Pre-Clinical Cancer Imaging

Exploring Metabolism in Cancer Through MRS

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Highlights

- MRS provides powerful means to explore and monitor molecular mechanisms underlying metabolic reprogramming, indicated as one of next generation cancer hallmarks.
- Combination of MRS with genomic and proteomic investigations on deregulated cellular bioenergetics and aberrant phospholipid metabolism allows identification of metabolic parameters and signatures of tumor progression and therapy response.
- The use of MRS can allow novel insights on integration of altered carbon and phosphorus fluxes involved in cancer metabolic rewiring.

Target audience: cancer cell biologists; oncologists; MRS technologists.

Who will benefit: users of molecular imaging approaches in cancer.

Genetic and epigenetic alterations lead malignant cells to a reprogramming of energy metabolism, a feature recently added to the list of general cancer hallmarks used to rationalize the complexities of neoplastic diseases [1,2]. Increasing evidence indicates that an even wider metabolic rewiring takes place during oncogenesis, providing fingerprints whose identification and use by molecular imaging approaches may improve cancer diagnosis, longitudinal monitoring of response to treatment and design of targeted therapies [3-5]. Magnetic resonance spectroscopy (MRS) provides powerful tools to preclinically explore and monitor molecular mechanisms and effects of deregulated cellular bioenergetics and aberrant phospholipid metabolism in cancer. By these means, MRS approaches allow the identification of a variety of metabolic parameters and signatures of tumor progression and therapy response [reviewed in 6-9].

Deregulated cellular bioenergetics. The capability of tumor cells to sustain a high consumption of glucose via aerobic glycolysis, first described by Otto Warburg 90 years ago, is only part of a more complex, oncogene-driven network of metabolic rearrangements in cancer. Besides an altered balance between pyruvate and lactate and the production of an acidic microenvironment, this bioenergetic rewiring also involves other metabolic changes such as an increased metabolic flux through the pentose phosphate pathway (PPP), a high glutamine consumption, elevated rates of lipid biosynthesis, and formation of nucleosides/nucleotides pools [3,10,11] . These evidences support the view that an enhanced glucose uptake and consumption through aerobic glycolysis contributes to the metabolic process of dividing cancer cells by fueling carbons into *de novo* biosynthesis mechanisms to generate the biomass needed for tumor growth [11]. [^{18}F]fluorodeoxyglucose-based PET has been the first molecular imaging method to implement these concepts in clinical settings. On the other hand, preclinical MRS combined with cell biology investigations progressively contributed to further elucidate some key molecular mechanisms whereby the enhanced aerobic glycolysis can be controlled by a network of oncogene-driven signaling pathways, such as the MAPK and PI3K-AKT-mTOR reaction cascades, under the action of mutated tumor suppression and transcription factors and the activation of the HIF-1-dependent hypoxia-sensitive system [6-9].

The tracer methods based upon ^{13}C MRS isotopomer analyses first developed for neurochemistry [12] can be adapted to investigate in cancer cells and tissues the fluxes of ^{13}C labels from ^{13}C-enriched substrates through aerobic glycolysis, TCA cycle and other biochemical pathways coupled to them. By allowing an over 10,000-fold increase in sensitivity, hyperpolarized ^{13}C MRS [13] opened new ways to image cell and tissue metabolism, including detection of the oxidative-to-glycolytic switch and tissue acidification in cancer, and the modified biodistribution of a variety of metabolites acting as probes of diseased conditions both pre-clinically and clinically [13-15].

Aberrant choline phospholipid metabolism. Compared with normal counterparts, cancer cells and tissues exhibit an aberrant choline phospholipid metabolism, reflected by an altered MRS profile of

water-soluble choline containing compounds. MRS-based evidence combined with genomic and proteomic investigations strongly suggested the inclusion of this abnormal choline phospholipid metabolism among the next generation cancer hallmarks [6,7]. The MRS-detectable choline metabolic profile, which mainly includes signals of phosphocholine (PCho), glycerophosphocholine (GPCho) and free choline (Cho), may vary according to cancer phenotypes and genomic subtypes. Alterations in the choline profile may occur as a consequence of modifications in tumor microenvironment and response to host-induced stressful conditions, and provide signatures of tumor response to conventional treatments and to therapies targeted against oncogene-activated signaling cascades [6-9]. Altered levels of components of the MRS choline profile result from modifications in the activities of multiple enzymes involved in the phosphatidylcholine (PtdCho) cycle [7,16] under the action of cell signaling pathways. Cancer cells and tissues also exhibit substantial alterations in the MRS profile of ethanolamine-containing compounds [16-18]. Further investigations are however needed to better elucidate the links between ethanolamine phospholipid metabolism and oncogene-driven signaling.

PCho may reach in breast, prostate and ovarian cancer cells levels even 10-15-fold higher than those of nontumoral counterparts [19-23]. High resolution analyses of tumor cells and surgical specimens allow interpretation of changes in the unresolved in vivo ^{1}H MRS "total choline" peak (tCho, 3.2 ppm) in patient cancer lesions [24-26].

PCho is produced by the first, choline kinase (ChoK)-mediated step in the *de novo* biosynthesis of PtdCho (CDP-choline or Kennedy pathway) essential in proliferating cells [27]. It is however becoming increasingly clear that PtdCho, the most abundant bilayer-forming phospholipid in mammalian cells, is an intermediate rather than an end product of the PtdCho cycle. Lipids produced during PtdCho turnover such as diacylglycerols, lysophospholipids, phosphatidate, lysophosphatidate and arachidonic acid act as second messengers, mitogens or substrates of key biological reaction cascades, while aqueous PtdCho breakdown products such as PCho, GPCho, glycerophosphate and choline can in turn affect lipid metabolism.

Accumulation in cancer cells of a large pool of PCho, generated by activation of choline kinase (ChoK) and PtdCho-specific phospholipases, cooperates with the deregulated tumor growth program. Recent studies showed that two enzyme isoforms directly responsible for PCho production, ChoK-alpha and PtdCho-specific phospholipase C PLC (66 kDa) can be recruited to the membrane of breast cancer cells by binding to receptors of the EGFR family [28,29]. Furthermore, PtdCho-PLC inhibition induces downmodulation of HER2 and EGFR from membrane [29] and also results into loss of mesenchymal traits in breast cancer cells [30].

PCho generated by biosynthetic and/or catabolic enzymes, reacts in the Kennedy pathway with a nucleotide (CTP), to produce a high-energy phosphorylated compound (pyrophosphate) and CDP-choline, immediate PtdCho precursor. Thus, the PCho pool in cancer cells can provide an important link between the enhanced carbon flux fueled by glycolysis and PPP into nucleotides' production and the oncogene-driven rewiring of phosphorus fluxes through the kinome and phosphatome (manuscript in preparation). In this context, MRS can allow novel insights on the integration of altered carbon and phosphorus fluxes involved in cancer metabolic reprogramming.

In conclusion, by adding information on the links among different pathways of cancer metabolic rewiring, MRS can further elucidate the effects of oncogene-driven cell signaling on the formation of abnormal metabolic profiles and lead to the identification of multiple targets for anticancer treatment.

References

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