Radiosynthesis of A Novel Antisense Imaging Probe Targeting LncRNA HOTAIR in Malignant Glioma

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Research Article

Keywords: LncRNA HOTAIR, antisense oligonucleotide probe, malignant glioma, SPECT imaging

Posted Date: May 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-152169/v2

Version of Record: A version of this preprint was published at BMC Cancer on January 18th, 2022. See the published version at https://doi.org/10.1186/s12885-022-09170-7.

Abstract

Background: Long non-coding RNA (lncRNA) HOTAIR was manifested amplifying in many human carcinomas, which could serve as a useful target for cancer therapy. The ^{99m}Tc radiolabeled antisense oligonucleotides (ASON) could visualize the expression of HOTAIR and provide a diagnostic value for malignant tumors. The aim of this study was to radiosynthesis ^{99m}Tc with HOTAIR ASON and investigate the in vivo imaging in malignant glioma xenografts.

Methods: The ASON targeting lncRNA HOTAIR as well as mismatched ASON probe (ASONM) were designed and modified. The radiolabeling of ^{99m}Tc with two probes were via the conjugation of bifunctional chelator HYNIC. Then probes were purified by Sephadex G25 and tested for their radiolabeling efficiency and purity, as well as stability by ITLC and gel electrophoresis. Then the radiolabeled probes were transfected with lipofectamine 2000 for cellular uptake test and the next experimental use. Furthermore, biodistribution study and SPECT imaging were performed at different times after probes were intravenously injected in glioma tumor-bearing mice models. All data were analyzed by statistical software.

Results: The labeling efficiencies of ^{99m}Tc-HYNIC-ASON and ^{99m}Tc-HYNIC-ASONM measured by ITLC were (91 ±1.5) % and (90 ±0.6) %, respectively, and the radiochemical purity were more than 89%. The probes showed good stability within 12 hours. Gel electrophoresis confirmed that the oligomers were successfully radiolabeled no significant degradation was found. Cellular uptake experiment showed that liposomes had the ability to carry probes into cells. Biodistribution study demonstrated that liposome coated ^{99m}Tc-HYNIC-ASON had significantly higher uptake in the tumor and higher T/M ratio than the mismatched group. Meanwhile, the tumor was clearly shown at 1-hour post probe injection of liposome coated ^{99m}Tc-HYNIC-ASON on SPECT/CT imaging, no tumor was seen in mismatched and blocking group.

Conclusion: The liposome encapsulated ^{99m}Tc-HYNIC-ASON probe can be used in the in vivo, real-time imaging of lncRNA HOTAIR expression in malignant glioma.

Background

Glioma is the most common proliferative and reversible primary brain tumor in human beings[1]. Approximately 80% of the malignant tumors of the central nervous system are gliomas[2]. Although treatment approaches such as surgery, radiotherapy, and chemotherapy have been widely used, the median survival time of glioma patients is still limited to about 14 months[3] [4]. Therefore, new and more effective approaches are urgently needed. LncRNA, a kind of mRNA-like transcripts as 200 nt to 100 kilobases (kb), have no protein-coding potential however its roles in gene transcription and translation, heredity, and epigenetic regulation in tumors has gained increasing attention in recent years[4]. It was found that LncRNA is widely expressed in human cancers, indicating that it may be associated with cancer onset and progression[5]. HOTAIR, as the first lncRNA with trans-regulatory function, is

overexpressed in glioma and silencing tumor suppressor genes by mediates recruitment of polycomb repressive complex 2 (PRC2) [6] [7]. previous literature reported that HOTAIR can affect the cell cycle, and the decrease of HOTAIR expression will lead to a significant increase in G0/G1 phase cells[3]. Meanwhile, HOTAIR is positively correlated with tumor cell proliferation, survival, invasion, and resistance to treatment, via molecules such as chromatin modifiers, ubiquitin ligases, and miRNAs[8]. These findings suggest that the high expression of HOTAIR is a biomarker of cancer diagnosis, metastasis, drug resistance, and poor prognosis. The fact that HOTAIR is stable and measurable in body fluids has diagnostic and prognostic value for glioma[9] [10].

Antisense imaging is an attractive and non-invasive method for detecting the expression of HOTAIR in tumors. It visually represents, characterizes, and quantifies biological processes at the cellular or subcellular level by linking radionuclides to targeted antisense oligonucleotides[11] [12].

Antisense oligonucleotide (ASON) technique uses a completely (or nearly complete) complementary single strand of 15 to 25 nucleotides that binds to the target RNA to produce DNA RNA heteroduplex, which can lead to the silencing of the target gene or interfere with the processing or translation of RNA[13, 14]. Preclinical studies have shown that after chemical synthesis and modification of ASON, the nuclease degradation rate is decreased, while enhanced the affinity of plasma binding protein, the tissue biological distribution is rapid, thus makes ASON a good biological effect and stability[15, 16], which can be used as a feasible human treatment strategy. As a molecular imaging technology, It has attracted much attention because of its high sensitivity, high resolution, and short acquisition time and detects the dynamic changes of tumor-related molecules in real-time, so it also has a great application prospect in early diagnosis [17].

Considering the role of HOTAIR in tumorigenesis and treatment, as well as the efficiency, specificity, and irreversibility of antisense oligonucleotides binding to the target, the objective of the present study was to explore the ^{99m}Tc labeled antisense oligonucleotide probe targeting HOTAIR mRNA, evaluate its characteristics in vitro, and observe whether it can be used in the imaging of gliomas.

Materials And Methods

The 19-base oligonucleotide probes were chemically synthesized and modified from Shanghai Shangon Bioengineering Co., Ltd. The ASON sequence was designed as 5'- AATTCTTAAATTGGGCTGG -3', which was completely complementary to the HOTAIR fragment, and the ASONM□mismatched antisense oligonucleotides□ sequence was 5'-AATACTTAGATTAGGCAGG-3' (The underlined part is the substituted nucleosides). Two probes were modified with 2' methylation (2'-O-methyl) at both ends of the sequence and two bases at each end were phosphonothioate modified to improve its stability, NH_2C_6 is connected to 5’ end. Ultimately, the synthetic structure was 5’-NH_2C_6-ASON/ASONM-3'. ^{99m}Tc is obtained from the ^{99m}Tc radionuclide generator produced by China Atomic Energy Research Institute.

Synthesis and labeling of the probe

0.2mg ASON dissolved in 50μL buffer (2mol/l NaCl, 0.5mol/l $NaHCO_3$, 2mmol/l EDTA), HYNIC (TriLink, US) dissolved in DMF solution (10mg/mL). Then mixed at a molar ratio of 25:1(HYNIC: ASON) and avoid light for 1 h. Then the mixture above were added with 60% methanol to the total volume of 500μL, using an ultrafilter tube (Sartorius, GER) 13000g to centrifuge for 10min (ensure that the volume after centrifugation was less than 50μL) to obtain HYNIC-ASON. Next, 100μL Tricine(100mg/mL), 20μL ^{99m}Tc(222MBq) as well as 4μL fresh $SnCl_2$•$2H_2O$ (1mg/mL) were added to the above HYNIC-ASON in turn and reacted for 60min. After the reaction, using Sephadex G25(GE, US) to separate and purify. 15 tubes of eluents were collected with 5 drops per tube, then measured the radioactivity counts and nucleic acid concentration of each tube. Take the peak tube for the following up experiment.

Serum stability

Fresh human serum is provided by volunteers in our department. The institutional review committee of General Hospital of XXX approved the study and all the volunteers obtained informed consent. ^{99m}Tc-HYNIC-ASON was incubated in saline and fresh human serum at 37℃ and room temperature, respectively (the volume ratio of the probe to serum/saline was 1:1). The radiochemical purity was detected by thin layer paper chromatography (ITLC) at 0, 2, 4, 6, 8, 12 h.

Agarose gel electrophoresis

To identify the integrity of ^{99m}Tc-HYNIC-ASON and eliminate the degradation after labeling. 1% agarose gel was configured, followed by an unbonded ASON sample, ^{99m}Tc, ^{99m}Tc-HYNIC-ASON before and after purification. The voltage was 120V, electrophoresis for 20 minutes, then the band was observed under UV.

Cell culture and transfection

U87 glioma cells were purchased from the Chinese Academy of Sciences, and cultured in DMEM (Invitrogen, US) medium containing 15% fetal bovine serum（ABW,CHN） and 1% antibiotics in a CO2 incubator at 37 ℃ for 24 hours, then passaged when the cell density reached 90%.

For transfection (Lip-^{99m}Tc-HYNIC-ASON and Lip-^{99m}Tc-HYNIC-ASONM). Liquid A: 10μg purified ^{99m}Tc-HYNIC-ASON/ ^{99m}Tc-HYNIC-ASONM were added to 500μL DMEM without serum and antibiotics; Liquid B: 25μL Lipofectamine 2000 (Invitrogen, US) were added to 475μL DMEM, and Liquid A and B were placed at room temperature for 5min respectively, then mixed two of them for 20min. After added Lip-^{99m}Tc-HYNIC-ASON/ Lip-^{99m}Tc-HYNIC-ASONM to the cells and cultured in a 37℃ incubator for 6 hours, the medium was replaced by DMEM containing 15% fetal bovine serum for 24-48 hours to complete transfection.

Cellular uptake

U87 cells were inoculated in a 12-well plate at 5×10^5 density and cultured overnight in DMEM containing 15% FBS without antibiotics. The cells were divided into liposome transfected and non-transfected

groups. In the transfection group, 200μL DMEM, 500ng ^{99m}Tc-HYNIC-ASON or ^{99m}Tc-HYNIC-ASONM (37kBq) and 3μL Lipofectamine 2000 were added into each well; 500ng ^{99m}Tc-HYNIC-ASON or ^{99m}Tc-HYNIC-ASONM (37kBq) in the non-transfection group. After cultured in a 37 ℃ incubator, each well of culture medium was collected at 0.5h,1h, 2h, 4h, and 6h, respectively, and washed three times with 100μL PBS collected into the same EP tube labeled Cout. Then the cell was collected with trypsin containing EDTA, 100μL PBS in each well was washed three times to EP tube labeled Cin. The radioactivity counts of Cin and Cout were measured by γ radioimmunoassay counter (Chinese Academy of Metrology), and the uptake rate of cells to the probe at each time was calculated. Calculation formula: cell uptake rate = Cin/(Cin+Cout) × 100%.

Animal xenograft model

BALB/c nu/nu mice (female, weight ±SD, 206.0g, age 3~4wk) were fed in the Experimental Animal Center of XXX. Malignant glioma U87 cells (5×10^{11}) were subcutaneously injected into the right fore axilla of each mouse. When the tumor reached 1.5-2.0cm, it was used in the follow-up experiment. All animal experiments have passed the ethical review of the Animal Experimental Center of XXX. and are conducted under the guidelines of the Animal Welfare Committee.

Biodistribution studies

Twenty nude mice were randomly divided into 5 groups with 4 mice in each group. Lip-^{99m}Tc-HYNIC-ASON 1μg, 2.59MBq (100μL) was injected into the tail vein. Then the mice were killed by cervical dislocation at 1, 2, 3, 4 and 6 hours after 100μL of blood was taken from the ophthalmic vein. After that, tissues like the heart, liver, spleen, kidney, stomach, small intestine, bladder, muscle, bone, and tumor were removed and weighed, then radioactivity count was measured. The distribution results were recorded as the percentage of radioactivity per gram of tissue (% ID/g).

SPECT imaging

Images were performed by a SPECT scanner at 1h, 2h, 4h, 6h, and 8h respectively after 4μg, 14.8MBq (150μL) Lip-^{99m}Tc-HYNIC-ASON/ Lip-^{99m}Tc-HYNIC-ASONM probe were injected into the tail vein. For blocking groups, 10μg liposome transfected unlabeled probes were injected 2 hours before Lip-^{99m}Tc-HYNIC-ASON injection. The collection counts were 100 KCounts and stored as a 64×64 matrix at 3.2 zooms, then T/M (Tumor/Muscle) and T/A (Tumor/Abdomen) ratio of regions of interest were calculated.

Statistical analysis

All data are processed by SPSS22.0 statistical software, variables are represented by $\bar{\chi}$ ±SD. T test was used for comparison between the two groups. $P<0.05$ is considered to be statistically significant.

Results

Synthesis and labeling of the probe

ITLC paper chromatography used saline as the developer, the radioactivity of the antisense probe before and after purification remained in the same position (1.18 Minutes) (Fig 1). The labeling rate of ^{99m}Tc-HYNIC-ASON and ^{99m}Tc-HYNIC-ASONM measured by ITLC were (91±1.5) % and (90±0.6) %, respectively, and both radiochemical purities were more than 89% after purification.

Radioactivity counts and nucleic acid concentration

In Fig 2b, the unbonded ASON fragments were filtered out by 1-4 tubes, and the eluent of 4-8 tubes were ^{99m}Tc-HYNIC-ASON, which coincided with the peak of radioactivity counts in Fig 2a.

Serum stability and agarose gel electrophoresis

The radiochemical purity of ^{99m}Tc-HYNIC-ASON reached more than 80% in 12 hours, and there was no significant difference between saline and fresh human serum (Fig 3a). Figure 3b shows brighter bands before and after purification, indicating that the oligonucleotide probe has integrity and there is no obvious degradation, no band was seen in ^{99m}Tc control group. The results showed that ^{99m}Tc-HYNIC-ASON had good tolerance and stability in serum at 37 ℃, which was similar to that in vivo.

Cellular uptake

At each time point, the uptake rate of transfection groups was higher than that of the non-transfection group and reached the peak at 2 h. The cellular uptake of Lip-^{99m}Tc-HYNIC-ASON at 2 h was 3.0%, while that of Lip-^{99m}Tc-HYNIC-ASONM was only 0.6%（P<0.01）(Fig 4).

Biodistribution studies

Lip-^{99m}Tc-HYNIC-ASON and mismatched probes had similar biological distribution after injection (listed in Tables 1 and 2). The two probes were mainly concentrated in the kidney and bladder, followed by the stomach and small intestine, indicating that the probes were cleared through the urinary and digestive systems. Secondly, for the organs with a rich blood supply, such as the heart, liver, spleen, and lung, the uptake of the two probes decreased gradually due to the effect of blood clearance. While the uptake rate of the probe by other organs such as skeletal muscle and bone is low. With the extension of time, after injection of Lip-^{99m}Tc-HYNIC-ASON, the radioactivity in the kidney decreased rapidly, from (6.25 ±1.31) % to (2.63 ±0.55) %, while the radioactivity in the tumor decreased slowly in 8 hours, from (1.73 ±0.13) % to (0.58 ±0.1) %. The results suggested that the Lip-^{99m}Tc-HYNIC-ASON targeted probe can be specifically aggregated in the tumor, which was also confirmed by the ratio of tumor to non-tumor (T/NT) of Lip-^{99m}Tc-HYNIC-ASON (Fig 5).

SPECT imaging

The static images were obtained within 8 hours after injection. According to the prediction of the experimental results of biological distribution, radioactivity is mainly concentrated in the abdomen. The tumor could be clearly demonstrated by SPECT imaging 1 hour after injection of Lip-^{99m}Tc-HYNIC- ASON, and the best acquisition time was at 2 hours post-injection. In contrast, the tumor was not shown within 8 hours after injection of the mismatched probe group and blocking group (Fig 6). The ratio of T/M and T/A in the antisense group was significantly higher than that in the mismatched group. 2h after injection, the maximum T/M ratio in the antisense group was 4.77, while that in the mismatched group was only 2.59. (Fig 7).

Discussion

With the successful application of antisense oligonucleotide technique combined with radionuclide tracer technique, precise tracking method at the molecular level is used for tumor localization diagnosis and therapy. In the present study, we successfully labeled antisense oligonucleotide probe targeting LncRNA HOTAIR with ^{99m}Tc to track the expression of HOTAIR in glioma cells. We found that, in the labeling experiment, the chemically synthesized and modified probes can successfully connect with ^{99m}Tc and obtain a higher labeling rate; At the cellular level, liposomes can effectively assist the probe to enter tumor cells; From imaging results, antisense probes can detect specific radioactivity uptake in the tumor site, while no radioactivity uptake was found in the mismatched group.

In this study, we try to adjust the ratio of HYNIC to ASON (25:1) and replace the buffer solution (2mol/l NaCl, 0.5mol/l $NaHCO_3$, 2mmol/l EDTA) that dissolves ASON to improve the labeling rate. The effect is obvious. The average labeling rate of the antisense probe and the mismatched probe is more than 90%, but the radiochemical purity is lower than that of other literature[18, 19].

One of the main factors affecting the binding of antisense probes is the low intracellular uptake of radiolabeled ASON probes [20]. In this study, liposomes were selected as carriers. As a cationic carrier, liposomes bind to anion oligomers by simple charge attraction, so that lipophilic oligonucleotides can easily pass through the cell membrane, thus the increase of antisense oligonucleotides can be observed in cells[21]. In this study, we compared the differences of the two probes between liposome transfection and non-transfection, we found that the uptake rate of the transfected antisense probe in tumor cells was the highest which indicated that the antisense probe sequence was specifically targeted. Meanwhile, this metastasis is not a one-way street, as the mismatched probes encapsulated by liposomes do not bind specifically, while it does not accumulate a large amount of radioactivity in the cells can explain this problem.

According to the biological distribution study, the antisense probe has specific uptake in the tumor, meanwhile, the most radioactivity is accumulated in the kidney and bladder, indicating that the probe is mainly excreted through the urinary and digestive system. However, there is not much radioactivity accumulation in the liver, which is different from other reports that the liver has the highest radioactivity uptake in all organs [11] [12] [19] [22]. The reason might be that the molecular of Lip-^{99m}Tc-HYNIC-ASON

probe is small and does not need to be digested by macrophages when passing through the liver, hence, caused a short retention time and mild potential toxicity to the liver.

In the imaging experiment, the tumor was clearly visualized in the antisense group, and there is a large amount of radioactivity in the abdomen and bladder, which is consistent with the biological distribution. No tumor imaging was seen in the blocking group within 8 hours, indicated that unlabeled ASON could block the binding of ^{99m}Tc-HYNIC-ASON to HOTAIR, and the binding was specific. The fact that there is no imaging in the mismatched group also illustrates this problem.

In summary, these results demonstrated that ^{99m}Tc-HYNIC-ASON can be successfully synthesized and used for glioma-specific imaging via transfected with lipofectamine. However, there are still some problems in this study that need to be addressed in future investigations. First of all, the high uptake of radioactive probes in the bladder and kidney will affect the image quality and blur the display of the tumor. The application of furosemide, a highly effective diuretic that has a strong diuretic effect that can dilate the blood vessels of the kidney quickly and briefly, may be helpful to accelerate the excretion of radioactivity. Secondly, the higher uptake of the stomach and small intestine, which will cause radioactive damage to tissue and lead to dysfunction, so we speculate whether the Furosemide drugs could help to reduce higher uptake and mucosal radioactive damage.

At the same time, there are some limitations and shortcomings. First of all, by trying to change the volume of the solution, the concentration of $SnCl_2 \cdot 2H_2O$ and the reaction time, the radiochemical purity of the probe is lower than that of other experiments. Secondly, Zhang et al [23] suggested that cationic liposomes should be used cautiously when delivering ASON to mammalian cells, because cationic liposomes can activate macrophages to produce NO and TNF- α, which will be toxic to phagocytes. Therefore, the antisense probe Lip-^{99m}Tc-HYNIC-ASON in this study is not suitable for further clinical transformation and human experiments. At the same time, this experiment lacks untransfected antisense probes for comparison. Saleem et al. [24] have successfully studied the biodistribution of ASO in tumors and normal tissues of cancer patients by using ^{11}C-labeled LY2181308, which is specially developed to inhibit Survivin inhibitor of apoptosis protein. Therefore, the next step of this study is to observe whether the untransfected antisense probe ^{99m}Tc-HYNIC-ASON can accumulate in the tumor site, then we can make a decision on whether the antisense probe can be used in human experiments.

Conclusion

The liposome coated ^{99m}Tc-HYNIC-ASON probe can be used for real-time imaging of lncRNA HOTAIR expression in malignant gliomas in vivo. The probe has good stability and targeting ability, which is a new type of non-invasive probe.

Declarations

1. **Ethics approval and consent to participate**

Approval for animal experiments

BALB/c nu/nu mice (female, weight ±SD, 206.0g, age 3~4wk) were purchased and fed in the Experimental Animal Center of Ningxia Medical University. All animal experiments have passed the ethical review of the Animal Experimental Center of Ningxia Medical University and are conducted in accordance with the guidelines of the Animal Welfare Committee. All the experimenters follow the ARRIVE guidelines. The experimental operation is described in detail in the method section of the article. All animals were killed by cervical dislocation.

Human Subjects

The institutional review committee of the General Hospital of Ningxia Medical University approved the study. The human serum came from volunteers, and all the volunteers obtained informed consent. All experiments involving humans are carried out in accordance with the Helsinki Declaration.

2. **Consent for publication**

Not applicable

3. **Availability of data and materials**

All data generated or analysed during this study are included in this published article [and its supplementary information files]**.**

4. **Competing interests**

There are no conflicts of interest to disclose to this article.

5. **Funding**

This study was supported by the National Natural Science Foundation of China (NSFC 81560292).

6. **Authors' contributions**

QZ designed the experiment. Material preparation, data collection were performed by JYR □XYZ□JC□JLT□ JL□YPY. Analysis were performed by QZ□JYR □FKW. The first draft of the manuscript was written by JYR. Final approval of the version to be published was performed by QZ, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

7. **Acknowledgements**

We thank He Yong and Li Chongjiao from the people's Hospital of Wuhan University for their help.

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Tables

TABLE 1. Biodistribution (%ID/g) of Lip-^{99m}Tc-HYNIC-ASON in Tumor-Bearing Mice					
Tissue	1h	2h	3h	4h	6h
Heart	1.68±0.38	1.73±0.33	1.32±0.1	0.82±0.15	0.59±0.21
Blood	1.37±0.5	0.81±0.58	0.56±0.4	0.66±0.17	0.18±0.27
Liver	1.22±0.42	1.28±0.52	0.83±0.32	0.88±0.08	0.61±0.29
Spleen	0.61±0.05	0.45±0.05	0.38±0.14	0.29±0.04	0.21±0.04
Lung	1.5±0.24	1.37±0.35	1.01±0.37	0.58±0.08	0.37±0.22
Kidney	6.25±1.31	5.36±1	5.38±1.52	3.95±0.67	2.63±0.55
Stomach	2.3±1.29	2.86±0.46	2.7±0.57	1.46±0.14	1.31±0.43
Small intestine	3.43±0.82	3.17±0.35	3.06±0.82	2.21±0.66	2.14±0.67
bladder	11.89±0.9	11.7±0.75	9.81±1.62	9.12±1.66	8.75±0.95
Skeletal muscle	0.81±0.06	1.73±0.23	2.68±2.35	1.01±0.48	1.76±2.37
Bone	0.63±0.32	1.96±1.53	0.46±0.32	0.87±0.6	0.66±0.6
Tumor	1.73±0.13	1.22±0.1	1.06±0.22	0.82±0.22	0.58±0.1
Each value represents average of 4 mice ± SD.					

TABLE 2. Biodistribution (%ID/g) of Lip-^{99m}Tc-HYNIC-ASONM in Tumor-Bearing Mice		
Tissue	2h	4h
Heart	0.31±0.13	0.35±0.16
Blood	0.03±0.01	0.06±0.04
Liver	0.52±0.25	0.6±0.13
Spleen	0.19±0.1	0.31±0.07
Lung	0.43±0.38	0.54±0.14
Kidney	3.21±0.54	2.73±0.48
Stomach	0.42±0.18	0.64±0.19
Small intestine	0.21±0.1	0.22±0.13
bladder	2.62±3.82	1.26±0.77
Skeletal muscle	0.2±0.13	0.45±0.13
Bone	0.31±0.14	0.3±0.19
Tumor	0.43±0.28	0.3±0.1
Each value represents average of 4 mice ± SD.		

Figures

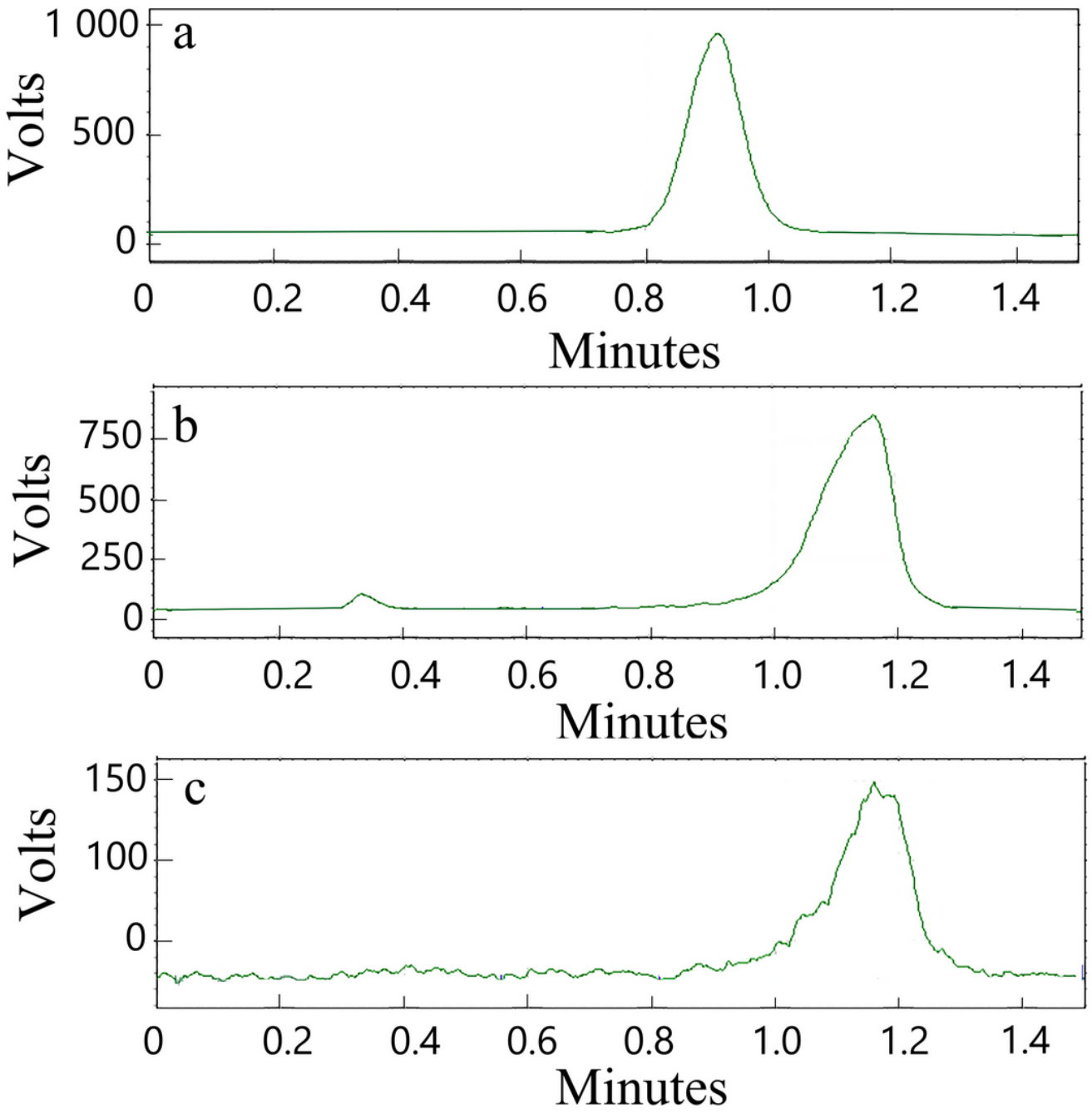

Figure 1

ITLC retention time of 99mTc (a); ITLC retention time of 99mTc-HYNIC-ASON before (b)and after purification by Sephadex G25 (c).

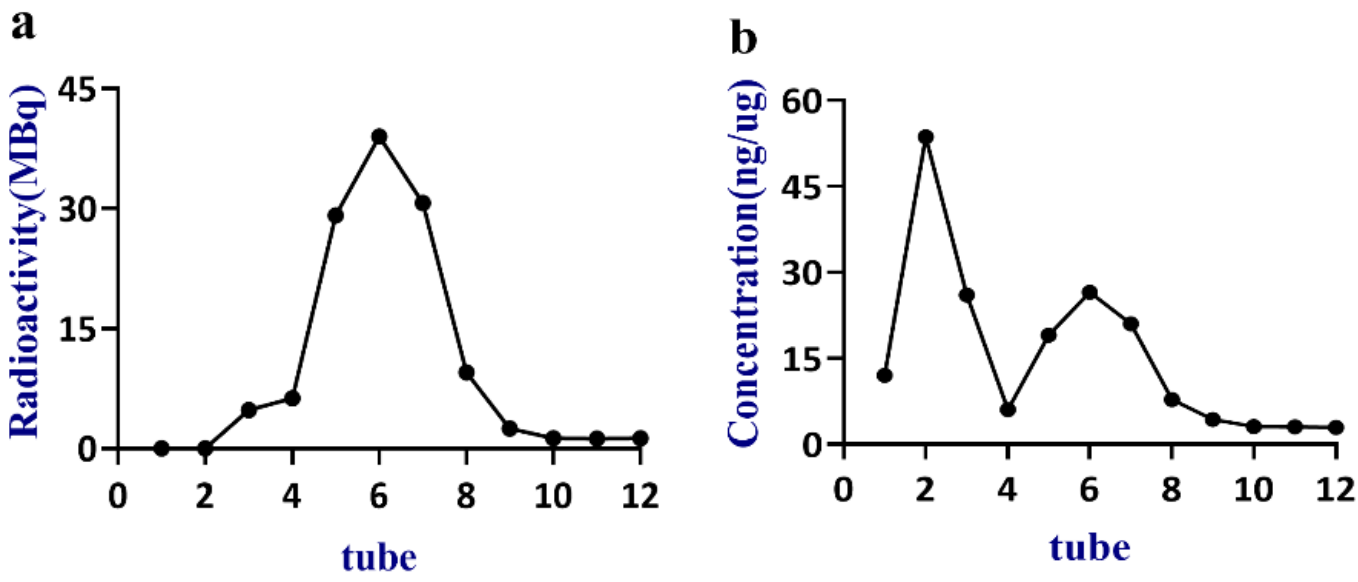

Figure 2

The radioactivity count (a) and nucleic acid concentration (b) of each tube of 99mTc-HYNIC-ASON purified by Sephadex G25.

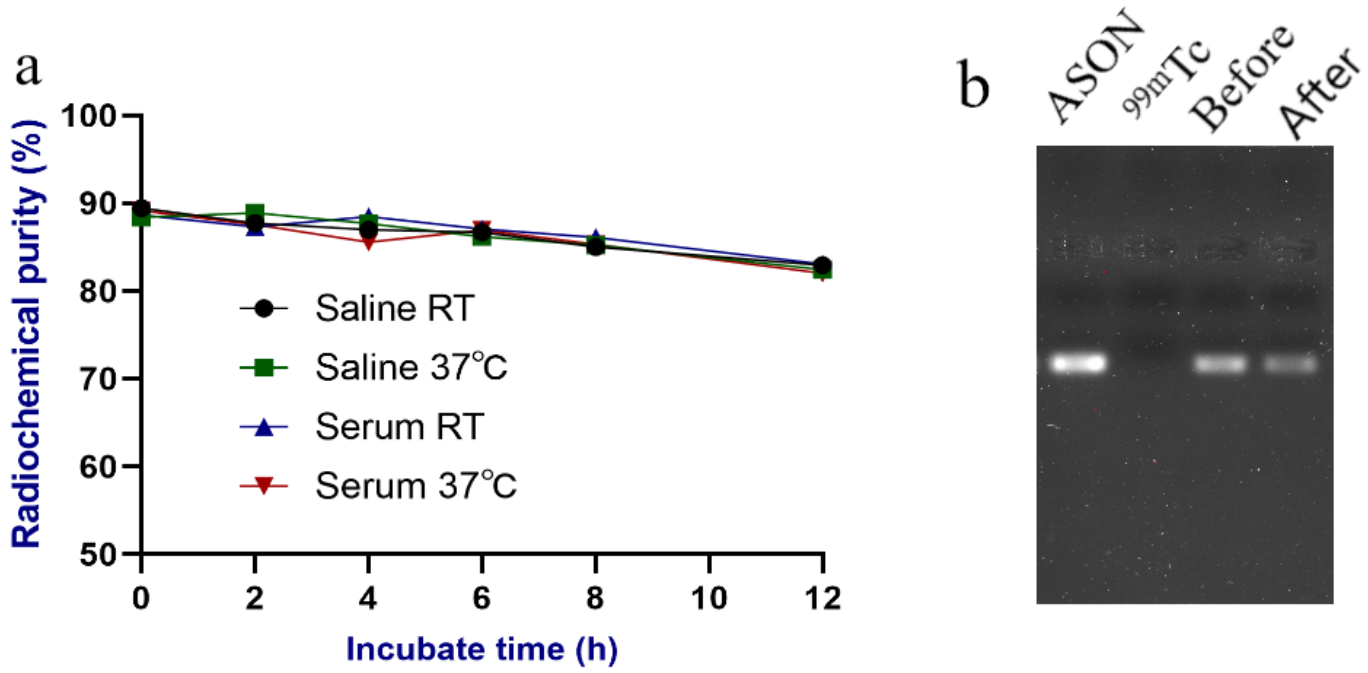

Figure 3

Probe stability identification. Radiochemical purity of 99mTc-HYNIC-ASON incubated in saline and fresh serum during 6h, at room temperature (RT) and 37°C(a). The probe integrity was detected by agarose gel

(from left to right: ASON sample, 99mTc, 99mTc-HYNIC-ASON before purification, 99mTc-HYNIC-ASON after purification)(b). (full-length gel is presented in Supplementary Figure 1.)

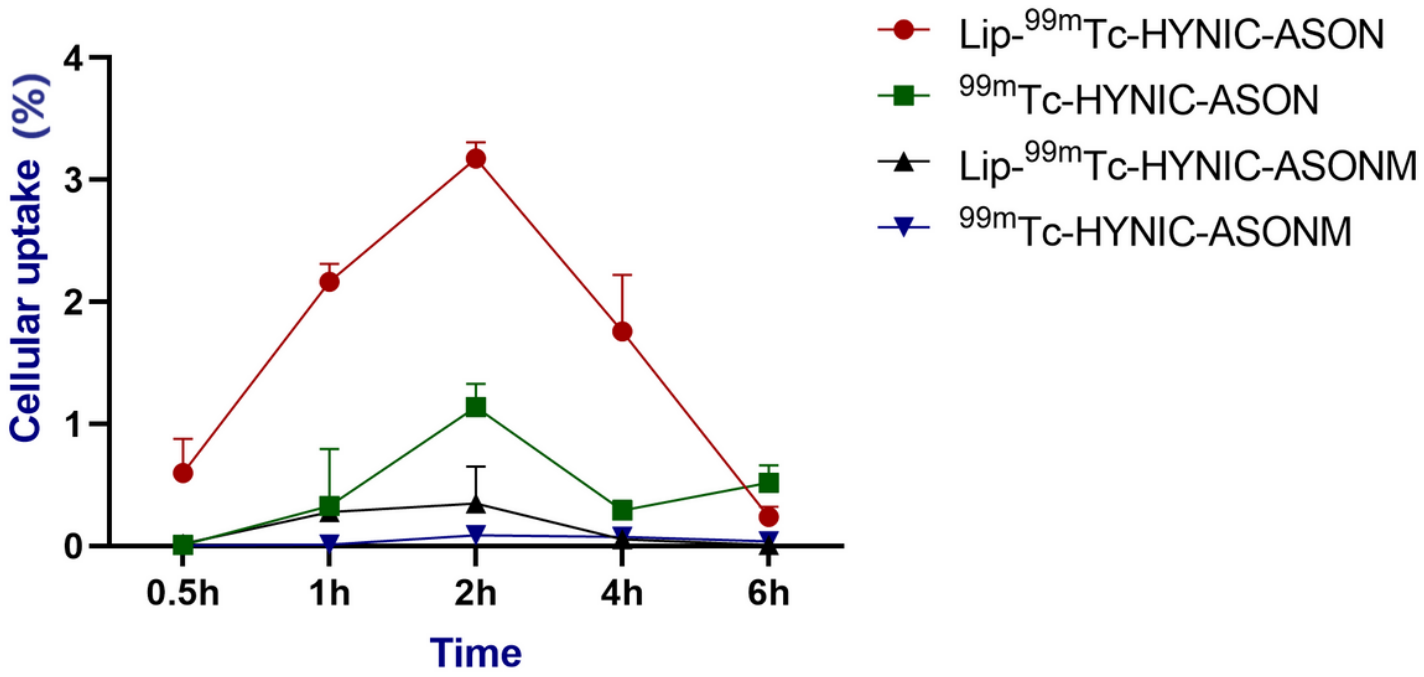

Figure 4

The in vitro cell uptake rates of 99mTc-HYNIC-ASON and mismatch groups with or without transfection at 30min, 1h, 2h, 4h and 6h .

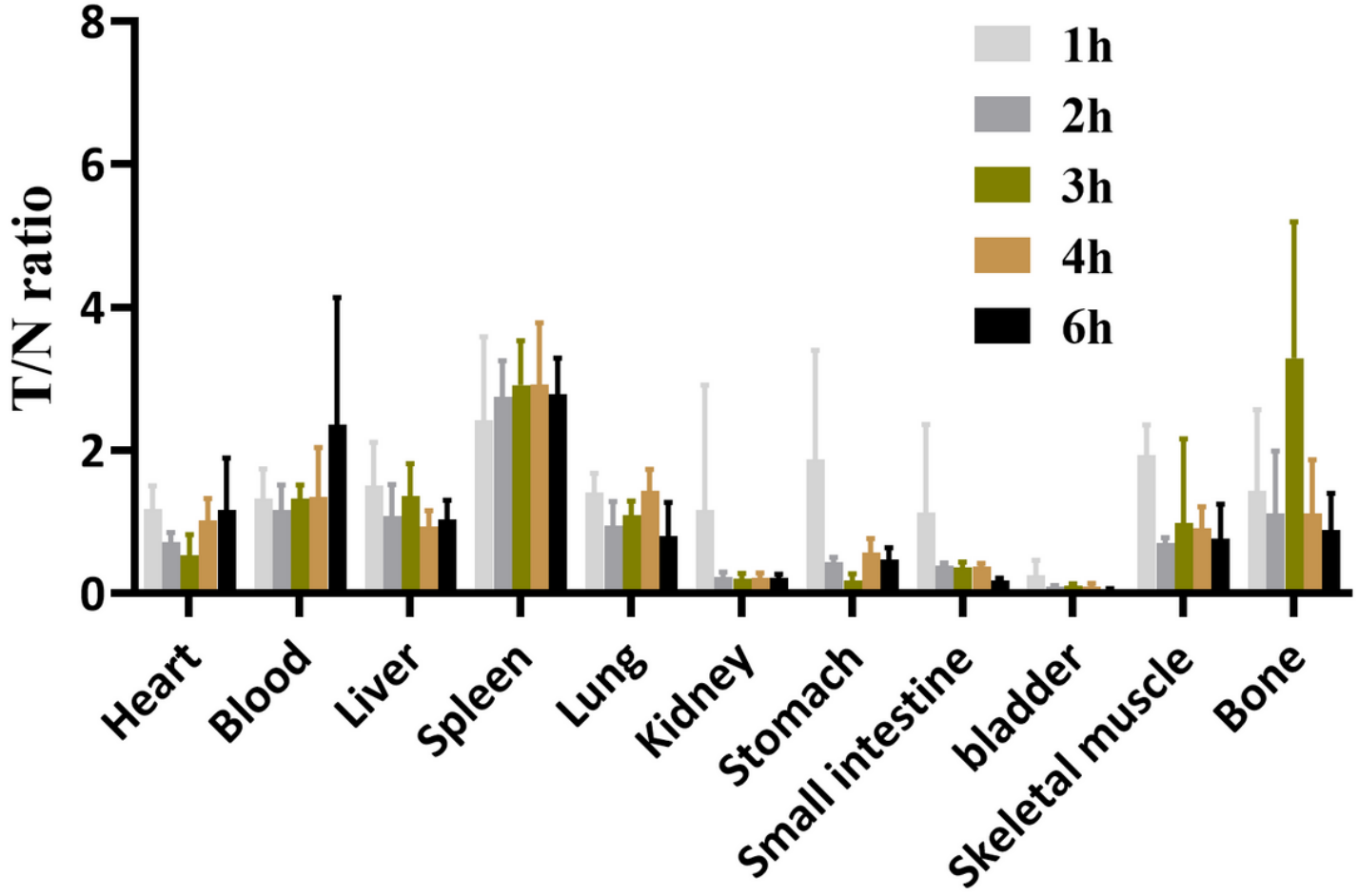

Figure 5

T/NT(Tumor and Non tumor tissues) ratio of Lip-99mTc-HYNIC-ASON in different tissues of glioma nude bearing mice xenografts at 1, 2, 3, 4, and 6 h after injection.

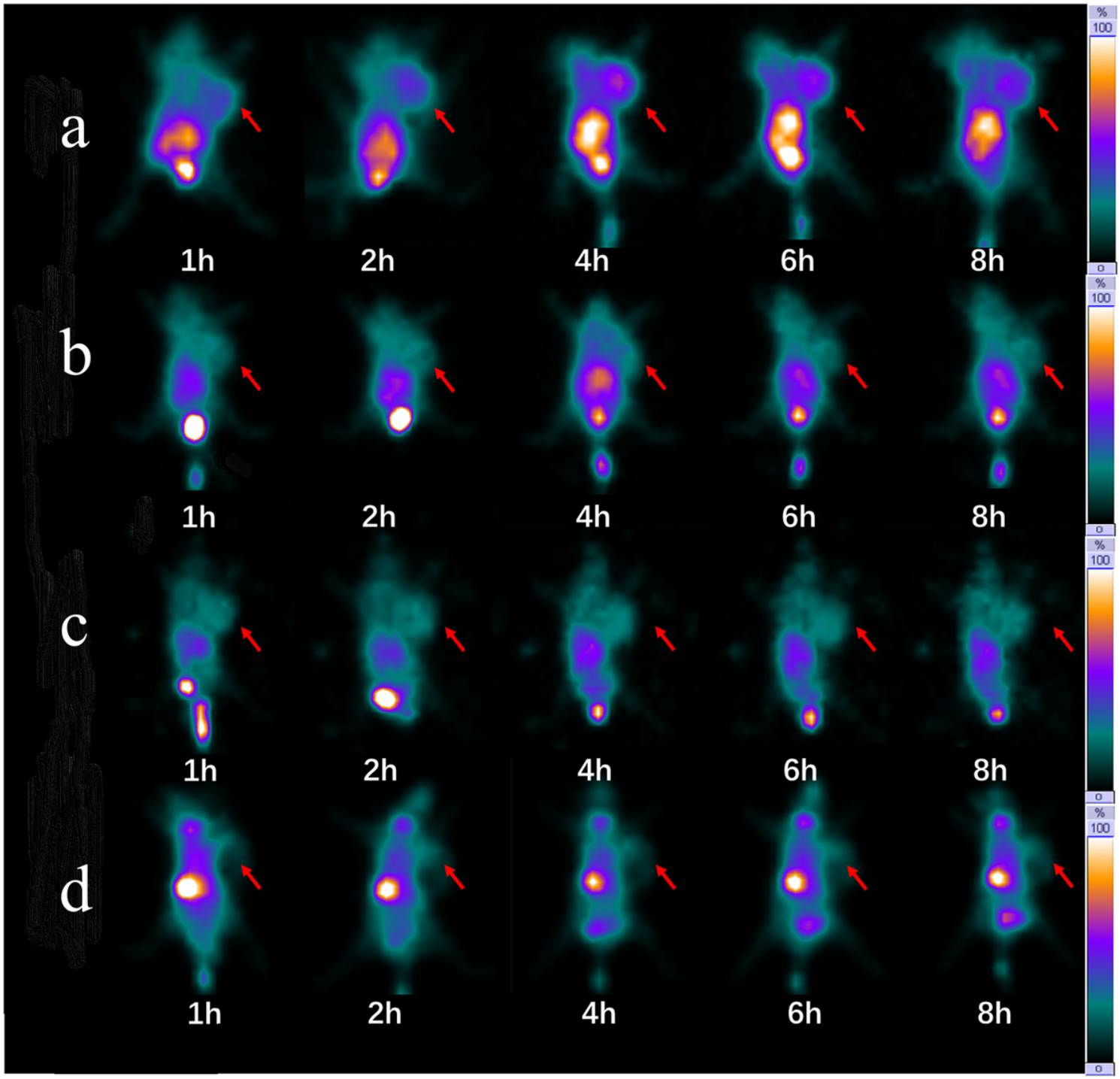

Figure 6

SPECT Imaging at 1 h, 2 h, 4 h, 6 h and 8 h after injected to glioma nude bearing mice xenografts. a: Lip-99mTc-HYNIC-ASON; b: Lip-99mTc-HYNIC-ASONM; c: Blocked; d: Tc-control. Tumor (arrow) was clearly visualized after injected with Lip- 99mTc-HYNIC-ASON, while is not visualized in mismatched□Blocked as well as Tc-control group.

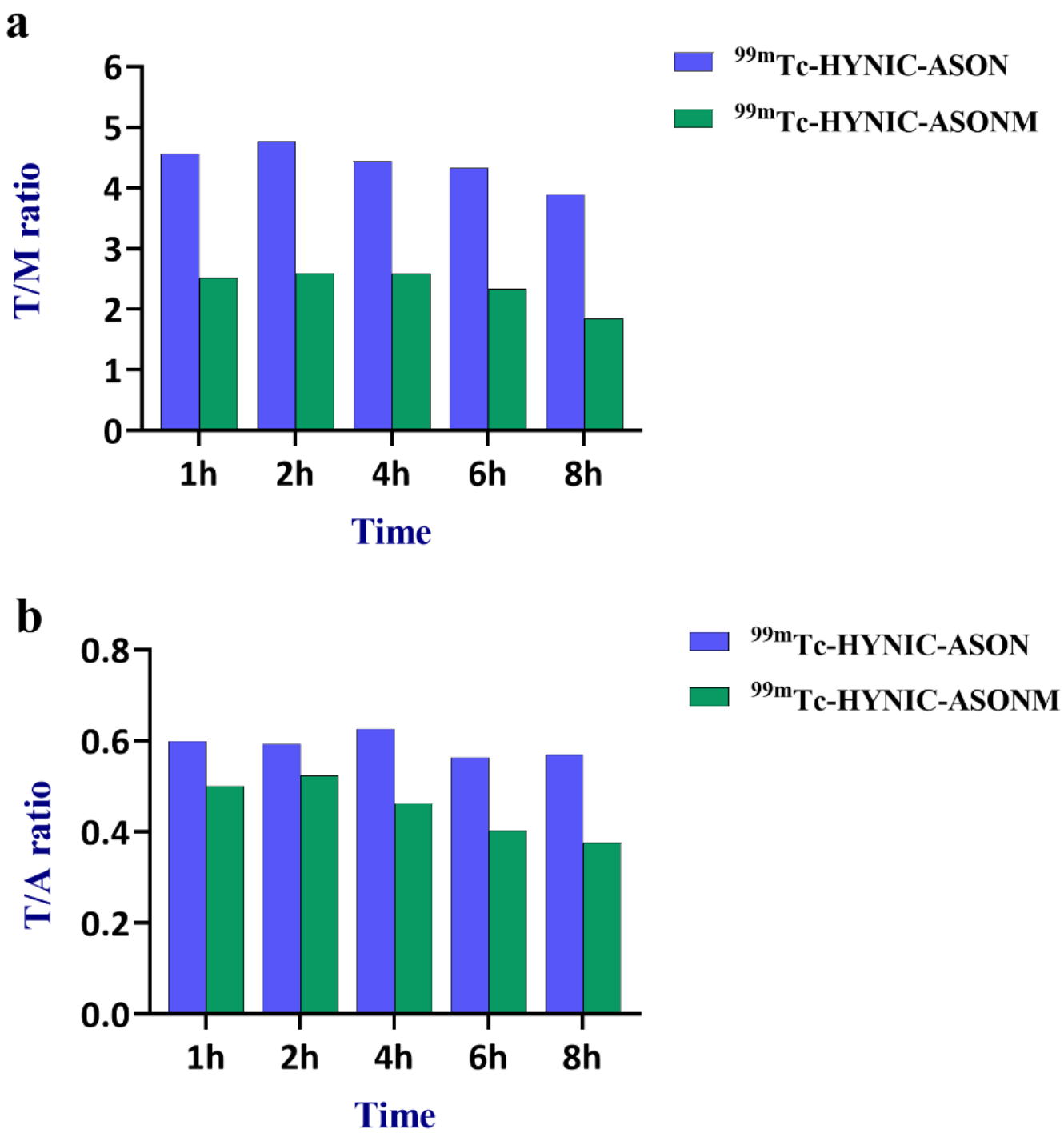

Figure 7

T/M(Tumor and Muscle)、T/A(Tumor and Abdomen) ratio of Lip- 99mTc-HYNIC-ASON and Lip-99mTc-HYNIC-ASONM. At all time, ratio of T/M and T/A is significantly higher in antisense groups than mismatched groups.

Supplementary Files

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- Supplementaryinformation.docx