## Tumor Lipid Metabolism

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**Target Audience:** Clinicians and basic researchers interested in MRS of tumor lipid metabolism.

### MRS of Lipid Metabolism

Multi-nuclear magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) are playing an important role in unraveling the aberrant lipid metabolism commonly observed in cancer cells and tumors. *In vivo* single-voxel $^{1}$H MRS and multi-voxel $^{1}$H MRSI detect several signals that are related to choline, ethanolamine, and lipid metabolism, such as the total choline (tCho) signal at 3.2 ppm, the methylene signal at 1.3 ppm, and the methyl signal at 0.9 ppm. The tCho signal consists of precursors and breakdown products of phosphatidylcholine, the most abundant phospholipid in cell membranes. Cellular choline phospholipid metabolism is altered in most cancers [1-5]. The methylene and methyl signals arise from $CH_2$ and $CH_3$ groups in mobile lipids located in the cytoplasm of intact cancer cells, or the intercellular space of solid tumors [6, 7]. The choline and ethanolamine metabolite profiles in tumors and cancer cells are significantly different from that in normal tissue, and they are characterized by an elevation of phosphocholine (PC), ethanolamine (PE), and total choline-containing metabolites (tCho). This has been demonstrated by numerous MRS studies in cancer cells and in solid tumors of animal models and patients [2-5]. In addition, a switch from high GPC and low PC to low GPC and high PC was observed with malignant transformation in breast [8] and ovarian [9] cancer cells (compare nonmalignant human mammary epithelial cells on left to breast cancer cells on right in Figure 1). Figure 1 demonstrates that *in vivo* $^{1}$H MRS detects a combined signal from the $-N(CH_3)_3$ groups of all water-soluble choline metabolism intermediates (tCho) at 3.2 – 3.3 ppm, as evident in the $^{1}$H MR spectra of a solid breast tumor xenograft or live perfused breast epithelial cells.

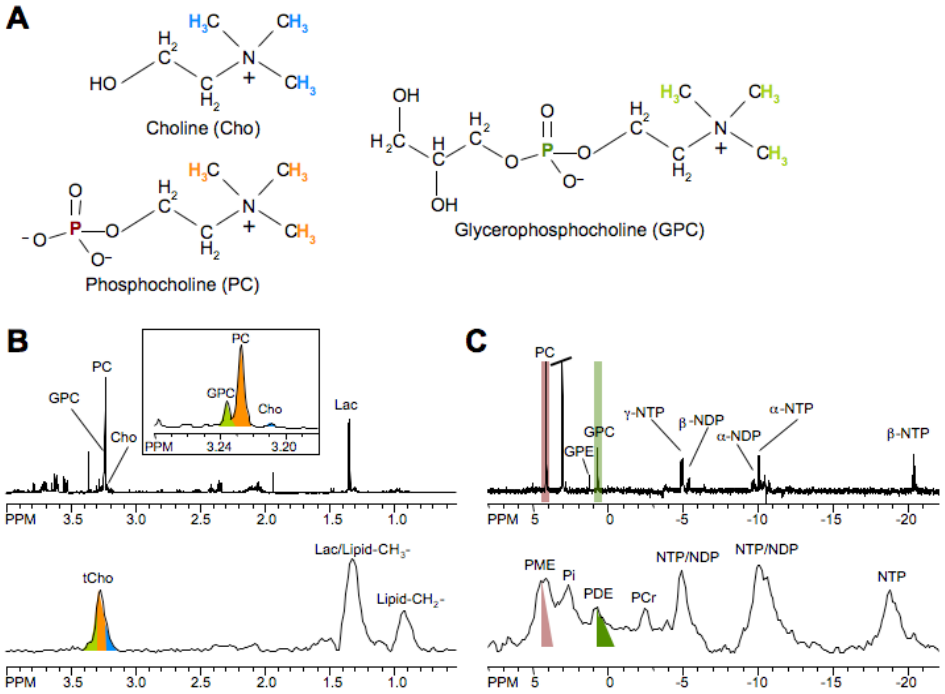


**Figure 1:** (A) Chemical structures of the choline phospholipid metabolites free choline (Cho), phosphocholine (PC), and glycerophosphocholine (GPC). (B) High-resolution *ex vivo* $^{1}$H MR spectra of triple-negative human MDA-MB-231 breast cancer cell extracts (top) and *in vivo* $^{1}$H MR spectra of the same cell line grown as orthotopic tumor (bottom). (C) High-resolution *ex vivo* $^{31}$P MR spectra of triple-negative human MDA-MB-231 breast cancer cell extracts (top) and *in vivo* $^{31}$P MR spectra of the same cell line grown as orthotopic tumor (bottom). Cho, free choline; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; DPDE, diphosphodiester; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate; Lac, lactate; Lipid-$CH_2$-, methylene groups of mobile lipids; Lipid-$CH_3$-, methyl groups of mobile lipids; PC, phosphocholine; PE, phosphoethanolamine; PCr, phosphocreatine; Pi, inorganic phosphate; tCho, total choline-containing compounds (Cho+PC+GPC). The $^{1}$H and $^{31}$P nuclei in Cho, PC, and GPC and their respective $^{1}$H and $^{31}$P signals in the MR spectra are color-coded to identify the MR signals that arise from the corresponding nuclei. Adapted from [10, 11].

The high-resolution $^{1}$H MR spectra of human MCF-12A mammary epithelial cell extracts (left) and human MDA-MB-231 breast cancer cell extracts (right) in the bottom panel of Figure 1 show that the tCho signal, which is visible in *in vivo* $^{1}$H MR spectra of live perfused cells (middle panel) and tumors (top panel), consists of three signals: Free choline (Cho) at 3.21 ppm, PC at 3.23 ppm, and GPC at 3.24 ppm, measured at pH 7.4 with 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid as a chemical shift reference. Proton MRS of a single voxel covering a region of interest, e.g. a tumor, or $^{1}$H MR spectroscopic imaging (MRSI) of one or multiple slice(s) through a tissue of interest can be performed to detect elevated tCho *in vivo*. Phosphorus-containing choline compounds such as PC at 3.9 ppm and GPC at 0.5 ppm, and ethanolamine compounds such as PE at 4.5 and glycerophosphoethanolamine (GPE) at -1.2 can also be detected with $^{31}$P MRS, using a reference compound such as methylene diphosphonic acid set to 18 ppm.

**Enzymes in Choline Metabolism**

A complex network of biosynthetic and breakdown pathways, with one or more enzymes acting per pathway, are at the core of choline phospholipid metabolism (Figure 2). Cancer-related molecular and genetic alterations as well as signaling pathways act on the expression and post-translational regulation of these enzymes, thereby generating changes in the overall choline metabolite profile [2-5]. For example, increased PC levels detected in cancer cells can directly arise from activity or expression changes in at least three enzymes: choline kinase, phosphatidylcholine (PtdCho)-specific phospholipase C, and CTP:phosphocholine cytidylyltransferase. Choline kinase generates intracellular PC in the CDP-choline pathway, which is the major biosynthetic pathway for *de novo* PtdCho synthesis in mammalian cells. PtdCho-specific phospholipase C produces PC from membrane PtdCho by a breakdown pathway. CTP:phosphocholine cytidylyltransferase utilizes PC as substrate, and thus, its activity inversely correlates with intracellular PC levels. PC itself has been reported to be mitogenic, and may act as a second messenger or mediator for the mitogenic activity of several growth factors [1].

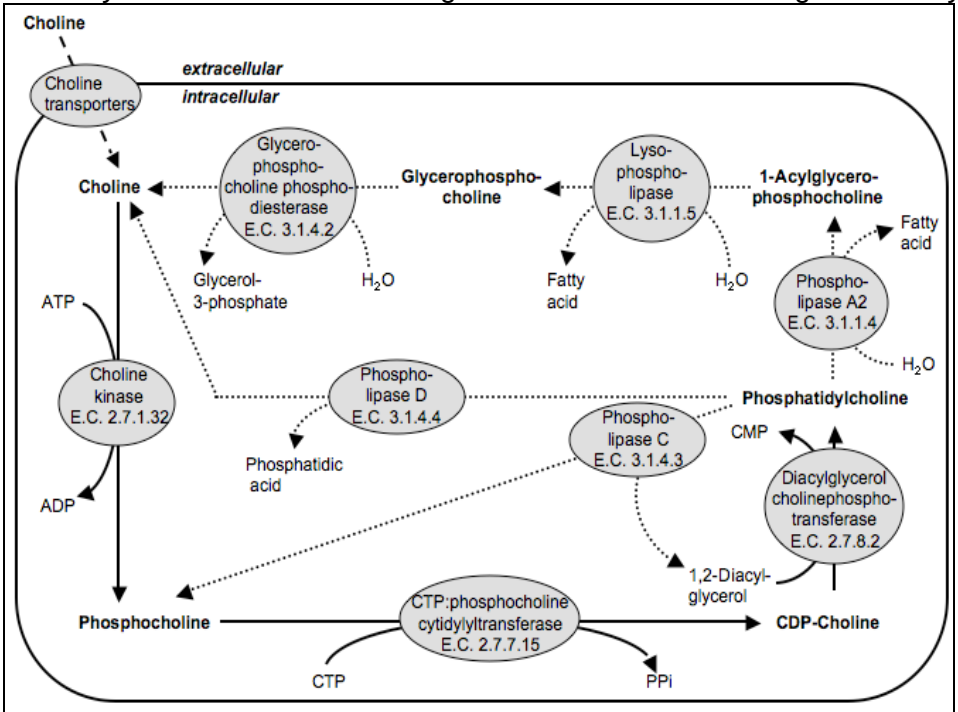


**Figure 2:** Biosynthetic (solid lines) and catabolic (dashed lines) pathways of choline phospholipids metabolism. Metabolites are given in bold letters and enzymes of choline phospholipids metabolism with their enzyme commission (E.C.) numbers are given in ellipses. CDP: Cytidine 5'-diphosphate; CMP: Cytidine 5'-monophosphate; CTP: Cytidine triphosphate; PPi: Pyrophosphate. Adapted from [12, 13].

**Mobile Lipid Droplets**

The lipid signals at 1.3 and 0.9 ppm detected in intact cells and tumors *in vivo* have been assigned to the fatty acid acyl chains in triacylglycerides that form mobile lipid droplets [6, 7]. The low mobility of membrane lipids limits their detection by MRS *in vivo*, and therefore membrane lipids do not contribute to these lipid signals at 1.3 and 0.9 ppm [6, 7]. These lipid signals overlap with various other signals, such as lactate at 1.3 ppm, and spectral editing, or other specialized MRS acquisition techniques, is required to separate these signals. Additional signals at 5.4 and 2.8 ppm can be assigned to mobile polyunsaturated fatty acyl chains to assess polyunsaturation of mobile lipids [7]. High-grade human gliomas displayed significantly higher levels of lipid than low-grade gliomas, suggesting that the lipid signal at 1.3 ppm may prove useful in tumor grading [6]. Lipid droplets in tumors have also been shown to correlate with drug resistance or response [6]. The cytoplasmic accumulation of

triacyglycerides in cancer cells and tumors has been attributed to such diverse biological processes as hypoxia, degeneration of mitochondria, differentiation, growth arrest, and apoptotic cell death [6, 7, 14]. Triacyglycerides are formed from increased diacylglycerol and triacyglycerol biosynthesis in lipid metabolism [7, 14].

This lecture will provide an overview of the biochemistry and metabolism of choline and ethanolamine compounds, as well as some of their upstream oncogenic molecular signaling pathways. It will emphasize molecular targets in choline and ethanolamine phospholipid metabolism and related signal transduction pathways, which may lead to novel anticancer therapies. This lecture will also discuss the different biological roles of MR-detectible mobile lipids in cancer and the corresponding metabolic pathways. It will outline the use of MRS and MRSI in discovering and monitoring novel molecular therapies, and in translating them into the clinic.

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