In situ fluorescence monitoring of diagnosis and treatment: a versatile nanoprobe combining tumor targeting based on MUC1 and controllable DOX release by telomerase†

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We have constructed versatile drug-loaded nanoprobes capable of responding to both MUC1 and telomerase and achieving intracellular drug release. Besides, the synthesized drug-loaded nanoprobes can realize the in situ imaging observation of the whole process of nanoprobes targeting the tumor cell membrane, the transmembrane entering the cytoplasm and the release of DOX into the cell nucleus.

Chemotherapy is the main means of cancer treatment, but there are common toxic side effects in clinical treatment. In order to reduce the side effects of drugs and improve their utilization efficiency, new targeted drug delivery systems are constantly being researched to improve the specificity of drugs for tumor cells and achieve precise targeted therapy of tumors. Tumor markers are biomolecules that specifically express in tumor cells, which are mainly found in biological fluids, circulating tumor cells and tumor tissues. In general, the target position of drugs in chemotherapy is mainly the tumor tissue. Therefore, the tumor markers that specifically express on tumor tissues have become important drug-loaded system targets and drug release switches.

Currently, the majority of the targeting sites and drug release switches on cells are the same biomarker, usually a highly expressed receptor on the cell surface. Although this can effectively distinguish between tumor tissues and normal tissues, most of the drugs are still released outside of cells. The toxic side effects of drugs still need to be reduced. Therefore, an ideal tumor marker as a drug release switch should be located within cells. In addition, corresponding to some versatile chemotherapeutic drugs, the selected markers should be ubiquitous in a variety of tumor cells. Telomerase is a reverse transcriptase present in cells and is normally inhibited in normal somatic cells. However, telomerase shows activity in most tumor cells and thus immortalizes the cancer cells. Therefore, telomerase is an important tumor intracellular universal biomarker. Currently, a variety of fluorescence methods and Raman scattering imaging are used to in situ detect the telomerase activity in tumor cells, which is further used to study the physiological role of telomerase activity in tumor progression and drug response. In contrast, since telomerase-targeted drug delivery systems are unable to selectively enter tumor tissues, there are few studies on targeted drug release based on telomerase. However, under the premise that the drug delivery system enters the cell, the telomerase-responsive chemotherapeutic drug delivery can achieve accurate intracellular release, thereby increasing drug bioavailability and reducing the damage caused by non-specific release in normal cells. Based on this, we chose telomerase as a switch for drug release in this study.

However, in order to effectively differentiate tumor tissues from normal tissues, especially paracancerous tissues, and to specifically target tumor cells before the drug delivery system enters the cell, a highly selective and high affinity ligand for targeting tumor cells is needed. MUC1 is a highly glycosylated transmembrane protein with a large extracellular domain. It exhibits abnormal glycosylation and significant overexpression in a variety of epithelial malignancies and some hematological malignancies. Therefore, it is an important epithelial tumor biomarker. In addition, the protein backbone exposed by the hypoglycosylation of MUC1 in tumor cells can become a specific binding site for ligands such as protein antibodies or aptamers. Among them, aptamers exhibit unique advantages in the detection of various chemical molecules due to their smaller size, high specificity and affinity to target proteins, low immunogenicity and high stability. Therefore, it is an effective way to design a drug delivery system that specifically targets tumor cells by utilizing the interaction of MUC1-aptamer and MUC1.

In this article, referring to the molecular characteristics and distribution of telomerase and MUC1, and taking advantage of nanomaterials in the integration of various biomolecules, cell transportation and signal conversion, we have developed versatile drug-loaded nanoprobes based on gold nanoparticles (AuNPs). The principle is shown in Scheme 1. The composite drug-loaded nanoprobes use AuNPs as carriers and bind nucleic acid probes

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that respond respectively to MUC1 and telomerase. Among them, the nucleic acid probes targeting MUC1 are MUC1-specific aptamers (MUC1 aptamer) and the Alexa Fluor 405 fluorophore is labeled. The nucleic acid probes that specifically recognize telomerase (telomerase probe) are DNA hybrids formed by the hybridization of 3’-notched molecular beacons with shorter telomerase primers (TSP). The molecular beacon is labeled with a FAM fluorophore. Besides, the telomerase probe can simultaneously serve as a drug carrier through combining drugs to the double strands. In our experiment, DOX was chosen as a model drug. DOX is a commonly used chemotherapeutic drug. The targeted delivery and controlled release of such drugs is of great significance to reduce their toxic side effects on normal tissues. In the initial state, the fluorescence of Alexa Fluor 405 and FAM can be quenched by AuNPs. As the probe approaches tumor cells, it interacts with MUC1 that expressed on the surface of the tumor cells. The hairpin structure of the MUC1 aptamer on the drug-loaded nanoprobes then opens and the fluorescence of Alexa Fluor 405 recovers. This results in the targeted binding to the cancer cells and the detection of surface MUC1 expression. More importantly, with the aid of MUC1-specific binding, the drug-loaded nanoprobes enter the cells through surface receptor-mediated endocytosis. The preparation procedure of drug-loaded nanoprobes is similar to that of drug-free nanoprobes (usually expressed as nanoprobes), but the telomerase probe is loaded with DOX (drug-loaded telomerase probe) in advance. The hydration radius and the UV-vis absorption peak of the prepared drug-loaded nanoprobes did not change significantly compared to those of the nanoprobes.

To verify the response of drug-loaded nanoprobes to MUC1 and telomerase, we tested the synthesized nanoprobes separately in MUC1 and telomerase solutions (Fig. 2). When the drug-loaded nanoprobes were added to the MUC1 solution, a strong fluorescence was observed, indicating that the aptamer and MUC1 acted to open the hairpin structures. Alexa Fluor 405 stays away from AuNPs and the fluorescence recovers. In the telomerase solution, TSP on the telomerase probe extended in

![Scheme 1](image)

**Scheme 1** Schematic illustration of drug-loaded nanoprobes for *in situ* fluorescence monitoring of diagnosis and treatment.
the presence of telomerase, so that the hairpin probe was opened and the fluorescence of FAM was observed at 500–550 nm. In addition, strong red fluorescence was also detected at 550–700 nm, demonstrating that DOX was released while the telomerase probes were opened. In order to demonstrate the specificity of the drug-loaded nanoprobes, we conducted controlled experiments using BSA protein and EpCAM protein as subjects. There is no obvious fluorescence signal, which indicates that the drug-loaded nanoprobes have good specificity. We then verified the performance of the probes in the mixed solution of MUC1 and telomerase (Fig. 2). The experimental results show that the nanoprobes respond well in the mixed solution and can achieve the joint detection of MUC1 and telomerase. The ability of the nanoprobes to quantify MUC1 and telomerase was also investigated. It can be seen from Fig. S2a and b (ESI†) that the fluorescence intensity of the probes is positively correlated with the increase of MUC1 and telomerase concentrations within a certain range. For the target MUC1 protein, there is a good linear range between 1 ng mL⁻¹ and 12 ng mL⁻¹, and the detection limit is 0.0748 ng mL⁻¹. For telomerase, there is a good linear range between 0.63 ng mL⁻¹ and 10 ng mL⁻¹ and the detection limit is 0.0326 ng mL⁻¹. The above results show that the high detection sensitivity of the nanoprobes we prepared can ensure the detection of biomarkers with a low content in cells.

The drug loading of nanoprobes and the release efficiency of drug-loaded nanoprobes are the main indicators of the therapeutic effect, so we tested the nanoprobes for DOX loading and release in solution. Firstly, the drug loading capacity of the nanoprobes was studied. As can be seen from Fig. 3a and Fig. S3 (ESI†), the fluorescence intensity no longer changes when the amount of telomerase probes is greater than 0.12 μM, indicating that 0.6 μM DOX is completely loaded into 0.12 μM telomerase probes and the drug loading amount of each telomerase probe is 5. The amount of telomerase probes loaded on each AuNP is converted from the fluorescence intensity of free telomerase probes obtained by mercaptoethanol competition method (ME).20 We then converted the fluorescence intensity of the competing telomerase probes (Fig. S4, ESI†) to the corresponding molarity via a standard curve (Fig. S5, ESI†). Calculations show that each AuNP is loaded with 35 ± 2 telomerase probes. Therefore, the number of DOX loaded on a single drug-loaded nanoprobe is approximately 175. We then measured the DOX release efficiency from the drug-loaded nanoprobes. The release of DOX gradually increases as the drug-loaded nanoprobes increase in response to the telomerase solution (Fig. 3b). When the reaction time reached 90 min, the DOX release efficiency reached the maximum of 54%. The above experiments show that drug-loaded nanoprobes have good drug loading and release properties.

We used HeLa cells as a model to verify that the nanoprobes can respond well to MUC1 and telomerase in cells and observe the intracellular drug release process. As shown in Fig. 4, when the incubation time was up to 15 min, MUC1-specific blue fluorescence began to appear on the cell membrane, indicating that the nanoprobes only reached the cell membrane and thus fluorescence was generated in response to MUC1 on the cell membrane. Simultaneously, there was no red fluorescence of DOX observed in the cells, indicating that the drug-loaded nanoprobes were stable and there was no non-specific extracellular drug release. After 30 min of incubation, MUC1-specific blue fluorescence and telomerase-specific green fluorescence appeared in the cytoplasm, indicating that the probe entered the cell. At the same time, the red fluorescence of DOX was also observed in the cytoplasm, indicating that the DOX embedded in telomerase was released after the drug-loaded telomerase probes were opened, and the intracellular release of DOX was achieved. As the incubation time prolonged, more drug-loaded probes entered the cell and the fluorescence intensity of the three fluorescent substances gradually increased. After 90 min of incubation, the intracellular fluorescence intensity reached its maximum. In addition, significant red fluorescence was observed in the nucleus, suggesting that DOX entered the nucleus in large numbers, thereby interfering with the DNA of the cell and eventually killing the cancer cells.

![Fluorescence spectra of (a) non-embedded DOX remained after DOX (0.6 μM) was mixed with different concentrations of MB (0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.14 μM). (b) Percentage of DOX released from DOX-loaded nanoprobes with telomerase for different incubation times (0, 10, 20, 30, 50, 70, 90, 110, and 130 min).](image)

![Fluorescence confocal images of HeLa cells after incubation with drug-loaded nanoprobes (1 nM) for different times (15, 30, 60, 90, 100, and 120 min), and HL-7702 cells after incubation with drug-loaded nanoprobes for 60 min.](image)
As a result, the drug-loaded nanoprobes successfully achieved the simultaneous detection of MUC1 and telomerase in cells as well as the imaging monitoring of the process of drug-loaded nanoprobes entering cells and intracellular drug release.

In order to further verify the specificity of drug release of drug-loaded nanoprobes, we used HL-7702 cells as a biological model for normal somatic cell experiments. As shown in Fig. 4, no specific fluorescence was observed in HL-7702 cells, indicating that our drug-loaded probes are able to specifically respond to MUC1 protein and telomerase in cancer cells, leading to the selective release of drugs in the cells, but not to normal cells.

To further verify the therapeutic effect of drug-loaded nanoprobes, we observed the apoptosis of cells using confocal imaging. As shown in Fig. S6 (ESI†), cells that had not been incubated with the drug-loaded nanoprobes exhibited normal spindle shapes. When the incubation time reached 4 h, a certain percentage of cells began to undergo apoptosis, which manifested as morphological changes caused by cell membrane shrinkage. After 16 hours of incubation, almost all cells were apoptotic, and the apoptotic cells became round and separated from the substratum, shrunk and underwent intense membrane blebbing. This shows that our synthetic drug-loaded nanoprobes can effectively act on cancer cells. As a control, the HL-7702 cells did not show obvious apoptotic morphology under the same incubation conditions (Fig. S7, ESI†), suggesting that the drug-loaded nanoprobes can achieve specific release. We also performed MTT assays on HeLa and HL-7702 cells. The cell survival rates of HeLa cells and HL-7702 cells incubated with drug-loaded probes show that the drug-loaded probes had a better killing effect on the HeLa cells, and underwent intense membrane blebbing. This shows that our nanoprobes entering cells and intracellular drug release.

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Conflicts of interest

There are no conflicts to declare.

Notes and references