An amplification-free method for the detection of HOTAIR long non-coding RNA

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HIGHLIGHTS
• An amplification-free electrochemical method for the detection of HOTAIR is described.
• HOTAIR sequences are extracted from ovarian cancer cells and plasma samples derived from ovarian cancer patients.
• Extracted sequences are detected amperometrically via a sandwich hybridization method at a screen-printed gold electrode.
• Catalytic enhancement of the signal is achieved using the hydrogen peroxide/horseradish peroxidase/hydroquinone system.

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ABSTRACT
The discovery of large transcripts of long RNAs that have limited protein coding capacity, known as long non-coding RNAs (lncRNAs) present new concepts on RNA-mediated gene regulation. Increasing evidence suggests that large intervening mRNAs regulate key pathways in cancer genesis and metastasis. Among the most characterized lncRNAs, homeobox (HOX) transcript antisense intergenic RNA (HOTAIR) acts as an oncogenic molecule in different cancer cells, and thus its expression level serves as a potential biomarker for diagnostic and therapeutic purposes in several human cancers, such as breast, prostate, liver and ovarian cancer. This paper reports a simple and sensitive sensor platform for the detection of HOTAIR. Extracted HOTAIR sequences from ovarian cancer cells and plasma samples derived from ovarian cancer patients were magnetically isolated and purified, followed by a sandwich hybridization event at a screen-printed gold electrode. This event was monitored by amperometry using the hydrogen peroxide/horseradish peroxidase/hydroquinone (H2O2/HRP/HQ) system. The catalytic enhancement of the amperometric signal enabled our assay to achieve a detection limit of 1.0 fM with a good inter-assay reproducibility (relative standard deviation (%RSD) = < 5.0%, n = 3). The method was used for the...
1. Introduction

Long non-coding RNAs are a heterogeneous class of transcripts that exceed 200 nucleotides in length and do not encode proteins. Previously, lncRNAs have been regarded as transcriptional noise due to their inability to translate into proteins [1]. However, increasing evidence shows that they exhibit a wide range of expression levels and distinct cellular localizations, and in association with human diseases [1]. They play a critical role in various epigenetic processes, including transcription, chromosome structure modulation, messenger RNA (mRNA) stability, mRNA availability and post-translational modification, cell cycle regulation, and differentiation regulation [2–6]. The interaction of lncRNAs with histone repressive proteins of the polycomb group family by recruiting them to gene loci, facilitates silencing [7]. Several studies have shown that lncRNAs are more nuclear localized than mRNA as a result of ineffective splicing and having a lower expression level, which is more specific to the cell type compared to protein coding RNAs while they can vary spatially, temporally or in response to stimuli [8,9].

HOTAIR is one of the first lncRNA discovered to be associated with tumorigenesis and has been reported as an oncogene in several cancers [10]. Transcribed in an antisense manner, HOTAIR is found within the HOXC gene cluster of chromosome 12 and has a length of 2158 nucleotides [11]. As a novel regulator of tumorigenesis, aberrant HOTAIR expression has been linked to cell metastasis in several cancers, including pancreatic [12], lung [13], gastrointestinal stromal [14], colorectal [9], and breast carcinomas [10]. Recently, a regulator of tumorigenesis, aberrant HOTAIR expression has been linked to cell metastasis in several cancers, including pancreatic [12], lung [13], gastrointestinal stromal [14], colorectal [9], and breast carcinomas [10]. Wu et al. demonstrated that rs4759314 and rs7958904 of HOTAIR predict the increased risk to develop epithelial ovarian cancer [15]. In addition, Zhang et al. has recently shown that aberrant HOTAIR was highly expressed in ovarian carcinoma and associated with proliferation and metastasis of ovarian cancer cells [16]. These progressive discoveries indicate that HOTAIR plays a critical role in regulating the cancer epigenome and therefore may be an important target for cancer diagnosis and therapy monitoring.

One of the major challenges associated with the detection of cancer biomarkers in biological fluids is that they are often found in low concentrations. The concentration of cancer biomarkers in biological fluids is usually several nanograms per millilitre. Henceforth, highly sensitive and specific technologies are required for their precise detection. The past few years have witnessed a steep rise in the development of detection technologies for lncRNA HOTAIR. Without any doubt, the emergence of sensitive, high-throughput genomic technologies such as microarrays, next-generation sequencing (NGS), northern blotting, microarrays and quantitative reverse transcription PCR (RT-qPCR) have provided opportunities for the detection of novel transcripts that are derived from none-protein coding genes. However, these technologies suffer from several limitations. Microarray based methods are limited to specific probes, specialized equipment and hybridization procedures which increase the cost and minimize the reproducibility between platforms [17]. RT-qPCR requires expensive instrumentation, smaller number of expressed genes, thus, not suitable for high-throughput lncRNA HOTAIR screening [18]. Although NGS is capable of simultaneous analysis of known and unknown sequences, it is a costly and time consuming method. The use of radioisotopes as labels in northern blotting presents numerous health and safety concerns for researchers and the environment [19].

To circumvent most of the limitations highlighted above, electrochemical biosensors have shown to be a cost-effective and simple alternative for biomolecular sensing. Over the past several decades, it has been demonstrated that electrochemical detection approaches hold a great promise for the rapid, simple and inexpensive detection of a range of nucleic acids based biomarkers including non-coding RNAs [20,21]. Enzyme labels have often been used to amplify signals in these approaches due to their high, steady and reproducible catalytic activity. Enzymatic catalytic reactions are often coupled with supplemental amplification processes (e.g., redox cycling) or using enzyme labels [22,23]. This enzymatic amplification generally describes a recurring process that produces or consumes signalling species (molecules or electrons) in the presence of reversible redox species, leading to enhanced signal amplification [24,25]. A possible alternative approach to achieve signal amplification is the application of an auto-inductive system using a distinct dendritic chain reaction (DCR). In this system, a single analyte molecule initiates a DCR that generates a strong detectable signal [26,27]. In the quest of designing a signal amplification method, we utilized the oxidation reaction of hydroquinone (HQ) to benzoquinone (BQ). Hydroquinone acts as an electron mediator between the electrode surface and redox centre of HRP.

Herein, we report an amplification-free platform for the amperometric detection of HOTAIR long non-coding lncRNAs using $\text{H}_2\text{O}_2$/HRP/HQ. Sequences of HOTAIR extracted from designated cells and plasma samples, and ovarian cancer patients were magnetically purified and isolated. Using well known avidin–biotin affinity, streptavidin coupled horseradish peroxidase (HRP) was attached to biotinylated capture probes, followed by a sandwich hybridization method where the target HOTAIR was hybridized with the second capture-probe immobilized onto a screen-printed gold electrode. This event was monitored amperometrically using the $\text{H}_2\text{O}_2$/HRP/HQ system. Standard RT-qPCR method was used to validate the method for the analysis of HOTAIR expression levels in patient samples. We envisage that the proof-of-concept assay demonstrated here could be a highly low-cost platform in routine clinical settings for screening of cancer-related lncRNAs.

2. Experimental

2.1. Preparation of RNA from cell line and ovarian cancer samples

RPMI-1640 growth medium (Life Technologies, Australia) supplemented with 10% fetal bovine serum (Life Technologies, Australia) and 1% penicillin/streptomycin (Life Technologies) were used to culture ovarian cancer cell lines (SKOV3 and OVCAR3) and non-cancerous cell line (Met-5A). This was performed in a
humidified incubator with 5% CO2 flow at 37 °C. All cells were harvested by standard trypsinization protocol after they reached 70–80% confluence. Briefly, cells were washed with 3 mL HBSS (Gibco) to remove enzyme inhibitors followed by 1–2 mL of TryPLe (Gibco) and incubation for 3 min at 37 °C. To neutralize the trypsin activity, 3–6 mL of cell culture media was added followed by centrifugation for 5 min at 2500 rpm. A cell pellet was collected for RNA extraction and stored at −20 °C until further processing. Plasma samples were collected according to the declaration of Helsinki and approved by the Ethics Committee of the University of Queensland (approval number 2016000300) and the Ochsner Medical Center (New Orleans, USA). Plasma was isolated from whole blood sample by centrifuging at 2000×g for 10 min and stored at −80 °C until analysis. Ovarian cancer samples were collected accordingly, assigned and classified based on their histotype (e.g. stage I and stage III), and stored at −80 °C in the Biobank units. In this study, only patients with epithelial ovarian cancer high-grade serous subtype (n = 3) and benign samples (n = 3) were utilized. miRNeasy Mini Kit (Qiagen, Australia) was used to extract RNA and the concentration was quantified using a SPECTROstar Nano Microplate Reader (BMG Labtech) operated by MARS data analysis software.

2.2. Magnetic purification and hybridization

For hybridization, 10 μL of RNA sample was mixed with 10 μL of 5X saline-sodium buffer (SSC) (pH 7.0) and 15 μL of 10 μM biotinylated capture probes (See Table S1). The mixture solution was heated at 55 °C for 2 min and placed on a thermomixer for 1 h at room temperature (25 °C) to allow hybridization of capture probe with target IncRNA. Next, 20 μL of streptavidin-labeled (MyOne Streptavidin C1, Invitrogen) magnetic beads were washed 3-times with 2X binding and washing (B&W) buffer (10 mM Tris-HCl, pH 7.5; 1.0 mM EDTA; 2.0 M NaCl) and resuspended in 20 μL of 2X B&W buffer. The prepared dynabeads were then dispersed into the solution containing biotinylated capture probe-IncRNA complex and incubated for 30 min at 25 °C to allow the formation of Dynabead/biotinylated capture probe-IncRNA complex. The dynabead bound IncRNA complex was separated using a magnet, washed three times with 2X B&W buffer, and resuspended in 9.0 μL of RNase-free water. The magnetically captured isolates were heated for 2 min at 55 °C in the Biobank to remove enzyme inhibitors followed by 1 h of 10 mM Tris(2-carboxyethyl)phosphine (TCEP) and incubated for 1 h at 25 °C in the dark to reduce the disulphide bond of the capture probe. This was followed by the addition of 70 μL of 1X PBS (pH 7.4) to the mixture and subsequent immobilization onto bare SP-Au surface (5 μL) and incubation for 2 h. The modified electrode was washed 3 times gently with 1X PBS to remove unbound probes. Following incubation and self-assembled monolayer formation, 2 mM of 6-mercaptohexanol solution was added to the modified electrode for 3 h in the dark at room temperature and washed 3 times with 1X PBS. Then, 5 μL of previously released HOTAIR target was adsorbed on the thiolated modified surface and incubated for 30 min on a thermomixer (300 rpm) at 25 °C to allow hybridization with the surface bound capture probe. This was followed by a washing step 3 times with PBS. Further incubation of biotinylated detection probe (5 μL of Cap 1, referred to as probe 2) was performed for 30 min at 25 °C to allow duplex hybridization on a thermomixer (300 rpm) and thereafter washing with 1X PBS. By taking advantage of the biotin-streptavidin affinity interactions, 5 μL of 10 ng/μL streptavidin conjugated HRP was immobilized on the modified electrode surface for 30 min at 25 °C. The modified electrode was washed profusely with 1X PBS pH 7.4. For electrochemical readout, 40 μL of 1 mM HQ was added onto the sensor surface and run chronoamperometry at −0.2 V vs Ag/AgCl. Then, 10 μL of 100 mM H2O2 was added onto the sensor surface and stabilized and the current generated was recorded until the steady-state current was reached. The amperometric responses recorded throughout the manuscript are proportional to the difference between the steady-state and the background currents and are the average of at least three repetitive experiments.

3. Results and discussion

3.1. Assay principle

In this study, HOTAIR IncRNA was selected as a model biomarker for ovarian carcinoma detection. Fig. 1 shows the schematic representation of steps involved in the isolation and detection of HOTAIR. Firstly, total RNA was extracted from cell lines and plasma samples obtained from patients with ovarian cancer. Complementary functionalized capture probes were dispersed into the sample solution to selectively bind to the HOTAIR strands. In the presence of streptavidin coated magnetic beads, captured HOTAIR targets were then magnetically purified with several magnetic washing steps and released from capture probes through heating. Finally, the released HOTAIR sequences were then detected via a sandwich hybridization method coupled with an enzyme catalyzed amplification step. The HOTAIR sequences were initially hybridized onto a thiolated capture probes modified SP-Au electrode which were further hybridized with HRP-functionalized detection probes (see Fig. 1 in Supplementary Information for stepwise successful attachments of these biomolecular layers). The amount of hybridized HOTAIR targets was then detected by measuring the cathodic current generated by the enzymatic reduction of H2O2 mediated by HQ. This current is proportional to the concentration of HOTAIR targets present in the sample. To check the assay functionality, we compared the assay performance for detecting 10 pM of synthetic IncRNA sample. As can be seen in Fig. 2, the total current density obtained with the presence (right bar) and absence (left bar) of 10 pM target were found to be 13.4 and 0.70 μA/cm2 respectively. The increased amperometric current response in the presence of target HOTAIR at the sensing surface could be related to the fact that a significant amount of redox active HRP could be attached within the duplex layers on the electrode surface. A slight response in the absence of the target could be related to the fact that a low amount of redox active HRP or HQ nonspecifically adsorbed on the sensor surface. The electrodes showed good reproducibility with less than
5.0% relative standard deviation (% RSD) between the inter-assay signals for three independent measurements (i.e., \( n = 3 \)).

### 3.2. Analytical performance of biosensor using the HQ/HRP/H2O2 system

The sensitivity and reproducibility of the assay was evaluated using the designated concentrations of synthetic HOTAIR (Fig. 3). An increase of the amperometric current density was observed with the increase of HOTAIR concentration in the range of 1.0 fM to 1.0 nM (Fig. 3a). The linear regression equation was estimated to be \( y = 2.48 \log C + 3.87 \) (\( y \) is the current density and \( x \) concentration of HOTAIR), and a correlation coefficient of \( R^2 = 0.9934 \) (Fig. 3b inset).

The limit of detection (LOD), which is clearly distinguished from that of the control (NoT in Fig. 3b), was estimated as 1.0 fm (S/N = 3) with a relative standard deviation (% RSD) of <5% for \( n = 3 \). The LOD clearly indicates the high sensitivity of the amperometric assay for HOTAIR long non-coding RNA detection. We believe that the duplex hybridization and signal amplification based on the HRP-catalytic reaction facilitated by the HQ/HRP/H2O2 system contributed to achieve this low LOD. A considerable amount of electrochemical non-coding RNA sensing strategies developed in recent years achieved femtomolar detection sensitivity which is comparable to our proposed assay [28–30]. (See Table S2) For example, the LOD is comparable to the recently reported label free electrochemical biosensor based on green l-cysteine electrodeposition and Au–Rh...
hollow nanospheres as tags by Liu et al. [31] It is also several hundred folds better as compared to recently reported LODs in the hairpin probe based circular exponential amplification with fluorescence (EXPAR) [32] and the quenched staunton-triggered probe-based rolling circle amplification (Q-STAR) [33] assays. Clearly, the reported LOD is higher in magnitude than those obtained in duplex-specific nuclease-actuated cyclic enzymatic repairing-mediated signal amplification [28] and gold-loaded nanoporous superparamagnetic iron oxide nanocubes based assays [34]. Our previously reported isothermal amplification based method for electrochemical quantification of HOTAIR also offers almost similar LOD [35]. The key advantages of our current assay is that it provides a novel amplification free detection of HOTAIR species using a single technique, and hence it avoids possible degradation of HOTAIR target as a result of amplification or target modification/s commonly used in the conventional assays or in the aforementioned assays. The acceptable range of reproducibility (% RSD = <5% for n = 3) is comparable or better than most of the existing electrochemical non-coding RNA sensors [36]. As IncRNAs and other related RNAs are often used as disease diagnostic biomarkers, the wide dynamic range of 1 fM to 1 nM for synthetic HOTAIR detection shows that the method could potentially be adopted to analyze HOTAIR in complex biological samples.

3.3. Specificity of the assay

The ability of a biosensor to distinguish closely related RNAs is important in diagnostic applications. The anti-interference properties of the developed assay were evaluated using solutions containing different RNA targets. Two small microRNA (miRNA) (miR-486 and miR-891) were selected to evaluate the specificity of the biosensor, each at a concentration of 1 pM. As can be seen in Fig. 4 (bars denoted as miR-486 and miR-891) resulted in 8.2% and 17.5% in current density signal (0.894 and 1.712 μA cm$^{-2}$ respectively when compared with NoT control (0%, 0.70 μA cm$^{-2}$). A significantly high response with respect to the NoT control was observed for the target HOTAIR (100%, 9.414 μA cm$^{-2}$). The similar response attained by non-complimentary targets with respect to the NoT control demonstrates that our assay exhibits a good specificity for HOTAIR detection. The reproducibility of amperometric measurements for HOTAIR was derived to be a RSD of 3.15% (n = 3), miR-891 was determined to be 3.93% (n = 3), miR-486 was found to have a RSD of 3.15% (n = 3). These results indicate that our developed method can clearly distinguish related non-coding RNAs.

3.4. Application of the assay in plasma sample

Motivated by the high sensitivity and specificity, the applicability of the developed assay in biological fluids was examined by performing the analysis in unpretreated and undiluted healthy human plasma spiked with increasing concentrations of HOTAIR synthetic sequences. As shown in Fig. 5, an amplified current density response was observed for a series of spiked samples with increasing concentrations of target HOTAIR. This shows that the developed assay can potentially be used to analyze complex serum sample. The catalytic redox cyclic current density measured by amperometry in the presence of H2O2/HRP/HQ allowed a dynamic range from 10 fM to 1 nM and an estimated minimum detectable concentration of 10 fM for the target determination. The reproducibility of the concentrations was examined on three independent electrodes and resulted in a RSD of <5% between the electrodes at each concentration. The LOD of the assay is comparable to existing electrochemical methods for non-coding miRNA detection. For example, our assay obtained a 3-fold better sensitivity than a recent approach reported by Fang et al. [37] To obtain a highly specific detection, Fang et al. utilized the selective binding of Zinc finger proteins (JAZ) to the DNA-RNA hybrid formed between a DNA capture probe and a target miR-21. It is also worth noting that when the sensitivity of spiked plasma sample was compared to that of the spiked buffer samples, a ten-fold decrease in sensitivity was obtained. This may be due to the complex nature of the plasma sample which contains millions of other nonspecific biomolecules that may potentially interfere as compared to the buffer system. More so, nonspecific hybridization in the capture and isolation step, and nonspecific adsorption during the detection process could have contributed towards the loss of sensitivity.

3.5. Analysis of IncRNA in ovarian cancer cell line samples

Following the establishment of the assay sensitivity and specificity, our developed approach was further evaluated for its specificity using biological cell line models. Total RNA extracts from SKOV3 and OVCAR3 ovarian cell lines and Met-5A non-cancerous cell line were used to isolate and detect HOTAIR IncRNA species (Fig. 6). Based on our assay results, HOTAIR IncRNA was expressed...
at varying levels in the different cell lines (Fig. 6b). Expression of HOTAIR was considerably higher in both the ovarian cancer cell lines compared to the non-cancerous Met-5A cells. Our assay results were in good agreement with the previous reports, which observed similar profile for HOTAIR expression levels in ovarian cell lines [38]. The % RSD of <4.12% for n = 3 was found in inter-assay signals which indicates the good reproducibility of the assay. HOTAIR expression levels in cell lines were validated by the RT-qPCR which showed a trend similar to our assay in all three cell lines (Fig. S2). This indicates that the electrochemical signals generated by our approach could discriminate the presence/absence of HOTAIR in ovarian cancer cell line. As high HOTAIR

Fig. 4. (a) Amperometric responses for 1.0 pM of miR-486, miR-891 and HOTAIR sequences. (b) Bars represent the corresponding current densities for the 1.0 pM of miR-486, miR-891 and HOTAIR sequences. Error bars represent the standard deviation of three repetitive experiments.

Fig. 5. (a) Amperometric responses corresponding to the designated starting concentration of HOTAIR sequences ranging from 10 fM to 1.0 nM in spiked plasma samples. (b) Corresponding current densities for the designated concentrations of HOTAIR. Inset, calibration plot showing concentration-current density relationship. Error bars represent the standard deviation of three repetitive experiments.

Fig. 6. Analysis of ovarian cancer and normal cell lines. (a) Amperometric responses for HOTAIR sequences in cell lines (SKOV3, OVCAR and MeT-5A). For comparison, no template control (NoT) data is used. (b) Corresponding current densities for cell lines and NoT. Error bars represent the standard deviation of three repetitive experiments.
expression is associated with increased metastatic potential of cancer cells and tumor progression [39,40], our developed approach may assist in detecting HOTAIR expression levels in cells collected from patients with various cancers.

3.6. Clinical sample analysis

The expression of cancer-related lncRNA HOTAIR in serum can be used as a biomarker of cancer progression [10,41,42]. The applicability of our assay in real clinical samples was tested using human serum samples of six newly diagnosed patients. The selected samples constituted three ovarian cancer high-grade serous subtype and three benign samples. Ovarian cancer is one of the leading causes of cancer death in women. It has been reported that the overexpression of HOTAIR in epithelial ovarian cancer patients is linked to an aggressive tumour phenotype and poor prognosis [43]. As shown in Fig. 7, the current density profile of high grade epithelial ovarian cancer samples (P1, P2, P3) showed 10 times higher response with respect to the NoT control suggesting an up-regulation of HOTAIR expression in ovarian cancer human serum. It is also noticeable that the current density obtained for benign samples (P4, P5, and P6) were two times lower than high grade serous subtype samples (P1, P2, and P3). The data obtained from these patient samples exhibited a good inter-assay reproducibility (RSD of 4.8%, n = 3) for the analysis of expression profiles of HOTAIR in different stages of ovarian cancers. Thus, the developed assay can directly measure HOTAIR expression levels in human serum without prior amplification or pretreatment and provide great potential in clinic diagnosis.

3.7. Advantages and limitations of the assay

Our developed assay offers several distinct merits towards the ultrasensitive detection of HOTAIR. Our assay results were consistent with existing reports which describes the presence of HOTAIR in ovarian cancer patients. The acceptable range of our assay reproducibility (RSD of 4.8%, n = 3) is comparable to existing lncRNA electrochemical biosensors. Our assay also uses single-use, disposable, and inexpensive screen-printed electrodes (<$3.0 AUD per electrode) which aid to overcome nonspecific response which is often caused by several surface reactions and excessive capacitive charge when using conventional disk electrodes. The elimination of time-consuming cleaning procedures and utilization of electrochemical cells associated with disk electrodes, reduce the assay time and enable decentralized HOTAIR analysis in point of care settings. Another useful aspect of our assay, the magnetic separation technology using dynabeads, provides rapid and gentle isolation and purification of HOTAIR targets and reduces the matrix effects of the biological sample, which further enhances the assay performance by minimizing the matrix effects of the biological sample. The utilization of duplex hybridization enables high specificity of the assay towards the target which subsequently improves the sensitivity. More so, the redox amperometric detection based on HRP and HQ/H2O2 system enables signal enhancement in the readout. The previously reported applications for RNA extracted from tumor tissues and cells from ovarian cancer patients using conventional methods required amplification of the genetic material. However, it is worth noting that our developed biosensor can be applied to detect HOTAIR directly in complex biological samples without prior PCR amplification, extraction and purification. The overall analytical performance (1.0 fm LOD, <5.0% RSD, 6-order dynamic range, clinical applicability) of the reported electrochemical method indicate its high potential for miniaturized, multiplexed and decentralized analysis of RNA biomarkers with high translational capacity. Despite the excellent performance of the assay, there are several limitations associated with the assay that still remain. One of the major limitations is the multistep fabrication process which is tedious and time consuming, and thus, complicates the biosensing assay. When immobilized on the sensor platform, lncRNAs tend to fold into various secondary or tertiary structures. This structural instability can reduce the analytical performance of the sensor. The sensing platform architecture still remains a key concern in determining the surface coverage of the target and the magnitude of non-specific interactions. However, many efforts have recently been devoted to the use of aromatic thiols to facilitate monolayer formation, which consequently enhance the assay sensitivity.

Although great efforts have been made in electrochemical sandwich hybridization sensors, further work is still required to translate them into portable point-of-care devices. One of the key areas of development includes probe design, which is essential for the overall selectivity and sensitivity of the assay. Effective and accurate probes’ design ensures high selectivity of the assay. Another key element of development is innovative sample pretreatment strategies, which could be coupled with the biosensors into automated platforms. This involves the integration of isolation, purification, immobilization and detection steps in a single device, and will be essential in routine clinical applications. More so,
coupling of these devices with amplification techniques in automated platforms, would pave way for accurate detection systems.

4. Conclusions

This work presents a sensitive and specific method for quantitative detection of HOTAIR expression levels in human cancer cells and serum samples without prior tedious pre-treatment or amplification steps. The self-assembled thiolated probes allow hybridization of target HOTAIR and enhanced amperometric quantification via HRP catalyzed HQ/H2O2 system. The developed assay shows detection limit as low as 1.0 fm HOTAIR with excellent reproducibility (% RSD < 5% for n = 3). The method shows great analytical performance and is comparable to conventional techniques and electrochemical biosensors previously reported. The developed method can be of great importance for the detection of HOTAIR or other RNA biomarkers in cancer patients.

CRedit authorship contribution statement

Narshone Soda: wrote the paper with the contribution from all authors. Surasak Kasetsirikul: wrote the paper with the contribution from all authors. Muhammad J.A. Shiddiky: conceived the idea and supervised the project. N.S. and M.J.A.S. designed the experiments and performed majority of the experiments with the help of M.U.J.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References