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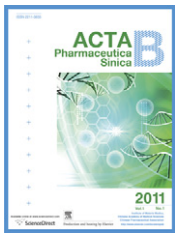

REVIEW

Anti-tumor targeted drug delivery systems mediated by aminopeptidase N/CD13

Xun Wang[a], Bin Wang[b], Qiang Zhang[a,*]

[a]*School of Pharmaceutical Sciences, Peking University, Beijing 100191, China*
[b]*Department of Endocrinology, Guang'an men Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China*

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Abstract Aminopeptidase N (APN)/CD13 is a transmembrane glycoprotein, which is over-expressed on tumor neovascular endothelial cells and most tumor cells, where it plays an important role in tumor angiogenesis. Peptides containing the Asn-Gly-Arg (NGR) motif can specifically recognize APN/CD13 allowing them to act as tumor-homing peptides for the targeted delivery of anti-tumor drugs to tumor neovascular endothelial cells and tumor cells. This article reviews the literature and recent developments related to APN/CD13, its role in tumor growth and some anti-tumor drug delivery systems containing NGR peptides designed to target APN/CD13.

*Corresponding author. Tel./fax: +86 10 82802791.
E-mail address: zqdodo@bjmu.edu.cn (Qiang Zhang)..

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1. Nomenclature and structure of aminopeptidase N

Aminopeptidases are proteinases that catalytically cleave amino acid residues from the NH_2-terminus of polypeptide chains. Aminopeptidase N (APN) is currently receiving considerable attention because of its participation in tumor growth, metastasis and immune regulation. APN is a type II transmembrane glycoprotein containing 967 amino acids with a molecular mass of 150 kDa.

APN was first considered to be a biomarker of normal or malignant myeloid cell subsets. Later it was found to be expressed on the surface of a wide variety of cells and organs of the hematopoietic system. Previously APN was known by various names including APM, P146, P161 and gp150. In 1988, the International Union of Biochemistry and Molecular Biology approved the name membrane alanyl aminopeptidase. In 1989, based on the determination of its amino acid sequence, APN was found to be identical to the cell surface differentiation antigen, CD13. Accordingly it is now commonly given the abbreviation APN/CD13.

APN/CD13 is a member of the Zn-binding metalloproteinase superfamily. Its active site contains a Zn^{2+} ion and its activity is Zn^{2+}-dependent. APN/CD13 referentially cleaves an oligopeptide segment with a neutral amino acid at the NH_2-terminus. Its activity can be blocked by metalloproteinase inhibitors through the binding of two monoclonal antibodies to its extracellular epitope. APN/CD13 is a membrane-bound protein comprising a short cytoplasmatic N-terminal domain (1–7 amino acid residues) with unclear function, a single transmembrane domain (8–29 amino acid residues) existing in an α-helix form, and an extracellular long and highly glycosylated domain with a 10-*N*-type sugar chain and an 11-*O*-glycan binding site[1], which determines its unique biological functions. APN/CD13 usually exists as a non-covalently bound homodimer, the dimerization of which occurs in the endoplasmic reticulum prior to glycosylation in the Golgi complex[1].

2. Aminopeptidase N and tumor growth

Tumor growth is a complex multi-step process, which includes tumor cell division, proliferation and angiogenesis. It requires an energy supply and transportation of metabolic products. APN/CD13 plays a role in each step of tumor growth particularly in primary and secondary tumor growth, invasion, metastasis and angiogenesis.

In order for tumor cells to translocate from their primary site to distant organs and tissues, they must first break through the basement membrane barrier into the surrounding tissue, spread with the circulation and finally implant and grow in particular parts of the capillary bed. The process of breaking through the basement membrane barrier depends not only on the action of various proteinases in the surrounding interstitium but also on the capacity of the tumor cells themselves to secrete proteinases. In malignant cells, APN/CD13 is generally evenly distributed on the monolayer cell surface. When cell cloning takes place, APN/CD13 molecules automatically migrate to cell–cell contact sites where they degrade extracellular matrix proteins. Thus, inhibiting the activity of APN/CD13 effectively prevents tumor invasion and metastasis.

When the diameter of a tumor reaches 1–2 mm, nutrients from the microenvironment can no longer penetrate in sufficient amounts to sustain the growth of tumor cells. Further tumor growth is then dependent on tumor angiogenesis in which there is a proliferation of blood vessels that penetrate into the tumor, supplying nutrients and oxygen and removing waste products. Angiogenesis is also the first step in tumor metastasis.

APN/CD13 plays an important role in angiogenesis. It is not expressed in endothelial cells of normal tissues but is found in malignant and nonmalignant effusions and intratumoral fluid[2]. In addition, its expression in tumor vascular endothelial cells is significantly increased by angiogenic signals such as hypoxia and growth factors (bFGF, VEGF, TNF-α, etc.). This indicates that APN/CD13 is a marker of angiogenesis. It facilitates the invasion of endothelial cells into surrounding tissues, enhances the invasiveness of tumor cells and acts as an auxiliary adhesion factor in cell–cell contact to enhance cell–cell fusion or act as a receptor in signal transduction. However, the expression of APN/CD13 is insufficient to reverse inhibition by Ras of tubular network formation in endothelial cells *in vitro* suggesting that APN/CD13 is mainly involved in the early stages of angiogenesis[1].

3. Aminopeptidase N mediated anti-tumor drug delivery systems

After the discovery of RGD peptide, Arap et al.[3] used phage display libraries to isolate peptides that specifically target tumor blood vessels. This screen led to peptides containing the Asn-Gly-Arg (NGR) motif. It appears that a unique form of APN/CD13, functionally active in mediating NGR binding, is present in tumor vasculature but not in other APN/CD13-rich tissues. However, the structural determinants of this selectivity remain unknown. It has been shown that the disulfide bond in NGR is essential to stabilize the structure of NGR-containing peptides and thereby enhance the efficiency of tumor targeting[4]. NGR-containing peptides can be used as ligands for targeted delivery of chemicals, peptides, cytokines, liposomes and polymeric micelles to activated blood vessels in tumors. The therapeutic effects can be improved while the toxicity and side effects can be reduced. NGR-containing peptides can also be used for tumor imaging.

Arap et al.[3] coupled the peptide Cys-Asn-Gly-Arg-Cys (CNGRC) to doxorubicin (DXR) and found that human MDA-MB-435 breast tumor-bearing mice treated with the conjugate exhibited significantly prolonged survival with reduced toxicity. They also designed short peptides composed of two functional domains: a tumor blood vessel homing motif (CNGRC) and a programmed cell death-inducing sequence. The peptides were designed to directly target angiogenic endothelial cells, disrupt their mitochondrial membranes and induce their apoptosis, without affecting other cells. The peptides showed stronger anti-cancer activity in nude mice than physical mixtures of peptides composed of the two functional domains[5].

Corti et al. made a significant contribution to our knowledge of NGR-containing peptides. They fused murine tumor necrosis factor (TNF) with CNGRC peptide (NGR-TNF) by genetic engineering and showed that it was 12–15 times more efficient than murine TNF in decreasing the tumor burden in lymphoma and melanoma animal models with no increase in toxicity. In addition, NGR-TNF made from human TNF

induced stronger anti-tumor effects than human TNF itself even at a 30-fold lower dose[6]. Human NGR-TNF is currently undergoing a phase II clinical trial[7]. These researchers also coupled NGR peptide to the surface of liposomal doxorubicin (NGR-SL[DXR]) and used it to treat orthotopic neuroblastoma (NB) xenografts in severe combined immunodeficient (SCID) mice. Pharmacokinetic studies indicated that the liposomes remained in blood for a long time. Uptake of NGR-SL[DXR] into NB tumor cells was at least 10 times higher than that of non-targeted SL[DXR] liposomes after 24 h. Histopathological analysis revealed pronounced destruction of the tumor vasculature with a marked decrease in vessel density and increased tumor cell apoptosis. Mice injected with NGR-SL[DXR] displayed rapid tumor regression as well as inhibition of metastatic growths. In contrast, mice treated with mismatched ARA-modified SL[DXR] peptide formed large well-vascularized tumors[8].

Corti et al. also prepared sterically stabilized immunoliposomes (SIL) loaded with DXR and modified with a monoantibody targeted to the disialoganglioside receptor GD2 [aGD2-SIL(DXR)]. They observed an additive anti-tumor effect of the combination of NGR-SL(DXR) (targeting tumor endothelial cells) and aGD2-SIL(DXR) (targeting neuroblastoma cells). A significant improvement in anti-tumor effects was seen in neuroblastoma-bearing animal models when treated with the combined formulation compared with control mice or mice treated with either tumor- or vascular-targeted liposomal formulations alone. The combined treatment resulted in a dramatic inhibition of tumor endothelial cell density. Long-term survival occurred only in animals treated with the combined formulation. The fact that the dual-targeting strategy of combining tumor targeting and tumor endothedial targeting was more effective and less toxic suggests that it has a promising future in cancer chemotherapy[9].

Chen et al.[10] designed PEGylated liposome-polycation-DNA (LPD-PEG) nanoparticles for efficient delivery of small interfering RNA (siRNA) to solid tumors in mice by modification with NGR peptide. LPD-PEG-NGR efficiently delivered siRNA to the cytoplasm and down regulated the target gene in CD13(+) HT1080 cells but not CD13(−) HT-29 cells. In contrast, nanoparticles containing a control peptide, LPD-PEG-ARA, produced only little siRNA uptake and gene silencing activity. LPD-PEG-NGR also efficiently inhibited HT1080 xenograft tumor growth *in vivo*.

Son et al.[11] synthesized a multifunctional polymer based on low molecular weight branched polyethylenimine (BPEI) thiolated with propylene sulfide and mixed it with alpha-maleimide-omega-*N*-hydroxysuccinimide ester polyethylene glycol (MAL-PEG-NHS, MW: 5000) and cyclic NGR (cNGR) peptide. Besides achieving efficient release of pDNA and long duration, the multifunctional gene carrier achieved efficient tumor targeting using the cNGR peptide. Cellular uptake of polymers was evaluated by confocal laser scanning microscopy (CLSM). CD13(+)B16F1 cells showed enhanced uptake of pDNA compared with CD13(−) MDA-MB-231 cells.

In our laboratory, the anti-tumor efficacy of PEG-b-PLA polymeric micelles (NGR-PM) decorated on their surface with NGR peptide was evaluated[12]. HT1080 cells (with high expression of CD13) were chosen as the model of positive tumor cells and HUVEC (with intermediate expression of CD13) as the model of tumor endothelial cells. The results showed that actively targeted PM produced stronger adhesion and better uptake than undecorated PM. HT1080 cells were also able to take up NGR-PM-DTX to a larger extent than HUVEC. After the PM was loaded with DTX, NGR-PM-DTX was found to be more cytotoxic than PM-DTX to HT1080 cells. NGR-PM-DTX also showed greater ability to inhibit the proliferation of HUVEC. In BALB/c mice bearing HT1080 tumor xenografts, stronger anti-tumor efficacy and less change in body weight were observed in the NGR-PM-DTX group with good *in vitro–in vivo* correlation[12].

Our research group also prepared NGR-modified DSPE-PEG micelles containing paclitaxel (PTX)[13]. These NGR-M-PTX micelles significantly depressed the proliferation of murine brain microvascular endothelial cells (BMEC) *in vitro*. The anti-tumor activity of NGR-M-PTX was also evident in C6 glioma tumor-bearing rats *in vivo* where glioma tumor growth was markedly inhibited compared with Taxol. The results demonstrate the antiangiogenic efficacy of NGR-M-PTX.

Oostendorp et al.[14] developed cNGR-labeled paramagnetic quantum dots (cNGR-pQDs) for the noninvasive assessment of tumor angiogenic activity using quantitative *in vivo* molecular magnetic resonance imaging (MRI). MRI results were validated using *ex vivo* two-photon laser scanning microscopy (TPLSM). The results showed that cNGR-pQDs were primarily located on the surface of tumor endothelial cells and to a lesser extent in the vessel lumen, supporting a high specificity of cNGR-pQDs for angiogenic tumor vasculature.

4. Future of aminopeptidase N mediated anti-tumor drug delivery systems

Aminopeptidase N/CD13-mediated anti-tumor targeted drug delivery systems show great promise in preclinical studies, and, as a result, NGR-containing peptides targeting APN/CD13 are receiving increasing attention. However, additional research is required to identify their mechanisms of action[15]. With more intensive research into therapeutic strategies, more small-molecule drugs, biological macromolecule drugs and some kinds of carriers are combined with NGR-containing peptide to produce a variety of novel tumor targeted drug delivery systems. NGR-containing peptides targeting APN/CD13 may also lead to improvements in tumor diagnosis and tumor blood vessel imaging.

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References

1. Shi E, Liao XL. The progress of CD13/APN biological function. *Foreign Med Sci (Section of Blood Transfusion and Hematology)* 2005;**28**:504–7.
2. Van Hensbergen Y, Broxterman HJ, Hanemaaijer R, Jorna AS, van Lent NA, Verheul HM, et al. Soluble aminopeptidase N/CD13 in malignant and nonmalignant effusions and intratumoral fluid. *Clin Cancer Res* 2002;**8**:3747–54.

3. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* 1998;**279**:377–80.
4. Majhen D, Gabrilovac J, Eloit M, Richardson J, Ambriović-Ristov A. Disulfide bond formation in NGR fiber-modified adenovirus is essential for retargeting to aminopeptidase N. *Biochem Biophys Res Commun* 2006;**348**:278–87.
5. Ellerby HM, Arap W, Ellerby LM, Kain R, Andrusiak R, Rio GD, et al. Anti-cancer activity of targeted pro-apoptotic peptides. *Nat Med* 1999;**5**:1032–8.
6. Curnis F, Sacchi A, Borgna L, Maqni F, Gasparri A, Corti A. Enhancement of tumor necrosis factor alpha antitumor immunotherapeutic properties by targeted delivery to aminopeptidase N (CD13). *Nat Biotechnol* 2000;**18**:1185–90.
7. Santoro A, Rimassa L, Sobrero AF, Citterio G, Sclafani F, Carnaqhi C, et al. Phase II study of NGR-hTNF, a selective vascular targeting agent, in patients with metastatic colorectal cancer after failure of standard therapy. *Eur J Cancer* 2010;**46**:2746–52.
8. Pastorino F, Brignole C, Marimpietri D, Cilli M, Gambini C, Ribatti D, et al. Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy. *Cancer Res* 2003;**63**:7400–9.
9. Pastorino F, Brignole C, Di Paolo D, Nico B, Pezzolo A, Marimpietri D, et al. Targeting liposomal chemotherapy via both tumor cell-specific and tumor vasculature-specific ligands potentiates therapeutic efficacy. *Cancer Res* 2006;**66**:10073–82.
10. Chen Y, Wu JJ, Huang L. Nanoparticles targeted with NGR motif deliver c-myc siRNA and doxorubicin for anticancer therapy. *Mol Ther* 2010;**18**:828–34.
11. Son S, Singha K, Kim WJ. Bioreducible BPEI-SS-PEG-cNGR polymer as a tumor targeted nonviral gene carrier. *Biomaterials* 2010;**31**:6344–54.
12. Wang X, Wang Y, Chen X, Wang J, Zhang X, Zhang Q. NGR modified micelles enhance their interaction with CD13-overexpressing tumor and endothelial cells. *J Control Release* 2009;**139**: 56–62.
13. Zhao BJ, Ke XY, Huang Y, Chen XM, Zhao X, Zhao BX, et al. The antiangiogenic efficacy of NGR-modified PEG-DSPE micelles containing paclitaxel (NGR-M-PTX) for the treatment of glioma in rats. *J Drug Target* 2011;**19**:382–90.
14. Oostendorp M, Douma K, Hackeng TM, Dirksen A, Post MJ, van Zandvoort MA, et al. Quantitative molecular magnetic resonance imaging of tumor angiogenesis using cNGR-labeled paramagnetic quantum dots. *Cancer Res* 2008;**68**:7676–83.
15. Corti A, Curnis F, Arap W, Pasqualini R. The neovasculature homing motif NGR: more than meets the eye. *Blood* 2008;**112**: 2628–35.