中国医学影像技术
CHINESE JOURNAL OF MEDICAL IMAGING TECHNOLOGY



朱高红,任炳秀,苏玉林,卫江亮,蔡静,陈礼林,宋彬.$^{99m}$Tc二步法标记人端粒酶逆转录酶反义寡核苷酸及体内实验[J].中国医学影像技术,2013,29(6):880~885

# $^{99m}$Tc二步法标记人端粒酶逆转录酶反义寡核苷酸及体内实验

# Two-step radiolabeling human telomerase reverse transcriptase antisense oligonucleotide with $^{99m}$Tc and in vivo experiment

投稿时间： 2012-09-26 最后修改时间： 2012-12-10

**DOI**:

中文关键词：锝 寡核苷酸,反义 同位素标记

英文关键词:Technetium Oligonucleotides, antisense Isotope labeling

基金项目:

| 作者 | 单位 | E-mail |
|---|---|---|
| 朱高红 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 任炳秀 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 苏玉林 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 卫江亮 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 蔡静 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 陈礼林 | 昆明医医科大学第一附属医院核医学科, 云南 昆明 650032 | |
| 宋彬 | 四川大学华西医院放射科, 四川 成都 610041 | cjr.songbin@vip.163.com |



中文摘要:

目的 为放射性核素活体内检测人端粒酶逆转录酶反义寡核苷酸(hTERT ASON)提供基础技术。方法 将DOTA、EDC、Sulfo-NHS按分子比(10:5:4)溶解于pH值5.5的磷酸缓冲液(PBS)中,室温下振荡反应30 min;将活化后的DOTA以摩尔比75:1逐滴加入hTERT ASON(66 μg溶解于0.6 ml三蒸水中),室温下振荡反应4 h,以pH值7.0的PBS为透析液透析纯化8 h;取市售MDP氯化亚锡药盒1支,以生理盐水1 ml溶解后,取10 μl加入DOTA-ASON(33 μg)中充分混匀,加入新鲜$^{99m}TcO_4^-$淋洗液370 MBq (10 mCi),37℃,pH值5.5,振荡反应45 min,采用薄层色谱分析法(TLC)及G25葡聚糖凝胶色谱柱分别测定$^{99m}$Tc-DOTA-ASON的放化纯度(RCP)及标记率,并对探针进行体外稳定性及血清学稳定性检测;最后进行细胞学摄取实验,以免疫组织化学染色法检测其对HEPG2细胞端粒酶活性。结果 $^{99m}$Tc-DOTA-ASON标记率(79.62±4.44)%,RCP可达(96.22±2.43)%;在室温下及37℃新鲜人血清中温育24 h后,$^{99m}$Tc-DOTA-ASON RCP可保持在90%以上,寡核苷酸的降解率低于15%;HepG2肿瘤细胞对探针的摄取率为(8.32±0.34)%,且明显高于人脐静脉内皮细胞[((5.10±0.41)%,$P$<0.001)]。结论 $^{99m}$Tc-DOTA-ASON标记率及放化纯度较高,并显示出良好的体外和血清学稳定性,可用于体内实验研究。

英文摘要:

**Objective** To offer a basic technique for detecting human telomerase reverse transcriptase antisense oligonucleotide (hTERT ASON) in vivo with nuclear medicine modality. **Materials** Firstly, 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA), 1-(3-dimethylaminoptoply)-3-ethylcarbodimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium (S-NHS) were mixed in pH 5.5 phosphate buffered solution (PBS) with a molecular ratio of 10:5:4, and stirred for 30 min at room temperature (RT). Then the activated DOTA and hTERT ASON were mixed (with a molecular ratio of 75:1) in pH 7.0 PBS and stirred for 4 h at RT. The mixed solution was purified through dialysis tube in pH 7.0 PBS for 8 h. Secondly, 0.3 ml (33 μg) of DOTA-ASON and 10 μl of prepared MDP kit (5 mg/ml) were mixed, and then 370 MBq of $^{99m}$Tc-pertechnetate elute (0.2 ml) was dropped into the mixed solution and stirred for 45 min at 37℃, thin layer chromatography (TLC) and G25- gel chromatography were applied to detect the labeling rate and radiochemical purity (RCP). Finally, in vitro stability and cellular test were performed, and immunohistochemical staining was used to measure the telomerase activity of HepG2 cells after incubating with antisense probes. **Results** The labeling rate of $^{99m}$Tc-DOTA-ASON reached (79.62±4.44)% with a high RCP of (96.22±2.43)%. The radiolabeled antisense probes showed perfect stability at 37℃ in fresh human serum over 24 h with RCP (higher than 90%) and degraded ASON (lower than 15%). Compared with human umbilical vein endothelial cell (HUVEC), HepG2 cells showed obviously higher uptake of $^{99m}$Tc-DOTA-ASON ([8.32±0.34]% vs. [5.10±0.41]%, $P$<0.001). **Conclusion** The high radio labeling rate and RCP of $^{99m}$Tc-DOTA-ASON and its good stability in vitro and in serum present that $^{99m}$Tc-DOTA-ASON can be used as a potential probe for nuclear imaging targeting telomerase iv vivo.

查看全文 查看/发表评论 下载PDF阅读器