Chemistry of Contrast Media

Biologicals

Protein and Oligonucleic Acid Scaffolds: Imaging Using Affibody Molecules

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Learning Objectives:

- Scaffold structure of Affibody molecules permits obtaining of robust binding proteins with subnanomolar affinities.
- Robust structure of Affibody molecules permits efficient labeling in harsh conditions (pH range of 3.6-11.5; temperature up to 100°C, presence of lipophilic solvents) without losing binding capacity.
- Small size (6-7 kDa) of Affibody molecules enables rapid extravasation and tumor penetration, and rapid clearance of unbound tracer; permitting high contrast imaging shortly after injection.
- Rich labeling chemistry permits optimizing of bio distribution of Affibody molecules and increasing of imaging contrast.
- Clinical studies demonstrated that Affibody molecules can image HER2-expressing breast cancer metastases with high sensitivity.

Affibody molecules are a new class of small (6-7 kDa) affinity proteins based on a three-helical scaffold. Randomization of 13 amino acids on the surfaces of helices 1 and 2 provides large (>10^{10} members) libraries enabling molecular-display selection of binders. The robust Affibody scaffold enables high affinity of selected proteins. Affibody molecules with picomolar affinity have been developed for binding to such therapeutic cancer associated targets as HER2, EGFR, HER3, IGF-1Rand PDGFRβ. Di- and multimeric forms of Affibody molecules and fusion proteins can be easily produced by recombinant expression in E. coli. Since the Affibody scaffold does not contain cysteine, a unique cysteine residue can be introduced in a desirable position allowing site-specific coupling of prosthetic groups or chelators using thiol-directed chemistry. Monomeric forms of Affibody molecules can be made by peptide synthesis allowing site specific incorporation of chelators or prosthetic groups as well as unnatural amino acids. One of the key features of Affibody molecules is very rapid (3 μS) refolding at physiological pH and molarity, permitting application of harsh conditions during labeling and purification (pH range of 3.6-11.5, temperature up to 95°C and the use of lipophilic solvents). During optimization of targeting properties, Affibody molecules were labeled with 16 different nuclides suitable for imaging (e.g. ^{18}F. ^{68}Ga, $^{99"}$'Tc, ^{111}In, ^{124}1) and therapy (e.g. ^{90}Y, ^{177}Lu, ^{131}I, ^{186}Re, ^{211}At), using over 50 different nuclide-chelator/linker combinations. The binding capacity was preserved in all cases.

In vivo, monomeric Affibody molecules clear rapidly via kidneys. In the case of abundant targets, such as e.g. HER2, a tumor uptake of 20-25% 10/g and tumor-to-blood ratio up to 200 can be obtained in mouse xenograft models at 4h after injection. However, good contrast is possible also in the case of a low target expression (< 30000 molecules/cells). Targeting is highly specific (as shown by the use of pre-saturation. by the use of non-expressing xenografts or non-specific Affibody molecules). An unspecific tumor accumulation due to EPR effect is negligible, at the level of less that 1% of specific uptake.

Genetic fusion of Affibody molecules with the albumin-binding domain (ABO. Albumod) enables appreciable (up to 80-fold) extension of their residence time in circulation and reduces renal uptake, which broaden their use for therapeutic applications. The use of near-infrared fluorescent dyes or fusion with fluorescent proteins facilitates optical in vivo imaging using Affibody molecules. Furthermore, Affibody molecules can also be used for specific targeting of different Nano carriers, such as liposomes or SPIO.

Preclinical studies have demonstrated a high potential of radiolabeled Affibody molecules for in vivo imaging of expression of therapeutic targets and for monitoring of response to targeted therapy. Clinical studies have demonstrated that the ^{111}In- and ^{68}Ga-labeled anti-HER2 Affibody molecules can image HER2-expressing metastases in breast cancer patients. So far, anti-Affibody molecule antibodies were not detected in clinics.