कैंसर का चयनात्मक लक्ष्यीकरण

3

कैंसर प्रभावी अंगों (स्थानों) की बेहतर स्थिरता और प्रभावी लक्ष्यीकरण के लिए पेप्टाइड रूपांतरण कार्यनीतियाँ

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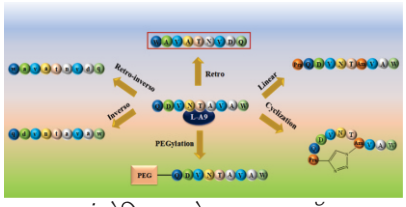

संशोधित A9 पेप्टाइड एनालॉग
(सबसे अच्छा एनालॉग, रेट्रो A9 बॉक्स में इंगित)

सारांश

पेप्टाइड्स, ट्यूमर कोशिकाओं पर उच्च घनत्व में निष्पीडित रिसेप्टर्स (अभिग्राही) के प्रति उत्कृष्ट रूप से उच्च चयनात्मकता वाले आकर्षक अणु हैं। हालांकि, पेप्टाइड्स की एंजाइमेटिक संवेदनशीलता चरणबद्ध निम्नीकरण की ओर ले जाती है जिसके परिणामस्वरूप कम लक्ष्य संचय का तीव्र निष्कासन होता है। यहाँ स्तन कैंसर में बढ़े हुए HER 2-रिसेप्टर स्तरों को लक्षित करने वाले मूल A9 पेप्टाइड को बेहतर चयापचय स्थिरता, कुशल लक्ष्यीकरण और अंततः कैंसर प्रभावित अंगों के आण्विक प्रतिबिंबन और चिकित्सा के लिए नैदानिक उपयोगिता हेतु विभिन्न कार्यनीतियों को अपनाकर रूपांतरित किया गया।

Selective Targeting of Cancers

3

Peptide Modification Strategies for Enhanced Stability and Effective Targeting of Cancer Sites

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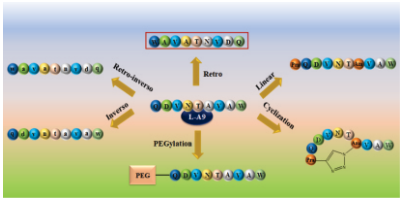

Modified A9 peptide analogs
(best analog, Retro A9 indicated in the box)

ABSTRACT

Peptides are excellently attractive molecules with high selectivity towards receptors expressed in high density on tumor cells. However enzymatic sensitivity of peptides leads to systemic degradation resulting in rapid clearance with low target accumulation. Here the original A9 peptide targeting elevated HER2-receptor levels in breast cancer was modified by adopting different strategies for improved metabolic stability, efficient targeting and ultimately clinical utility for molecular imaging and therapy of cancer sites.

KEYWORDS: *HER2, Lu-177, D-peptide, Retro peptide, Click chemistry*

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Introduction

Rapid rise in cancer cases worldwide is an alarming situation demanding essential development of cancer specific molecules for precise molecular imaging and treatment of the disease. Breast cancer is the most prevalent cancer in human population accounting for highest mortality (15.5%) amongst women [1]. However, detection at an early stage through radiolabeled peptides targeting elevated receptor (HER2) levels shall expedite treatment thereby improving the chances of survival [2].

Peptides serve as wonder probes by virtue of their high selectivity towards the target (receptors) and favorable biological profile (high tumor penetration, quick blood clearance, low toxicity) [3]. Besides, simple synthetic methodologies with options allowing suitable modification, is an added advantage of peptides. However, peptides are highly sensitive towards enzymatic degradation leading to rapid kidney clearance thereby reducing bioavailability and target accumulation limiting their clinical applicability. To overcome these limitations several modification strategies can be adopted to confer enzymatic resistance to peptides: (i) introduction of polyethylene glycol (PEG); (ii) cyclization; (iii) incorporation of D-amino acids (inverso peptide); (iv) arrangement of D-amino acids in reverse order (retro-inverso peptide); (v) arrangement of L-amino acids in reverse order (retro peptide). In this regard, A9 peptide (QDVNTAVAW) targeting HER2-receptors overexpressed in breast cancer was synthesized and above-mentioned modifications were conferred to the original peptide.

Materials and Methods

Peptides, DOTA-A9, DOTA-PEG_4-A9 (pegylated), DOTA-c[Tz]A9 (cyclic), DOTA-D-A9 (inverso), DOTA-rD-A9 (retro-inverso) and DOTA-rL-A9 (retro) were synthesized manually by Fmoc solid-phase peptide synthesis (Fig. 1).

Cyclic peptide was prepared by introduction of azide functional group bearing azidoalanine (Aza) and alkyne bearing propargyl glycine (Pra): *Pra*-Gln-Asp-Val-Asn-Thr-*Aza*-Val-Ala-Trp-NH_2. Cyclization of the linear peptide [Pra, Aza]A9 was performed on solid phase by Cu(I)-catalyzed 'click chemistry' reaction. The inverso peptide was synthesized by substituting L-amino acids with D-amino acids. The retro- and retro-inverso A9 peptide variants were prepared by coupling of L-amino acids and D-amino acids respectively in reverse order (swapping of C- and N-terminal). Purified and characterized peptides were radiolabeled with Lu-177. CD spectra were recorded to obtain information about the secondary structure stabilization of modified peptides. Molecular docking studies were performed to analyse the change in interaction of the peptide sequence with the receptor. Biological efficacy was tested in HER2-positive SKBR3 cells and SKBR3 tumor-bearing SCID female mice xenografts.

Results and Discussion

The original A9 peptide, [^{177}Lu]Lu-DOTA-A9 exhibited quite low tumor uptake, rapid urinary clearance and poor metabolic stability. Introduction of PEG_4 moiety could not improve the tumor uptake or metabolic stability to significant levels [4]. Hence with an aim to impart conformational rigidity and improve the pharmacokinetic pattern the A9 peptide was cyclized and triazole as peptide bond isostere was introduced. Enhanced conformational stability was confirmed by CD spectral studies (Fig. 2a) and molecular modelling studies established retention of binding efficiency towards the receptor. Improved metabolic stability and higher retention in the tumor was observed for [^{177}Lu]Lu-DOTA-c[Tz]A9. To further improvise the pharmacokinetic features all L-amino acids were replaced by D-amino acids. D-amino acids having chirality

Fig.1: Solid Phase Peptide Synthesis Facility Set-Up.

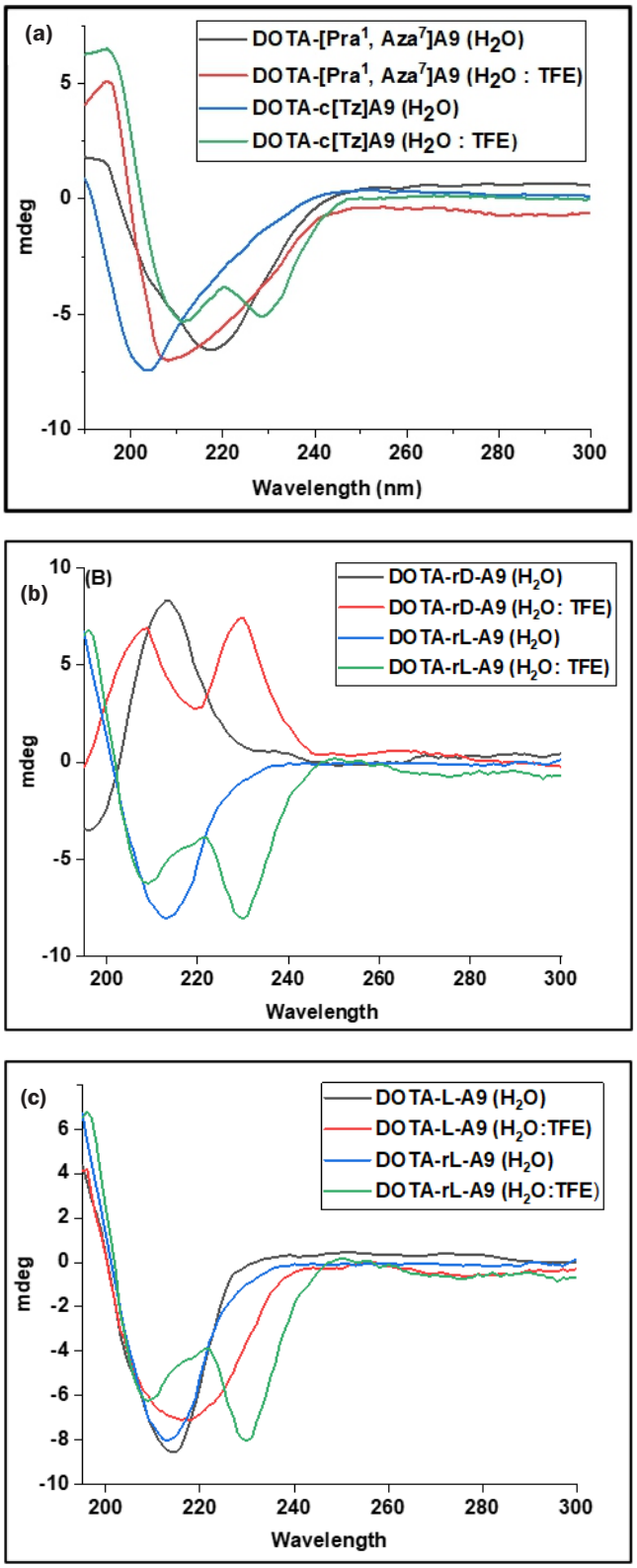

Fig.2: CD spectra of (a) DOTA-c[Tz]A9, (b) DOTA-rD-A9, (c) DOTA-rL-A9.

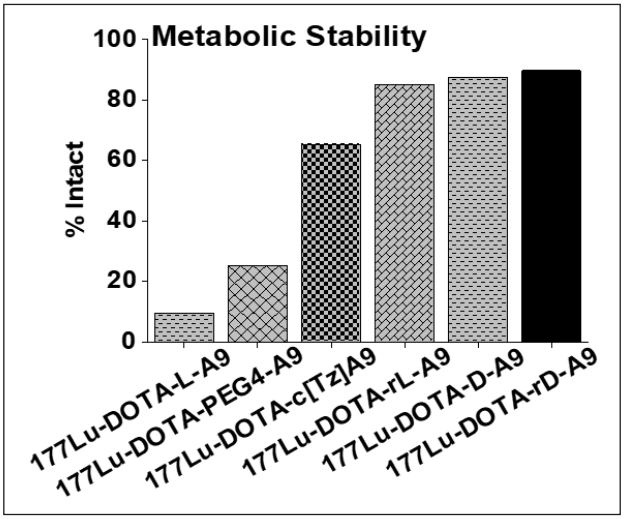

Fig.3: Metabolic stability of modified A9 peptide analogs.

opposite to that of L-amino acids are not recognized by enzymes and hence confer resistance towards enzymatic degradation leading to enhanced metabolic stability. The CD spectra of D-amino acids incorporated peptides, DOTA-D-A9 and DOTA-rD-A9 was observed to be mirror image of their respective L-peptides, DOTA-L-A9 and DOTA-rL-A9 highlighting the opposite chirality of D- and L-amino acids (Fig. 2b). The inverso analogue, [^{177}Lu]Lu-DOTA-D-A9 despite being metabolically stable, did not exhibit any improvement in tumor accumulation over the original L-peptide. The retro-inverso analog, [^{177}Lu]Lu-DOTA-rD-A9 however exhibited high metabolic stability along with enhanced tumor accumulation.

The retro A9 analogue, DOTA-rL-A9 demonstrated enhanced conformational stability (CD spectra, Fig. 2c) and significantly enhanced receptor interaction (molecular modeling) [5]. Arrangement of L-amino acids in reverse direction results in topochemical similarity to the D-peptide hence leading to higher metabolic stability than the original L-peptide. The retro A9 analogue, [^{177}Lu]Lu-DOTA-rL-A9 demonstrated excellent pharmacokinetic features (high tumor uptake and retention, rapid clearance) amongst all the analogues studied resulting in a promising and an efficient radiopharmaceutical.

Conclusion

Amongst the several strategies adopted for modification of HER2-targeting A9 peptide for boosting the metabolic stability (Fig. 3) and bioavailability, best attributes were

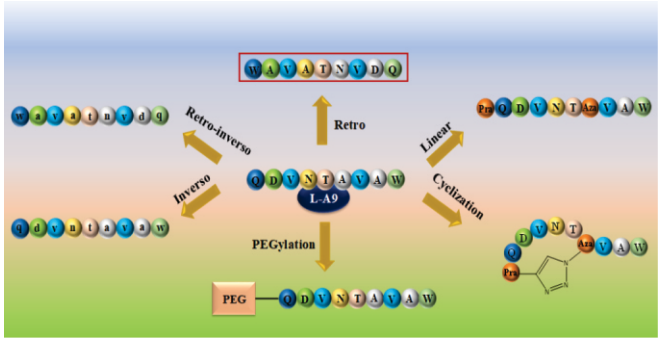

Fig.4: Modified A9 peptide analogs (best analog, Retro A9 indicated in the box).

demonstrated by the retro peptide, rL-A9 (Fig. 4). Arrangement of L-amino acids in reverse manner alters the backbone of peptides resulting in change in bond angles and bond lengths affecting the overall conformation. Thus, better pharmacokinetic features of retro peptide indicate plausible involvement and interaction of the peptide backbone amide groups with the receptor whereby enhanced conformational stability might have induced better receptor fitting.

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