Commentary

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The first inhibitor-based fluorescent imaging probe for aminopeptidase N

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ABSTRACT: Höltke and his co-workers firstly reported the synthesis and characterization of an inhibitor-based fluorescent imaging probe for aminopeptidase N. This fluorescent probe demonstrated high binding affinity to APN and could specifically bind to APN high expressed cells, thus revealed the distribution of APN. As a milestone, this outstanding work provided a useful tool to understand APN pathophysiology.

Keywords: Aminopeptidase N, affinity-based, fluorescent probe, cell imaging

Aminopeptidase N (APN/CD13) is a zinc-dependent exopeptidase positioned on the surface of diversified cells, such as the brush border membranes of kidney, synaptic membranes in the central nervous system, mucosal cell of the small intestine, as well as the fibroblasts, monocytes and myeloid progenitors (*1-3*).It is up-regulated on a number of carcinoma cells formediating the angiogenesis and metastasis of the tumor (*4*,*5*). Moreover, APN participates a criticalrole in cancer stem cells since it has become a diagnostic or prognostic biomarker for cancers (*6*). As a result, it is meaningful to image the distribution of APN *in cellulo*. Among the current imaging strategies, fluorescent imaging approach reflects the distinctive advantage, such as reasonable sensitivity, high spatiotemporal resolution and noninvasive feature to be employed in real-time examination (*7*,*8*).

Our group have reported a ratiometric fluorescent probe that can image APN activity in living cells (*9*). However, after being hydrolyzed by APN, the obtained fluorophores went into the whole cells, so it failed to capture the accurate location of APN. CNGRC (Cys-Asn–Gly–Arg-Cys) is a tumor homing peptide that can specifically bind to APN in tumor vasculature (*10*). Although numerous CNGRC based imaging probes has been documented throughout the literature, the binding affinity of CNGRC to APN was moderate (*11*). It's well known that inhibitors often have appropriate binding affinity to their enzymatic target, therefore the connection of a inhibitor with a fluorophore at the appropriate position, which does not affect the inhibitor-target interaction, may acquire an efficient and specific probe. The first inhibitor-based fluorescent APN probe, bestatin-linker-fluorescein, was synthesized by the Green baum lab (*12*). However, they did not report the imaging result of this probe on cells.

The first successful example for APN cell imaging was recently reported by Höltke and his colleagues (*13*). As depicted in Figure 1, this imaging probe (**Cy5.5-23**) includes three portions *i*) the inhibitor moiety that can tightly bind to APN; *ii*) a fluorescent reporter; and *iii*) a short polyethylene glycol (PEG) spacer that can reduce the hindrance of interaction between APN and fluorophore. Compared to the lead inhibitor, **Cy5.5-23** revealed even better inhibitory activity to APN. Additionally, **Cy5.5-23** displayed low cytotoxicity, which is essential for an imaging probe. Cell binding assays disclosed that **Cy5.5-23** could bind to APN-positive BT-549 cells but not to APN-negative BT-20 cells, and this binding could be significantly reversed by 100-fold excess of bestatin (a positive APN inhibitor).

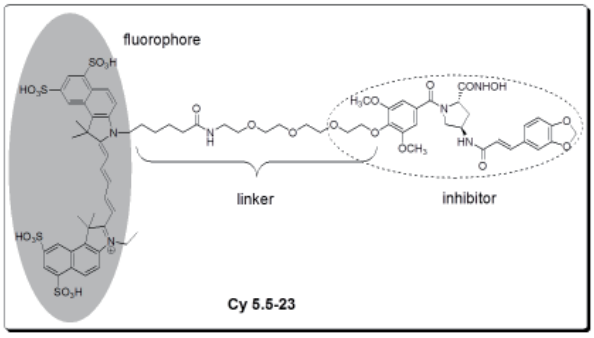

Figure 1. The structure of probe Cy 5.5-23.

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Based on the above-mentioned superiorities, **Cy5.5-23** provides a significant breakthrough for surveying the distribution of APN. Furthermore, considering that the near-infrared light emitted by Cy5.5 has exceptional deep tissue penetration capability, **Cy5.5-23** may be facilitated to image APN distribution in tissues and even in living animals.

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