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Editorial

CAIX-targeted PET imaging agents based on acetazolamide small molecule for clear cell renal cell carcinoma

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Abstract: Clear cell renal cell carcinoma (ccRCC), accounting for 65%-70%, is the most common subtype of renal cell cancers. Contrast-enhanced CT and MRI are still the predominant diagnostic modalities for renal carcinoma in clinical practice, but they cannot provide accurate diagnosis and staging, and molecular information related to tumor microenvironment. Carbonic anhydrase IX (CAIX), a transmembrane metalloenzyme on the cell surface and associated with hypoxia within the tumor, is emerging as a potential molecular target for both diagnosis and therapy in ccRCC. CAIX-targeted molecular imaging enables non-invasive visualization of ccRCC and guides treatment decision-making.

Keywords: Clear cell renal cell carcinoma, carbonic anhydrase IX, small-molecule inhibitor, positron emission tomography/computed tomography (PET/CT)

Introduction

Renal cell carcinoma (RCC) ranks as the eighth most common cancer, with an annual incidence increase of 2%-3% (1.5% to localized-stage disease). In 2024, an estimated 81,610 new cases and 14,390 deaths are expected in the United States [1]. Clear cell renal cell carcinoma (ccRCC) is the predominant subtype, accounting for 65%-70% of cases, followed by papillary RCC (13%-20%), chromophobe RCC (5%), and other rare types (~5%). At present, the diagnosis and treatment monitoring of ccRCC mainly rely on contrast-enhanced CT and MRI. However, these conventional imaging modalities cannot provide detailed biological insight into tumor status. PET/CT, which integrates functional and anatomical imaging, has gained prominence in oncology [2, 3]. The metabolic imaging agent 2-deoxy-2-[^{18}F]fluoro-*D*-glucose ([^{18}F]FDG) is widely used in clinical practice for tumor detection and management [4, 5]. Despite its utility, [^{18}F]FDG is not a tumor-specific tracer and exhibits significant variability in accumulation with renal masses of uncertain nature. Many primary and metastatic lesions of ccRCC appeared false-negative on [^{18}F]FDG PET/CT, limiting its applicability. As a result, [^{18}F]FDG is not recommended for RCC in the current National Comprehensive Cancer Network (NCCN) guidelines. This limitation underscores the need to develop target-specific imaging agents for ccRCC. Such advancements could significantly enhance clinical staging, risk stratification, therapeutic decision-making, treatment response evaluation, and outcome prediction.

Carbonic anhydrase IX (CAIX) is a cell surface transmembrane zinc metalloenzyme consisting of 459 amino acids with a molecular weight of 54 kDa. It is upregulated under hypoxic conditions and catalyzes the reversible hydration of carbon dioxide [6]. Hypoxia, a key regulator of tumor microenvironment and heterogeneity, induces the overexpression of CAIX via hypoxia-inducible factor-1 (HIF-1). By converting high concentration carbon dioxide to hydrogen and bicarbonate ions, CAIX facilitates cancer cell adaptation to hypoxia conditions. Its role is closely related to cancer progression, metastasis, aggressiveness, and drug resistance. CAIX is specifically expressed in a variety of hypoxic tumors, including breast, renal, pulmonary, colorectal and pancreatic cancers. For normal tissues, CAIX is predominantly expressed in the gastrointestinal tract, whereas in other normal tissues its expression is almost negligible [7]. Therefore, CAIX has emerged as a highly attractive and potential diagnostic and therapeutic target.

Approximately 95% of ccRCCs overexpress CAIX due to inactivation of the Von Hippel-Lindau (VHL) tumor suppressor gene. VHL regulates the ubiquitination of HIF-1α through E3-ubiquitin ligase, leading to the degradation of HIF-1α. The genetic mutation of VHL prevents its interaction with HIF-1α, resulting in stabilization and activation of HIF-1α. As the most prominent regulator, HIF-1α ultimately induces the overexpression of CAIX via activated *CA9* gene under hypoxia conditions [6]. More than 10 CAIX-targeted radiopharmaceuticals, including radionuclide labeled monoclonal antibodies, antibody fragments, affibodies, single-domain antibodies, peptides, and small molecules, have been evaluated for RCC diagnosis in preclinical and clinical studies [8]. In a multicenter phase III clinical trial (NCT03849118) involving 284 patients with renal masses, PET/CT imaging with the CAIX-targeted tracer [^{89}Zr]Zr-girentuximab showed favorable diagnostic

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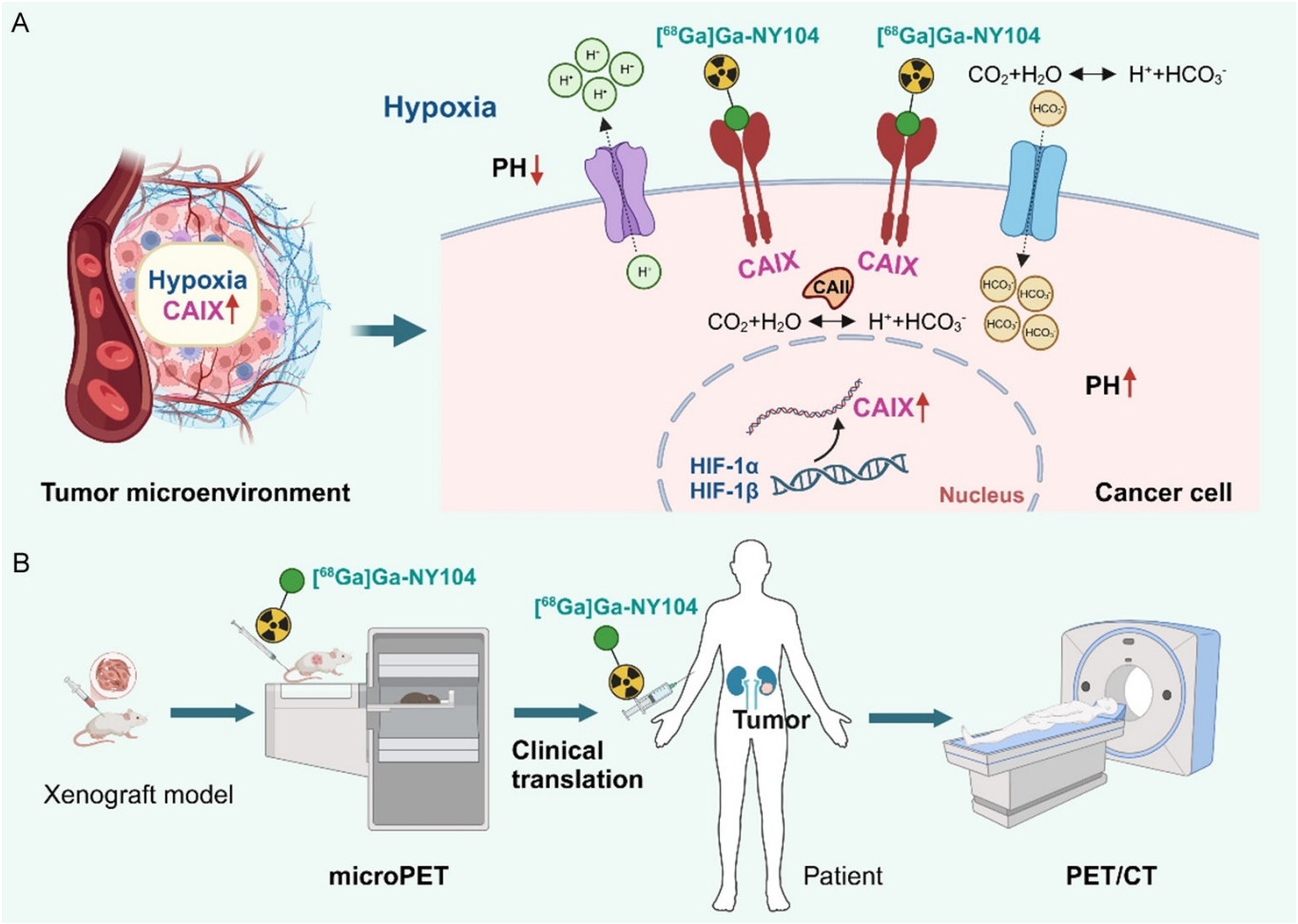

Figure 1. Diagram of imaging mechanism of [^{68}Ga]Ga-NY104 targeting carbonic anhydrase (CAIX) (A) and corresponding imaging procedures in preclinical study and clinical trial (B).

efficacy with sensitivity of 85.5% and specificity of 87.0% [9]. Unlike ccRCC, papillary and chromophobe RCC lack VHL mutations and hypoxia-associated biomarkers. This distinction enables CAIX-targeted tracers to differentiate ccRCC from other RCC subtypes, as confirmed by immune-PET imaging with the iodine-124-labeled chimeric G250 ([^{124}I]I-cG250) antibody [10]. Despite these promising results, monoclonal antibody-based imaging agents face limitations such as prolonged imaging wait times and low imaging contrast, restricting their clinical applicability. In contrast, small-molecule and peptide tracers offer significant advantages, including same-day imaging and high tumor-to-background ratios. For example, the derivative of acetazolamide, named [^{99m}Tc]Tc-PHC-102, exhibited excellent imaging contrast and favorable detection performance in five ccRCC patients [11]. As a result, acetazolamide-based small molecules have gained substantial interest as viable alternatives for CAIX-targeted imaging.

Literature highlight

Huo and coworkers introduced [^{68}Ga]Ga-NY104 (NY-M005), an acetazolamide-based CAIX-targeting tracer, for imaging ccRCC in preclinical and pilot clinical studies [12]. **Figure 1** shows the mechanism of action between [^{68}Ga]Ga-NY104 and CAIX, and the imaging workflow. Small molecule NY104 consists of an acetazolamide core and a hydrophilic arm, and conjugated to Gallium-68 using the chelator NOTA. The tracer demonstrated a radiochemical yield of 65%, radiochemical purity more than 95%, and an excellent binding affinity (Kd) to CAIX of 5.75 nM. In preclinical evaluations, [^{68}Ga]Ga-NY104 showed substantial tumor uptake in an OS-RC-2 (CAIX-positive human renal cell carcinoma cells) xenograft mouse model. The %ID/g valves were 20.05 ± 5.70 at 5 min post-injection, increasing to 27.06 ± 4.90 and 29.29 ± 6.82 at 1 h and 3 h, respectively. The specific high uptake of [^{68}Ga]Ga-NY104 was confirmed through blocking experiments and in a negative control model using PC-3 xenograft tumors (CAIX-negative) (**Figure 2**). Immunohistochemical analyses corroborated high CAIX expression in OS-RC-2 tumors, while PC-3 tumors showed no detectable expression. In addition, some normal tissues and organs, including the kidney, lung, liver, heart, and stomach, showed high tracer accumulation in the OS-RC-2 xenograft mouse model, with maximum %ID/g of 18.67

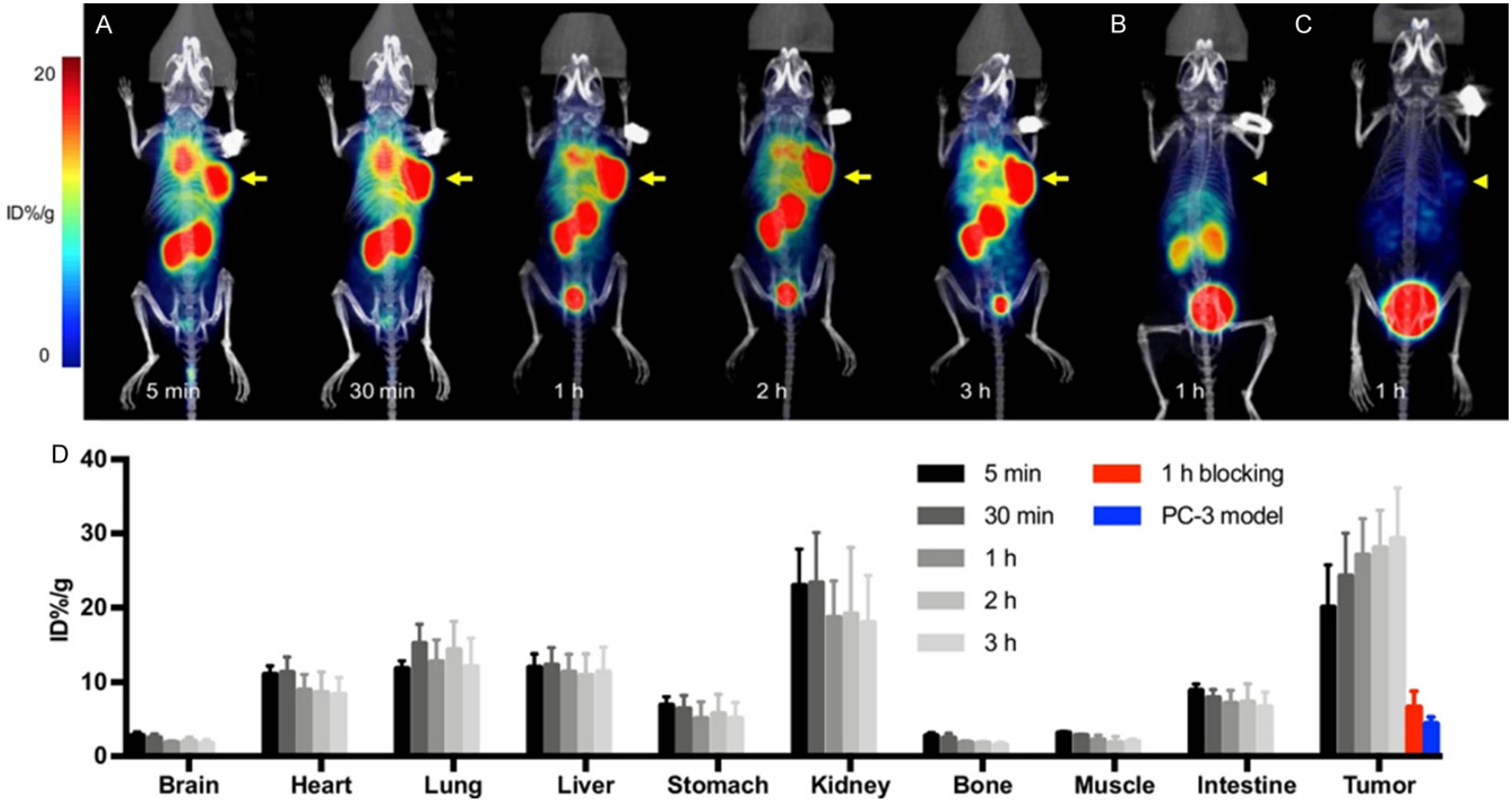

Figure 2. MicroPET/CT imaging shows the distribution of [^{68}Ga]Ga-NY104 in xenograft tumor models. A. CAIX-positive OS-RC-2; B. CAIX-negative PC-3; C. Blocking with NY104 in OS-RC-2 tumor; D. Detailed uptake information of tumors and normal tissues and organs. Reprinted with permission from [12]. © EJNMMI.

± 4.92, 12.78 ± 2.92, 11.36 ± 2.39, 8.94 ± 2.14, and 5.12 ± 2.26, respectively.

Following promising preclinical results, a pilot clinical trial involving three patients (NCT05728515) was conducted [12]. [^{68}Ga]Ga-NY104 was well-tolerated, with no adverse event observed. The first patient, a 67-year-old man with progressive ccRCC following pazopanib treatment, underwent [^{68}Ga]Ga-NY104 PET/CT, which not only detected all known lesions (primary tumor, hilar lymph node and lung metastases, and tumor thrombus in inferior vena cava), but also revealed two additional lesions (abdominal lymph node and femoral metastases), with SUVmax values of 13.8, 14.6, and 16.0 at 0.5, 1, and 2 h post-injection, respectively. The second patient, a 65-year-old woman with a history of left kidney cancer and radical nephrectomy 13 years ago, [^{68}Ga]Ga-NY104 PET/CT detected multiple pancreatic metastases, with an SUVmax of 37.4 and the corresponding tumor-to-blood and tumor-to-liver ratios of 31.2 and 10.4, respectively, at 1 h post-injection. The third patient, a 30-year-old man who received radical nephrectomy and was confirmed as left kidney ccRCC, [^{68}Ga]Ga-NY104 PET/CT identified a true-negative lesion adjacent to the left abdominal wall, which had been identified as a false-positive nodule on [^{18}F]FDG PET/CT. Histopathological analysis confirmed the lesion as inflammatory fibrous tissue. Interestingly, biodistribution in humans differed from preclinical results. For example, the stomach showed the highest uptake of [^{68}Ga]Ga-NY104, followed by the kidney, pancreas, and liver in humans, whereas moderate uptake of the stomach was observed in mice. This marked accumulation of [^{68}Ga]Ga-NY104 in the stomach may reflect CAIX expression in the gastric mucosal epithelium. Notably, high renal background uptake, caused by binding to extracellular CAIX in normal renal parenchyma, could hinder the detection of small lesions. These findings in this pilot study highlighted the potential of [^{68}Ga]Ga-NY104 for imaging ccRCC. However, further studies with large sample are warranted to validate its diagnostic performance in primary and metastatic ccRCC.

Recently, the same group published a large-scale clinical study (NCT05902377) assessing the diagnostic performance of [^{68}Ga]Ga-NY104 PET/CT imaging of ccRCC [13]. Their study included a cohort of 47 patients, in which 20 individuals with primary renal mass and 27 with suspected or confirmed metastatic ccRCC. The study demonstrated an overall sensitivity, specificity, and accuracy of 81% (30/37), 78% (7/9), and 80% (37/46) on a per-patient analysis. However, the tracer's detection efficiency for identifying primary renal lesions was suboptimal, with a sensitivity of 62% (10/16) and specificity of only 33% (1/3). The high renal background uptake, attributed to spill-over effects from normal kidney parenchyma expressing CAIX, likely masked visualization of renal masses. Despite these limitations, [^{68}Ga]Ga-NY104 PET/CT exhibited exceptional performance in identifying metastatic lesions, with a sensitivity of 95% (20/21), specificity of 100% (6/6), and accuracy of 96% (26/27), respectively. Furthermore, [^{68}Ga]Ga-NY104 PET identified additional metastatic lesions in 67% (14/21) of cases compared to conventional imaging. A representative case is shown in **Figure 3**. These encouraging findings are essential for the precise identification and localization of metastatic lesions and subsequent clinical management. Besides the kidney, physiological uptake of [^{68}Ga]Ga-NY104 was observed in the stomach wall, pancreas, and lungs, consistent with CAIX expression in these normal tissues to varying degrees. Importantly, it should be noted that tyrosine kinase inhibitor (TKI) treatment may affect [^{68}Ga]Ga-NY104 PET imaging outcomes of ccRCC. To minimize interference, a 7-day washout period before PET imaging is recommended for patients undergoing TKI treatment. Overall, this study features the exceptional diagnostic accuracy of [^{68}Ga]Ga-NY104 PET/CT for metastatic ccRCC, providing critical insights for precise lesion identification and clinical decision-making.

Perspectives

The diagnostic potential of [^{68}Ga]Ga-NY104 in ccRCC is promising; however, it still had some limitations. Notably, high uptake in the kidney and gastric wall may hinder the visualization of lesions in these areas and affect subsequent therapeutic applications. In addition, cross-reactivity with other CA family members (e.g., CAXII) needs to be investigated, which may reduce tracer selectivity and specificity. Building on [^{68}Ga]Ga-NY104, two novel imaging agents based on acetazolamide ([^{18}F]AlF-NYM005 and [^{68}Ga]Ga-NYM046) were introduced through labeling strategy refinement and molecular modification, which significantly improved the diagnostic efficacy of primary renal tumors [14, 15]. Besides small molecular inhibitor, a peptidomimetic-based CAIX-targeted tracer, [^{68}Ga]Ga-DPI-4452, showed exceptional tumor uptake with SUVmax of 6.8-211.8, high tumor-to-background contrast, and reduced renal accumulation in a clinical trial involving three ccRCC patients [16]. Furthermore, a macrocyclic peptide-based CAIX-targeting agent, [^{64}Cu]Cu-PD-32766, developed by PeptiDream, exhibited excellent binding affinity and optimal tumor visualization, with xenograft tumor uptake of 88%ID/g and renal uptake of 23%ID/g at 4 hours post-injection in preclinical studies [17]; A corresponding clinical trial in ccRCC patients (jRCTs031240046) is currently underway. Recently, Wei and colleagues introduced a novel class of specific tracers based on single-domain antibodies (sdAbs) targeting cluster of differentiation 70 (CD70). Their studies demonstrated the diagnostic value and specificity of tracers such as [^{18}F]RCCB6 and [^{68}Ga]Ga-NOTA-RCCB6 in preclinical and pilot clinical investigations. These findings highlight the potential of CD70-targeted imaging as a complementary approach to CAIX-targeted strategies in ccRCC [18-20].

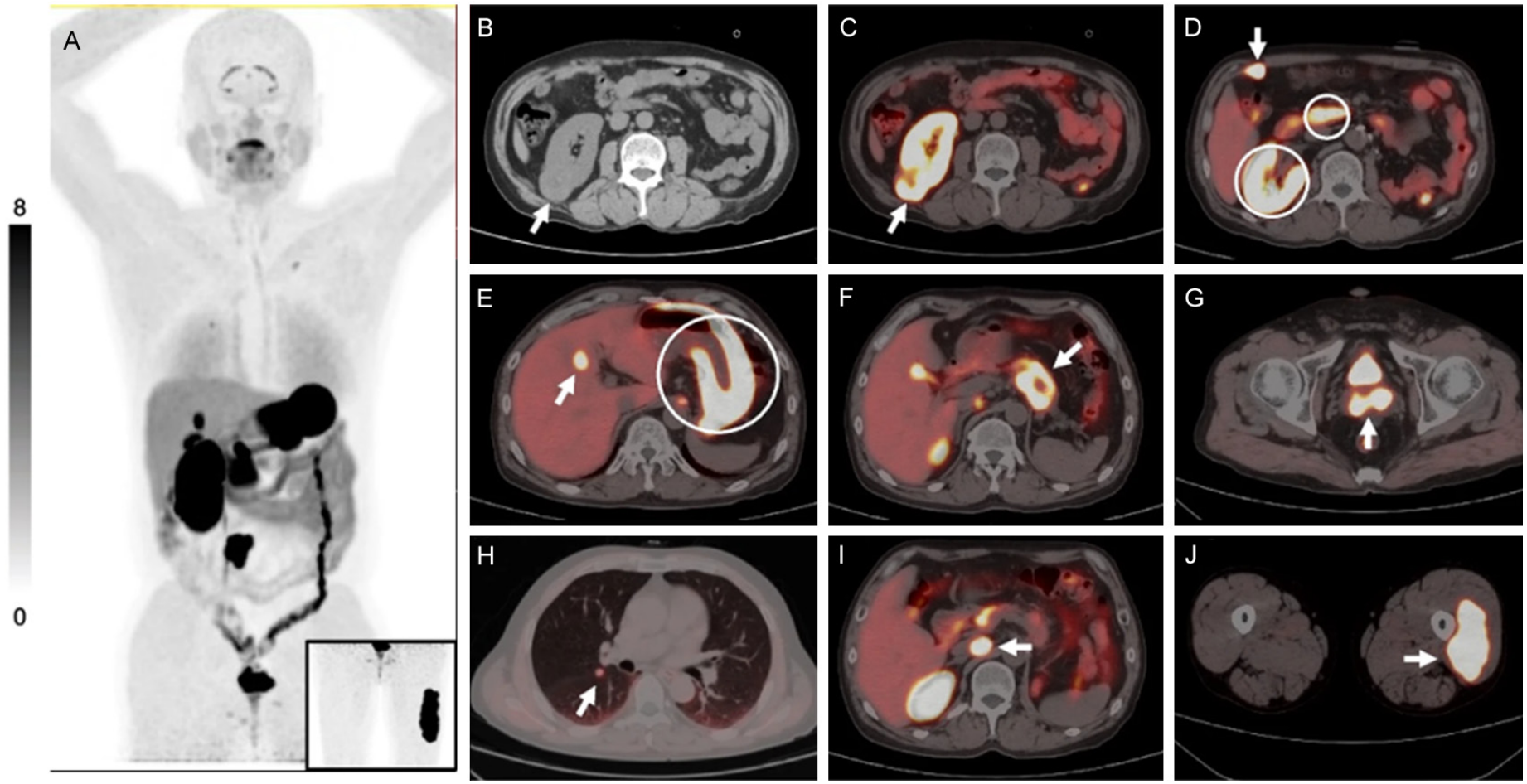

Figure 3. [^{68}Ga]Ga-NY104 PET/CT was performed for restaging in a 54-year-old male patient who had a history of radical nephrectomy due to left kidney ccRCC 14 years ago. All the lesions (A) were observed to be CAIX-overexpression, including the right kidney (B and C), abdominal wall (D), liver (E), pancreas (F), seminal vesicle (G), lung (H), paraaortic lymph node (I), and muscle of the left thigh (J) metastases (arrows). The abdominal wall lesion was confirmed to be metastasis from ccRCC by biopsy pathology. Note. The normal renal parenchyma, pancreas, and stomach wall showed intensive physiological uptake (circles in D and E). Mild physiologic uptake can also be observed in the lungs (A). Reprinted with permission from [13]. © EJNMMI.

CAIX represents a promising target for both diagnostic and therapeutic applications in ccRCC. Since ccRCC is not sensitive to conventional chemotherapy and radiotherapy, CAIX-targeted theranostics, based on paired radiopharmaceuticals combining diagnostic and therapeutic approaches, provides a valuable strategy for cancer management for patients with locally advanced or metastatic ccRCC. CAIX-targeted [^{177}Lu]Lu-labeled girentuximab has demonstrated therapeutic efficacy in phase I/II clinical trials, with 64% to 74% of patients achieving stable disease within three months of treatment [21, 22]. In a recent preclinical study, peptide-based [^{177}Lu]Lu-DPI-4452 targeting CAIX also exhibited promising therapeutic effect in ccRCC xenograft models, and clinical trial related to [^{177}Lu]Lu-DPI-4452 is currently underway [23]. Macrocyclic peptide-based [^{177}Lu]Lu-PD-32766 also showed strong tumor growth inhibition in a preclinical study [17]. Further research is needed to explore whether target radioligand therapy (TRT) derived from small molecule inhibitor targeting CAIX can provide comparable benefits for ccRCC patients. The encouraging findings from the clinical trials of acetazolamide-based imaging tracers provide strong evidence supporting the development of therapeutic radiopharmaceuticals targeting CAIX to optimize the clinical management of ccRCC. Further prospective multicenter studies with larger patient cohorts are necessary to confirm the diagnostic value of acetazolamide-based tracers. Concurrently, efforts should focus on enhancing the pharmacokinetics and pharmacodynamics of acetazolamide-based tracers to increase its detection efficiency for primary ccRCC, decrease non-specific uptake in the gastrointestinal tract, and minimize potential nephrotoxicity associated with TRT.

Disclosure of conflict of interest

None.

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