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Frontiers in Radiologic Imaging Techniques for Visualizing Protein Metabolism: A Focus on Hepatocellular Carcinoma

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Abstract

Protein metabolism constitutes a central regulatory mechanism underpinning normal hepatic physiological function. In Hepatocellular Carcinoma (HCC) and other hepatic pathologies, critical processes governing protein homeostasis—including ribosomal synthesis, chaperone-mediated folding, post-translational modifications, and regulated degradation pathways—frequently exhibit profound dysregulation that actively drives carcinogenesis and disease progression. Contemporary radiological imaging has undergone a transformative evolution beyond conventional anatomical delineation toward multidimensional function-molecular-metabolism integration, enabling unprecedented *in vivo* quantification of spatiotemporal proteomic flux. This paradigm shift is primarily driven by two synergistic technological pillars: Positron Emission Tomography (PET) utilizing radiolabeled amino acid analogs to dynamically map transporter-mediated uptake kinetics and ribosomal incorporation rates, and Chemical Exchange Saturation Transfer—Magnetic Resonance Imaging (CEST-MRI) exploiting endogenous proton exchange phenomena to spatially resolve protein concentration gradients and microenvironmental parameters. These advancements offer novel pathways for early HCC diagnosis, prognostic evaluation, and assessment of hepatic functional reserve. Nevertheless, widespread translational adoption faces persistent barriers, including insufficient probe specificity for HCC molecular subtypes, quantification standardization deficits across imaging platforms, and substantial cost-accessibility constraints within resource-limited settings, necessitating multidisciplinary innovation spanning molecular biology, biomedical engineering, and health economics to realize personalized HCC management paradigms.

Keywords

Protein Metabolism, Radiologic Imaging Technique, HCC, PET, MRI

1. Introduction

Proteins serve as central executors of life activities, providing not only structural support for cells but also playing indispensable roles in maintaining cellular functional homeostasis and survival [1]. Given their multifunctionality—including acting as biocatalysts, structural components, and signaling regulators—proteins exhibit universal biological significance in living systems [2]. From a dynamic metabolic perspective, protein metabolism encompasses a series of biochemical reactions (e.g., synthesis and degradation) within organisms. These processes constitute the material basis for sustaining life activities by supplying energy and biosynthetic building blocks [3]. Notably, dysregulation of protein metabolism is strongly associated with disease pathogenesis, as exemplified by aberrant protein aggregation and impaired degradation in autosomal dominant neurohypophyseal diabetes insipidus [4].

Under physiological conditions, the liver acts as the primary regulatory organ for protein and amino acid metabolism, maintaining systemic proteostasis [5]. However, hepatic pathologies frequently disrupt protein metabolic networks. Studies demonstrate significant downregulation of Thioredoxin (TRX) expression during acute hepatitis and hepatic fibrosis, with its plasma levels serving as a clinical biomarker for hepatitis and Nonalcoholic Steatohepatitis (NASH) [6]. Furthermore, Glypican-3 (GPC3), a membrane-anchored oncofetal proteoglycan expressed in the fetal liver but silenced in the healthy adult liver, shows dual overexpression at both gene and protein levels in Hepatocellular Carcinoma (HCC) patients, correlating significantly with poor prognosis [7].

Building upon these pathological targets, molecular imaging technologies enable dynamic monitoring of *in vivo* spatiotemporal expression profiles of target proteins, offering novel dimensions for disease progression assessment and early diagnosis [8]. This review systematically elaborates on recent advances in protein-targeted molecular imaging techniques, with a focus on their diagnostic and therapeutic applications in HCC management.

2. Fundamentals of Protein Metabolism and Imaging Targets

Protein translation serves as the central regulatory process of gene expression, determining cellular proteome composition through the conversion of mRNA nucleotide sequences into functional polypeptide chains [9]. This tightly coordinated process comprises initiation, elongation, and termination phases. During initiation in eukaryotes, 80S ribosome assembly is initiated with 40S small subunit binding to the mRNA 5' terminus, followed by start codon (AUG) scanning mediated by initiation factors. Subsequent 60S large subunit integration positions initiator

methionyl-tRNA at the Peptidyl site (P-site) via codon-anticodon pairing, ensuring precise decoding of the open reading frame [10] [11]. Following initiation complex assembly, elongation commences with ribosomal progression along the mRNA 5' → 3' direction. Aminoacyl-tRNA (aa-tRNA) entry into the Aminoacyl site (A-site) enables peptidyl transferase-catalyzed peptide bond formation between the P-site peptide chain and the A-site amino acid. Subsequent translocation relocates tRNA from the A-site to the P-site and finally to the Exit site (E-site), vacating the A-site for subsequent elongation cycles to progressively extend the polypeptide chain [12]. Upon ribosomal progression to mRNA termination regions, termination is activated when stop codons (UAA/UAG/UGA) occupy the A-site, triggering release factor recognition that catalyzes ester bond hydrolysis between the P-site tRNA and the nascent peptide, culminating in polypeptide release and ribosomal subunit dissociation [12].

Newly synthesized proteins undergo functional maturation, where Post-Translational Modifications (PTMs) play critical roles. Through covalent side-chain modifications (e.g., ubiquitination, phosphorylation, methylation), PTMs dynamically regulate protein folding conformations, subcellular localization, stability, and interaction networks [13]. Notably, these modifications exhibit bidirectional regulatory functions in physiological homeostasis maintenance and disease pathogenesis, significantly expanding proteome functional diversity [14] [15]. Beyond synthetic modifications, proteostasis maintenance relies heavily on precisely regulated degradation pathways. The Ubiquitin-Proteasome System (UPS), as the primary selective degradation route, employs an E1-E2-E3 enzymatic cascade to covalently conjugate ubiquitin C-terminal glycine to substrate lysine ε-amino groups. Polyubiquitinated substrates are recognized by the 26S proteasome—its 19S regulatory particle mediates ubiquitin chain disassembly and substrate unfolding, while the 20S core particle executes ATP-dependent hydrolysis to achieve programmed substrate degradation [16]-[19]. System precision originates from the dynamic equilibrium between ubiquitinating enzymes and Deubiquitinases (DUBs) through reversible substrate stability regulation [20]. Complementarily, the autophagy pathway represents an evolutionarily conserved degradation mechanism whereby autophagosome formation encapsulates cargo for lysosomal targeting, facilitating hydrolytic breakdown and product recycling to maintain intracellular homeostasis [21].

Proteins thus constitute the fundamental molecular machinery of human physiology, with their proper function being essential for health, while dysregulation through aberrant expression, misfolding, modification imbalances, or clearance deficiencies directly contributes to disease pathogenesis [22]. PET with radiolabeled amino acids primarily measures the "translation" step, while CEST-MRI provides information on overall protein concentration resulting from the balance of synthesis and degradation. Contemporary protein imaging technologies leverage three principal mechanisms for *in vivo* visualization: Molecular tracer imaging employs radionuclide-labeled amino acids or analogues to quantify protein synthesis kinetics through positron annihilation detection; chemical exchange im-

aging exploits magnetic resonance frequency differences of exchangeable protons to map protein concentration and microenvironment via saturation transfer effects on water signal; targeted probe imaging utilizes engineered high-affinity ligands to detect specific protein localization and expression levels. Radiolabeled peptides represent an expanding class of molecular probes exhibiting exceptional specificity and structural diversity—from small cyclic peptides to antibody fragments and intact immunoglobulins—that can be precisely tailored to engage biological targets while accommodating diagnostic imaging and therapeutic applications [23].

3. Applications of Radiological Imaging in Protein Metabolism Assessment

Dynamic visualization of protein metabolism represents a critical window for elucidating tumorigenesis mechanisms. Contemporary radiological imaging technologies—notably Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), and multimodal systems—are continuously advancing the spatiotemporal resolution limits for *in vivo* protein metabolic analysis through innovative tracer development and imaging sequences. These advancements provide unprecedented insights for early cancer diagnosis and precision therapeutics.

3.1. PET

PET, as a functional metabolic imaging modality based on molecular events, operates on the core principle of utilizing radionuclide-labeled specific bioactive molecules (*i.e.*, tracers). It achieves a multidimensional assessment of the biological characteristics of malignant tumors by detecting paired γ-photons generated from the annihilation of positrons emitted during the distribution and metabolic processes of these tracers within the body. A key strength of PET lies in its ability to detect early pathophysiological alterations before significant structural changes occur in tissues [24]. This technique enables the noninvasive and quantitative delineation of tumor cell metabolic activity, viability, expression levels of specific proteins/receptors, aggressiveness, and metastatic potential.

Among amino acid-based tracers targeting protein synthesis, carbon-11-labeled methionine ([^{11}C]Methionine, [^{11}C]MET) holds significant value due to its direct involvement in key biological pathways, including protein synthesis, methylation reactions (as a precursor to S-adenosylmethionine), and polyamine metabolism. Its uptake level within tissues directly reflects cellular amino acid transport capacity and protein synthesis rate, and typically exhibits a significant positive correlation with cellular proliferative activity—a characteristic enabling its use as an effective marker for rapidly proliferating tissues [25]. [^{11}C]MET PET not only allows for the quantitative assessment of tissue protein anabolic rates but also facilitates high-contrast precise localization and visual imaging of lesions exhibiting abnormally elevated metabolism [25] [26]. Currently, although most amino acid- and glucose-based PET tracers were initially developed for brain tumor imaging [27]

owing to the low physiologic background in normal brain tissue, their application in HCC is more challenging. The liver exhibits intrinsically high metabolic activity and abundant expression of glucose and amino acid transporters, resulting in substantial physiological uptake that diminishes tumor-to-liver contrast and limits the sensitivity of tracers. To overcome these limitations, hepatocyte-targeted or lipid-metabolism-related tracers have shown superior performance in differentiating HCC lesions from surrounding parenchyma by exploiting altered lipid synthesis and oxidative pathways characteristic of malignant hepatocytes. Nonetheless, background hepatic metabolism and inter-tumoral heterogeneity remain key challenges for accurate quantitative imaging of HCC.

Addressing the specific demands of brain tumor diagnostics, the structurally modified fluorine-18-labeled amino acid tracer O-(2-[^{18}F]fluoroethyl)-L-tyrosine (^{18}F-Fluoroethyltyrosine, ^{18}F-FET) has emerged as an important clinical tool for glioma diagnosis, grading, differential diagnosis, and recurrence monitoring. Its advantages include a high tumor-to-background ratio, relatively stable *in vivo* metabolic properties, and uptake that is less influenced by the degree of blood-brain barrier disruption [28]. This further expands the applications of PET molecular imaging in the field of precision oncology diagnosis and treatment. Furthermore, other amino acid analogues, such as ^{18}F-fluorodihydroxyphenylalanine (^{18}F-DOPA), also play a role in protein metabolic imaging for specific diseases. As a clinically promising radiopharmaceutical, ^{18}F-DOPA demonstrates excellent performance in imaging the assessment of dopaminergic neuron function, brain tumors, neuroendocrine tumors, and congenital hyperinsulinism [29].

3.2. MRI

Nuclear Magnetic Resonance (NMR), a physical phenomenon where atomic nuclei absorb specific radiofrequency energy within a static magnetic field, undergo energy level transitions, and generate detectable electromagnetic signals, has sustained significant interest from the scientific community and translational medical research due to its broad potential in chemical structure characterization, biomolecular sensing, and medical imaging [30]. MRI, developed based on this principle, generates anatomical and functional images by detecting the spatial distribution of radiofrequency signals from water protons (^{1}H) or other nuclear magnetic moments within biological tissues. Renowned for its exceptional soft-tissue contrast resolution, diverse multi-contrast mechanisms, absence of ionizing radiation risk, and outstanding capability for multi-modal information co-localization [31], MRI has become a cornerstone imaging tool for the clinical evaluation of diseases affecting the central nervous system, abdomen (particularly the hepatobiliary system), and musculoskeletal system. Its powerful translational potential was vividly demonstrated early on by Haun *et al.*, who developed a handheld miniaturized NMR device enabling rapid, highly sensitive cancer diagnosis of fine-needle aspiration biopsy samples [32].

In recent years, Chemical Exchange Saturation Transfer (CEST) MRI has emerged

as a pivotal breakthrough in molecular imaging. By exploiting the chemical exchange effect between exchangeable protons (e.g., amide-NH, amine-NH_2, hydroxyl-OH) and surrounding bulk water protons, CEST MRI significantly enhances the capability of MRI to quantitatively probe biomolecule concentrations, pH, and metabolic microenvironments. Amide Proton Transfer (APT) imaging, a vital branch of CEST technology, specifically targets exchangeable protons within the amide groups (-CONH-) of protein backbones. The application of saturation pulses at the resonance frequency of amide protons saturates their magnetization, which is then transferred to the large pool of water protons via chemical exchange, resulting in a measurable reduction in the water signal. The magnitude of this signal reduction is closely associated with tissue protein concentration and local pH. APT imaging has demonstrated substantial clinical utility in the diagnosis and management of gliomas, encompassing noninvasive tumor grading [33], determination of IDH mutation status [34], identification of 1p/19q co-deletion [35], early prediction [36] and dynamic monitoring of treatment response [37], and prognostic assessment [38]. The core advantage of this technique stems from its dual sensitivity to tissue microenvironment pH and free protein/peptide concentration [39], providing a unique window for *in vivo* molecular pathological analysis. Research into its applications for liver diseases, particularly HCC, is rapidly expanding.

Beyond APT, CEST technology can target other molecules, such as glutamate. Glutamate-weighted CEST (gluCEST) MRI serves as one of the key techniques currently enabling the noninvasive visualization of glutamate concentration changes and their spatial distribution within the human brain. Over the past decade, gluCEST has progressed from proof-of-principle experiments conducted in phantoms and model systems to an increasingly mature tool for revealing aberrations in fundamental human neurometabolism—particularly in patients with seizure disorders and across the spectrum of psychiatric illnesses [40].

3.3. Multimodal PET/MRI Systems

PET/CT technology has rapidly gained widespread adoption in clinical oncology practice due to its ability to precisely fuse PET functional metabolic information with CT anatomical localization. However, this technique possesses inherent limitations, including relatively low soft tissue contrast resolution and additional radiation exposure [41]. Integrated PET/MRI systems, employing simultaneous acquisition and deep integration at both physical and software levels, represent a key technological breakthrough addressing these limitations. They combine the high sensitivity of PET for molecular functional metabolic imaging with the superior soft tissue resolution, multiparametric functional imaging capabilities, and radiation-free advantage of MRI, enabling truly "one-stop" multimodal assessment. The anticipated significant advantages of PET/MRI include: significantly enhanced detection sensitivity and biological characterization capabilities for lesions in soft tissues such as the brain, breast, liver, prostate, pancreas, and musculoskeletal sys-

tem; optimized local T-staging assessment for specific tumors; utilization of MRI data for motion artifact correction, thereby improving PET image quality; elimination of the additional ionizing radiation associated with CT, offering particular benefits to pediatric and young patients, as well as those requiring repeated examinations; improved patient comfort and workflow efficiency through the integration of all PET and MRI information in a single examination; and the potential to shorten total examination time through optimized scanning protocols by enabling multiparametric imaging. Early large-scale comparative studies indicated equivalent overall clinical efficacy between PET/MRI and PET/CT for tumor staging and restaging [42]. However, more recent accumulating evidence more clearly indicates that the significant advantages of PET/MRI are primarily concentrated in specific anatomical regions (e.g., brain, head and neck, liver, pelvis) and specific tumor types, and its impact on overall clinical decision-making still requires confirmation through further prospective studies [43]. The most definitive and recognized improvements relate to enhanced detection sensitivity and qualitative accuracy for liver lesions, brain pathologies, and bone metastases.

The advantage of PET/MRI in improving tumor staging diagnostic efficacy primarily stems from three synergistic mechanisms: Firstly, the superior soft tissue contrast and functional sequences of multiparametric MRI offer significantly better soft tissue resolution and a wealth of functional sequences compared to CT. This is crucial for the detection and characterization of lesions within abdominal organs such as the liver and pancreas [41]. Secondly, technical synergy enhances image quality: MRI sequence acquisition times are typically long. In simultaneous PET/MRI systems, PET data acquisition can fully utilize this extended time for prolonged counting, significantly increasing photon counts. This results in PET images of higher quality, particularly beneficial for detecting lesions with low radiotracer uptake [44]. Thirdly, dedicated liver imaging protocol optimization: PET/MRI protocols specifically designed for the liver enable high-count, low-noise PET acquisition focused on the liver region, effectively reducing image noise and significantly enhancing the conspicuity of small metastases or primary lesions [44].

At the level of liver tumor applications, the advantage of the MRI component in lesion characterization is well-established: its exceptional soft tissue resolution and Diffusion-Weighted Imaging (DWI) capabilities effectively differentiate small hepatic cysts, hemangiomas, focal nodular hyperplasia, and metastases that are difficult to distinguish using conventional CT or non-contrast PET/CT. Practical clinical studies confirm specific advantages of PET/MRI in liver tumor evaluation: 1) it can detect liver metastases identifiable only by the MRI component [45]; and 2) its Dynamic Contrast-Enhanced (DCE) component provides rich tumor hemodynamic parameters. When combined with the ADC values from DWI and metabolic parameters from PET, this significantly optimizes the comprehensive analysis of lesion biological behavior, holding critical value for the early diagnosis of metastatic liver cancer, the search for primary tumors, and the formula-

tion of individualized treatment plans.

4. Clinical Applications of Protein Metabolic Imaging in Hepatocellular Carcinoma

Protein metabolic imaging is essential for early tumor detection, differentiation from regenerative nodules and benign hepatic lesions, accurate staging, recurrence identification, and treatment response assessment. In the domain of tumor detection, Tang *et al.* pioneered an innovative CEST-based pH mapping technique using iohexol as an activatable contrast agent [46]. Their methodology employed asymmetric magnetization transfer ratio (MTRasym) analysis to isolate pH-sensitive CEST contributions, establishing a specificity ratio for quantitative validation. In a clinical study of 15 HCC patients, they demonstrated significantly lower mean pH values within tumor tissue (6.66 ± 0.19) compared to adjacent non-tumorous parenchyma (7.31 ± 0.12, $p < 0.0001$). This tumor microenvironment acidosis likely results from glycolytic metabolism and upregulated proton pump activity, providing a novel metabolic imaging biomarker for HCC detection. Regarding histological grading prediction, Lin *et al.* established amide proton transfer-weighted (APTw) MRI as a valuable complement to diffusion-weighted imaging [47]. Their research demonstrated that combining APTw and Apparent Diffusion Coefficient (ADC) values significantly improved accuracy in discriminating high-grade (Edmondson-Steiner grade III) from low-to-intermediate grade (grade I/II) HCC. The area under the receiver operating characteristic curve (AUC) reached 0.814 for APTw alone and 0.745 for ADC alone. Both imaging parameters exhibited significant inverse correlations with histological grade (APTw: $r = -0.52$, $p < 0.01$; ADC: $r = -0.48$, $p < 0.01$), indicating that increased protein content/microenvironment alterations and elevated cellular density collectively characterize aggressive HCC phenotypes. For treatment response evaluation, Jia *et al.* developed a predictive model based on pre-treatment APT signal characteristics for stratifying response to Transarterial Chemoembolization (TACE) [48]. Their quantitative MTRasym (3.5 ppm) analysis demonstrated excellent measurement reproducibility within tumor regions (intraclass correlation coefficient > 0.85) and revealed significant differences in baseline APT values among tumor tissue, peritumoral parenchyma, and distant normal liver ($p < 0.001$). Crucially, specific pre-treatment tumor APT signatures correlated significantly with subsequent pathological tumor necrosis extent following TACE, suggesting that protein metabolic imaging may provide earlier and more biologically relevant assessment of treatment efficacy compared to conventional size-based criteria or contrast-enhanced imaging.

5. Current Challenges and Technical Bottlenecks

Among currently recognized HCC-specific molecular targets, Glypican-3 (GPC3) stands out as a prototypical oncofetal protein highly expressed on malignant hepatocytes but absent in normal liver tissue. This unique expression pattern ren-

ders GPC3 an attractive candidate for both diagnostic imaging and therapeutic intervention. However, the translation of GPC3-targeted molecular probes into clinical application remains limited, and further efforts are warranted to design high-affinity, low-background imaging agents that can achieve reliable lesion detection and treatment monitoring in HCC. Despite considerable promise, the clinical integration of radiological protein metabolic imaging for HCC management faces four substantial translational barriers. Foremost is the critical scarcity of liver-specific molecular probes with high binding affinity and metabolic stability. Current radiolabeled amino acid tracers predominantly target fundamental metabolic pathways rather than HCC-specific molecular signatures such as mutant protein isoforms, dysregulated signaling kinases, or HCC-associated neo-antigens. Developing novel radiopharmaceuticals targeting validated HCC biomarkers and designing tumor-activated CEST probes represent urgent research priorities. Second, the absence of standardized quantitative methodologies severely compromises cross-institutional data comparability and hinders multicenter validation studies. Protein metabolic kinetic parameters exhibit significant variability due to differences in instrumentation sensitivity, magnetic field homogeneity, radiofrequency pulse calibration accuracy, image reconstruction algorithms, and post-processing workflows. Establishing consensus imaging protocols, implementing standardized phantom calibration systems, and conducting rigorous cross-platform validation are essential prerequisites for clinical adoption. Third, limited clinical accessibility and unfavorable cost-effectiveness profiles present significant practical barriers to widespread implementation. Integrated simultaneous PET/MRI systems require capital investments exceeding $1.5 million USD per unit, substantially higher than standalone PET/CT or MRI systems. Furthermore, per-examination costs typically range from 5 to 8 times those of contrast-enhanced CT, compounded by limited insurance reimbursement in many healthcare systems. These economic factors impose prohibitive burdens for HCC patients requiring longitudinal metabolic monitoring throughout their disease course. Fourth, insufficient integration persists between emerging metabolic imaging biomarkers and established clinical assessment frameworks. Algorithmic correlations between quantitative protein metabolic parameters and conventional liver function indices remain poorly defined. Consequently, metabolic imaging data often exist in isolation from routine serum biomarkers within clinical decision support systems, failing to realize their potential for comprehensive multidimensional assessment. To enhance clinical applicability, future studies should explore integrated predictive models that combine quantitative metabolic imaging metrics with established biochemical indicators. For instance, correlating CEST-MRI-derived amide proton transfer ratios or exchange rate constants with serum alpha-fetoprotein (AFP) levels could enable a multimodal risk-stratification system, improving prognostic precision beyond either modality alone. Such an integrative approach may bridge the current gap between imaging-derived molecular information and routine clinical biomarkers, facilitating personalized manage-

ment strategies in HCC. Collectively, these technological, methodological, and economic constraints currently confine advanced protein metabolic imaging primarily to academic research settings and specialized tertiary referral centers. Overcoming these translational bottlenecks necessitates sustained multidisciplinary collaboration across radiology, hepatology, oncology, molecular biology, medical physics, and health economics, coupled with strategic investment in probe development, standardization initiatives, and health technology assessment.

6. Conclusion

The confluence of metabolic PET, molecular CEST, and hybrid PET/MRI technologies is fundamentally redefining protein metabolism visualization capabilities in hepatocellular carcinoma, transitioning from anatomical surrogate observation toward mechanistic interrogation of underlying biological processes. Robust validation across the hepatocellular carcinoma continuum confirms clinical utility for precision diagnosis, molecular stratification, prognostication, and therapeutic personalization. For instance, PET tracers that quantify amino acid uptake and protein synthesis, such as ^11C-methionine or ^18F-fluoroleucine, can non-invasively identify tumors exhibiting hyperactive translational machinery and mTOR pathway activation, thereby predicting enhanced responsiveness to mTOR inhibitors like everolimus. Such imaging-guided therapeutic stratification exemplifies how protein metabolic imaging can directly inform precision treatment selection in HCC. Nevertheless, overcoming residual translational barriers demands concerted innovation in probe chemistry, computational analytics, reimbursement frameworks, and healthcare delivery models. Realizing the full potential of these technologies requires sustained multidisciplinary collaboration across radiology, hepatology, molecular oncology, medical physics, and health economics, ultimately establishing protein metabolic imaging as the cornerstone of next-generation precision hepatology paradigms that seamlessly integrate molecular profiling, functional assessment, and targeted intervention throughout the hepatocellular carcinoma care continuum.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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