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Aptamers Selected by Cell-SELEX for Molecular Imaging

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Abstract

Conventional diagnostics for cancer rely primarily on anatomical techniques. However, these techniques cannot monitor the changes at the molecular level in normal cells, which possibly signal the onset of cancer at its very earliest stages. For accurate prediction of the carcinogenesis at the molecular level, targeting ligands have been used in combination with imaging probes to monitor this biological process. Among these targeting ligands, aptamers have high binding affinity to various targets ranging from small molecules to whole organisms, and, hence, exceptional recognition ability. Many recent studies have been reported on aptamer-based molecular imaging, clearly indicating its clinical and diagnostic utility. In this review, we will discuss some key results of these studies.

Keywords

Aptamer; SELEX; Molecular imaging; Cancer; Targeting ligands

Introduction

Molecular imaging has been described as the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems, and, as such, it has attracted enormous attention over the last decade (Mankoff 2007). Conventional diagnostic techniques for cancer primarily rely on the morphological changes of normal cells into tumors (Massoud and Gambhir 2003). However, these techniques are limited to assessing anatomical changes or nonspecific glucose

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Compliance with Ethical Standards

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metabolism not the molecular level changes arising in normal cells. Such changes can now be observed with the aid of targeting ligands for in vivo molecular imaging. Commonly used molecular imaging modalities in the clinic include optical imaging, magnetic resonance imaging (MRI), ultrasound imaging and positron emission tomography (PET), or single photon emission computed tomography (SPECT) (Hong et al. 2011).

As mentioned above, targeted recognition is combined with molecular imaging to realize molecular-level visualization of cellular and subcellular events, in particular, incipient carcinogenesis. Although a number of biological targeting molecules like antibodies and peptides have been conjugated with imaging probes for molecular imaging studies, aptamers stand out as the probe of choice.

Aptamers are single-stranded RNA or DNA oligonucleotides with unique intramolecular conformations that hold distinct binding properties to a wide range of targets such as proteins, phospholipids, sugars, nucleic acids, and whole cells, among others (Daniels et al. 2003; Ellington and Szostak 1990; Tuerk and Gold 1990). As a relatively new type of targeting ligand, aptamers have recently emerged with superb potential for diagnostic and therapeutic applications (Fang and Tan 2015). In the molecular imaging field, aptamers display several intrinsic advantages. First, aptamers can be derived from a process termed “systemic evolution of ligands by exponential enrichment”(SELEX) against various targets after several rounds of selection (Ellington and Szostak 1990; Tuerk and Gold 1990). Unlike antibodies produced by in vitro or in vivo operations, aptamers can be obtained through an economical chemical synthesis process with high yields (Hayakawa et al. 1990). Furthermore, in contrast with antibodies, aptamers afford better resistance toward thermal or pH change. Such high stability allows aptamers to be easily modified with imaging probes through covalent conjugation (Hu and Zhang 2013). Finally, aptamers are much smaller (ca. 5–40 kDa) than antibodies (ca. 150 kDa). Their small size can improve tissue penetration and increase systemic clearance. The development of aptamers for biomedical applications has greatly expanded since their inception approximately two decades ago (Keefe et al. 2010). For example, an aptamer that specifically binds to human vascular endothelial growth factor (VEGF) has been approved by the United States Food and Drug Administration (FDA) for the clinical treatment of age-related macular degeneration (Ng et al. 2006).

A newer process termed cell-SELEX is used to produce aptamers that specifically bind to a certain cell line, or, particularly, a cell-surface molecule, based on unique extracellular characteristics, as shown in Fig. 1 (Shangguan et al. 2006). Based on its tight binding to target cells, aptamer-based molecular imaging promises enormous potential application in preclinical cancer diagnostics (Fang and Tan 2010). In this review, we will briefly summarize the advancements of aptamers selected by cell-SELEX as targeting ligands combined with imaging agents for targeted molecular imaging.

Optical Imaging

Optical imaging, which includes fluorescent and bioluminescent imaging, provides real-time, noninvasive, cost-efficient, high-resolution imaging for epithelial tissue (Sokolov et al.

2003). The combination of confocal microscopy and near-infrared dyes has improved the resolution of optical imaging and allows for deeper penetration into tissue (Mao et al. 2013).

With the direct conjugation of a fluorescent probe with an aptamer, targeted optical imaging can be realized (Cai et al. 2008). Compared with activatable probes, directed optical imaging has lower signal-to-background ratios when used in biological imaging (Kemin et al. 2009). Activatable optical probes, on the other hand, are unique in the field of molecular imaging in that they can be turned “on” in particular environments, but otherwise remain in the “off” state (Li et al. 2002; Tan et al. 2004).

Molecular beacons (MBs) are representative of DNA-based probes (Kemin et al. 2009; Li et al. 2002; Tan et al. 2004). An MB is a single-stranded DNA molecule having a stem-and-loop structure with a fluorophore at one end and quencher at the other. The loop is complementary to the target DNA. In the absence of target, the fluorophore and quencher are in close proximity, and fluorescence is quenched (“off” state). Upon binding to target DNA, the probe opens, separating the fluorophore and quencher and fluorescence is restored (“on” state). MBs are characterized by simple operation and high sensitivity and specificity. Based on the programmability of DNA strands, a novel molecular aptamer beacon was developed by replacing the loop moiety with an aptamer sequence (Hui et al. 2011; Qiu et al. 2013; Yang et al. 2005). Molecular aptamer beacons retain the same properties as MBs, but expand response to different kinds of targets.

Tan’s group designed a self-delivered MB for photoinitiated real-time imaging and detection of messenger RNA (mRNA) in living cells via direct hybridization of an extended internalizing aptamer and a MB (Qiu et al. 2013). With this fluorescent aptamer as an internalizing carrier, the MB can be efficiently delivered into the cytoplasm of target cells, and its internalized cargo, as well as its intracellular distribution, can be tracked before photoactivation. To expand their repertoire of aptamer-based in vivo cancer imaging probes, Shi et al. first reported a molecular aptamer beacon for tumor imaging in mice, shown in Fig. 2, (Hui et al. 2011) which could effectively bind tumors with high sensitivity and specificity, thus establishing the efficacy of fluorescent molecular aptamer beacons for diagnostic applications. As expected, molecular aptamer beacons could be activated by target cancer cells with rapid restoration of fluorescence achieved at the tumor site, but not other areas. Next, to further improve the resistance against various nucleic acids degradation enzymes in blood, a series of chemically modified aptamers were designed and optimized (Ke et al. 2012; Shi et al. 2014). This resulted in the development of aptamer probes with increased stability against nuclease digestion and, hence, more suitability for tumor imaging.

Activated aptamer-based molecular imaging probes can realize targeted cancer imaging by receptor-mediated endocytosis. However, many aptamers display relatively weak binding affinity to targets. Therefore, in some cases, aptamers have been modified on the surface of nanoparticles to increase their binding affinity through multiplex interaction (Huang et al. 2008). As previously reported, aptamers conjugated with gold-silver nanorods yield at least 26-fold affinity enhancement compared to that of a single aptamers behave. Based on this principle, various such aptamer-functionalized conjugates have been applied to targeted molecular imaging studies.

The past several years have seen several advances in the fields of synthesis and characterization of nanomaterials. These kinds of nanomaterials exhibit large surface area, unique size and shape, as well as composition-dependent physical and chemical properties (Bruchez et al. 1998; Daniel and Astruc 2004; Kelly et al. 2003; Michalet et al. 2005). In addition, most nanomaterials are adaptable to further modification with functional molecules for specific applications (Medintz et al. 2005; Rosi and Mirkin 2005).

Gold nanoparticles (AuNPs) have unusual optical and electronic properties, high stability, and biological compatibility, controllable morphology and size dispersion, and facile surface functionalization (Barnes et al. 2003; Daniel and Astruc 2004; Kelly et al. 2003; Sun and Xia 2002). Aptamer-labeled AuNPs have been identified as a powerful system for targeted molecular recognition, detection, imaging, and therapy (Liu and Lu 2006a, b; Pavlov et al. 2004). An example can be cited from the Mirkin's group which employed an assembly and disassembly assay to detect DNA. Specifically, two designed pieces of DNA, which were each complementary to part of the target DNA, were immobilized onto the surface of the AuNPs. Target DNA acted as a crosslinker, leading to aggregation of the AuNPs, which, in turn, resulted in color change from red to purple, which could be seen by the naked eye (Elghanian et al. 1997). The introduction of aptamers has allowed the monitoring of many more analytes using this platform, albeit with different mechanisms, to realize molecular imaging. Medley et al. constructed an aptamer-AuNP-based colorimetric assay for the direct imaging of cancer cells (Medley et al. 2008). After incubation with target cancer cells, aptamer-AuNP conjugates anchor on the surface of cell membrane, leading aggregation of AuNPs, resulting in the alteration of solution color from red to blue, as shown in Fig. 3. Indeed, many such aptamer-AuNPs against a variety of targets for targeted molecular imaging have been reported (Wang et al. 2008b).

Besides AuNPs, other noble metallic nanomaterials have been functionalized as an effective molecular imaging system by modifying multiple aptamers. Li et al. reported a one-pot synthesis of aptamer-functionalized silver nanoclusters with high fluorescence quantum yields of about 40 % (Li et al. 2012). Confocal laser scanning microscopy and Z-axis scanning results confirmed that the AS1411-functionalized silver nanoclusters could be internalized into MCF-7 human breast cancer cells and that they could stain cell nuclei with red color. Based on the ease of synthesis and specific target recognition, this fluorescent platform has the potential to broaden the applications of silver nanoclusters in biological imaging. Another aptamer, sgc8c, which targets CCRF-CEM cancer cells, has also been functionalized with silver nanoclusters with a cyto-sine-enriched terminal sequence for targeted cell imaging (Jinjin et al. 2012).

Lanthanide-doped upconversion nanoparticles (UCNPs) can emit light in the visible range following photoexcitation with near-infrared light, usually at 980 nm. Compared with traditional stokes imaging probes, this anti-stokes characteristic makes UCNPs an ideal biomedical imaging platform because of its stronger tissue penetration ability and lower phototoxicity (Chatterjee et al. 2008; Wang and Liu 2009; Wang et al. 2005). Yuan et al. combined the beneficial features of a DNA aptamer with near-infrared light-irradiated UCNPs to develop a smart cancer-specific imaging and activatable photodynamic therapy nanopatform. The photosensitizer TMPyP4 (Mesotetra(N-methyl-4-pyridyl)porphine) can

intercalate into a G-quadruplex structure, which is covalently conjugated to nearby UCNPs, as shown in Fig. 4 (Yuan et al. 2013). Without near-infrared light, the photosensitizer cannot produce singlet oxygen to suppress tumor cell proliferation. However, upon irradiation with near-infrared light, obvious inhibition of cancer growth was observed. In another paper, Wang et al. combined UCNPs with a DNA aptamer to image a universal fingerprint compound, providing an easily performed strategy for latent fingerprint imaging under nearinfrared light excitation (Wang et al. 2014). This approach also applied to fingerprints on different surfaces, thus indicating the great practicality of this method. Other inorganic nanomaterials, such as silica nanoparticles, quantum dots, carbon nanotubes, and graphene have also been conjugated with DNA aptamers for targeted molecular imaging (Chen et al. 2009; Estevez et al. 2009; Jie et al. 2014; Medley et al. 2011; Tan et al. 2012; Wang et al. 2010; Zhong et al. 2011).

DNA is one of the most promising materials for nanobiotechnology. The base sequence in DNA encodes tremendous structural and functional information into the biopolymer. Nucleic acid bases constitute the building blocks of the biopolymer and provide instructions that indicate the structure and functions of nucleic acids. The basic Watson–Crick complementarity of A-T and C-G base-pairing leads to the formation of duplex DNA structures. The unique features of DNA hybrids provide building blocks for the “bottom-up” assembly of nanoarchitectures with programmed functionalities and properties emerging from the one-, two-, or three-dimensional ordering of the systems. Such nanostructures can partially mimic cellular functions and may serve as scaffolds for the secondary templated synthesis of nanodevices.

Based on this design principle, A series of switchable aptamer molecular beacon micelle nanoparticles for intracellular adenosine triphosphate (ATP) (Wu et al. 2013) and mRNA imaging (Tao et al. 2013) have been reported. In the presence of ATP or mRNA, the conformation of the aptamer molecular beacon is altered, thereby separating fluorophore away from quencher, and, in consequence, restoring fluorescence. Thus, the presence of the analyte can be demonstrated, showing exceptional promise for molecular imaging.

Adapted from DNA micelles, scientists has also reported a series of DNA-based building blocks, such as DNA nanoflowers, DNA nanodevices and so on, assembled through hybridization chain reaction (HCR) or rolling circle replication (RCR). Fluorescent DNA nanodevices target living cell membrane surfaces by anchoring preformed model nanodevices and by in situ self-assembly of nanodevices through specific aptamer-target interaction, as shown in Fig. 5 (Guizhi et al. 2013). This work was followed by another report describing the use of this DNA nanodevice for targeted anticancer drug delivery by inserting DOX molecules into double-stranded(dsDNA) at GC base pair positions (Zhu et al. 2013b).

Zhu and the co-authors also reported a facile approach to construct aptamer-conjugated FRET (fluorescent resonance energy transfer) nanoflowers (NFs) through RCR for multiplexed cellular imaging. The combination of multi-fluorescence emissions by a single wavelength excitation and traceable targeted drug delivery provides a novel system for

applications in multiplex fluorescent cellular imaging, effective screening of drugs, and therapeutic protocol development (Hu et al. 2014; Zhu et al. 2013a).

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) probes the variation in relaxation rates of protons or certain other nuclei in a tissue of interest. The variation in relaxation rates of nuclei results from molecule interactions, and different tissues have different relaxation times, resulting in endogenous contrast in MRI (Winter et al. 2006). Additionally, exogenous contrast agents can further enhance the MRI signal by selectively shortening either the T₁ (longitudinal) (Zhang et al. 2005) or T₂ (transverse) (Pathak et al. 2004) relaxation time, allowing for visualization and discrimination between tissue types with higher contrast. This makes MRI a powerful molecular imaging modality for early clinical diagnosis of cancer and management of malignant tumors.

Aptamers have been conjugated to paramagnetic molecules, including paramagnetic metal chelates (used to enhance T₁ contrast) and super-paramagnetic iron oxide nanoparticles (SPIONs, used to increase T₂ contrast), for magnetic resonance-based molecular imaging (Wang et al. 2008a).

SPIONs, which are well-known MRI contrast media, have been incorporated with aptamers, affording the added ability to distinguish abnormal cells from healthy cells. For example, Chen et al. reported a smart multifunctional nanostructure (SMN) constructed from a porous hollow magnetite nanoparticle, a heterobifunctional PEG ligand and sgc8 aptamer, as shown in Fig. 6 (Chen et al. 2011). Sgc8 aptamer recognizes CCRF-CEM cancer cells by specifically binding to the cell membrane protein receptor PTK7 with high affinity and selectivity (Shangguan et al. 2008). Upon reaching the lysosomes of target cancer cells through receptor-mediated endocytosis, the relatively low lysosomal pH level results in corrosion of the porous hollow magnetite nanoparticles, facilitating the release of doxorubicin to kill the diseased cell. Moreover, T₂ relaxation measurements and T₂*-weighted MRI images demonstrated that this nanostructure showed great potential for use as a T₂ contrast agent, which may enable real-time monitoring of cancer treatment progress.

Based on a similar design, the A10 RNA aptamer, which can specifically bind the extracellular domain of prostate-specific membrane antigen (PSMA), was covalently conjugated with SPION by classical coupling chemistry. This novel multifunctional SPION-aptamer bioconjugate with diagnostic and therapeutic capabilities was identified as an MRI contrast agent and therapeutic carrier for sensitive and selective targeting of PSMA on prostate cancer cells (Wang et al. 2008a).

These examples show the potential of aptamer-conjugated SPIONs as effective MRI contrast agents with highly selective cancer cell-targeting detection ability. However, as a single modality, optimal capabilities are limited, whereas combining multiple molecular imaging modalities can offer an advantageous synergism (Hong et al. 2011). A case in point involves MRI and optical imaging. Once fluorophores are attached to the MRI contrast agents to create dual-modality imaging agents, the resulting assembly can offer the high spatial and

temporal resolution, as well as deep tissue penetration, of MR imaging combined with the rapid response and sensitivity of optical imaging. Based on this principle, Li et al. developed a gold-coated iron oxide ($\text{Fe}_3\text{O}_4@\text{Au}$) nanorose about 70 nm in diameter with five distinct functions that integrate aptamer-based targeting, MRI, optical imaging, photothermal therapy, and chemotherapy into one single probe (Li et al. 2014). Cell-specific aptamers modified on the surface provide selective recognition of cancer cells. DOX drug molecules, which intercalate into DNA strands, provide responsive drug delivery, and optical imaging. The iron oxide core and incorporated gold shell act as MRI agents and allow laser-irradiated photothermal therapy. Thus, combination MRI and optical imaging has the potential to improve the specificity of cancer cell diagnosis and facilitate therapeutic drug monitoring.

It is difficult to completely clear the nonspecific binding of aptamer probes. These “always on” probes may cause poor target-to-background signal ratios, thus critically limiting image contrast. Inspired by the design principle of activated fluorescent probes, (Hong et al. 2011) Zhao et al. developed a novel dual-activatable fluorescence/MRI bimodal platform via an MnO_2 nanosheet-aptamer nanoprobe (Zhao et al. 2014). As illustrated in Fig. 7, MnO_2 nanosheets behave like an intracellular GSH-activated MRI contrast agent and a fluorescence quencher of Cy5-labeled aptamers. Both fluorescence signaling and MRI contrast of the nanoprobe are quenched in the absence of target cells. In the presence of target cells, however, aptamers specifically bind to the target cells, allowing release from the MnO_2 nanosheet, which, in turn, results in fluorescence recovery and illumination of target cells. Endocytosed MnO_2 nanosheets can be reduced by intracellular GSH, thus allowing further recovery of fluorescence signal and generation of large amounts of Mn^{2+} ions for MRI. This MnO_2 nanosheet-aptamer nanoprobe shows promise for use as a target-cell-activated fluorescence and intracellular GSH-activated MRI contrast agent with low background and high sensitivity.

Other Imaging Modalities

Computed Tomography is a medical imaging method whereby digital geometry processing is used to generate a 3D image of an object's interior from a large series of 2D X-ray images taken around a single axis of rotation (Mortele et al. 2002). Currently available CT contrast agents, which are based on small iodinated molecules, present a number of limitations, including lack of targeted molecular imaging, short imaging time and renal toxicity (Kim et al. 2010). Inspired by previous research of anti-body-modified AuNPs for targeted CT imaging (Popovtzer et al. 2008; Reuveni et al. 2011). Kim et al. conjugated A10 aptamer with AuNPs to target PSMA. AuNPs have unique physical, chemical, and biological properties, making them ideal candidates for CT contrast agents. Investigators showed that A10-targeted nanoparticles could bind to PSMA-expressing prostate cancer cells with high sensitivity and specificity, leading to more than fourfold greater CT intensity for targeted LNCaP cells compared to non-targeted PC3 cells (Kim et al. 2010).

Given its intrinsic properties of safety, low cost, ease of use, and wide availability, ultrasound imaging is the most commonly used clinical modality (Kaufmann and Lindner 2007; Reuveni et al. 2011). However, it is only recently that targeted ultrasound imaging, which uses an aptamer as the targeting ligand, has been reported. Nakatsuka et al.

engineered a novel aptamer-crosslinked microbubble which was designed to show ultrasound activation only at levels of thrombin associated with clot formation. The ex vivo experimental results indicated that these stimulus-responsive contrast agents can be useful probes for thrombosis imaging (Nakatsuka et al. 2012).

Superb tissue penetration and high quantitative capability afford radionuclide-based imaging techniques, such as SPECT or PET, with much higher clinical potential than other molecular imaging modalities (Massoud and Gambhir 2003). Labeling of aptamers with radioisotopes can result in valuable radiopharmaceuticals with promising applications. In the first study reporting on this technology, ^{99m}Tc -labeled aptamers were used for in vivo imaging of inflammation in a rat model. When compared with anti-body-labeled agents, the aptamer probe achieved a higher target-to-background ratio (fourfold) than that of antibody probe (threefold) (Massoud and Gambhir 2003). This difference was attributed to clearance of unbound aptamer probes, which is more rapid than that of antibody probes. Almost ten years later, another aptamer, named TTA1, which binds tightly to tenascin-C, an extracellular matrix protein overexpressed in many solid tumors, was prepared with a radiolabel for tumor imaging (Winnard et al. 2008). Biodistribution and imaging results indicated that ^{99m}Tc -labeled TTA1 exhibited rapid blood clearance with a circulation half-life of less than 2 min and tumor penetration when performed in several xenograft models. The durable tumor retention in combination with fast blood clearance yielded an excellent tumor-to-blood ratio, and various tumors were clearly visualized by planar scintigraphy.

Conclusions and Perspective

As a relatively new category of targeting ligands, aptamers have made a significant impact on clinical medicine. Current research and clinical translation efforts are focused on developing aptamers for therapeutics and molecular diagnostics. On the other hand, aptamers possess characteristics that are quite suitable to molecular imaging. Noninvasive molecular imaging of various biomarkers with aptamer-based probes has potential clinical applications in lesion detection, patient stratification, new drug development/validation, and therapy monitoring and dose optimization. However, it should be noted that the successful clinical translation of aptamer-based molecular imaging probes will also require the development of aptamers against disease markers that have high clinical significance.

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Fig. 1.

Schematic illustration of the cell-based aptamers selection. Briefly, the single-strand DNA(ssDNA) pool is incubated with target cells. After washing, the bound DNAs are eluted and then incubated with negative cells for counter-selection. After centrifugation, the supernatant is collected and the selected DNA is amplified by PCR. The PCR products are separated into ssDNA for next-round selection or cloned and sequenced for aptamer identification in the last-round selection. Reproduced with permission from (Shangguan et al. 2006). Copyright 2006 Proceeding of the National Academy of Sciences

Fig. 2.

Schematic illustration of the novel strategy for in vivo cancer imaging using activatable aptamer probe based on cell membrane protein-triggered conformation alteration. This activatable probe consists of three segments: a cancer-targeted aptamer sequence (A-strand), a poly-T linker (T-strand), and a short DNA sequence (C-strand) complementary to a part of the A-strand, with a fluorophore and a quencher attached at either terminus. In the absence of a target, the probe is hairpin structured, resulting in quenched fluorescence. When the probe is bound to membrane receptors of the target cancer cell, its conformation is altered, thus resulting in an activated fluorescence signal. Reproduced with permission from (Hui et al. 2011). Copyright 2011 Proceeding of the National Academy of Sciences

Fig. 3. Schematic illustration of the aptamer-conjugated AuNPs-based colorimetric assay. Before bind with target cells, aptamer-conjugated gold nanoparticles disperse in solution and display *red* color. Upon incubation with target cells, aptamers bind with membrane proteins of interest, leading aggregation of gold nanoparticles, as a result, the color of gold nanoparticles turns to *blue*. (Medley et al. 2008) (Color figure online)

Fig. 4. Schematic illustration of targeted photodynamic therapy system based on combination of UCNPs and aptamer-guided G-quadruplex DNA carrier. UCNPs convert near-infrared light to visible light, this visible light then stimulate the production of singlet oxygen by activating photosensitizer TMPyP4 (Yuan et al. 2013)

Fig. 5. Schematic illustration of construction of fluorescent DNA nanodevices on target living cell surfaces by in situ self-assembly of nanodevices. First, aptamers bind with membrane proteins on the cell surface. Addition of monomer strands M1 and M2 to culture medium, leading in situ self-assembly of nanodevices. FRET between intercalated and labeled dyes lightens cell membrane. M1, M2: monomer 1, 2 (Guizhi et al. 2013)

Fig. 6. Schematic illustration of SMNs for targeted chemotherapy and magnetic resonance imaging to cancer cells. *Left figure:* porous hollow magnetite nanoparticles modified with PEG ligands and aptamers for specific binding and uptake by cancer cell. Upon reaching the lysosomes, the relatively low lysosomal pH level results in the faster release of doxorubicin to kill the target cancer cells. *Right figure* T2*-weighted MRI images of SMNs, SMNs incubated with CEM cells (target cells), and SMNs incubated with Ramos cells (control cells). Reproduced with permission from (Chen et al. 2011). Copyright 2011 American Chemical Society

Fig. 7. Schematic illustration of the activation mechanism of the MnO_2 nanosheet-aptamer nanoprobe for fluorescence/MRI bimodal tumor cell imaging. The nanoprobe exhibits low fluorescence signal and MRI contrast in the absence of target cells. In the presence of target cells, binding of the aptamer to its target weakens the adsorption of the aptamer on the MnO_2 nanosheets, causing partial fluorescence recovery, illuminating the target cells and also facilitating the endocytosis of MnO_2 into target cells. The reduction of the endocytosed MnO_2 nanosheets by intracellular GSH can further activate the fluorescence signal and generate large amounts of Mn^{2+} ions suitable for MRI. Reproduced with permission from (Zhao et al. 2014). Copyright 2014 American Chemical Society