In Vivo Detection of Cancer Stem Cells by Dual Mode CT/Fluorescence Using Immunotargeted Nanoparticle Probes

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Statement of Purpose: The high mortality and poor prognosis for women diagnosed with ovarian cancer is mainly due to late diagnosis. Improved detection of primary tumors and recurring tumors after chemotherapy is, therefore, crucial to reduce ovarian cancer mortality and improve progression-free survival. However, current clinical screening and diagnostic imaging methods are limited by low sensitivity and/or specificity. Contrast-enhanced computed tomography (CT) and spectral (multi-energy) CT have the potential to enable molecular imaging with CT as a lower cost and higher resolution alternative to PET and MRI [1]. Therefore, we have developed a modular approach for the design and scalable synthesis of immunotargeted core-shell nanoparticle (NP) probes enabling bimodal imaging (e.g., fluorescence, CT).

Methods: Au@SiO_2 core-shell NPs (~10 nm Au core, 2-4 nm shell thickness, Fig. 1A) were prepared and loaded with fluorophores (CY5) for bimodal imaging by fluorescence and CT using previously established methods [2]. Antibodies were conjugated to the silica shell (Fig. 1B) to enable cell surface receptor targeting using CLICK chemistry [3]. *In vitro* immunotargeting was investigated by incubating Au@SiO_2(CY5)-anti-CD133 NPs with CD133(+) SKOV3-IP cells, which were imaged over 24 h using confocal fluorescence microscopy and flow cytometry [3]. Au@SiO_2(CY5)-IgG and Au@SiO_2(CY5) NPs were used as controls. *In vivo* immunotargeting was investigated in a murine xenograft model [4] of ovarian cancer using CD133(+) SKOV3-IP cells. The tumor site was imaged longitudinally over 48 h by *in vivo* CT and fluorescence. Tumor structure and CD133 expression levels were assessed in tumor explants by histology and immunohistochemistry (IHC). CD133(-) SKOV3-IP cells were used to generate control tumors to assess non-specific binding *in vivo*.

Results: Anti-CD133 was conjugated to Au@SiO_2 NPs with ~77% efficiency. Au@SiO_2(CY5)-anti-CD133 NPs exhibited immunotargeting to CD133(+) SKOV3-IP cells (Fig. 1C), which are known to be over-expressed in chemoresistant ovarian cancer tumors [5]. Flow cytometry revealed that ~15-16% of SKOV-3-IP cells overexpressed CD133. Quantitative fluorescence imaging confirmed that the CD133(+) cells were targeted *in vitro* with a specificity an order of magnitude greater than control cells. The intracellular distribution of NPs was characterized spatiotemporally at single NP sensitivity using confocal microscopy (Fig. 1C). After *i.v.* delivery, NPs exhibited >12 h of circulation in the blood pool of mice. CD133(+) xenograft tumors exhibited significantly greater X-ray contrast ($\Delta HU > 35$) compared with CD133(-) control tumors at 12-24 h after delivering the immunotargeted NPs (Fig. 1D). *In vivo* fluorescence and X-ray contrast at the tumor site appeared to be co-localized with clear delineation of tumor margins. Histopathology revealed the absence of metastatic sites. IHC confirmed overexpression of CD133 at select clusters in the tumor site.

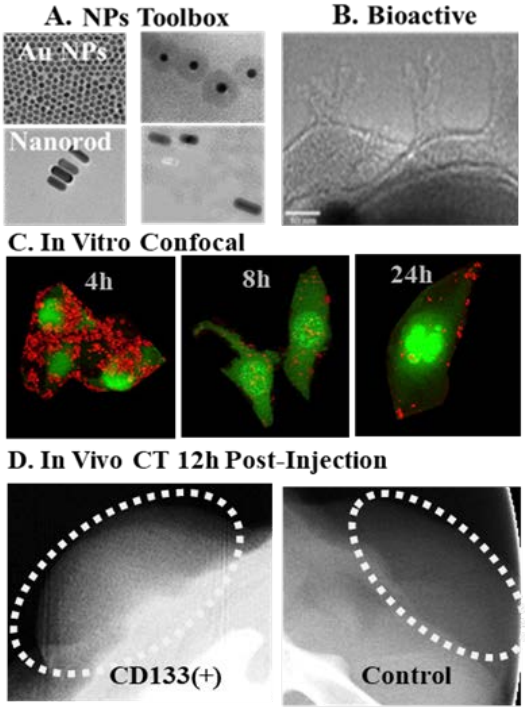

Figure 1. (A) TEM micrographs showing Au@SiO_2 core-shell NPs and **(B)** Negatively-stained TEM micrographs showing antibodies bioconjugated to Au@SiO_2 NPs. Scale bar = 5 nm. **(C)** Fluorescence images showing Au@SiO_2 NPs (red) targeted to CD133(+) SKOV3-IP cells *in vitro*. **(D)** *In vivo* micro-CT images showing enhanced tumor contrast due to Au@SiO_2 NPs targeted to xenograft CD133(+) tumors in mice

Conclusion: Au@SiO_2(Cy5)-anti-CD133 NPs exhibited immunotargeting and enabled contrast-enhanced detection of CD133(+) ovarian cancer cells by fluorescence and CT in both *in vitro* cell and *in vivo* tumor models. The modular approach used to assemble immunotargeted core-shell NPs can be readily extended to include other targeting molecules, therapeutic molecules, and imaging modalities.

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References:

1. Roeder RK, *et al.*, *Proc. SPIE*, 2017;10132:101320X.
2. Nallathamby PD, *et al.*, *J. Mater. Chem. B*, 2016;4:5418-28.
3. Huang T, *et al.*, *Anal. Chem.*, 2007;79:7708-18.
4. Bobbs A, *et al.*, *Canc. Growth Met.*,, 2015;8:29-36.
5. Sahlberg SH, *et al.*, *PLoS One*, 2014;9:e9462.1.