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PET-MRI Imaging of Cellular Trafficking of Lipid Metabolism and Its Association with Inflammatory and Genetic Markers in Breast Cancer

REVIEW

Shaharudin Shazreen[1], Fathinul Fikri Ahmad Saad[1], Shahrun Niza Muhd Suhaimi[2]

ABSTRACT

The tumour landscapes are varied, and depending on the orchestration of specific molecular pathways, signaling of its early manifestation could be understood. The conventional ways to signal the metabolic expression of cancers are notably via recognizing the altered glycolytic pathways in the cells whereby the transport of glucose is unchecked by the high demand of the ATP production by the cancer cells. It is notwithstanding that by relying on the glucose analogue (flurodeoxyglucose, FDG) signaling in cells, there are other cellular mechanism deemed to be explored, that is, the cell membrane or lipid metabolism. Molecular imaging–based nanoparticles are proving useful for cell trafficking studies, whereas radionuclide- and optical-based molecular–genetic reporters are yet to be determined. On the other hand, for receptor- or enzyme-based imaging or for studying the pharmacokinetic disposition of chemotherapeutic agents, the radionuclide-based techniques predominate. This review highlights the utility of the molecular imaging techniques in expressing the altered lipid metabolism, that is, choline transport in cells as an index of tumour aggressiveness in breast cancer.

Keywords: Genetic markers, inflammatory markers, lipid metabolism, PET-CT, PET-MRI, 18F-FCH

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[1]Centre for Diagnostic Nuclear Imaging, University Putra Malaysia, Selangor, Malaysia

[2]Department of Surgery, UKM Medical Centre, Jalan Yaakub Latiff, W.P, Kuala Lumpur

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Correspondence
Fathinul Fikri Ahmad Saad, Centre for Diagnostic Nuclear Imaging, University Putra Malaysia, Selangor, Malaysia
Phone: +60126930794
e.mail: ahmadsaadff@gmail.com

INTRODUCTION

Tumours vary depending on the regulation of specific molecular pathways; non-invasive molecular signaling of the its early clinical manifestation could lead to a better understanding of factors that underpin its cellular reprogramming. The conventional approaches to studying the metabolic expression of cancers depend on recognizing changes in glycolytic pathways and typically rely on the glucose analogue fluorodeoxyglucose. However, other cellular mechanisms for the study of cancer are now being explored, including cell membrane and lipid metabolism. Nanoparticles are proving useful for cell trafficking studies, but radionuclide- and optical-based molecular–genetic reporters of cell trafficking remain relatively underdeveloped. However, radionuclide-based techniques are widely used for receptor- or enzyme-based imaging and for studying the pharmacokinetic disposition of chemotherapeutic agents. Here, we highlight the usefulness of molecular imaging techniques for examining altered lipid metabolism. We focus on choline transport as an index of tumour aggressiveness in breast cancer.

Choline in breast cancers

Choline levels are markedly higher in human breast cancer cells than in normal mammary epithelial cells (1). Choline transport and phosphorylation are increased in human breast cancer cells. Progression of human mammary epithelial cells from a normal to a malignant phenotype is associated with an induced overexpression of choline kinase (CK) that catalyzes the phosphorylation of choline to form phosphocoline, followed by generation of phosphatidylcholine in the tumour cell membranes (2). In earlier studies, increased choline uptake in tumour cells was typically accounted for by an upregulation of CK due to an increased demand of membrane constituents. However, recent studies have found that cancer development is caused by choline intake (3). In addition, other studies have shown that CK and phosphorylcholine (PCho) production are increased in growth factor–induced mitogenic signaling of primary human breast epithelial cells in response to insulin or hydrocortisone (4).

Cellular trafficking of choline transport

Imaging of tumour cell metabolism has been remarkably successful in the recent years. In the region of the mammary gland, total choline is considered the most important metabolite for proton magnetic resonance (MR) spectroscopy. It has been reported that the degree of elevation in choline-containing compounds is related to tumour grade, with higher levels in high-grade than in low-grade lesions (5). Also, malignant lesions are more likely to show high levels of choline-containing compounds than benign or normal breast tissues. However, there have been mixed reports about choline-derived cancer cells and their significance for differentiating between benign and ma-

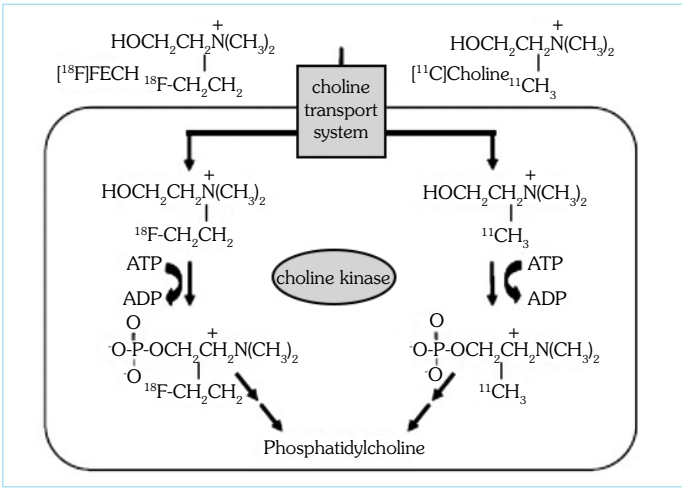

Figure 1. Schematic presentation of [18F] FECH and [11C] choline uptake mechanism. Both tracers are transported into the cell via choline transport systems. In the cell, choline kinase phosphorylates the compounds resulting in the corresponding phosphorylcholine derivatives

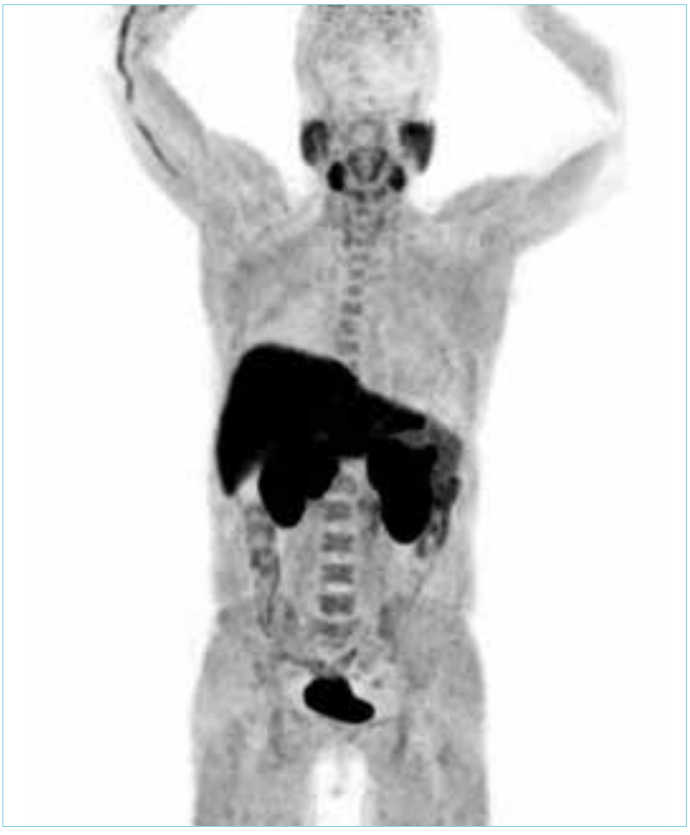

Figure 2. 18F-Flurocholine PET showing normal uptake in organ systems

lignant tumours (6). It is expected that 3-Tesla magnetic resonance imaging (3T MRI) will lead to the development of new surrogate molecular markers for aggressive lesions.

Positron emission tomography (PET) and MR methods are clinically translatable; non-invasive imaging techniques are increasingly being used to detect physiologic changes and tumour responses to target therapy. There is a significant correlation noted between standardized uptake value (SUV) max (3.06±2.34 g/dL) and the creatinine (3.39±0.54 U) and N-acetyl Aspartate (NAA) MRI parameters (6). The pattern of the metabolites concentration showed that the highest mean being creatinine followed by N-acetyl aspartate (NAA) (mean: 2.84±0.99 U) and choline (mean: 2.46±0.70 U).

Choline imaging agents

In early approaches, choline was initially labeled with an 11-carbon, producing an isotopic tracer. Synthesis of [11 C]choline can be done by C-11 methylation (7). Depending on the method used, radiochemical yields of up to 95% can be achieved. [11 C] choline can be used to visualize a variety of tumours, including prostate cancer (8). However, for improved half-life, 18F-labeled choline derivatives have been developed. 18F labeling results in the analogue tracer [18 F] fluoromethylcholine ([18F] FCH). Unlike [11C] choline production, [18F] FCH synthesis is a two-step process (Figure 1).

High levels of pCho have been found in many cancers, with low levels in the corresponding normal tissue. pCho is the first intermediate in the incorporation of choline into phospholipids via the Kennedy pathway (9, 10). However, whether the corresponding CK reaction or an upstream transporter are principally responsible for this tracer accumulation is currently uncertain. It has been demonstrated that the choline transport systems tolerate synthetic analogues.

Inflammatory marker (CD47) in breast cancer

Cluster of Differentiation 47 (CD47) was originally identified in association with the integrin αvβ3, hence, its alternative name of integrin-associated protein (IAP). The human CD47 is a transmembrane protein belonging to the V-type Ig-like extracellular domain possessing immunoglobulin superfamily. CD47 partners with membrane integrins and binds the ligands thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα) (11, 12). CD47 is involved in a range of cellular processes, including apoptosis, proliferation, adhesion, and migration. CD47 also plays a key role in immune and angiogenic responses. CD47 is ubiquitously expressed in human cells and is overexpressed in various tumour cell types. Erythrocytes lacking CD47 expression are rapidly removed from the bloodstream by splenic red pulp macrophages (13). SIRPα is a transmembrane glycoprotein and acts as a novel intracellular signal transducer when it is engaged by its ligand, CD47. CD47-carrying normal peripheral blood red cells can circumvent elimination by binding to SIRPα (14). The interaction of CD47 with SIRPα occurs between host-derived cells and is mostly related to cell signaling in the immune and nervous systems (15).

Recently, it has been shown that macrophages can eliminate tumour cells via a highly regulated immunosurveillance mechanism referred to as programmed cell removal (PrCR) (16). However, cancer cells often express CD47 and other antiphagocytic (protective) signals that protect them from PrCR-mediated recognition and phagocytosis. Unlike normal cells, many cancer cells express prophagocytic (non-protective) signals (e.g., calreticulin or CRT) that interact with the Tec non–receptor protein tyrosine kinase family, which plays a vital role in the regulation of the innate immune response. Recently, it has been shown that macrophages express CRT and that Btk-mediated Toll-like receptor signaling results in trafficking of CRT to the cell surface, where this non-protective signal can contribute to the PrCR of cancer cells (17) (Figure 2).

The production of CD47 (a cell surface protein) enables cancer cells to avoid destruction by macrophages and promotes further mitotic proliferation. CD47 levels are typically high in aggressive tumours. Reduction of CD47 levels in breast cancer cells increases killing by macrophages and depletion of cancer stem cells. There is increasing evidence indicating that CD47 expression is required to avoid innate immunosurveillance and elimination by phagocytosis in various human cancers (18). Hypoxia, a critical microenvironmental stimulus in advanced breast cancers, induces HIF-dependent expression of CD47, leading to decreased phagocytosis of cancer cells by macrophages and induction of the breast cancer stem cells (CSC) phenotype, which promotes cancer progression and patient mortality (19). However, an association between CD47 expression and choline metabolism has yet to be investigated. In breast cancer cases, carcinogenicity may promote the CD47-SIRPα cell signaling system in bone marrow and in peripheral blood, thereby creating cancer-specific microenvironments that support micrometastasis (20).

Overexpression of CD47 is associated with poor prognosis of patients with breast cancer because of activation of the CD47-SIRPα signaling pathway in circulating cells (20). Recent studies have shown that CD47 specifically inhibits phagocytosis and that there is a significant correlation between CD47 transcript levels and leukemia, hematopoietic stem cells, and tumour-initiating cells of bladder cancer (21). CD47 could work as a marker of self on cancer cells, and breast cancer cells may express high levels of CD47. The mean ratio of CD47/GAPDH mRNAs in the high-expression group of cancer cases was found to be three to five times higher than in non-cancer cases (21). This may indicate that CD47 has various functions and that CD47 expression levels are affected by the cell environment, rather than by the number of cancer cells. Therefore, the high expression of CD47 in the bone marrow and peripheral blood of breast cancer patients might represent the characteristic appearance of breast cancer and, thus, provide evidence for a cancer-specific mechanism in the bone marrow and peripheral blood of patients with breast cancer.

Overexpression of CD47 in bone marrow and peripheral blood has been reported to correlate with breast cancer aggressiveness. Therefore, it will be important to clarify CD47 expression levels in the bone marrow and peripheral blood of patients with breast cancer in order to determine whether micrometastasis is occurring. Thus, CD47 may be a novel biological marker capable of predicting the number of highly malignant circulating tumour cells that escape from the immune systems in patients with breast cancer (22).

Recent studies have found that high CD47 expression correlates with high CK19 expression in the bone marrow and peripheral blood of patients with breast cancer. The expression of CD47 in circulating tumour cells increases exponentially during cancer progression. Thus, isolated tumour cells (ITC)-derived CD47 might be an upregulating factor of breast cancer (23).

Genetic probe with choline in breast cancer

Breast cancer is not a single disease with variable morphologic features but rather a group of molecularly distinct neoplastic disorders (24). According to gene expression–based intrinsic classifications, breast carcinomas can be categorized into at least five subtypes: luminal A, luminal B, normal breast-like, human epidermal growth factor receptor 2 (HER2)-enriched, and a basal-like subtype. In addition to the distinctly different gene expression patterns, the subgroups also show significantly different clinical outcomes, likely due to alterations in specific cellular pathways. Moreover, tumours that appear to have similar diagnostic features do not always respond to treatment in the same way. This can be caused by differences in their mutational profile, signaling redundancy, and the particular tumour microenvironment, as well as by other factors (25).

Gene amplification often occurs in breast cancer cells, affecting multiple genomic regions. One of the most studied amplifications is located in chromosomal region17q12 and involves the ERBB2 gene. ERBB2 encodes a transmembrane tyrosine kinase receptor of the ERBB/EGFR family, also frequently referred to as HER2 (from human epidermal growth factor receptor or Her2/neu) (26). Amplification or overexpression of the ERBB2 gene occurs in approximately 15%–30% of breast cancers and is associated with increased disease recurrence and poor prognosis (27). These genes are observed in 18%–20% of breast cancers and can be used as selection criteria for HER2-targeted therapies. Patients with ErbB2-overexpressing breast cancer have substantially lower overall survival rates and shorter disease-free intervals than patients whose cancer does not overexpress ErbB2. Moreover, overexpression of ErbB2 leads to increased breast cancer metastasis (28). The important roles of ErbB2 in cancer progression render it a highly attractive target for therapeutic interventions of breast cancer. The associations between choline metabolism and ErB2 are not entirely understood, although it has been reported that transfection of human mammary epithelial cells with the erbB2 oncogene causes a significant increase in phosphocholine (PC) levels (29).

miRNA and breast cancer

miRNAs can have either oncogenic or tumour suppressor capabilities (30). miR-21 can downregulate a variety of tumour suppressor proteins. Among the targets of miR-21 are BCL2, PTEN, and tropomyosin 1, which are proteins implicated in proper cell-cycle progression and regulation of apoptosis (31). It has also been shown that specific cancer types have specific miRNA expression profiles and that tumour miRNA patterns can be used to predict the effectiveness of cancer treatments and prevention strategies. Circulating microRNAs (miRNAs) are considered stable miRNAs in the serum/plasma. These miRNAs represent potential biomarkers for evaluating cancer, and many circulating miRNAs indicative of breast cancer have been identified (32). For example, the combination of miR-145/miR-15a and miR-451 has been shown to be useful for breast cancer detection (33, 34).

MRI spectroscopy choline in breast cancer types

Choline-containing compounds are the major components of cell membrane required for structural stability and cell proliferation. Elevated levels of total choline are primary due to an increase in choline metabolites, such as PC. By MR spectroscopy, elevated levels of total choline have been detected in several cancers, including breast, prostate, colon, and brain cancers. Thus, elevated choline can be used as an *in vivo* biomarker for malignant disease (34). In line with this, a reduction in total choline has been suggested as an *in vivo* marker for response to treatment. In cultured breast cancer cells, high choline levels were shown to contribute

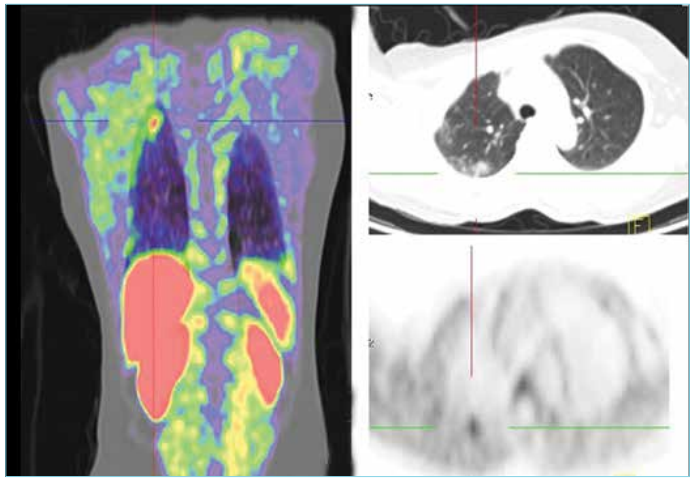

Figure 3. Fused 18F-Flurocholine PET-CT (left), axial CT (right upper), and FCH-PET (right lower), showing the FCH uptake of the metastatic breast lesion in the right lung apex

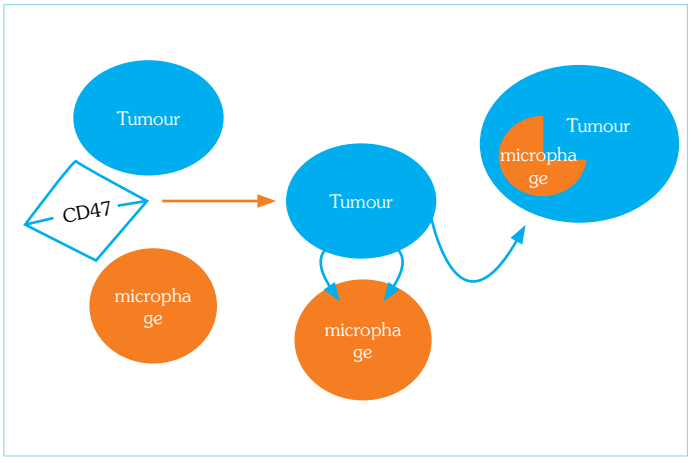

Figure 4. Protective signal: cancer cells protect themselves against phagocytosis or programmed cell removal by overexpression of CD47. Interaction of these protective signals molecules with SIRPα on the cell surface of macrophages protects cancer cells from phagocytosis. Non-protective signal: inhibition of the interaction between CD47 and SIRPα and translocation of calreticulin from the ER to the cell surface promote phagocytosis

to high levels of pCho and PC. Also, high levels of glycerophosphocholine (GPC) were detected in human breast cancer biopsies and xenograft (35).

Given that cancer tissues contain high amounts of choline, radiolabeled choline and choline analogues are promising tools for cancer detection (36). Several fluorine 18–labeled choline analogues have been used as precursors for the biosynthesis of cellular membrane phospholipids (e.g., phosphatidylcholine). An increase in cell proliferation or CK activity is associated with an increase in choline levels in cancer cells. Although choline-PET has been reported to be a good diagnostic technique for many tumour types, in practice, its clinical value is mostly limited to prostate cancer (36).

Human breast cancer cells and tumours exhibit consistently elevated levels of PC, allowing total choline levels to be used to discriminate between malignant and benign lesions. In addition, progressively elevated levels of total choline and PC were observed in immortalized, oncogene-transformed, and tumour-derived breast epithelial cells. Aberrant choline phospholipid metabolism in breast cancer cells has been associated with increased choline transport and CK activity, as well as increased phospholipase D and phospholipase A2 activity (37).

In a study of 184 breast cancer patients, Shin et al. (38) have shown that the use of spectroscopy-determined absolute choline-containing compound peak integral, normalized choline-containing compound integral, and the signal-to-noise ratio can help with the differentiation of invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS). These same parameters could also be useful in determining tumour aggressiveness. In the same breast cancer patients, Mitsuhiro et al. found that the normalized choline signal correlated with the peak standardized uptake value ($r=0.52$; $p<0.0001$) in both the semi-quantitation of the choline levels (as measured using 1H MR spectroscopy) and FDG uptake (as measured using PET/CT). For patients with breast cancer with an invasive ductal carcinoma of 1.5–3 cm in size, the total choline levels in tumours measured by 1H-MR spectroscopy was highly correlated with the standardized 18F-FDG uptake values obtained by PET/CT (Figure 5-6). These measurements were also supported by histologic prognostic parameters (nuclear grade, estrogen receptor status, and triple-negative lesion status). The sensitivity

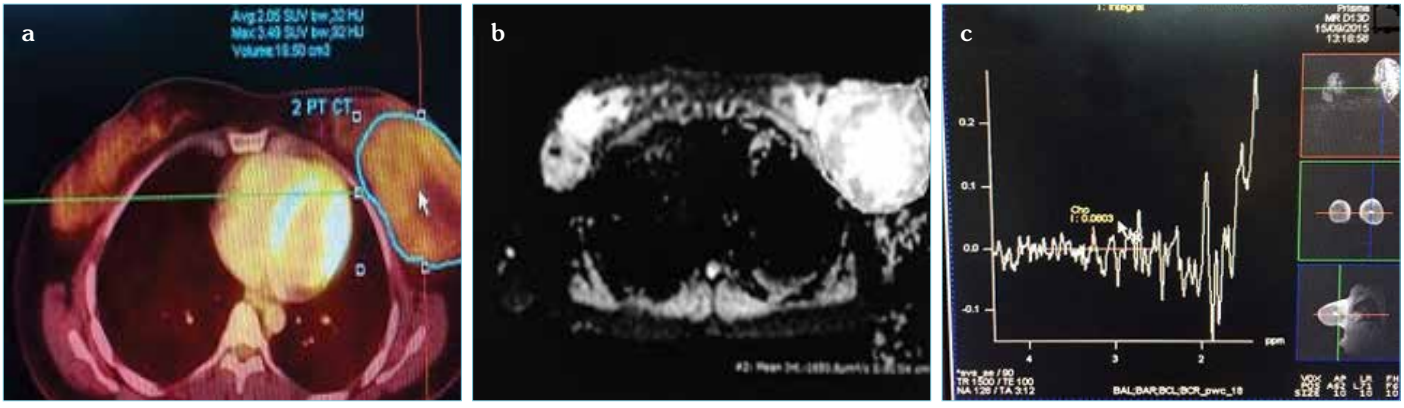

Figure 5. a-c. (a) Circular region of interest (diameter, 1.5 cm) was placed over the tumour in the slice with the maximum SUV :3.49 µg/dL. (b) Circular region was drawn at the breast lesion with b value of 50 showed non-restricted with ADC value of 1.65 mm2/s which were benign. (c) Peak of tCho with 1.41ppm and integral at 0.08 in benign tumour

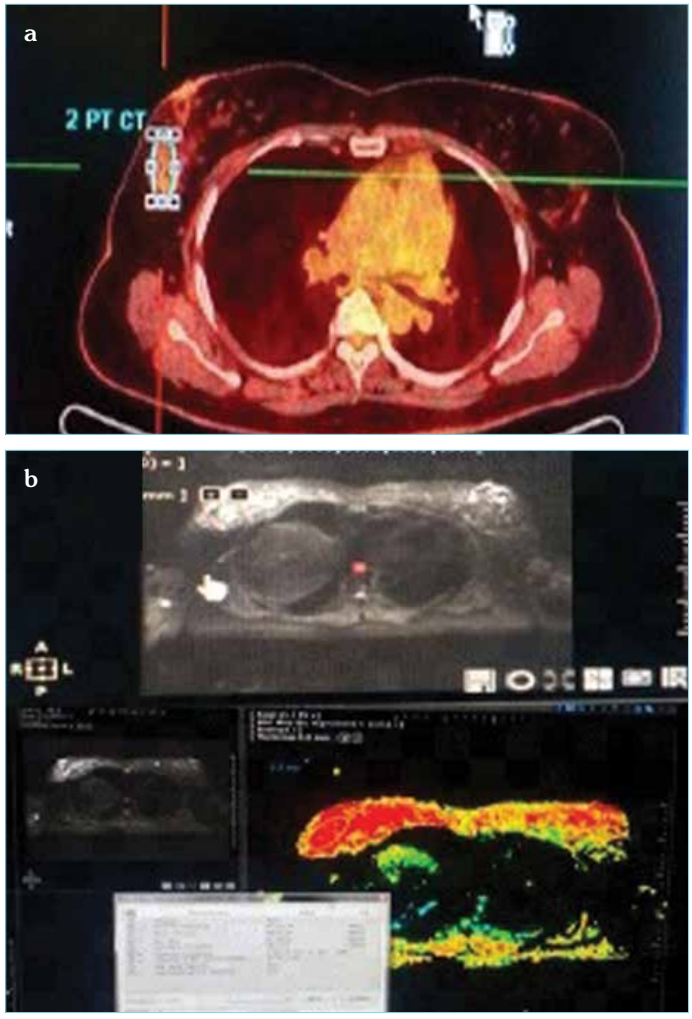

Figure 6. a,b. (a) Circular region of interest (diameter, 1.5 cm) was placed over the tumour in the slice with the maximum SUV: 2.33. (b) Circular region was drawn at the breast lesion with b value of 50 showed non-restricted whereas the b value of 900 showed restricted with ADC value of 0.66 which were malignancy

and specificity of the total choline level (as determined by 1H-MR spectroscopy) for detecting breast cancer were 83% and 85%, respectively, and both values could be as high as 92% after technical exclusions (39).

Choline and fluorodeoxyglucose

Generally, malignant cells have enhanced glucose metabolism due to accelerated tumour growth, as well as an increase in glucose transporter proteins compared to non-malignant cells, and thus an increased glycolytic activity. This high glycolytic activity eases the detection of malignant cells by FDG-PET imaging. Unfortunately, 18F-FDG is not a cancer-specific tracer because it also has an increased uptake in inflammatory and infectious lesions and even in a significant number of physiologic processes, such as brain glucose uptake or muscle uptake (40). Although many radiotracers have been developed for PET imaging, most breast cancer imaging studies have been performed with FDG. FDG is a glucose analogue transported via glucose transporters into the cells, where it is phosphorylated by hexokinase. FDG becomes metabolically trapped in tumour cells at a rate proportional to glucose utilization and therefore, glucose metabolism (41). Many studies have demonstrated the high sensitivity and specificity of FDG-PET for the detection of primary large and palpable breast tumours (42). Nevertheless, this sensitivity decreases when the lesions are small and non-palpable, low-grade, or non-invasive neoplasms. As mentioned above, FDG-PET has been used in breast cancer for diagnosis, staging, and re-staging, and treatment response evaluation. In PET imaging, 18F-FDG may hamper detection of certain breast cancer types (e.g., lobular type) because of their low glucose metabolism. Thus, 18F-FDG has limited value in workup studies (43–45). 18F-FDG and 18F-fluorocholine (18F-FCH) combination are potentially better than a single tracer signaling of the 18F-FDG in improving the sensitivity of the detection rates.

There is little evidence to suggest that choline PET can be useful for the detection of breast cancer. Increased choline metabolism has been noted during the transition of normal human mammary epithelial cells to immortalized, oncogene-transformed, and non-metastatic and metastatic cancers. There is a switch from predominantly higher intracellular metabolite levels of GPC (degradation pathway) to predominantly higher PC levels (biosynthetic pathway) early during cell transformation (46). PC levels then increase throughout disease progression (46). Studies in mammary epithelial cells have shown that aberrant increases in PC metabolite levels are due to the expression of the biosynthetic enzyme choline kinase-α (46).

Breast cancer cells have an increased uptake of choline, and 11C-choline PET/CT has been used to accurately localize malignant tumours (47). The relatively long half-life of 18F- FCH (110 min) means that it can be produced off-site, which is a major practical advantage for its use in imaging (48). Also, 18F- FCH is already being used in the evaluation of prostate cancer and is, therefore, more widely available than other radiotracers (49).

18F-FCH PET-CT is a promising tool and is being used to detect prostate cancer with greater accuracy than the semi-quantitative analysis of the degree of inherent choline uptake (SUVmax). (50–52).

CONCLUSION

Conventional clinical molecular imaging, that is, with radionuclide-based probes, has been practiced for many years and is a growing field with the development of ever more selective receptor-, enzyme-, and transporter-based imaging agents. We are beginning to see the first applications of MR-PET genetic-based clinical molecular imaging, particularly for cell trafficking studies to unveil the potential association of the lipid metabolism of the cell membranes and the associated expression of genetic and inflammatory molecular markers as discussed to discover a potential mechanism that underpin the cellular reprogramming of an aggressive breast cancers.

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Peer-review: Externally peer-reviewed.

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