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EDITORIAL

Albumin binding improves nanobody pharmacokinetics for dual-modality PET/NIRF imaging of CEACAM5 in colorectal cancer models

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Monoclonal antibodies (mAbs) are usually highly specific to a particular antigen or epitope. Consequently, mAbs have become valuable tools in biomedical research, diagnostics, and therapeutics [1, 2]. However, their larger size limits their ability to penetrate solid tumors, resulting in poor tissue distribution and slow uptake [3]. It typically takes several days for full antibodies to reach peak enrichment in tumors, making them unsuitable for use as same-day imaging agents [4, 5]. As a result, smaller antibody fragments, such as nanobodies, have emerged as promising alternatives [6]. Nanobodies, also known as single-domain antibodies, have a molecular weight of around 15 kDa and a diameter of less than 4 nm, making them capable of penetrating deeply into tumor tissues while maintaining their antigen recognition ability [7, 8]. In contrast to other antibody-like scaffold proteins, nanobodies have several advantages, including low immunogenicity and ease of screening and humanization for theranostic purposes [9]. These properties have drawn attention to nanobodies, especially after the FDA approval of caplacizumab, a humanized single-variable-domain immunoglobulin developed for the treatment of adults experiencing thrombotic thrombocytopenic purpura [10, 11].

A significant disadvantage of nanobodies is their low tumor accumulation and quick blood clearance [12]. Researchers have been exploring various strategies to overcome these limitations, such as PEGylation, fusion with other functional domains, and modifying the tumor microenvironment to enhance the penetration and retention of nanobodies [13–15]. For instance, PEGylation can slow circulation clearance from the bloodstream, leading to increased bioavailability and improved pharmacokinetics. Another promising strategy involves fusing nanobodies with an albumin-binding domain (ABD) to extend their cycle time and increase tumor uptake, leading to improved imaging contrast [16]. Albumin and its interacting moieties have been widely used to increase the in vivo half-life of small biotherapeutics by increasing their size beyond the renal filtration threshold and enabling neonatal Fc receptor (FcRn)-mediated recycling [17, 18]. However, this approach may negatively impact the exceptional tissue distribution of nanobodies. To address this issue, non-covalent interactions with albumin can be achieved by fusing nanobodies with ABD [19]. ABD is a small protein domain that binds with high affinity to albumin and has been shown to be successful in improving the pharmacokinetics of therapeutic molecules [20]. Incorporation of ABDs into nanobodies will enable the fusion proteins simultaneously binding to endogenous albumin, thereby extending their cycle time and increasing tumor uptake, leading to improved imaging contrast [21]. Moreover, studies have shown that ABD does not interfere with the penetration of nanobodies into tumor tissues, further supporting their potential for clinical use [22, 23].

In one study, Xenaki et al. evaluated the efficacy of HER2-targeted nanobody (11A4) fused with ABD in solid tumors [24]. After fusion with ABD, the serum half-life of the molecule increased by a factor of 14.8. Additionally, fusion with ABD resulted in prolonged accumulation of 11A4-ABD in HER2-expressing xenografts while maintaining uniform distribution within the tumors. These findings suggest that monovalent internalizing ABD-fused nanobodies have the potential to be highly effective nanobody-drug conjugates. Meanwhile, Fleming et al. constructed a panel of

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immunotoxins by combining HN3 (human nanobody targeting GPC3) with deimmunized toxin fragments for the treatment of hepatocellular carcinomas [25]. The immunotoxins were modified to improve serum half-life and reduce immunogenicity, resulting in high-affinity and potent therapeutics. Notably, the addition of a streptococcal albumin-binding domain (ABD) to HN3-T20 resulted in a 45-fold increase in serum half-life in mice and was associated with a tenfold decrease in therapeutic dose in treating Hep3B xenografts. These findings suggest that ABD-containing deimmunized HN3-T20 immunotoxins have great potential as high-potency therapeutics for liver cancer treatment. Taken together, these studies provide valuable insights into strategies for improving the distribution and efficacy of antibody–drug conjugates in solid tumors using ABD nanobody fusion proteins.

In this issue of *European Journal of Nuclear Medicine and Molecular Imaging*, Xiao et al. demonstrated the use of carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)-targeted nanobodies for non-invasive imaging of CRC using positron emission tomography (PET) and near-infrared fluorescence (NIRF) imaging [26]. CEACAM5 is a member of the carcinoembryonic antigen (CEA) family, which is an interesting colorectal cancer (CRC) target due to its high expression levels in CRC and limited expression in normal tissues. The CEACAM5-targeted nanobody (Nb41) fused with ABD exhibited excellent in vitro binding properties, with a dissociation constant (Kd) of 0.21 nM, and significant accumulation in CRC subcutaneous xenografts and lymph node metastases in vivo. Fluoride-18 labeled Nb41-ABD gradually accumulated in the tumor, with relatively low uptake in peripheral organs and tissues. At 4 h post-injection, tumor uptake was quantified as 6.53 ± 0.99 %ID/g, which was 1.64 times higher than that of the Nb41 without ABD fusion. Similarly, in the lymph node metastasis model, compared with ^{18}F-FB-Nb41, the uptake of ^{18}F-FB-Nb41-ABD was higher. To investigate the prolonged tumor retention of Nb41-ABD, the researchers conjugated it with IRDye800CW to form Nb41-ABD-IR800. As it exhibited slow blood clearance, it produced excellent tumor-to-background ratios compared to Nb41-IR800. These findings suggest that CEACAM5-targeted ABD fusion nanobodies are potential tools for the early detection and diagnosis of CRC.

The short circulation time of nanobodies in the blood and their high renal reabsorption after glomerular filtration have also limited their use in targeted radionuclide therapy (TRT). To address these challenges, Hu et al. have fused a targeted nanobody with ABD. The resulting dual-targeting nanobody, MIRC213-709, is capable of binding to both HER2 receptors and endogenous IgG in plasma, resulting in an extended half-life [27]. ^{177}Lu-MIRC213-709 has demonstrated excellent tumor uptake and prolonged blood circulation time, with a tumor uptake of 6.59 ± 1.67, 27.65 ± 7.66, 30.82 ± 7.29, 19.95 ± 1.76, and 17.61 ± 2.63 %ID/g at 1, 24, 48, 72, and 120 h post-injection, respectively ($n = 4$). TRT with ^{177}Lu-MIRC213-709 demonstrated significant tumor growth inhibition in the NCI-N87 tumor model. The tumor size of the control group was 3.6 times that of the 9 MBq single injection of ^{177}Lu-MIRC213–709 group. These findings suggest that the use of nanobodies fused with ABD represents a promising approach to improving the metabolic properties of nanobodies for TRT, potentially leading to more effective cancer treatment options. Another study from our group demonstrated that fusing the CD47-targeting nanobody C2 with ABD035 significantly prolonged the circulation time and increased the tumor uptake, which further facilitated TRT with ^{177}Lu-DOTA-ABDC2 [28]. However, the treatment protocols need to be streamlined to balance the therapeutic efficacy and treatment-related toxicity.

In conclusion, nanobodies connected to ABDs offer an exciting new approach in molecular imaging, addressing some of the limitations of traditional imaging agents [29]. The use of ABDs may overcome these limitations by sustaining the binding of nanobodies to endogenous albumin, thus adjusting their serum clearance rates. This strategy retains the antigen-binding capabilities of nanobodies while overcoming their limitations, such as limited tumor deposition. Apart from circulation and the associated tumor uptake, high kidney accumulation is another bottleneck to be addressed when developing therapeutic radiopharmaceuticals. As such, researchers are exploring new approaches to improve this. One promising direction is modifying the sequence at the C or N-terminus to make it more resistant to renal reabsorption, thereby reducing kidney uptake [30–32]. This modification could make it possible to increase the therapeutic dose and potentially improve treatment efficacy, while minimizing the toxicity risks. While there are still challenges to overcome, engineering nanobodies for cancer theranostics is among the most promising strategies in the era of precision medicine [33]. Accumulating evidence has supported the ABD incorporation strategy in improving both the diagnostic and therapeutic efficacies of radiolabeled nanobodies, but clinical data are needed to confirm the benefit of the strategy. While radiolabeled singly valent nanobodies are favored agents for same-day immunoPET imaging [34], nanobody derivatives are among the powerful vectors for developing next-generation therapeutic agents. Continued research and development in this regard will pave the path for inventing theranostic pairs for a wide range of tumors.

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Declarations

Studies with human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest Weibo Cai declares conflict of interest with the following corporations: Actithera, Inc., Rad Source Technologies, Inc., Portrai, Inc., rTR Technovation Corporation, and Four Health Global Pharmaceuticals Inc. All other authors declare that they have no conflict of interest.

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