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Brief Communication

Trop2-targeted immunoPET ligands

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Abstract: Trop2 is overexpressed in various tumors and serves as a key biomarker. Targeted immunoPET ligands, mainly developed from Trop2 monoclonal antibodies and nanobodies, provide the landscape of heterogeneous expression of Trop2 in tumors, which has great potential in improving accuracy of cancer diagnosis and staging, as well as decision-making in therapy.

Keywords: Trophoblast cell-surface antigen 2 (Trop2), immunoPET ligand, nanobody, tumor, in vivo imaging

Introduction

Trophoblast cell-surface antigen 2 (Trop2) is a transmembrane glycoprotein and a member of tumor-associated calcium signal transducer (TACSTD) family. It has been established as a pan-cancer biomarker, widely expressed in various tumors, and implicated in tumorigenesis through diverse signaling pathways [1]. As illustrated in **Figure 1**, the phosphorylation of Trop2 leads to the hydrolysis of 4,5-diphosphate phosphatidylinositol (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG), and IP3 stimulates the release of calcium ions that are stored in the endoplasmic reticulum (ER), which regulates the cell proliferation, division, and cycle progression. Additionally, Trop2 is hydrolyzed into intracellular domain (ICD), which co-localizes with β-catenin and controls cell self-renewal, proliferation, and tissue hyperplasia. Moreover, Trop2 induces the accumulation of cytoplasmic receptor for activated c kinase 1 (RACK1) at the cell membrane, reducing fibronectin and integrin β binding. This decrease in the adhesion of tumor cells promotes tumor metastasis. Trop2 also up-regulates the expression of the proliferative marker Ki-67, thereby promoting tumor cell proliferation. In summary, Trop2 promotes tumor cell proliferation, growth, and metastasis by altering calcium signaling pathway, β-catenin-related cyclin expression, and reducing fibronectin adhesion through RACK1 signaling. Furthermore, Trop2 is overexpressed in many human cancers but exhibits much lower expression in normal tissues, which empowers its tumor prognostic and therapeutic significance [2].

Trop2 immunoPET ligand development

Positron emission tomography (PET) imaging is gaining increasing attention in tumor diagnosis and treatment efficacy assessment [3-5]. Given the importance of Trop2 in tumor biology, various forms of therapeutics, including antibodies and antibody-drug conjugates (ADCs), are under development, which highlights the need for optimized PET ligands to characterize the heterogeneous and dynamic expression of Trop2. In this context, three monoclonal antibody ligands were initially developed for Trop2-targeted immunoPET imaging, including [^{89}Zr]hRS7 [6], [^{89}Zr]DFO-AF650 [7], and [^{64}Cu]hIMB1636 [8]. Compared with the antibodies, nanobody ligands offer advantages such as rapid tumor accumulation, fast blood clearance, and reduced radiation exposure, making them suitable for same-day immunoPET imaging [9]. Several nanobody-based radioligands have been evaluated for Trop2 PET imaging. Among them, [^{68}Ga]Ga-NOTA-RTD98 and [^{68}Ga]Ga-NOTA-RTD01 effectively identified differential Trop2 expression in human-derived pancreatic cancer models but exhibited high liver accumulation [10]. Subsequently, [^{68}Ga]Ga-NOTA-T4 and [^{68}Ga]Ga-NOTA-T5 were developed with improved association and dissociation kinetics, and [^{68}Ga]Ga-NOTA-T4 advanced to a pilot clinical trial, demonstrating its potential in visualizing Trop2 expression in patients with solid tumors [11]. Furthermore, a translational study with the novel nanobody PET ligand [^{68}Ga]MY6349 highlighted its precision in quantifying Trop2 expression across multiple cancer types, underscoring its promise for guiding Trop2-targeted therapies [12].

Literature highlight: [^{18}F]AlF-RESCA-T4 and [^{18}F]AlF-RESCA-RT4

Recently, building on the nanobody [^{68}Ga]Ga-NOTA-T4, Huang et al. developed the first series of ^{18}F-labeled Trop2 PET ligands: [^{18}F]AlF-RESCA-T4 (His-tagged) and [^{18}F]AlF-RESCA-RT4 (His-tag-free) [13]. These nanobodies showed high binding affinities to immobilized human Trop2 antigen, with K_D values of 965.9 for T4 and 915.7 pM for RT4. The radioligands [^{18}F]AlF-RESCA-T4 and [^{18}F]AlF-RESCA-RT4 were prepared by an established Al[^{18}F] restrained complexing agent (RESCA) method with high radiochemistry yields of 22.4 and 61.9%, respectively. In Trop2-positive T3M-4 models, [^{18}F]AlF-RESCA-T4 exhibit-

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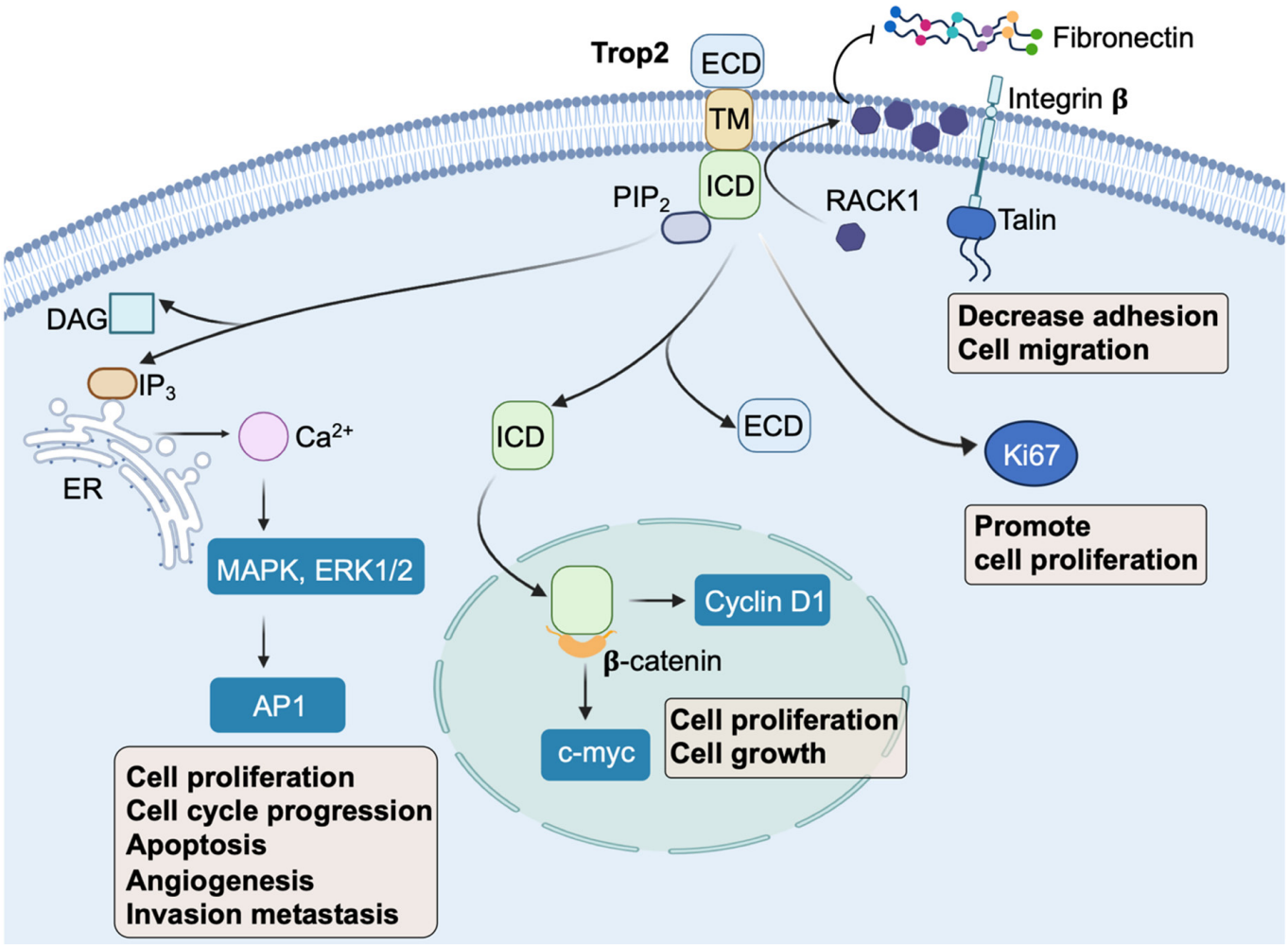

Figure 1. Trop2-mediated intracellular and extracellular signaling pathways. AP-1, activator protein 1; DAG, diacylglycerol; ECD, extracellular domain of Trop2; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; ICD, intracellular domain of Trop2; IP3, inositol triphosphate; MAPK, mitogen-activated protein kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; RACK1, receptor for activated c kinase 1; TM, transmembrane helix of Trop2.

ed higher tumor uptake than [^{18}F]AlF-RESCA-RT4 (11.13 *vs.* 8.83% ID/g) during PET region-of-interest (ROI) analysis within the first hour. Self-blocking experiments using the corresponding cold nanobodies reduced the PET signal by 79% and 76% for T4 and RT4, respectively, indicating their high in vivo specific binding to Trop2 (**Figure 2A**). Both radioligands showed favorable pharmacokinetics, characterized by fast clearance from non-target tissues/organs such as blood, liver, and bone, resulting in a low-background signal during PET imaging. However, high kidney accumulation was observed, with values of 125.60 and 48.88 %ID/g for [^{18}F]AlF-RESCA-T4 and [^{18}F]AlF-RESCA-RT4, respectively. This suggests that the removal of His-tag may be a simple but effective strategy to mitigate renal accumulation. [^{18}F]AlF-RESCA-T4 was utilized in a pilot clinical trial for immunoPET imaging. The results revealed that the uptake of [^{18}F]AlF-RESCA-T4 correlated well with positive Trop2 expression in patients of primary lung cancer (**Figure 2B**) and lung adenocarcinoma (**Figure 2C**). Notably, this ligand also showed potential in differentiating tumors from inflammatory diseases such as tuberculosis. Further clinical trials with larger patient cohorts are necessary to validate these promising findings and establish the clinical utility of [^{18}F]AlF-RESCA-T4 in Trop2-targeted imaging.

Conclusion

Trop2 is overexpressed in various malignancies compared to normal tissues, rendering its noninvasive imaging crucial for tumor detection and treatment monitoring. ImmunoPET imaging using Trop2 radioligands derived from low-molecular-weight vectors - characterized by high affinity, exceptional stability, and versatile engineering potential - offers significant advantages. Noninvasive quantification of Trop2 expression levels and distribution has revolutionized Trop2-targeted cancer treatment, yielding remarkable advancements in cancer management, including identifying patients most likely to benefit from Trop2-targeted drugs, monitoring therapeutic efficacy, and detecting early resistance. This personalized approach can optimize therapeutic outcomes while minimiz-

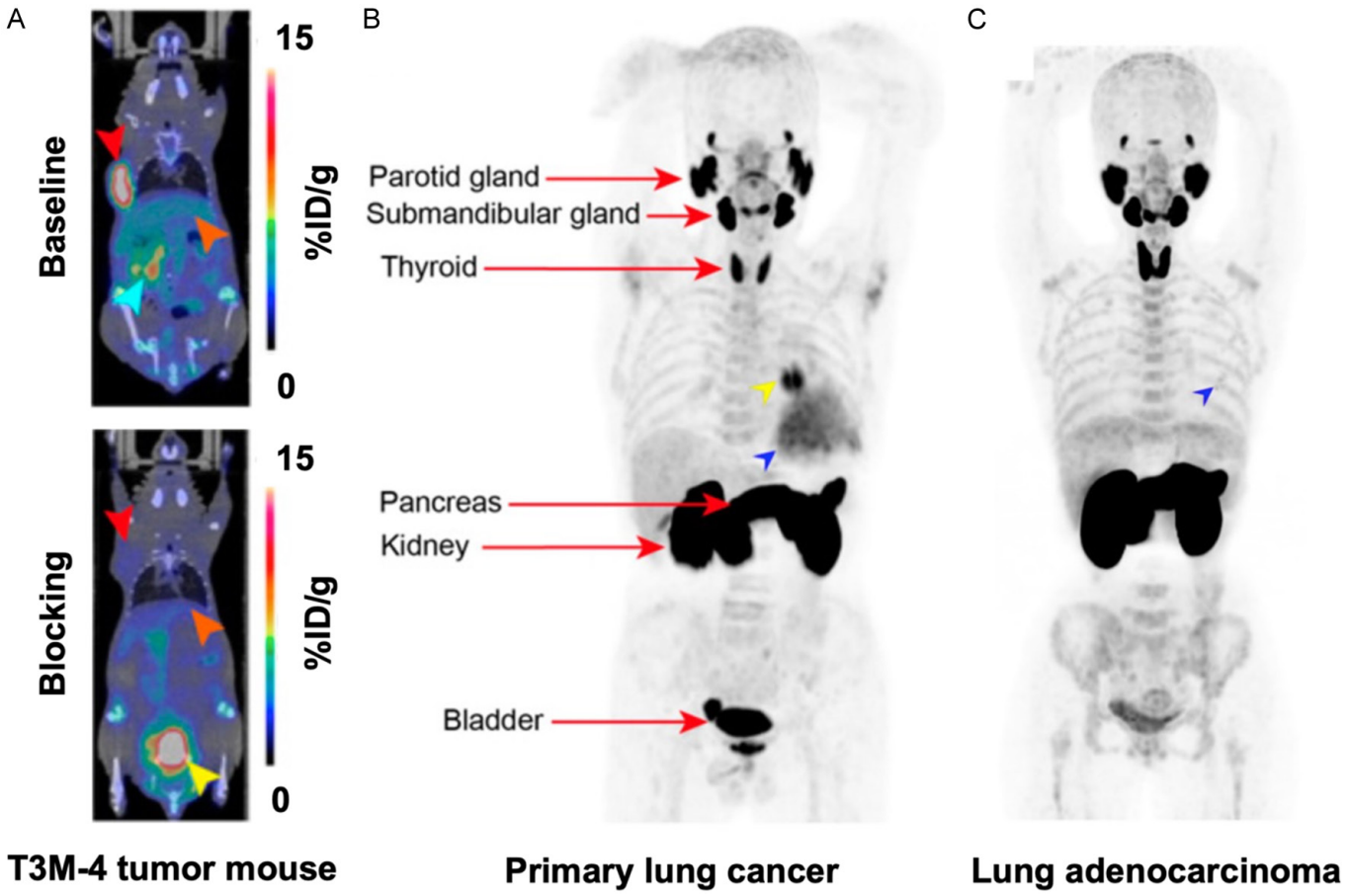

Figure 2. [^{18}F]AlF-RESCA-T4 immuno-PET/CT imaging in Trop2-positive T3M-4 models (A) and patients of primary lung cancer (B) and lung adenocarcinoma (C). Red arrowhead in (A) indicates the tumor of T3M-4 model; yellow arrowhead in (B) indicates left hilar occupancy and lymph node; blue arrowheads in (B and C) indicate distal lung atelectasis and left lung nodule, respectively; Reprinted with permission from [13]. © SNMMI.

ing unnecessary adverse effects in non-responders. However, these radioligands still exhibit high renal uptake, which hinders the detection of tumors in or near the kidneys, limits the administrable "safe dose" in radioligand therapy, and potentially induces nephrotoxicity. Thus, further structural modifications in developing more valid Trop2 radioligands should focus on minimizing the kidney retention. Key strategies include reducing tracer size (e.g. utilizing peptide- or small molecule-based scaffolds), enhancing hydrophilicity, incorporating renal-cleavable linkers (e.g. Met-Val-Lys), and modifying molecular charge. Such optimized radioligands would enhance the accuracy of cancer diagnosis and staging, thereby supporting decision-making for Trop2-targeted therapies.

Disclosure of conflict of interest

None.

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