypoxia-inducible factor 1 (HIF1). Recent studies point to a reciprocal interaction between choline cycle enzymes and oncogenic signalling, where modulation of the enzymes can contribute to enhancing oncogenic signalling.
- The therapeutic response of tumours can be monitored non-invasively by MRS of the tCho signal because treatment with conventional chemotherapeutic agents results in a decrease of tCho levels in responding, but not in non-responding, tumours.
- Inhibition of oncogenic signalling pathways with targeted anticancer drugs results in altered choline-containing metabolite levels. Therefore, these metabolites could provide downstream metabolic readouts of the effective inhibition of these pathways.
- New molecularly targeted therapeutic opportunities arise from targeting choline cycle enzymes such as CHKα, which is currently being tested in Phase I clinical trials.

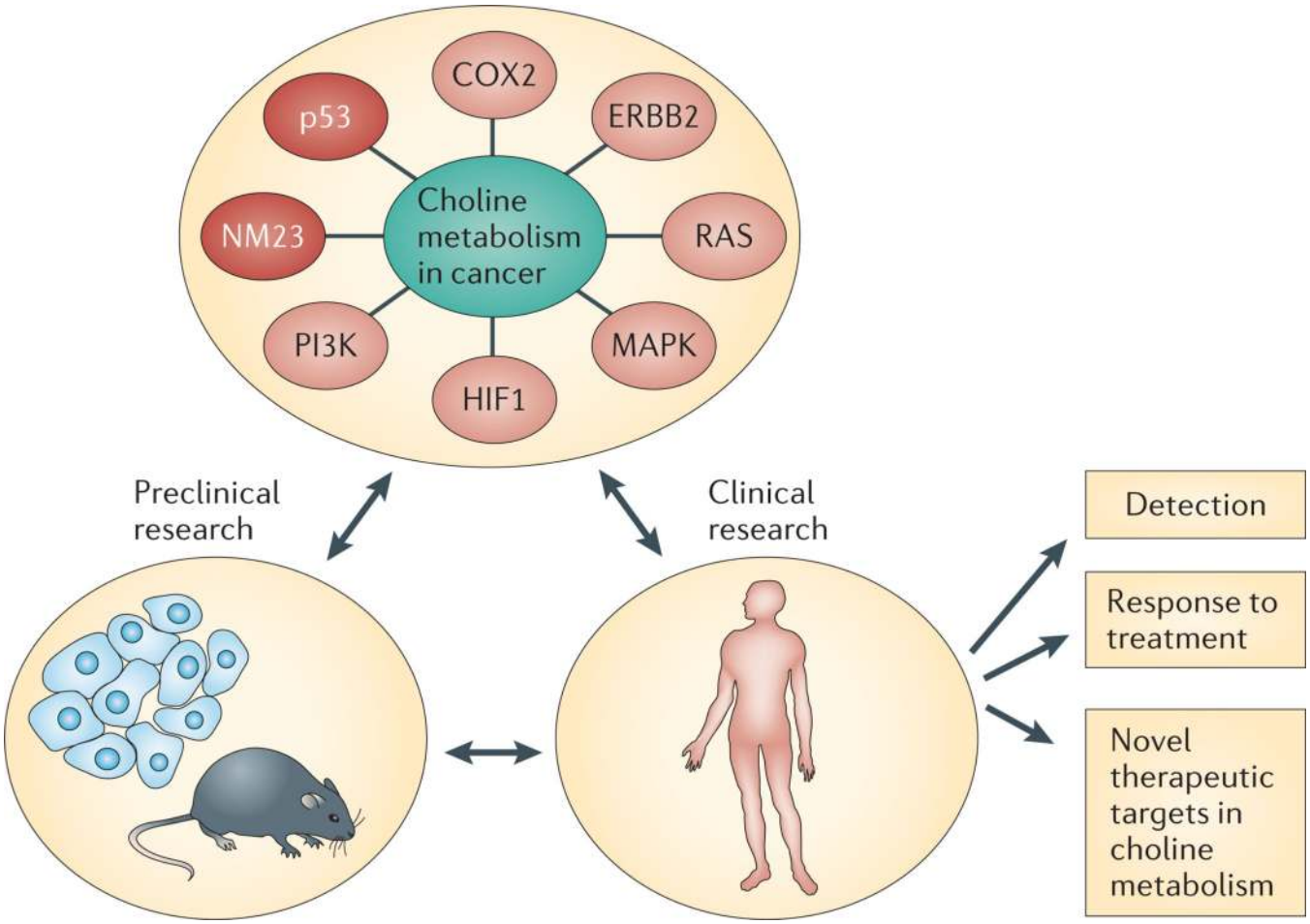


Figure 1. Overview of deregulated choline metabolism in cancer

Magnetic resonance spectroscopy (MRS) studies with cells, animal models and human studies have revealed deregulated choline metabolism in cancer. Oncogenic pathways (shown in light red), tumour suppressor pathways (dark red) and molecules that are associated with choline metabolism (green) in cancer are shown in the upper circle. These studies have led to the use of MRS for cancer detection and for following the treatment-induced changes in total choline-containing compounds (tCho) to determine response to therapy. The association of choline metabolism with cancer has also led to the development of inhibitors that target enzymes in the pathway, and a Phase I clinical trial with a choline kinase-α (CHKα) inhibitor has been initiated. COX2, cyclooxygenase 2; HIF1, hypoxia-inducible factor 1.

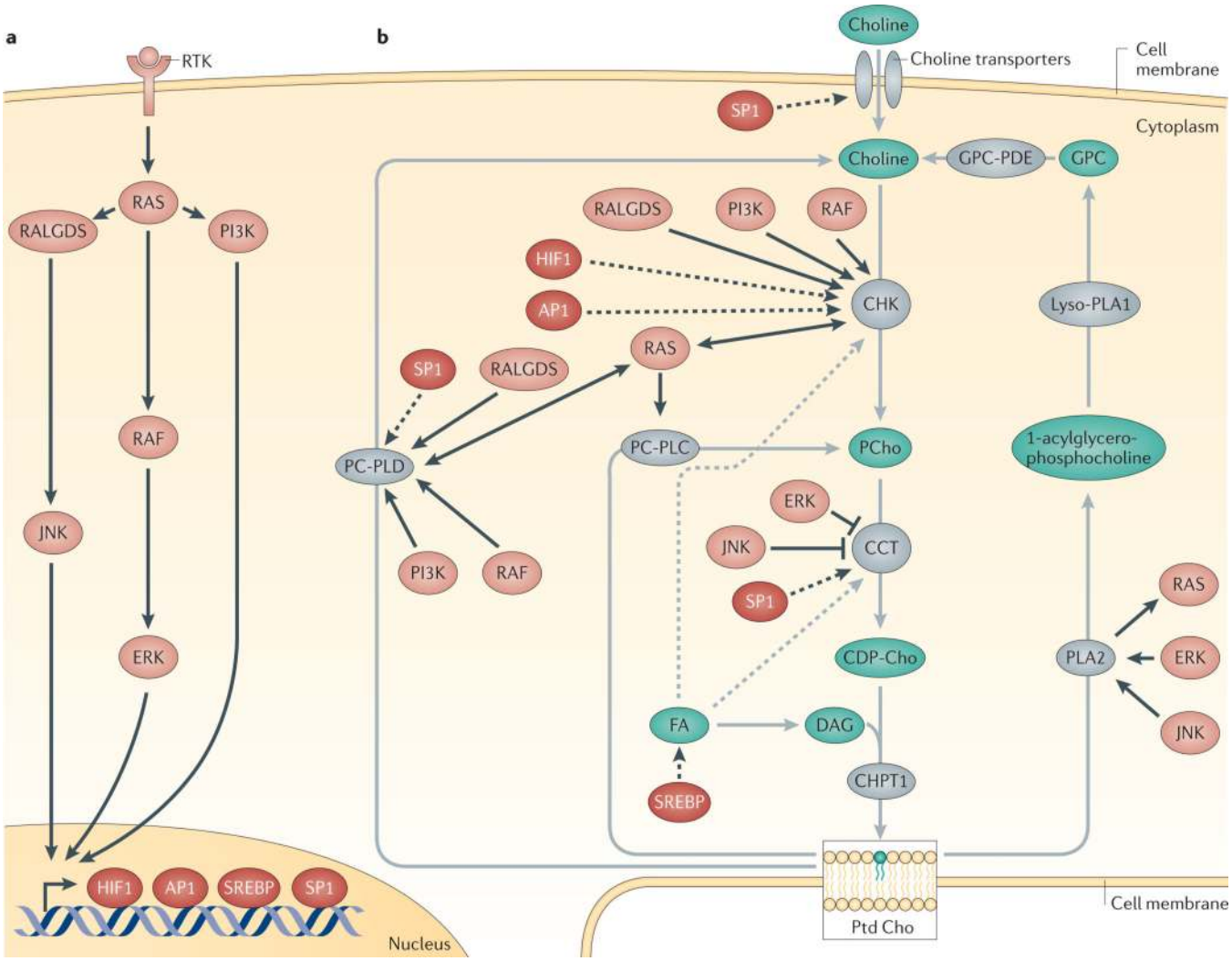


Figure 2. Control of choline metabolism by oncogenic signalling pathways

Oncogenic signalling pathways (part **a**) that interact with the choline metabolic pathway (part **b**) are shown. Grey arrows represent the choline metabolism pathway, proteins in grey catalyse the reaction that is depicted by the corresponding grey arrow. Dashed grey arrows indicate the regulation of enzyme activity in the choline metabolism pathway. Black arrows indicate connections to the oncogenic signalling pathways shown in part **a**. Solid black arrows indicate increased or decreased enzyme activity, dashed black arrows indicate increased gene transcription. AP1, activator protein 1; CHK, choline kinase; CHPT1, diacylglycerol cholinephosphotransferase 1; CCT, CTP:phosphocholine cytidylyltransferase; DAG, diacylglycerol; FA, fatty acid; GPC, glycerophosphocholine; HIF1, hypoxia-inducible factor 1; JNK, JUN N-terminal kinase; PC-PLC, phosphatidylcholine-specific phospholipase C; PC-PLD, phosphatidylcholine-specific phospholipase D; PLA2, phosphatidylcholine-specific phospholipase A2; PCho, phosphocholine; PtdCho, phosphatidylcholine; RALGDS, RAL GTPase guanine nucleotide dissociation stimulator; RTK, receptor tyrosine kinase; SREBP, sterol regulatory element binding protein.

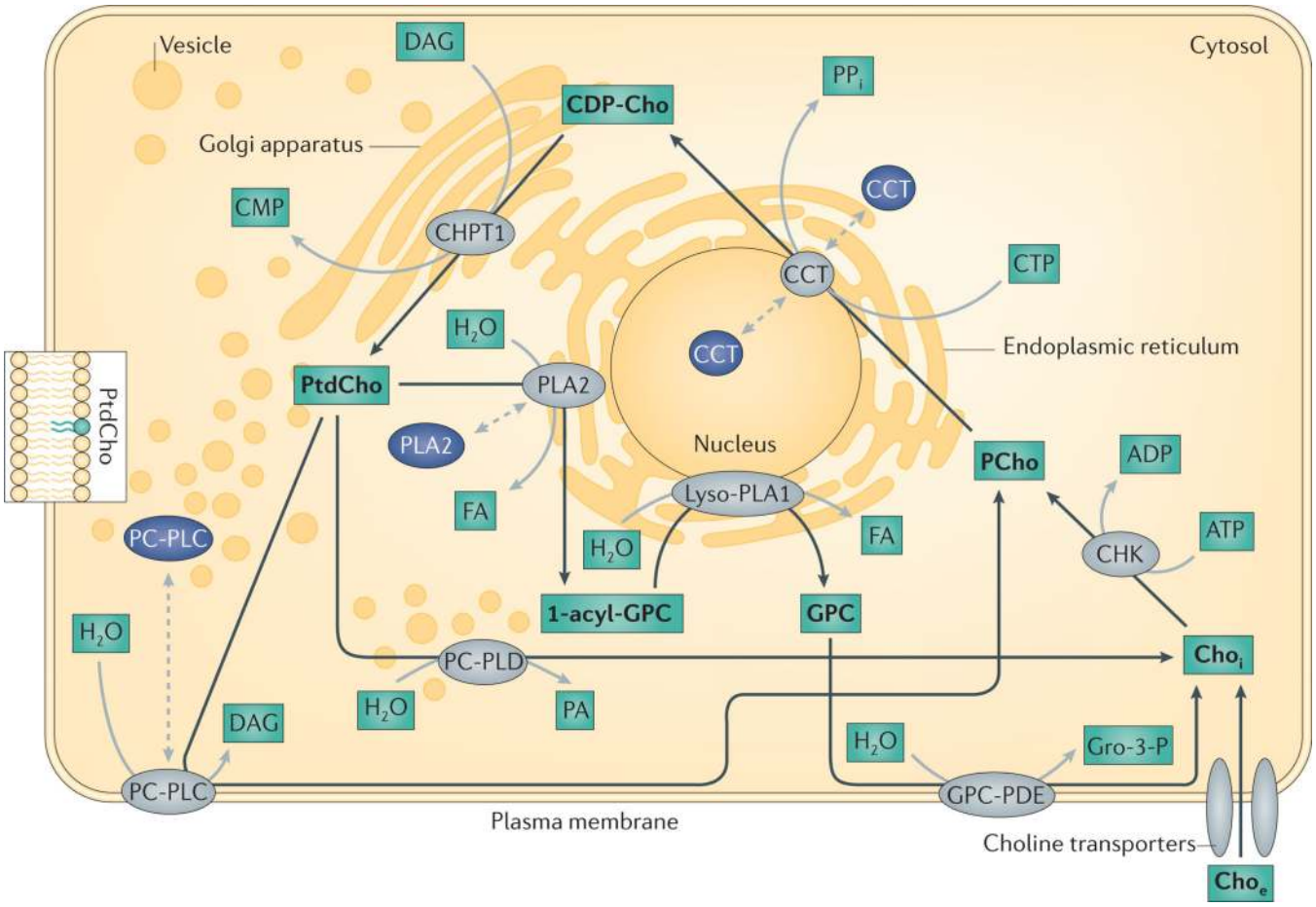
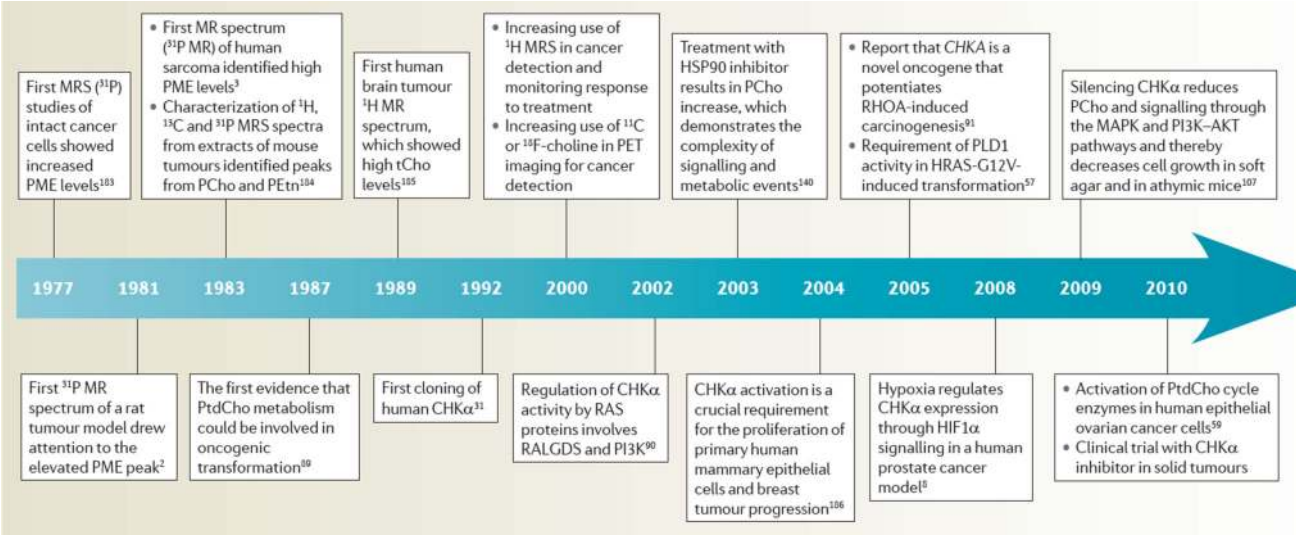


Figure 3. The major enzymes involved in choline phospholipid metabolism in the cell

Enzymes shown in grey indicate active choline cycle enzymes, which are shown in the organelle in which they are active. Enzymes shown in dark blue indicate the location of choline cycle enzymes that are deactivated by translocation to a different organelle. Black arrows represent the choline metabolism pathway, proteins in grey catalyse the reaction that is depicted by the corresponding black arrow and choline cycle metabolites are shown in bold. Dashed grey arrows show translocation to different subcellular locations, which can deactivate (dark blue) or activate (grey) the enzyme. CCT, CTP: phospho-choline 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, glycerophospho-choline 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_i, diphosphate.



Timeline. Deregulated choline metabolism in cancer

CHKα, choline kinase-α; HSP90, heat shock protein 90; MRS, magnetic resonance spectroscopy; PCho, phosphocholine; PET, positron emission tomography; PEtn, phosphoethanolamine; PLD1, phospholipase D1; PME, phosphomonoester; PtdCho, phosphatidylcholine; RALGDS, RAL GTPase guanine nucleotide dissociation stimulator; tCho, total choline-containing compounds.

Table 1

Choline metabolite concentrations and choline enzyme expression and activity levels in human cancers

Cancer site	PCho		GPC		tCho		Enzyme expression	Enzyme activity
	Normal	Cancer	Normal	Cancer	Normal	Cancer		
Bladder	ND	ND	ND	ND	ND	ND	CHKα ↑[176]	CHKα ↑[176]
Brain	0.48 ±0.08 (REF. 177)	0.91 ±0.20 (REF. 177)	0.86 ±0.11 (REF. 177)	0.68 ±0.17 (REF. 177)	0.64* ±0.10 (REF. 178) and 1.79‡ ±0.24 (REF. 177)	1.19§ ±0.30 (REF. 178) and 1.88 // ±0.33 (REF. 177)	ND	ND
Breast	0.03 ±0.03 (REF. 42)	0.79 ±0.55 (REF. 42)	0.04 ±0.04 (REF. 42)	0.28 ±0.20 (REF. 42)	0.07 ±0.07 (REF. 42)	1.07 ±0.75 (REF. 42)	CHKα ↑[38], CHT1 ↑[28] OCT2 ↑[28] and PLD1 ↑[61]	CT ↑[28] CHKα ↑[41], CCT inconclusive[28] and PLD ↑[60,61]
Colon	ND	ND	ND	ND	ND	ND	CTL1 ↑[24,27] CHKα ↑[38,40], CCT ↑[50] and PLD2 ↑[64,65]	CT ↑[27], CHKα ↑[40], CCT ↑[50], PLD2 ↑[64] and PC-PLC↓,[50]
Liver	0.17 ±0.11 (REF 49)	1.36 ±0.50 (REF 49)	2.46 ±0.37 (REF 49)	0.59 ±0.15 (REF 49)	ND	ND	CCT ↑[48]	CT ↑[179], CCT ↑[48] and PC-PLC ↑[78]
Lung	Increased in tumour tissue versus normal tissue[180,181]		Increased in tumour tissue versus normal tissue[181]		Increased in tumour tissue versus normal tissue[180,181]		CHKα ↑[38,39] CTL1 ↑[26] and OCT3 ↑[26]	CHKα ↑[39]
Ovary	Increased in tumour tissue versus normal tissue[59]		Increased in tumour tissue versus normal tissue[59]		Increased in tumour tissue versus normal tissue[59]		CHKα ↑[59] OCT3 ↓[9], CTL3 ↑[59] CCT ↓[59] and PC-PLC↑[59]	CHKα ↑[59] PC-PLD ↑[59] and PC-PLC ↑[59]
Prostate	0.02 ±0.07 (REF. 182)	0.39 ±0.40 (REF. 182)	0.29 ±0.26 (REF. 182)	0.57 ±0.87 (REF. 182)	0.31 ±0.33 (REF. 182)	0.96 ±1.27 (REF. 182)	CHKα ↑[38]	CT ↑[29]

CCT, CTP:phosphocholine cytidylyltransferase; CHKα, choline kinase-α; CHT1, choline transporter 1; CT, choline transport; CTL, choline transporter-like; GPC, glycerophosphocholine; ND, not determined; OCT, organic cation transporter; PCho, phosphocholine; PC-PLC, phosphatidylcholine-specific phospholipase C; PLD, phospholipase D1; tCho, total-choline-containing compounds.

*Cortex.

‡White matter.

[§] Glioblastoma.

[//] Astrocytoma grade IV.