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REVIEW

Emerging Applications of EpCAM-Targeted Nuclear Medicine Probes: Current Research and Future Perspectives

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Abstract: Molecular imaging in nuclear medicine has been employed extensively in recent years for tumor-targeted diagnosis and treatment that is attributed to its non-invasive property, which enables visualized functional localization. This functionality relies on the development of radionuclide molecular probes designed with the objective of identifying specific targets on the surface of tumors. Epithelial cell adhesion molecules (EpCAM) are considered to be a promising target as an antigenic marker for its widely present and integral to the processes associated with tumor occurrence and progression. This paper provides a comprehensive review of recent advancements in EpCAM-targeted radionuclide probes, focusing specifically on a range of primary ligands, including DARPins (Designed Ankyrin Repeat Proteins), antibodies, and aptamers, and evaluates the benefits and challenges, potential future directions of these radioactive probes.

Keywords: epithelial cell adhesion molecule, nuclear medicine imaging, molecular imaging, tumor targeted imaging

Introduction

Molecular imaging has made significant strides in clinical translational applications,[1,2] such as facilitating personalized treatment approaches,[3] enhancing drug screening processes,[4–6] and assessing therapeutic efficacy and prognosis,[7–9] which pertaining to the precision diagnosis and treatment of tumors in recent years. Notably, nuclear medicine imaging technologies such as single-photon emission computed tomography (SPECT) and positron emission computed tomography (PET) offer distinct advantages, including non-invasive whole-body scanning, dynamic quantitative analysis, and high sensitivity.[10–12] These technologies have proven particularly effective in addressing tumor heterogeneity.[13–15] Central to their efficacy is the advancement of specific radionuclide molecular probes that target unique markers on the tumor surface, enabling precise localization of tumor foci through targeted imaging techniques.

EpCAM (epithelial cell adhesion molecule), classified as a type I transmembrane protein of around 40 kDa,[16] plays critical roles in cell-cell interactions.[17] It was first identified in 1979 as a tumor surface antigen in colorectal cancer (CRC) through antibody screening.[18] Initially referred to by various names, including leukocyte differentiation antigen 326 (CD326),[19] tumor-associated calcium signal transduction molecule 1 (TACSTD-1),[20] trophoblast cell surface antigen (TROP-1),[21] and carcinoma-associated glycoprotein (KS1/4),[22] a consensus was established in 2007 to adopt EpCAM as the primary designation.[23] Human EpCAM is a polypeptide consisting of 314 amino acids,[24] located on chromosome 2 at position 2p21, with a total size of approximately 14 kb. The structure of EpCAM includes an extracellular domain (EpEX), which constitutes about 80% of its total length and is primarily encoded by exons 1–6, a single transmembrane segment (23 amino acids, encoded by exon 7), and a cytoplasmic domain (26 amino acids, encoded by exons 8 and 9, EpICD).[25,26] The extracellular domain features two epidermal growth factor (EGF)-like domains (EA and EB) that characterized by two pairs of disulfide bonds (Cys-Cys bonds)

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to sustain their structural stability, and a cysteine-poor region known as the TY domain. Within the EA domain, the hypervariable region (HVR) motif serves as a functional site associated with cell adhesion. The transmembrane domain consists of a single α-helix, while the intracellular domain contains a short tail with conserved phosphorylation sites (Figure 1). Similar to many other transmembrane proteins, EpCAM possesses a signal peptide that is cleaved by a signal peptidase, resulting in a 6 kDa peptide which most monoclonal antibodies (mAbs) specifically bind to that highlighting its significant immunogenicity,[27,28] remains covalently linked to the 32 kDa portion via disulfide bonds.

Main Location and Cell Adhesion

The EpCAM is predominantly produced by the basement membrane in healthy tissues and is primarily localized laterally in embryonic epithelial cells and most normal adult epithelial cells, including those in the liver and skin.[16] In cancerous tissues, EpCAM is generally distributed uniformly across the cell surface.[29] This distribution may be influenced by the differential expression of various proteins that interact with EpCAM, as well as the distinct glycosylation patterns of EpCAM itself.[30] EpCAM is involved in intercellular adhesion among epithelial cells; however, it lacks both sequence and structural similarities to any recognized cell adhesion molecules, thereby excluding it from the traditional classification of adhesion molecules. Its function in the regulation of adhesion is predominantly that of a modulator of adhesion strength, rather than serving as a facilitator of epithelial cell aggregation or the establishment of junctional complexes.[31] Nevertheless, it continues to be referred to as an epithelial cell adhesion molecule to reflect its limited expression to epithelial cells.

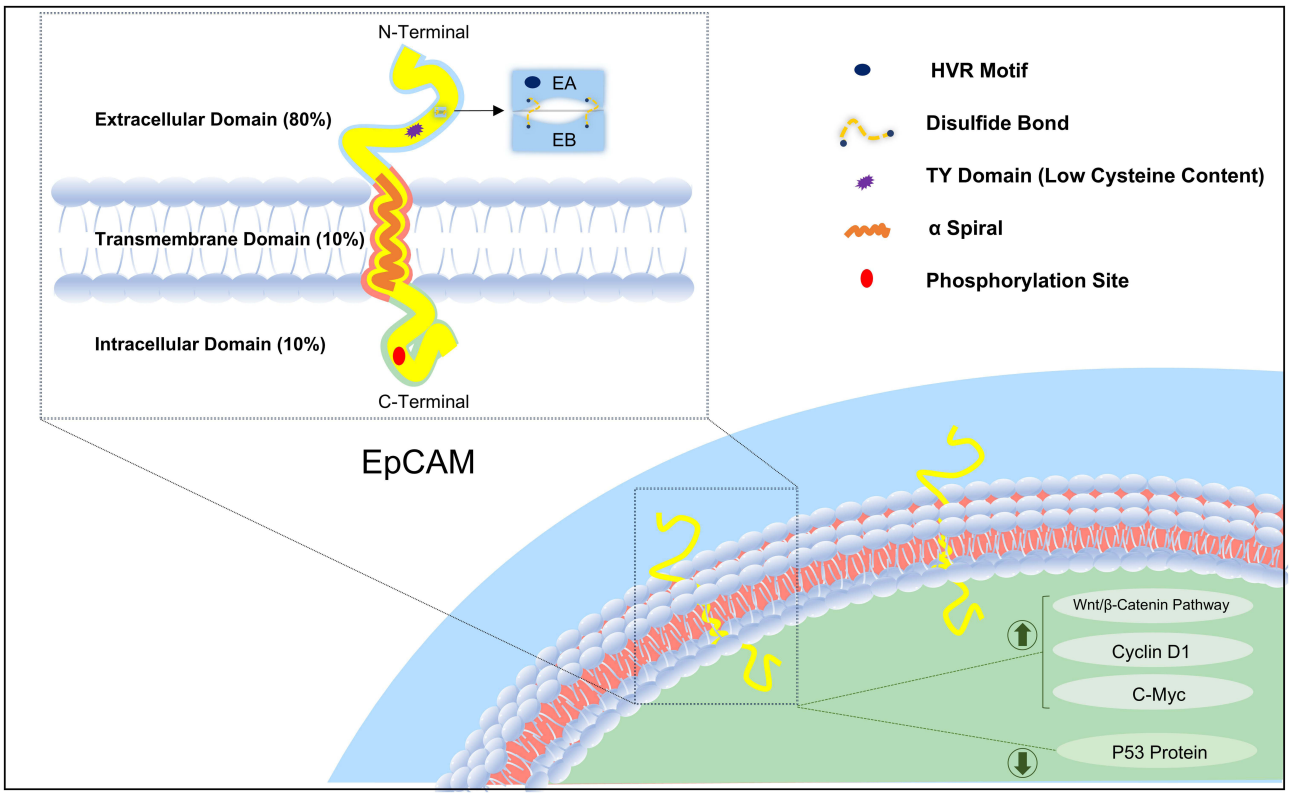

Figure 1 Structural organization of EpCAM. Schematic representation of the 314-amino acid transmembrane glycoprotein EpCAM, spanning from the extracellular N-terminus to the intracellular C-terminus. Extracellular domain (EpEX) is approximately 80% of the total length and comprises two epidermal growth factor (EGF)-like domains (EA and EB) stabilized by disulfide bonds (Cys-Cys linkage), and a cysteine-poor TY domain. The EA domain harbors a hypervariable region (HVR) critical for cell adhesion. Transmembrane domain (about 23 amino acids) have a single α-helix anchoring EpCAM to the plasma membrane. Intracellular domain (EpICD, about 26 amino acids) has short cytoplasmic tail containing conserved phosphorylation sites for regulatory signaling. The upward arrow indicate activation/positive regulation of EpCAM-mediated signaling pathways and the downward arrow denote suppression/negative regulation of downstream pathways.

Involvement in Cancer

EpCAM has been recognized as a prominent signature antigen in various including breast,[32] hepatic,[33] gastric,[34] ovarian,[32,35] prostate,[36] lung cancer,[37] and other epithelial tumors, where its overexpression has been documented. This overexpression is observed in metastatic lesions, malignant effusions, cancer stem cells (CSCs), undifferentiated embryonic stem cells, and circulating tumor cells (CTCs), indicating a significant correlation with the diagnosis and classification of these diseases (Figure 2). Furthermore, EpCAM overexpression serves as an independent prognostic marker linked to diminished patient survival and is indicative of invasive and metastatic potential, typically correlating with unfavorable outcomes in cancers such as pancreatic, urothelial, and gallbladder cancers. Notably, exceptions exist in renal and thyroid cancers, where elevated levels of EpCAM have been associated with improved survival rates. EpCAM is implicated in various cellular processes, including signal transduction, migration, proliferation, and differentiation in cancer cells.[38,39] The underlying mechanisms are complex and involve the activation of the Wnt/β-catenin signaling pathway,[40] the ablation or downregulation of the tumor suppressor protein p53,[41] the rapid upregulation of the oncogenic transcription factor c-Myc,[42,43] the upregulation of cyclin D1.[44] In summary, the extensive tumor-specific overexpression of EpCAM, along with its evident role in tumorigenesis and metastasis, positions it as a promising target for surveillance and therapeutic interventions.

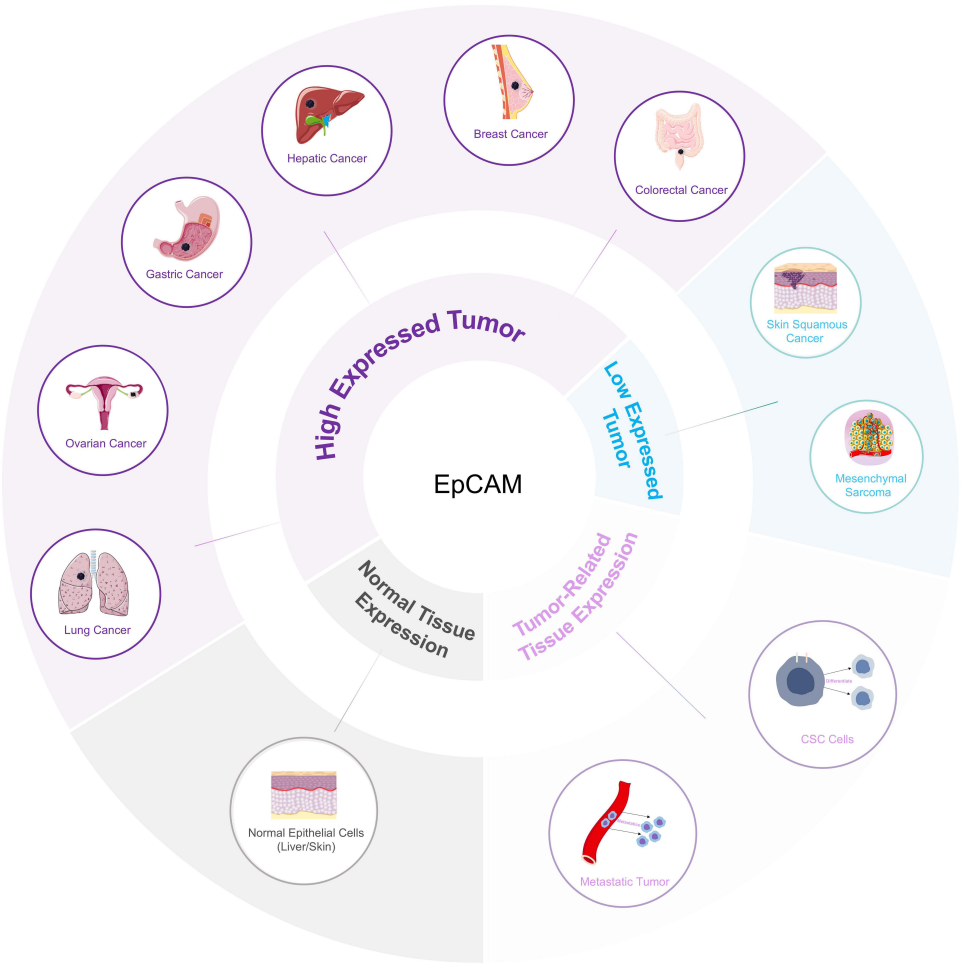

Figure 2 EpCAM expression patterns in normal and neoplastic tissues. In healthy tissues, EpCAM is basolaterally localized in epithelial cells (eg, liver, skin) and produced by the basement membrane. High EpCAM expression is observed in epithelial-derived malignancies, including colorectal, breast, hepatic, gastric, ovarian, and lung cancers, as well as in tumor-associated niches such as cancer stem cells (CSCs) and metastatic lesions. Low expression occurs in cutaneous squamous cell carcinoma and mesenchymal tumors (eg, sarcoma).

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Tumor Heterogeneity and Molecular Imaging of Nuclides

The heterogeneity of tumor targets presents a significant challenge in the monitoring and targeted therapy of neoplasms, with EpCAM demonstrating variability across numerous tumors characterized by overexpression.[45] Nuclear molecular imaging offers a non-invasive method for the quantitative visualization of molecular targets, as well as a comprehensive systemic assessment of patients both pre- and post-treatment.[46] This imaging modality is noted for its high sensitivity and is regarded as a robust tool for patient stratification and addressing target heterogeneity. Consequently, it has gained considerable traction in the targeted diagnosis and treatment of oncological conditions in recent years. The core of its application lies in the advancement of nuclide molecular probes, which primarily focus on nuclear imaging and the targeting of specific tumor cell domains.[47–49] These probes may include antibodies, fragments, proteins, or aptamers that are either directly conjugated or linked via chelating agents, and are utilized in conjunction with SPECT or PET imaging systems for tumor localization, metabolic assessment, or therapeutic monitoring.[50–52] With ongoing improvements in nuclear medicine technology and algorithms, as well as advancements in the development and labeling of nuclide probes, research into molecular probes targeting EpCAM is also progressing. Recent representative studies in this area are illustrated (Figure 3). This paper aims to synthesize research findings in the domains of SPECT and PET with the objective of providing a reference for related fields, thereby fostering the development of pertinent molecular probes and enhancing the precision of targeted tumor diagnosis and treatment.

Molecular Probes Using Antibodies and Fragments as Ligands

Antibody molecules are a class of engineered scaffold proteins with established diagnostic, therapeutic, and biotechnological potential.[53] Their high affinity and specificity for binding to specific protein targets make antibodies a popular choice for molecular imaging.[54,55] Studies have demonstrated that the rapid in vivo pharmacokinetics of antibody molecules allow for compatibility with nuclides that have varying half-lives, facilitating their application in different tumor models.[55–57] Radionuclide-labeled antibody molecules can provide highly specific and sensitive imaging on the day of injection, with enhanced sensitivity for delayed imaging in certain targets.[50]

In June 2010, Matthias et al[58] systematically compared the PET imaging performance of four anti-EpCAM antibody fragments (IgG, trimer, dimer, scFv) labeled with ^{68}Ga (Gallium-68, $T_{1/2}$ = 68 min) in mice bearing EpCAM-positive CRC. PET results indicated that full-length IgG (150 kDa) showed the highest uptake (150 kDa, 3.54 ± 2.01%ID/g), followed by the triplet (75 kDa, 3.01 ± 1.23%ID/g), dimer (51 kDa, 1.87 ± 0.50%ID/g), and scFv (30 kDa, 0.62 ± 0.24% ID/g), and the dimer was identified as the preferred probe for ^{68}Ga immunoPET due to its balanced targeting efficacy and pharmacokinetics. Limitation of this study is the relatively low absolute tumor uptake compared to other bispecific antibodies, potentially attributed to EpCAM internalization or antigen saturation. This study highlighted the need for optimizing antibody fragments targeting alternative tumor-associated antigens.

In 2012, Hall et al[59] conducted a ^{64}Cu (Copper-64, $T_{1/2}$ = 12.7 h)-DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic Acid)-labeled anti-EpCAM antibody probe for PET/CT imaging in a hormonal mouse model of PC3 prostate cancer, results indicated that 50% for primary tumors and 83% for metastatic lymph nodes (LNs), achieving 89% overall sensitivity, aligned with near-infrared fluorescence imaging, confirming the probe's reliability for non-invasive LN metastasis diagnosis and image-guided staging. In a follow-up study, the research team[60] optimized monoclonal antibodies (mAbs) using the same platform, identifying a high-affinity mAb for improved metastatic LN detection.

In February 2015, Ghosh et al[61] compared two EpCAM-targeted immunoconjugates (^{64}Cu-DOTA-mAb7 vs ^{64}Cu-NODAGA (1,4,7-Triazacyclononane,1-glutaric Acid-4,7-acetic Aci)-mAb7) in PC-3 prostate cancer mice models. The findings indicated that the immunoreactivity and tumor uptake (13.44 ± 1.21%ID/g vs 13.24 ± 4.86%ID/g) was comparable of two conjugates while ^{64}Cu-NODAGA-mAb7 exhibited higher tumor-to-prostate ratios and reduced normal tissue uptake, demonstrating that NODAGA as a preferred chelator for EpCAM-targeted probes, and ^{64}Cu-NODAGA-mAb7 offer superior specificity in detection and holds potential for application in other types of cancers that express EpCAM.

In 2016, Warnders et al[53] conducted the inaugural non-invasive preclinical imaging study utilizing ^{89}Zr (Zirconium-89, $T_{1/2}$ = 78.4 h)-labeled bispecific T cell engaging antibody AMG110 (Bispecific T-cell Engager, BiTE), which facilitates T cell-mediated cytotoxicity against tumor cells by cross-linking EpCAM and human CD3ε. The findings

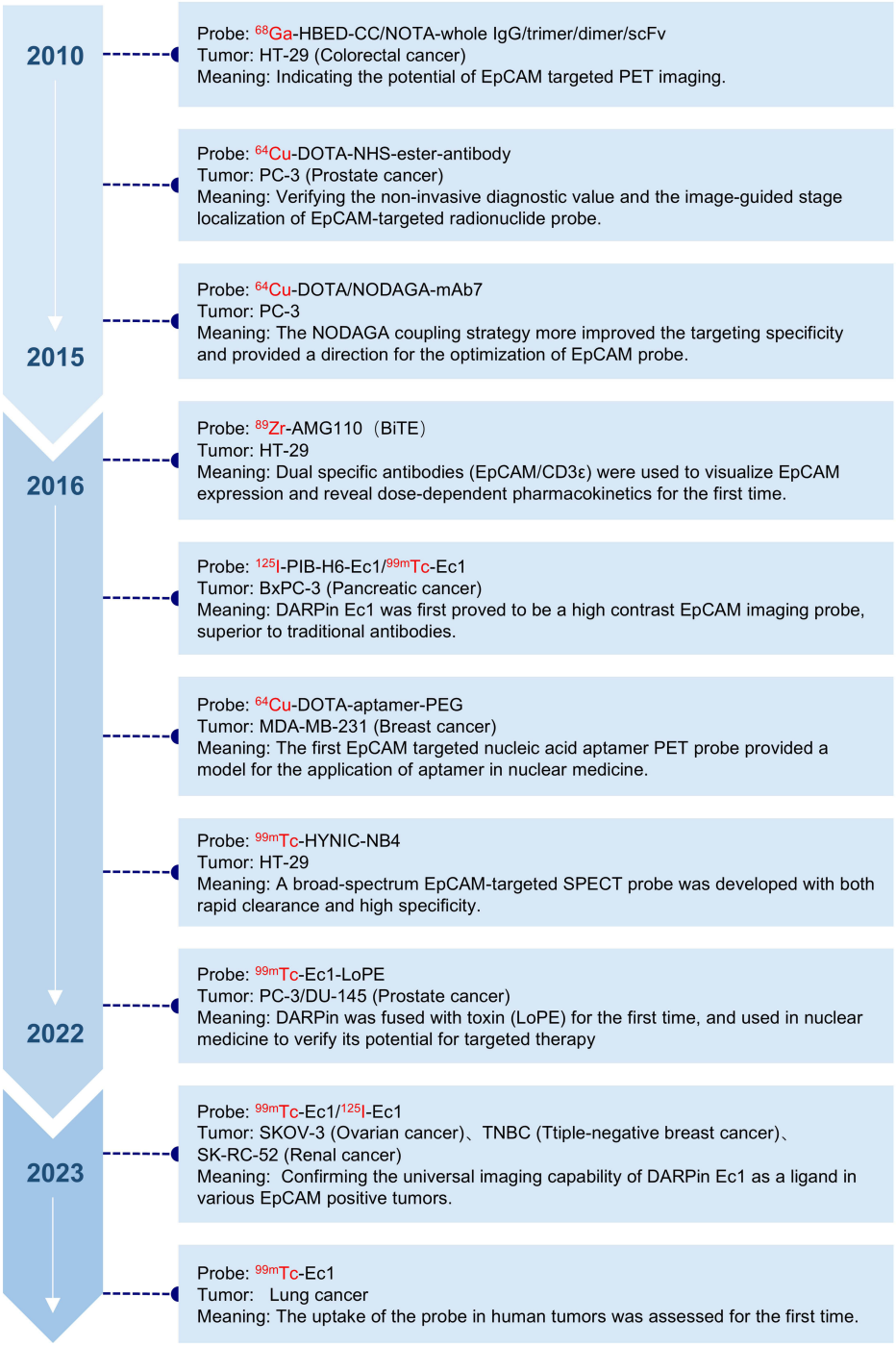

Figure 3 Main explore research of EPCAM targets in the field of nuclear medicine over the years are partly listed.

indicated that the tumor uptake values of ^{89}Zr-AMG 110 in xenograft models were positively correlated with the levels of EpCAM expression. PET effectively demonstrated significant differences, with the highest uptake observed in HT-29 tumors (CRC, high expression, 5.3 ± 0.3%ID/g), followed by FaDu tumors (squamous cell carcinoma of the head and neck, moderate expression, 2.7 ± 0.6%ID/g) and HL-60 tumors (promyelocytic leukemia, negative expression, 0.8 ± 0.2%ID/g). Notably, there were no significant differences in the uptake of normal organs among the mice with different xenograft tumors. The study further revealed that 40 μg dose achieved prolonged tumor retention (5.3 ± 0.3%ID/g at

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24 h) and peak tumor-to-blood ratio of 65.1 ± 15.5. Additionally, HT-29 xenograft tumors exhibited specific retention of ^{89}Zr-AMG110, in contrast to the non-EpCAM-binding ^{89}Zr-Mec14 bispecific T cell engager (BiTE). These results underscoring the potential of visualizing EpCAM-positive tumors using nucleic acid-labeled BiTE antibody constructs, thereby facilitating their clinical development. In May of the same year, Rodriguez et al[62] developed a bioorthogonal ^{18}F-labeling method to resolve half-life mismatch challenges, enabling sequential EpCAM-targeted PET imaging with reduced heterogeneity interference, it is feasible to administer a different antibody the following day to the same tumor that enhance the precision of predicting the in vivo distribution of therapeutic antibodies.

In May 2022, Liu et al[63] labeled the nanoantibody NB4 with ^{99m}Tc (Technetium-99m, $T_{1/2}$ = 6.02 h), which showed high EpCAM specificity both in vivo and ex vivo. SPECT/CT visualization showed that ^{99m}Tc-NB4 was rapidly cleared from the blood and normal organs (except kidneys), and the tumor uptake was increased from 3.77 ± 0.39%ID/g (0.5 h) after injection to 5.53 ± 0.82%ID/g at 12 h with clear visualization of tumor-draining lymph nodes, demonstrated that ^{99m}Tc-NB4 is a broad-spectrum, specific, and sensitive SPECT nuclear tracer for noninvasive visualization of EpCAM expression tumors. Hugo et al[64] established a pretargeted PET platform using ^{18}F-labeled bispecific antibodies (bsAbs), enabling sequential imaging to address tumor heterogeneity. Other imaging studies include Ghosh et al[65] pioneered a NIR/nuclide (^{68}Ga/^{64}Cu) dual-model EpCAM-targeted imaging platform, validated precise tumor localization and pharmacokinetics in prostate cancer models.

Probes with DARPins as Ligands

Designed ankyrin repeat proteins (DARPins, which can also be abbreviated as EVDs) are a class of stable protein structural domain molecules produced by Escherichia coli and consisting of multiple motifs (each of about 33 amino acids).[66] As a novel engineered scaffold protein,[67] its specificity and affinity can be comparable to that of antibodies,[68,69] but the DARPins (14–18 kDa) system is much smaller than the fragment of antibodies is smaller even when it fused with toxins, which is structurally more easily modified, and it can also extravasate and diffuse very quickly in the extracellular gap of tumors, accumulating inside the tumors (< 30 min) to deliver the cytotoxic payloads more efficiently.[70] In addition, DARPins have many advantages such as good solubility, thermal stability and protease resistance, high expression and yield, and thus are widely used in various tumor-targeted imaging and therapeutic studies.[71] Due to the high affinity with EpCAM (K_D = 68 pM)[72] and the fact that tumor uptake tends to be EpCAM-dependent after the two are combined and there is no non-specific accumulation in non-targeted tissues,[73] DARPins have been widely used in EpCAM-positive tumors for targeting studies in recent years. Specific DARPins, mainly screened from combinatorial libraries by phage display and ribosomal display,[74] are highly cytotoxic[75–77] to a wide range of EpCAM-positive tumor cell lines and produce a strong antitumor response in athymic mice.[75] The main mechanism may be efficient internalization by tumor cells via receptor-mediated endocytosis for delivery of antitumor drugs. Among the specific DARPins, the binding body named Ec1 has the highest affinity for EpCAM, and is therefore the most widely used in subsequent probe development.[32,78,79]

In 2020 Vorobyeva et al[80] labeled different variants of Ec1 with ^{125}I (Iodine-125, $T_{1/2}$ = 59.6 d) and ^{99m}Tc for use in the BxPC-3 pancreatic cancer model, which showed that homozygous mice showed a certain degree of tumor uptake of both nuclide probes, demonstrating high specificity and affinity. Among them, ^{125}I-PIB (Polyisobutylene, PIB, polyisobutylene)-H6-Ec1 showed significantly lower retention in normal tissues and higher target uptake by tumors, displaying high imaging contrast, higher than that of any other EpCAM imaging agent to date, suggesting that transnucleotide-labeled DARPin Ec1 is the best choice to visualize EpCAM-positive tumors It demonstrated that DARPin Ec1 is a feasible and explorable probe for the visualization of EpCAM-positive tumors. Since then, in May and October of the same year, and in November 2022, the team has used the DARPin Ec1 nuclide probe in EpCAM-positive SKOV-3 and OVCAR-3 ovarian cancers,[32] MB-468 triple-negative breast cancer (TNBC),[47] and SK-RC-52 renal carcinoma[81] in a murine model with loaded tumor, SPECT showed high tumor uptake of all these nuclide probes. In one of the ovarian cancer-related studies, the tumor/blood ratios of ^{125}I-PIB-Ec1 were 30 ± 11 and 48 ± 12, respectively, 6 h after injection, which formed a high contrast with other organs. In the triple-negative breast cancer-related study, the tumor uptake values of ^{99m}Tc(CO)$_3$-Ec1 and ^{125}I-PIB-Ec1 were 2.6%ID/g and 1.5% ID/g, respectively, 6h after injection, and 1.7%ID/g and 0.27%ID/g, respectively, 24 h. This is in agreement with

the results of the biodistribution in mice, which are in comparison to those of the ^{99m}Tc(CO)3-Ec1, ^{125}I-PIB-Ec1 uptake was significantly lower in normal organs with higher tumor/organ ratios. In the renal cancer-related study, the mean tumor uptake of ^{99m}Tc(CO)3-Ec1 and ^{125}I-PIB-Ec1 was 5.6 ± 1.4%ID/g, with ^{125}I-PIB-Ec1 showing more favorable abdominal metastases of renal cell carcinoma. The above findings demonstrate that DARPin Ec1 can be used as a potential non-residual marker in the visualization of a wide range of EpCAM-positive tumors in nuclide imaging.

After proving the imaging value of DARPin Ec1 in nuclear medicine, Vorobyeva's team[82] did further mapping to optimize the radiolabeling of the protein, mainly investigating the labeling position (DARPin Ec1 has a shielded hydrophobic protein core at both ends N- or C-terminal terminal cysteines) and its compositional on Ec1 targeting and imaging performance, as a good labeling method can increase the tumor-to-organ ratio by an order of magnitude and effectively improve the imaging success rate.[82] They used the nuclides ^{68}Ga, ^{111}In (Indium-111, $T_{1/2}$ = 2.8 d), and ^{57}Co (Cobalt-57, $T_{1/2}$ = 17.5 d) to label Ec1 tumors via the chelator DOTA to specifically concatenate two variants of Ec1, as well as localized radioiodination using (4-hydroxyphenyl)-ethyl) maleimide (HPEM) (which provides binding sites for proteins). Among them, ^{57}Co, ^{111}In, and ^{125}I-labeled Ec1 variants showed high stability in reaction with Ethylene diamine tetraacetic acid (EDTA), while ^{68}Ga-labeled Ec1 variants were slightly less stable in PBS, but acceptable These eight nuclide-labeled Ec1 probes were imaged by SPECT or PET in DU145 prostate cancer-loaded nude mice, and the results showed that after 3 h, ^{68}Ga-labeled DARPin Ec1 variants showed higher uptake in tumors, whereas ^{57}Co and ^{111}In-labeled uptake was lower, and after 24 h, ^{125}I-HPEM-labeled DARPin Ec1 variant showed higher uptake in tumors and lower uptake of ^{57}Co and ^{111}In labeling, but ^{57}Co could be used for delayed imaging. The ^{125}I-HPEM marker showed the highest tumor/muscle and tumor/bone ratios and was more suitable for EpCAM-positive early prostate cancer imaging. Of the radioactive metals, ^{111}In has the highest tumor/blood ratio, tumor/lung and tumor/liver ratio and can be used for advanced prostate cancer imaging. Biodistribution results showed that labeling the C-terminus resulted in the best tumor/organ ratio. In conclusion, this study not only optimized the radiolabeling of Ec1, but also broadened the spectrum of nuclide probes targeting EpCAM by using multiple nuclide markers, which is more conducive to translation to the clinic.

Therapeutic applications of DARPin focus on its role as a carrier for cytotoxic agents, notably Pseudomonas Exotoxin A (PE) derivatives, a potent immunotoxin mainly composed of antibody fragments that bind to tumor cells and bacterial toxin fragments that can kill cells, and its variants are widely used in tumor-targeted therapies, including EpCAM-targeted immunotherapies,[83–85] in which some of them are coupled with antibodies or antibody fragments to enter the clinical trial stage. Couplings with antibodies or antibody fragments are in clinical trials, such as MOC31 PE (Pseudomonas Exotoxin A)[86] coupled to mouse mAb-MOC31, and VB4-845 (otolizumab)[87] coupled to a single-chain antibody fragment (4D5MOCB). LoPE (Low-Immunogenicity PE Variant) is an A variant of PE, ie, de-immunized with the C-terminal catalytic subunit (25 kDa), which can be used to form a fusion protein with the DARPin Ec1 Ec1-LoPE (43 kDa), a conjugate with low immunogenicity and toxicity, which delivers cytotoxic drugs into EpCAM-expressing cells by triggering receptor-mediated endocytosis, and has been shown to inhibit a wide range of tumors, including breast[32,74] and ovarian cancers.[72] These approaches highlight DARPin's potential to enhance precision in toxin delivery for EpCAM-expressing tumors.

In 2022, Xu et al[88] demonstrated that the ^{99m}Tc-labeled Ec1-LoPE fusion protein exhibited effective internalization and high specificity in EpCAM-positive prostate cancer models (PC-3/DU-145), confirming its potential for both targeted imaging and cytotoxic therapy. Building on this, the team further explored its therapeutic synergy in 2023 by combining ^{99m}Tc(CO)3-Ec1-LoPE with MM-121, a HER3 (Human Epidermal Growth Factor Receptor 3)-targeting monoclonal antibody, in BxPC3 pancreatic cancer models.[89] Results revealed retained EpCAM-binding efficiency of the probe without interference from MM-121, while in vitro cytotoxicity assays demonstrated synergistic effects between the two agents. In vivo studies in dual EpCAM/HER3-expressing xenografts validated the feasibility of this dual-targeting strategy, validating that dual-targeting molecules targeting EpCAM are also promising for exploration in the nuclide field.

Probe with Aptamers and Other Molecules as Ligands

Nucleic acid aptamers are ssDNA or RNA structures that can specifically bind to target molecules, possessing a unique folded three-dimensional structure, and are generally obtained by screening using Systematic evolution of ligands by exponential enrichment (SELEX) technology.[90] The aptamers have high affinity and specificity, a wide range of target molecules, and can be structurally modified, and can be used for tumor-specific imaging when combined with nuclides to provide useful information for cancer staging and to detect therapeutic responses. Compared with antibodies and DARPins, aptamers have the advantages of small molecular weight (generally 25–80 bases), low immunogenicity,[91] and rapid tissue-targeting aggregation ability.[92] However, due to their small molecular weight and susceptibility to hydrolysis by nucleic acid endonucleases and exonucleases, aptamers have short plasma half-life and poor stability in vivo, although they can be chemically modified, combined with nanomaterials[93] to resist enzyme degradation, or optimized for pharmacological behaviors,[94] which leads to relatively limited application and clinical translation. Nuclide-labeled aptamers have been used in the diagnostic and therapeutic imaging of many diseases, including tumors,[51] but in the field of EpCAM target-related nuclide labeling, the relevant research is far less extensive than that of antibodies and DARPins, suggesting that there is a large gap in this area.

A representative study was conducted by Li et al[95] in 2021, who used ^{64}Cu to perform targeted PET imaging of EpCAM-positive breast cancer with DOTA-labeled aptamer-polyethylene glycol (PEG), demonstrating specific binding to target cells in vitro cellular uptake assays and a 3-fold higher tumor uptake (2.4%ID/g) compared to EpCAM-negative controls (0.75%ID/g) at 24 h post-injection, with rapid hepatic/renal clearance suggesting reduced off-target toxicity. In addition, the rapid clearance of the PEGylated nucleic acid aptamer tracer in the liver and kidney may reduce the risk of adverse side effects and provide useful information for identifying patients suitable for EpCAM-targeted therapy that confirms the feasibility of the use of nucleic acid-labeled aptamers in PET imaging of EpCam-positive tumors. Complementing these imaging advances, Marshall et al[96] reported in 2022 a therapeutic approach using EpCAM antibody-functionalized poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with ^{131}I as the core, achieving targeted cytotoxicity against MCF-7 breast cancer cells with efficacy rates of 69.11% (24 h), 77.84% (48 h), and 74.6% (72 h). This nanoplatform, leveraging erythrocyte membrane encapsulation for biocompatibility, validated the dual utility of EpCAM-targeted strategies in both diagnostic imaging and radiotherapy while addressing safety concerns in heterogeneous tumor models.

Comparative Analysis of the Three Ligands

The principal attributes of the EpCAM-targeted radionuclide probes associated with the three ligands previously mentioned are delineated in Figure 4. In terms of molecular weight, conventional antibodies and their fragment counterparts are relatively large, ranging from 30 to 150 kDa,[97,98] which constrains their ability to penetrate tumors effectively.[99] Conversely, DARPins, with a molecular weight of 14 to 18 kDa, and aptamers, ranging from 8 to 25 kDa, are smaller in size, thereby enhancing their capacity to infiltrate solid tumor tissues. In relation to binding affinity, DARPins exhibit superior binding strength, exceeding that of certain monoclonal antibodies (0.1 nM to 10 nM), with affinities ranging from 0.1 to 1 nM.[68,100] Aptamers, on the other hand, demonstrate a greater variability in affinity ranging from 1 nM to 1 μM,[101] due to their reliance on specific tertiary structural conformations. Significant pharmacokinetic differences are also observed. Antibodies possess extended half-lives (spanning days to weeks), making them suitable for delayed imaging applications, such as those utilizing ^{89}Zr labeling, however, they may result in elevated background signals. In contrast, DARPins (with half-lives of hours to 1 day)[102] and aptamers (with half-lives of less than 1 hour)[103] necessitate the use of short-lived radionuclides, such as ^{68}Ga and ^{18}F, for rapid imaging purposes. Regarding clinical applicability, antibody probes have progressed into various clinical trials, whereas DARPins and aptamers remain in the preclinical validation phase, necessitating further resolution of stability challenges (such as protease resistance in DARPins) and enhancements in in vivo delivery efficiency (including chemical modifications of aptamers).

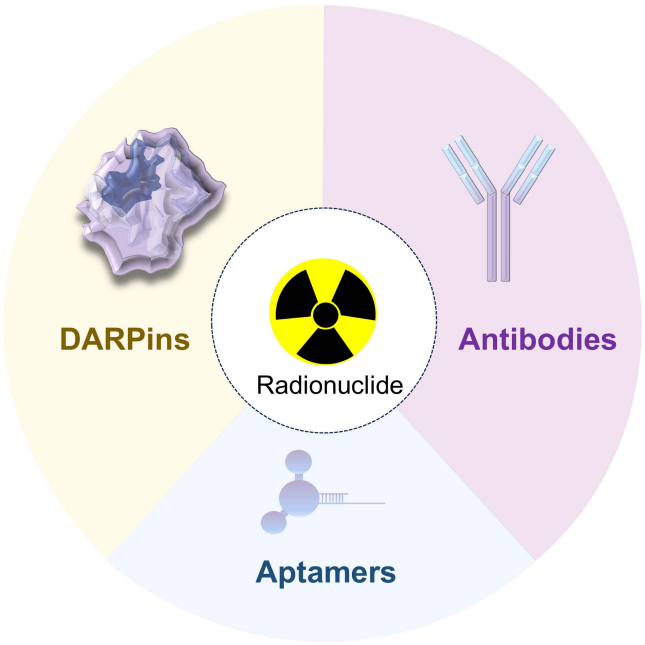

Figure 4 Comparative analysis of EpCAM-targeted radioligands in nuclear medicine. Schematic evaluation of three principal probe classes, namely antibodies, designed ankyrin repeat proteins (DARPins), and aptamers. Key distinguishing features include their molecular properties: antibodies possess a high molecular weight (30–150 kDa), demonstrate a strong binding affinity (0.1–10 nM), and exhibit a prolonged plasma half-life (ranging from days to weeks). DARPins, in contrast, are characterized by a smaller molecular size (14–18 kDa), exhibit an even higher binding affinity (0.1–1 nM), and have a moderate plasma half-life (hours to days). Aptamers present an intermediate molecular size (8–25 kDa), a broader binding affinity range (1 nM–10 μM), and a notably shorter half-life (minutes to hours). The efficiency of tumor penetration differs among these classes: antibodies show low penetration, DARPins achieve high penetration, and aptamers demonstrate moderate penetration. Clinically, antibodies are undergoing multiple trials owing to their high target specificity, though their utility is constrained by slow blood clearance. DARPins are primarily in preclinical phases, offering advantages such as rapid tissue penetration and metabolism, though further optimization of protease stability is needed. Aptamers are in the early stages of investigation, notable for their low immunogenicity; however, their application is currently limited by susceptibility to nuclease degradation.

Comparison of EpCAM with Other Targets

EpCAM distinguishes itself from other tumor-associated antigens, such as Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor Receptor 2 (HER2), Prostate-Specific Membrane Antigen (PSMA), due to its widespread expression across epithelial tissues and its dual functionality as both a diagnostic biomarker and a therapeutic target. In contrast to PSMA's limited specificity for prostate cancer and HER2's variable expression in breast cancer, EpCAM is consistently overexpressed in approximately 80% of epithelial tumors, exhibiting lower levels of intratumoral heterogeneity. Its extracellular localization facilitates probe binding more effectively than intracellular targets like KRAS. While fluorodeoxyglucose positron emission tomography (FDG-PET) remains the standard imaging modality in oncology, EpCAM-targeted probes offer distinct advantages in the detection of CTCs and micrometastases that are not identifiable through metabolic imaging. Ongoing clinical trials are assessing EpCAM's efficacy in stratifying gastrointestinal cancers, demonstrating its superiority over Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5/6 (CEACAM5/6). However, the development of therapeutic radionuclide applications, such as ^{177}Lu/^{225}Ac conjugates, has not progressed as rapidly as PSMA-targeted alpha therapies, indicating a significant potential that necessitates further optimization of probe pharmacokinetics. Despite the promising prospects of EpCAM as a target, its clinical application, particularly in therapeutic radionuclide contexts, remains underdeveloped compared to other tumor targets, underscoring the need for additional preclinical investigations to substantiate its utility.

Challenges of Clinical Translation About EpCAM Targets

The clinical translation pathway pertinent to the target is illustrated in Figure 5. Despite the encouraging preclinical findings, the clinical advancement of EpCAM-targeted radiopharmaceuticals is predominantly confined to the initial three stages and encounters a variety of challenges in the translation process. These challenges encompass model discrepancies, limitations associated with ligands, and target heterogeneity.[104,105] Specifically, xenograft models fail to accurately

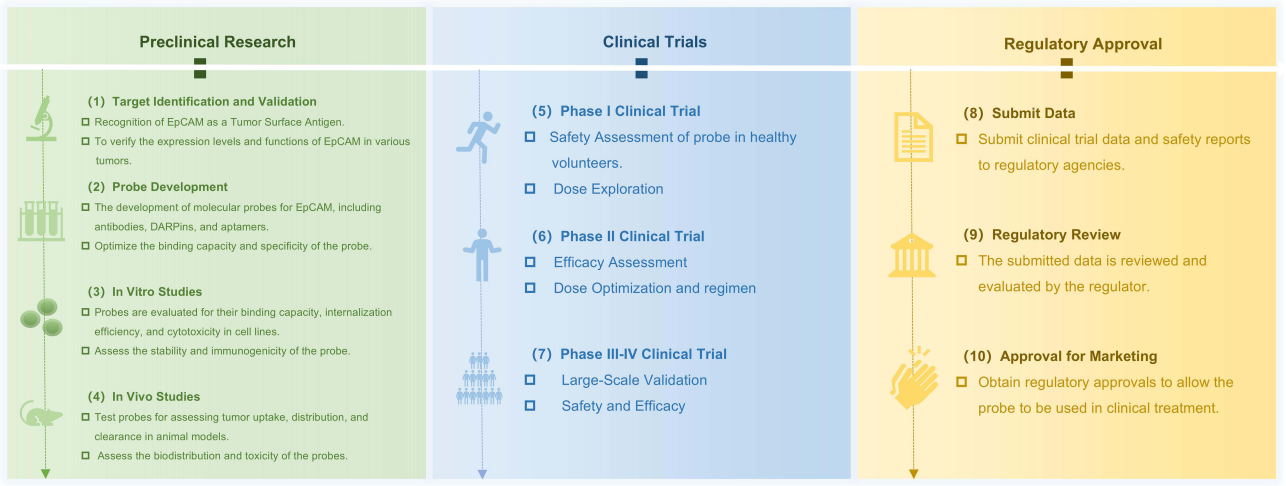

Figure 5 Clinical Translational Pathway of EpCAM-targeted probe. A flowchart showing the EPCAM-targeted nuclear medicine clinical translational pathway, including preclinical studies, clinical trials, and regulatory approvals.

replicate the interactions between human tumor stroma and the dynamics of EpCAM shedding[106,107] associated with its biological intricacy. The EpEX undergoes cleavage by metalloproteinases, resulting in soluble fragments that enter the circulatory system and affect the uptake of tumor-targeting agents.[40] In certain metastatic lesions, hypermethylation of the EpCAM promoter leads to a loss of expression, which can result in false-negative diagnostic outcomes.[108,109] Moreover, while EpCAM is known to facilitate epithelial-mesenchymal transition through the activation of the Wnt/β-catenin signaling pathway, this process concurrently downregulates its expression on the cell membrane[110,111] Additionally, conventional xenograft models do not accurately mimic the interactions between human tumors and stroma involving EpCAM and cadherins,[112] and certain immunodeficient models may further expedite the clearance of probes. Additionally, the prolonged circulation time of antibodies results in elevated background levels. Certain imaging modalities may necessitate execution 4–7 days post-injection to attain clinically acceptable contrast and sensitivity, further complicating the research trial timeline. The sensitivity to tumors is suboptimal, and some tumors that do not express the target may not exhibit specific uptake, thereby heightening the risk of false-positive diagnoses. The undefined internalization pathways of DARPins pose challenges for dosimetry, while rapid renal clearance and nuclease degradation of aptamers restrict tumor uptake, even with PEGylation. Furthermore, the target heterogeneity observed in metastatic lesions demonstrates resistance to monospecific probes.

Potential solutions that are emerging include site-specific modifications in ligand engineering, such as 2′-fluoro/2′-O-methyl modifications,[113] and nanocarrier modification, such as DNA tetrahedron[114] and poly lactic acid-co-glycolic acid nanoparticle[115] assembly, to enhance the serum stability of aptamers, as well as the exploration of multimodal probes.[116] The optimization of antibody probes primarily aims to reduce their half-life. For example, the generation of antibody fragments and the attainment of targeted fusions can significantly diminish the half-life of these probes while simultaneously lowering background signals[117] Additionally, the advancement of bispecific antibody models contributes to an increased penetration depth of the probes.[118,119] Current research is quantitatively assessing the endocytosis rate of Ec1-LoPE fusion proteins to refine appropriate dosage levels.[88] Future clinical trials should emphasize combinatorial targeting strategies, such as targeting both EpCAM and CD133, alongside the implementation of AI-driven dosimetry models to effectively address spatial heterogeneity.

Future Development

The radionuclides used in the relevant effective studies in this review are mainly ^{99m}Tc, while the future advancement of EpCAM-targeted imaging agents will focus on the development of multimodal theranostic probes that incorporate various radionuclides with optimized pharmacokinetic profiles, such as positron-emitting isotopes (eg, ^{68}Ga), other therapeutic radionuclides, such as ^{177}Lu/^{225}Ac, are also gaining attention, but related research may be limited by cost, since the conditions for labeling are stringent, and there exists a significant demand for chelating agents.[120,121] In the next

place, innovations in scaffold engineering, including the conjugation of nanobodies, the creation of bispecific DARPin constructs, and the optimization of glycosylated aptamers, will aim to overcome existing challenges related to tumor penetration and target heterogeneity. This review highlights that pertinent research has demonstrated the efficacy of optimized nanostrips, including nanoantibodies, bispecific antibodies, and Ec1-related protein probes. However, it also indicates that there remains significant potential for further optimization in this area. Next, the integration of artificial intelligence (AI) with quantitative SPECT/PET imaging facilitates predictive modeling of EpCAM expression patterns through the extraction of radiomic features, which may assist in the identification of occult metastases that are undetectable by conventional imaging methods. Furthermore, novel platforms that combine EpCAM-targeted probes with immune checkpoint inhibitors, such as anti-PD-1 (Programmed Death-1), may allow for real-time visualization of the dynamics within the tumor-immune microenvironment during combination therapies. The clinical translation of these advancements will be enhanced by microfluidic systems designed for the capture of CTCs, which will validate the specificity of probes against various subpopulations of EpCAM expressing cells. Additionally, further research is required to establish EpCAM as a reliable detection antigen for epithelial tumors and as a biomarker for evaluating responses to radioimmunotherapy.

Conclusion

EpCAM, recognized as one of the most prominent and extensively expressed tumor surface antigens, has been regarded as a reliable prognostic marker for cancer and a potential therapeutic target in the realm of molecular imaging. Although there are certain challenges associated with the development and transformation of clinical probes targeting EpCAM, its effectiveness as a superior imaging target for tumor detection remains indisputable Recent progress in nuclear instrumentation and computational algorithms is driving substantial advancements in molecular imaging, thereby positioning EpCAM to assume an increasingly vital role in precision theranostics, non-invasive tumor detection, real-time treatment monitoring, and personalized patient stratification through the optimization of probe designs and the integration of multimodal imaging techniques. By systematically comparing ligand-specific probe designs, analyzing clinical translation barriers, and highlighting emerging strategies (eg, dual-targeting DARPins), this review provides an integrated framework to accelerate EpCAM-based precision nuclear medicine.

Abbreviations

SPECT, Single-Photon Emission Computed Tomography, PET, Positron Emission Tomography, EpCAM,Epithelial Cell Adhesion Molecule, CRC, Colorectal Cancer, CD326, Leukocyte Differentiation Antigen 326, TACSTD-1, Tumor-Associated Calcium Signal Transducer 1, TROP-1, Trophoblast Cell Surface Antigen 1, EpEX, Extracellular Domain of EpCAM, EpICD, Intracellular Domain of EpCAM, EGF, Epidermal Growth Factor, HVR, Hypervariable Region, mAbs, Monoclonal Antibodies, CSCs, Cancer Stem Cells, CTCs, Circulating Tumor Cells, %ID/g, Percentage Injected Dose per Gram, DOTA, 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic Acid, NODAGA, 1,4,7-Triazacyclononane,1-glutaric Acid-4,7-acetic Acid, BiTE, Bispecific T-cell Engager, DARPins, Designed Ankyrin Repeat Proteins, PE, Pseudomonas Exotoxin A, LoPE, Low-Immunogenicity PE Variant, HER3, Human Epidermal Growth Factor Receptor 3, PLGA, Poly Lactic-co-Glycolic Acid, SELEX, Systematic Evolution of Ligands by Exponential Enrichment, PEG, Polyethylene Glycol, EDTA, Ethylene Diamine Tetraacetic Acid, PD-1, Programmed Death-1, AI, Artificial Intelligence, FDG-PET, Fluorodeoxyglucose Positron Emission Tomography, EGFR, Epidermal Growth Factor Receptor, HER2, Human Epidermal Growth Factor Receptor 2, PSMA, Prostate-Specific Membrane Antigen, CEACAM5/6, Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5/6.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Disclosure

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