Molecular Imaging Evaluation of a Novel Claudin18.2 Specific Monoclonal Antibody Labeled with Radionuclide

Hua Zhu[1], Jin Ding[1], Xiaoyi Chong[2], Congcong Ji[2], Cheng Zhang[2], Fei Teng[3], Yi Gu[3], Xueming Qian[3], Zhi Yang[1], Lin Shen[2], Jing Gao[4]

[1]Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing, China; [2]Department of Gastrointestinal Oncology, Peking University Cancer Hospital & Institute, Beijing China; [3]Research, Mabspace Biosciences (Suzhou) Co. Ltd, Suzhou, China; [4]National Cancer Center/National Clinical Research Center for Cancer, Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen, China.

ABSTRACT

BACKGROUND: Claudin18.2 (CLDN18.2), a member of tight junction protein family, is strictly limited to differentiated epithelial cells of gastric mucosa and is overexpressed in multiple tumor types, such as gastric, esophageal and pancreatic cancers [1-2]. We have generated a novel species cross-reactive CLDN18.2 specific antibody, and labeled it with "Next Generation" radionuclide I-124 (^{124}I-18B10).

METHODS: I-124 was produced by the medical cyclotron using ^{124}Te (p, n) ^{124}I reaction. In the cell-based assay, the uptake of ^{124}I-18B10 in MKN45-CLDN18.2 (CLDN18.2+ cell line) and MKN45 (CLDN18.2- cell line) were detected at 10, 30, 60 and 120 min, and the blocking group using cold 18B10 antibody to block the uptake was also evaluated. PDX-bearing mice, which were selected by immunohistochemical (IHC) method and assessed as CLDN18.2+ or CLDN18.2-, were injected with either 18.5 MBq ^{18}F- fluorodeoxyglucose (^{18}F-FDG), or ^{124}I-18B10, or ^{124}I-hIgG via the tail vein, and Micro-PET/CT images were taken at 2, 60 and 120h post injection.

RESULTS: The specific activity of ^{124}I-18B10 was 0.62 mCi/mg antibody and the labeling rate was higher than 95%. The cell-based assay showed that specific uptake by the MKN45-CLDN18.2 cells was significantly higher than that of by the MKN45 cells (23.51±0.47 % vs 8.69±0.35 % at 2 h, P<0.05). Both uptake assay and competitive binding assay in the MKN45-CLDN18.2 cells showed that cold 18B10 antibody could significantly reduce the uptake and binding of ^{124}I-18B10 (15.33±0.82 % at 2 h, P<0.05). As expected, the uptake of ^{124}I-hIgG was low (5.21±0.29 % at 2 h). In PDX bearing mice, the uptake of ^{18}F-FDG in the tumor sites was low. The distribution of ^{124}I-18B10 in CLDN18.2+ PDX bearing mice was increasingly enriched in the tumor sites over time. The uptake signals of ^{124}I-18B10 in CLDN18.2- PDX bearing mice in all tissues and tumors remained similar at different time points.

Note: MKN45 was purchased from Chinese Academy of Medical Sciences; MKN45-CLDN18.2 was generated in house by transfection.

METHODS

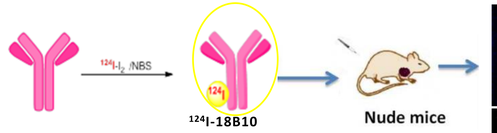

Figure 1. ^{124}I was labeled to CLDN18.2 targeted monoclonal antibody 18B10 by N-bromosuccinimide (NBS) reaction. After purification by PD-10 column, ^{124}I-18B10 was injected to PDX (gastric tumor) bearing mice. The biodistribution and accumulation of ^{124}I-18B10 probe was detected by Micro-PET imaging at different timepoints after injection.

RESULTS

Radiolabeling rate and radiochemical purity of ^{124}I-18B10

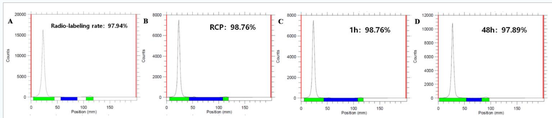

Figure 2. (A) Radiolabeling rate of ^{124}I-18B10 after purification (measured by Radio-TLC); (B) Radiochemical purity of ^{124}I-18B10 after purification (measured by Radio-HPLC); (C) Radiochemical purity of ^{124}I-18B10 after 1 h in PBS; (D) Radiochemical purity of ^{124}I-18B10 after 48 h in PBS.

Specific uptake of ^{124}I-18B10 in CLDN18.2+ cells

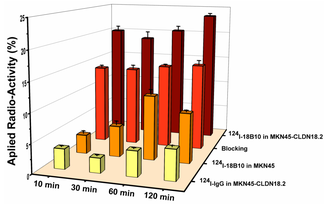

Figure 3. The uptake of ^{124}I-18B10 and its isotype control ^{124}I-IgG in MKN45-CLDN18.2 or MKN45 cells. Cells were cultured with 1mCi ^{124}I-18B10 or ^{124}I-IgG for 10 min, 30 min, 60 min and 120 min, respectively. In blocking group, 0.5 mg/well cold precursor 18B10 was added in addition to 1mCi ^{124}I-18B10. The uptake signals were measured by γ counter.

Biodistribution of ^{124}I-18B10 in normal mice

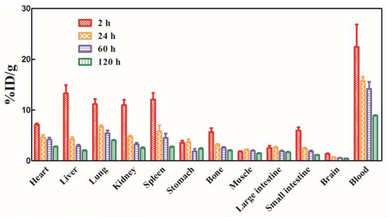

Figure 4. Biodistribution of ^{124}I-18B10 in normal mice. The organs were isolated at 2 h, 24 h, 60 h, 120 h after injection. After grinding, the uptake signals were collected and analyzed by γ counter.

Biodistribution and trend of ^{124}I-18B10 in PDX bearing mice

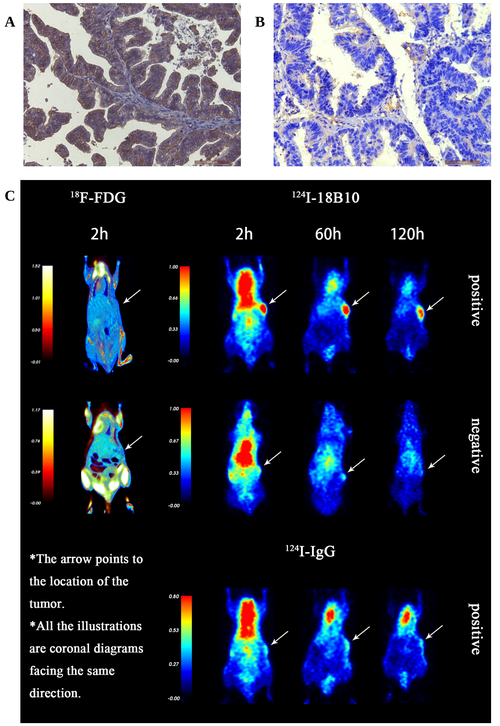

Figure 5. Micro-PET imaging of ^{124}I-18B10 and ^{124}I-IgG in PDX bearing mice. By immunohistochemical (IHC) method, a CLDN18.2+ PDX (intensity: 3+) and a CLDN18.2- PDX were selected for inoculation. (A) IHC photo of CLDN18.2+ PDX; (B) IHC photo of CLDN18.2- PDX; (C) Micro-PET imaging 2 h, 60 h and 120 h after ^{18}F-FDG, 18.5 MBq ^{124}I-18B10 and 18.5 MBq ^{124}I-hIgG injection. "positive" means CLDN18.2+ PDX, "negative" means CLDN18.2- PDX.

^{124}I-18B10 could be blocked by cold 18B10 in CLDN18.2+ PDX mice

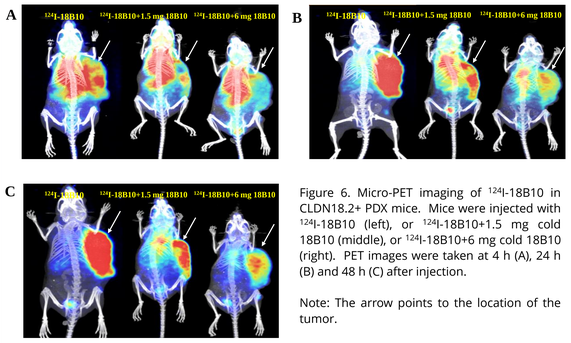

Figure 6. Micro-PET imaging of ^{124}I-18B10 in CLDN18.2+ PDX mice. Mice were injected with ^{124}I-18B10 (left), or ^{124}I-18B10+1.5 mg cold 18B10 (middle), or ^{124}I-18B10+6 mg cold 18B10 (right). PET images were taken at 4 h (A), 24 h (B) and 48 h (C) after injection.

Note: The arrow points to the location of the tumor.

^{124}I-18B10 was increasingly enriched at the tumor sites over time. At 24 h and 40 h after injection, high concentration and signal in tumor led to difficulty of complete blockade by cold precursor 18B10 at the tumor sites. Only partial blocking could be observed.

CONCLUSIONS

- The ^{124}I-18B10 antibody has excellent radio-chemical characteristics and stability.
- The antibody probe is shown highly specific to CLDN18.2 in both cell based uptake assay and competitive binding assay.
- Micro-PET images of PDX bearing mice showed that ^{124}I-18B10 was enriched in the lesion of CLDN18.2 positive tumors rather than negative tumors or normal tissues.
- The 18B10, a novel CLDN18.2 specific antibody, has great potential to be further developed as an imaging agent for CLDN18.2 positive cancer patients, indicated by the results of preclinical studies above.

REFERENCES

[1] Ö. Türeci et al. Gene 481 (2011) 83–92.
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Contact Author: Dr. Hua Zhu
Contact Email: zhuhuabch@pku.edu.cn