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Toward early cancer detection: Focus on biosensing systems and biosensors for an anti-apoptotic protein survivin and survivin mRNA

Magdalena Stobiecka*, Katarzyna Ratajczak, Slawomir Jakiela**

Department of Biophysics, Warsaw University of Life Sciences (SGGW), 02776, Warsaw, Poland

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ABSTRACT

The development of biosensors for cancer biomarkers has recently been expanding rapidly, offering promising biomedical applications of these sensors as highly sensitive, selective, and inexpensive bioanalytical tools that can provide alternative methodology to that afforded by the advanced hyphenated-instrumental techniques. In this review, we focus particularly on the detection of a member of the inhibitor of apoptosis proteins (IAP) family, protein survivin (Sur), a ubiquitous re-organizer of the cell life cycle with the ability to inhibit the apoptosis and induce an enhanced proliferation leading to the unimpeded cancer growth and metastasis. Herein, we critically evaluate the progress in the development of novel biosensing systems and biosensors for the detection of two survivin (Sur) biomarkers: the Sur protein and its messenger RNA (Sur mRNA), including immunosensors, electrochemical piezo- and impedance-sensors, electrochemi-luminescence biosensors, genosensors based on oligonucleotide molecular beacons (MBs) with fluorescent or electrochemical transduction, as well as the microfluidic and related analytical platforms based on solution chemistry. The *in-situ* applications of survivin biomarkers' detection technologies to equip nanocarriers of the controlled drug delivery systems with MB-based fluorescence imaging capability, apoptosis control, and mitigation of the acquired drug resistance are also presented and critically evaluated. Finally, we turn the attention to the application of biosensors for the analysis of Sur biomarkers in exosomes and circulating tumor cells for a non-invasive liquid biopsy. The prospect of a widespread screening for early cancers, based on inexpensive point-of-care testing using biosensors and multiplex biosensor arrays, as a means of reducing the high cancer fatality rate, is discussed.

1. Introduction

In the battle with ever expanding and largely incurable cancer growth and metastasis, plaguing the human population today, early detection of carcinogenesis is vital and brings hope of survival to patients. Since the early cancer detection needs to be carried out in a widespread testing at the points-of-care and doctor offices, the traditional diagnostic procedures involving advanced instrumentation should be augmented with inexpensive and widely available testing platforms. Hence, the development of biosensors capable of detecting cancer biomarkers is highly desirable. Many biomarkers specific to various cancers have been discovered and can be used in cancer diagnostics. Some of the most common to all types of cancers are the markers of the inhibitor of apoptosis proteins (IAP) family and, in particular, survivin which became a forefront cancer biomarker (Altieri, 2001; 2008b; Fan and Chen, 2017; He et al., 2018; Nguyen et al., 2019; Wang et al., 2017; Xu et al., 2018).

Survivin (Sur) is the smallest member of the IAP family which includes eight members (Altieri, 2008b; Ebrahimiyan et al., 2018; Schimmer, 2004): cIAP1, cIAP2, NIAP, IAP-like protein 2 (ILP2), X-linked inhibitor of apoptosis (XIAP), survivin, livin, and BRUCE. The wild type survivin splice WT S (16.5 kDa) consists of four exons and three introns (Fig. 1) and forms a chain of 142 amino acids (Ambrosini et al., 1997). In solution, it forms a bow tie-shaped homodimer (Altieri, 2008a; Chantalat et al., 2000; Muchmore et al., 2000; Verdecia et al., 2000), as illustrated in Fig. 1A. The role of Sur in cancer biology and the methods of Sur gene detection have recently been evaluated in a series of excellent reviews (Andersen et al., 2007; Chen et al., 2016; Chiou et al., 2003; Dallaglio et al., 2012; Fangusaro et al., 2006; Johnson and Howerth, 2004; Li et al., 2018; Sah et al., 2006; Wheatley and Altieri, 2019). Sur plays a crucial role in regulation of cell division, proliferation and the cell cycle control (Altieri, 2003; Khan et al., 2009; Mita et al., 2008; Wolanin and Piwocka, 2007). It is strongly expressed in embryos and malignant tumors but very weakly in the normal

* Corresponding author.
** Corresponding author.
E-mail addresses: magdalena_stobiecka@sggw.pl (M. Stobiecka), slawomir_jakiela@sggw.pl (S. Jakiela).

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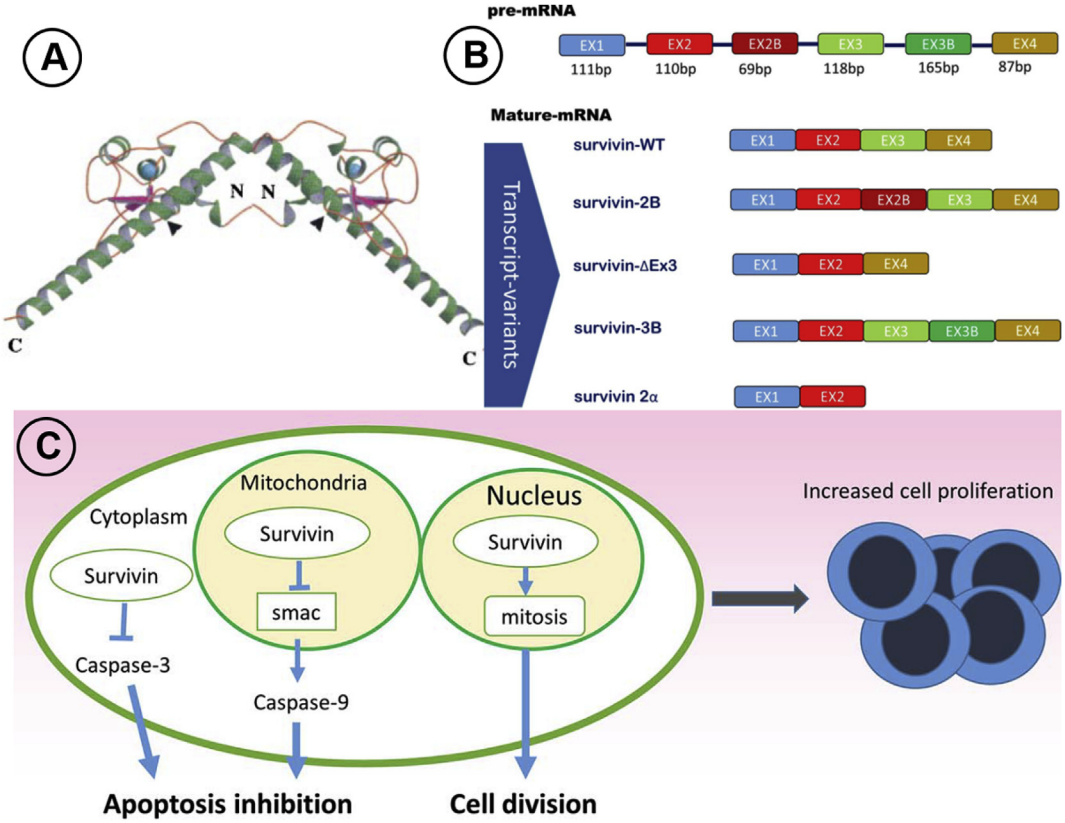


Fig. 1. (A) Structure of survivin dimer. Reprinted with permission from (Chantalat et al., 2000); (B) Five splice variants of survivin; (C) Schematic exhibition of survivin roles in apoptosis and cellular proliferation. Reprinted with permission from (Ebrahimiyan et al., 2018).

differentiated cells, evidently showing that Sur is involved in the rapid growth and proliferation of cells. The inhibition of apoptosis and enhancement of cell proliferation distinguish cancer cells from normal cells. Due to its active role in tumor growth and metastasis, Sur became a prime cancer biomarker (Gleichenhagen et al., 2018; Khan et al., 2012; Konopka et al., 2009; Rödel et al., 2012; Su, 2016). In Fig. 1C, the basic effects of survivin on cell life are characterized. It has been demonstrated that knocking Sur out of cells would cause an apoptosis process (Olie et al., 2000). Hence, the Sur protein and Sur mRNA have become extensively investigated in view of their potential utilization as selective targets for cancer therapy (Blanc-Brude et al., 2003; Ferrario et al., 2007; Frassanito et al., 2019; Garg et al., 2016; Ghadimi et al., 2012; Kanwar et al., 2011; Mazur et al., 2018; Mobahat et al., 2014; Pennati et al., 2008; Ryan et al., 2009; Smolewski and Robak, 2011).

This review summarizes the achievements of the scientists and clinicians in the field of optical and electrochemical biosensing systems and biosensors utilized for the detection of Sur protein and Sur mRNA biomarkers for most common human cancers based on antigen-antibody interactions and DNA hybridization processes. Particular attention has been paid to the methods of detection and the design of molecular beacon probes, genosensors based on solid electrodes and nanoparticles, nanogravimetric and chemiluminescence survivin immunosensors, as well as the microfluidic systems. The reports published on the development of biosensors and biosensing systems for the detection of Sur biomarkers are highly advanced but non-frequent, likely because the number of research groups involved in developing the biosensors for Sur protein and Sur mRNA is now only beginning to increase. Hence, more developments will likely be published soon. The assessment of the prospective biosensors and biosensing systems, proposed until now, indicates that the biosensor technology for the detection and monitoring of Sur biomarkers is ready to enter the stage of expansion and may be able to offer new solutions for a widespread, non-invasive, early cancer detection initiatives which are one of the key elements of a successful treatment of neoplasia. This new technology may also be able to help in diagnosing and therapy of pre-metastatic cancers utilizing non-invasive procedures based on exosomes.

2. Prospective biosensing platforms

The anti-apoptotic IAP proteins, and especially Sur, are overexpressed in all types of malignant tumors. In Table 1, recent studies reporting the expression of Sur in different cancer cell lines are listed and the methodology utilized in the detection process, together with conditions of the detection, are presented. The listing includes also the limit of detection (LOD) achieved. As can be seen, most of the methods used for survivin detection are based on molecular biology techniques, including reverse transcription polymerase chain reaction (RT-PCR) (Chu et al., 2012; Fu et al., 2008; Han et al., 2012; Jin et al., 2019), enzyme-linked immunosorbent assay (ELISA) (Chen et al., 2018; Gleichenhagen et al., 2018; Kappler et al., 2003), Western blot (WB) (Yang et al., 2003), and immunohistochemical (IHC) (Jakubowska et al., 2016; Li et al., 2019; Lorenzettia et al., 2019) methods.

The early cancer detection requires testing of many high-risk patients, including those exposed to carcinogens or radiation, patients carrying genetic inheritance, having lowered immune defenses or otherwise of a high risk due to the lifestyle or vulnerable phenotype. Therefore, a range of prospective biosensing platforms must be considered to narrow down the choices to the most effective, low cost, and robust platforms which are simple to operate in the environment of highly accessible points-of-care and doctor offices. Here, we describe and evaluate different approaches based on the detection of Sur protein and Sur mRNA biomarkers which are by far the most common signature of the malignant tumors. In comparison with the current molecular biology techniques and methods based on sophisticated instrumental

Table 1
Expression of survivin in various cancer cell types and published methods of Sur protein and Sur mRNA detection.

No.	Types of cancer	Cell lines	Sur target	Method of detection[b-h]	Quantification/LOD*,[a]	Ref.
1	Oral squamous carcinoma	H376	protein	ELISA	70 ± 28 nM (5.8 ± 2.3 ng/mg protein in lysate)	Konopka et al. (2009)
		HSC-3	protein	ELISA	146 ± 35 nM (12.0 ± 2.9 ng/mg protein in lysate)	
		H413	protein	ELISA	153 ± 11 nM (12.6 ± 0.9 ng/mg protein in lysate)	
2	Astrocytoma	U87 MG	protein	Nanogravimetric immunosensor	1.87 ± 0.7 nM in cell lysate 1.7 ± 0.8 nM in 10 mM PBS	(Stobiecka et al., 2016a,b)
3	Nasopharyngeal carcinoma	CNE-2	mRNA	RT-PCR	2.48×10^{-3} relative expression level	Fu et al. (2008)
4	Prostate cancer	PC-3	protein	ELISA	4.44 pM (73.3 pg/mL)	Khan et al. (2009)
5	Ovarian cancer	n.s.	protein	IHC	n.s.	Turan et al. (2014)
6	Breast cancer	MCF-7	cDNA	MB-GN	3 nM	Piao et al. (2012)
			protein	ELISA	3.8 pM (63 pg/mL)	Khan et al. (2009)
7	Pancreatic cancer	MIAPaCa-2, BXPC-3, Panc1	protein	WB	n.s.	Yang et al. (2003)
		CapanI	protein	ELISA	15.0 pM (246.9 pg/mL)	Khan et al. (2009)
		Panc1	protein	ELISA	1.24 pM (20.4 pg/mL)	
8	Colorectal cancer	LoVo, SW480, Colo 320, HT-29	mRNA, protein	RT-PCR WB	n.s.	Chu et al. (2012)
		SW480	mRNA	RT-PCR	n.s.	Sarela et al. (2000)
		SW480	tDNA	MB	16 nM in buffer	(Ratajczak et al., 2018a,b)
9	Neuroblastoma	CHP134, IMR32, SH-SY5Y, SK-N-AS	mRNA	NB	n.s.	Islam et al. (2000)
10	Bladder cancer	5637	protein	ELISA	395 nM (32.6 ng/mg of protein)	Kappler et al. (2003)
		SW1710	protein	ELISA	73 nM (6.0 ng/mg of protein)	
		HT1197	protein	ELISA	108 nM (8.9 ng/mg of protein)	
11	Rhabdomyo-sarcoma	A-204	protein	ELISA	34 nM (2.8 ng/mg of protein)	Kappler et al. (2003)
12	Hepatocellular carcinoma	n.s.	protein	IHC	n.s.	Jin et al. (2014)
13	Malignant melanoma	A375	mRNA	RT-PCR	n.s.	Carpi et al. (2014)
14	Cervical cancer	SiHa, HeLa	mRNA	MB	n.s.	Xue et al. (2011)
		HeLa	protein	ELISA	295 ± 35 nM (24.3 ± 2.9 ng/mg protein)	Konopka et al. (2009)
15	Gastric cancer	n.s.	protein	IHC	n.s.	Dizdar et al. (2017)
		SGC 7901	protein mRNA	WB RT-PCR	n.s.	Yang et al. (2004)

* Quantification/LOD is provided in molar concentration and it is recalculated from other units assuming that the average content of proteins in mammalian cells is 0.2 g/mL of cell volume (concentrations in original units are also provided).
[a] Detection limit: n.s. – not specified.
[b] ELISA - enzyme-linked immunosorbent assay.
[c] MB-GN - molecular beacon with spherical graphite nanoparticle.
[d] MB - molecular beacon.
[e] RT-PCR – reverse transcription polymerase chain reaction.
[f] WB - Western blot.
[g] NB - Northern blot.
[h] IHC - immunohistochemical method.

techniques, such as mass spectrometry, the prospective biosensor-based methodologies, discussed in this section, provide alternative, lower cost, more robust, faster, competitively sensitive, and more scalable devices which are easier to handle by untrained personnel and are suitable to detect Sur protein and Sur mRNA biomarkers in widespread early cancer screening framework.

2.1. Solid electrodes and nanoparticles-based biosensors for the detection of Sur mRNA

One of the most effective methods of detection of the overproduction of Sur protein in the framework of *in vivo* or *in vitro* measurements is based on an indirect testing involving the expression of Sur mRNA. The massive expression of Sur mRNA results in overproduction of Sur protein in the intracellular process of mRNA translation into a protein. Thus, the high level of Sur mRNA is indicative of overproduction of Sur protein. The detection of Sur mRNA is performed using Sur mRNA genosensors (or: Sur genosensors, in brief). These genosensors consist of oligonucleotide probes with antisense sequences encoding a fragment of Sur mRNA. The probes are immobilized in the active recognition layer of the biosensors. The most common sequences of the oligonucleotide survivin probes and targets are presented in Table 2.

Sur mRNA in cells has recently been analyzed by the Xu's group (Liu et al., 2012) using an “on-off-on” switchable electrochemical biosensor based on labeled stem-loop DNA probes attached via thiolate groups to a gold electrode. The Authors have shown that ferrocene (Fc) tagged DNA probes can be used for survivin mRNA detection inside human hepatoma cells line SMMC-7721 using electrochemical techniques. Upon DNA duplex formation the ferrocene moiety was moved to a distal location from the surface of the electrode resulting in a decrease of the electrochemical signal. In the presence of calcium ions, the interaction of the duplex with cells caused further decreasing of the signal. After the lysis of cells with Triton X-100 lysing buffer, only the Fc tagged DNA probes remained on the electrode. The advantage of this method is that two opposing signals, electrochemical and fluorescence, are generated, thereby increasing the method reliability. However, the requirement of cell capture on the sensor surface may lead to a higher noise due to the varying number of cells attached. On the other hand, different kinds of cells could also be distinguished using these biosensors (Fig. 2).

Liu and coworkers have developed an electrochemical DNA biosensor for the detection of Sur mRNA (Liu et al., 2013a). The biosensor was based on a sulfhydryl modified capture probe, self-assembled on a

Table 2
Sequences of oligonucleotide probes and targets used for survivin mRNA recognition in biosensors and fluorescence essays in solution.

No.	Oligonucleotide sequence for targeting survivin	Method	LOD	Ref.
1	MB 5'-(ATTO647N)CGACGGAGAAAGGGCTGCCACGXCG(BBQ)-3′ X = C6-dT Thio Target 5′-CCCCTGCCTGGCAGCCCTTTCTCAAGGACC-3′ Random sequence 5′-ATCGGTGCGCTTGTCG-3′ Linear probe 5'-(ATTO647N)GAGAAAGGGCTGCCA(Thiol)-3′	Optical fiber sensor	0.57 nM	Giannetti et al. (2015)
2	Sur MB 5′-Joe-*CCTGGC* CCA GCC TTC CAG CTC CTT *GCCAGG*-Dabcyl-3′ Complementary target 5′-CAA GGA GCT GGA AGG CTG GG-3′	Fluorescence MB	16 nM	Ratajczak et al. (2018b)
3	MB1 5′-FITC-AGACAGTTGAGAAAGGGCTGCTG TCTAAAAAA- (CH2)3-SH -3 DNA target 1 (MB1 perfectly matched) 5′-CAGCCCTTTCTCAA -3′ DNA target 2 (MB1 single-base mismatched) 5′-CAGCCCTGTCTCAA -3′	Fluorescence MB	n.s.	Qiao et al. (2011)
4	Probe 5′-6-FAM-CCT GGC CCA GCC TTC CAG CTC CTT GCC AGG-Dabcyl-3′ Target 5′-CAA GGA GCT GGA AGG CTG GG-3′	Fluorescence MB	2 nM	Stobiecka and Chalupa (2016)
5	Probe DNA 5′-Ferrocene-C6-CCGGCACAAGG AGCTGGAACCATGCCGG-C6-HS-3′ Antisense oligonucleotide 5′-6-FAM-CCCAGCCTTCCAGCTCCTTG-3' (5 phosphatic bases from both endings were modified by thiosulfates) Target in human SMMC-7721 CAAGGAGCTGGAAGGCTGGG. Target in mouse MEF TAAGGAATTGGAAGGCTGGG	DPV	n.s.	Liu et al. (2012)
6	forward 5′- CCACTGCCCCACTGAGAAC-3′ reverse 5′-TGGCTCCCAGCCTTCCA-3′	RT-PCR	n.s.	Peng et al. (2005)
7	Target Survivin cDNA from 27 to 43 nucleotide Survivin cDNA from 33 to 50 nucleotide probe 5′-Cy3-CTGAGAAAGGGCTGCCAGTC TCAG-Dabcyl-3′ 5′-FITC-TGGTCCTTGAGAAAGGGCGA CCA-Dabcyl-3′	Fluorescence MB	n.s.	Yang et al. (2005)
8	Donor MB: 5'-/Cy3/GAGAAAGGGCTGCCATTCTC/BHQ-2/-3′ Acceptor MB: 5'-/BHQ-3/ACCACGTAGAGATGCGGTGGT/Cy5/-3′ Target: 5′-ATGGGTGCCCCGACGTTGCCCCCTGCC TGGCAGCCCTTTCTC aagg ACCACCGCATCTCTAC ATTCAAGAACTGGCCC-3′	Dual FRET MB	n.s.	Santangelo et al. (2004)
9	Target: 5′-ATGGGTGCCCCGACGTTG CCCCCTGCC TGGCAGCCCTTTCTC aagg ACCACCGCATCTCTAC ATTCA AGAACTGGCCC-3′ Donor MB: 5′-/Cy3/GAGAAAGGGCTGC CATTCTC/BHQ-2/-3′ Acceptor MB: 5′-/BHQ-3/ACCACGTAGAGATGCGGTGGT/Cy5/-3′ Donor MB: 5′-/FAM/GAGAAAGGGCTG CCATTCTC/BHQ-2/-3′ Acceptor MB: 5′-/BHQ-3/ACCACGTAGAGATGCGGTGGT/Cy3/-3′	Dual FRET MB	n.s.	Wang et al. (2011)
10	antisense oligonucleotide for cell viability and apoptosis detection (S1) 5′ CCCAGCCTTCCAGCTCCTTG3′ antisense oligonucleotide labeled with FITC (F–S1) 5′FITC –CCCAGCCTTCCAGCTCCTTG3′ chemical synthesized target survivin strand (S2) 5′CAAGGAGCTGGAAGGCTGGG3′ random oligonucleotide labeled with FITC (F–S3) 5′FITC –TCTCCCCAGGACGCTCTCCT3′	Microfluidic system with fluorescence assay	$(4.8 \pm 1.8) \times 10^6$ copies 2.1–6.5 μM	Li et al. (2013)
11	Probes 5′-GTT CTT GGA TGT AGA GAT GC-3′ 5′-GCT TCT TGA CAG AAA GGA A-3′ 5′-CAA CGT CGG GGC ACC CAT-3′	Flow cytometry	n.s.	Yang et al. (2004)
12	Probe 5′-Cy3-CGACGGAGAAAGGGCTGCCACG/thiol-dT/CG-BHQ2-3′	Peptide-linked molecular beacons	n.s.	Nitin et al. (2004)
13	Probe Recognition sequence: 5′-CTT GAG AAA GGG CTG CCA AAA AA-SH-3′ Reporter sequence: 3′-CCC GAC GGT T-Cy5-5′ Target: 3′-GAA CTC TTT CCC GAC GGT-5′	Fluorescent nano-flares	n.s	Seferos et al. (2007)
14	SurMB-Joe 5′-Joe-CCTGGC CCA GCC TTC CAG CTC CTT GCCAGG-Dabcyl-3′ St 5′-CAA GGA GCT GGA AGG CTG GG-3′ St-1 5′-CAA GGA GCT GCA AGG CTG GG-3′ St-2 5′-CAA GGA GCT CCA AGG CTG GG-3′ St-nc 5′-CCC AGC CTT CCA GCT CCT TG-3′	Fluorescence MB	26 nM	Ratajczak et al. (2018a)
15	SurMB 5′-FAM - CCT GGC CCA GCC TTC CAG CTC CTT GCC AGG - Dabcyl-3′ St 5′-CAA GGA GCT GGA AGG CTG GG-3′ St-1 5′-CAA GGA GCT GCA AGG CTG GG-3′ St-2 5′-CAA GGA GCT CCA AGG CTG GG-3′	Fluorescence MB	24 nM	Stobiecka et al. (2016b)

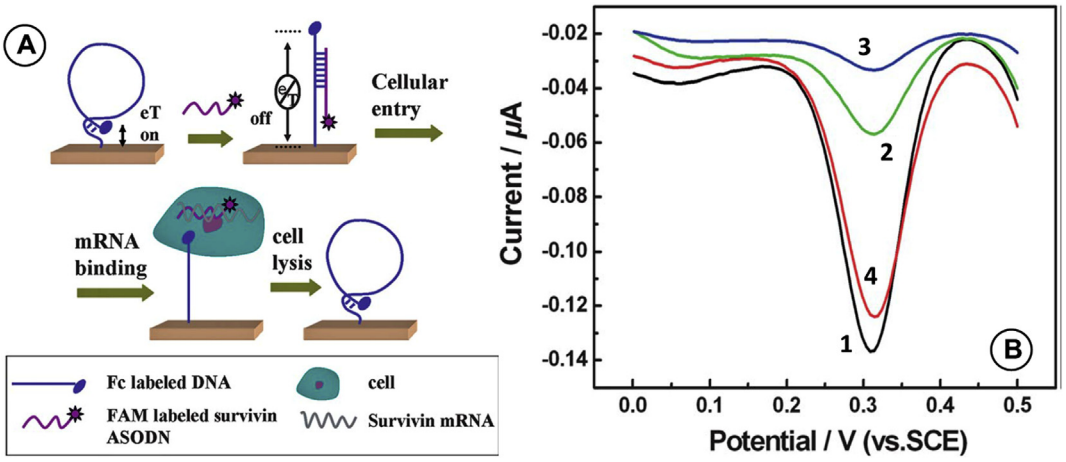


Fig. 2. (A) A circular 'ON-OFF-ON' Fc-DNA-based electrochemical switch for mRNA sensing. (B) DPVs obtained in 10 mM PBS (pH 7.4) with (1) Fc-DNA, (2) DNA duplex, (3) after reaction with SMMC-7721 cells in the presence of Ca^{2+}, (4) after treatment with lysis buffer. Adopted with permission from (Liu et al., 2012).

three-dimensional nanostructure of a graphene-gold nanocomposite which was deposited on the surface of a glassy carbon electrode. Upon capture, the target was flanked by the capture and biotinylated reporter probes creating the "sandwich-type" detection strategy. The electrochemical current signal was enhanced by horseradish peroxidase (HRP) enzyme conjugated with streptavidin attached to the biotinylated reporter when target was captured on the electrode. This highly amplified DNA biosensor has shown excellent sensitivity and selectivity with LOD = 3.4 fM. However, the disadvantage of the amplifying strategy based on electrochemical enzymatic currents is the limitation of this genosensor usage to the cell lysates. The genosensor was successfully applied for the analysis of PCR samples.

High signal amplification in the detection of Sur mRNA was also investigated by Li et al., (2015). The Authors have utilized the ability of the duplex-specific nuclease (DSN) enzyme to cleave a double-stranded DNA or a DNA in DNA:RNA heteroduplexes causing the bio-bar-code probe with cadmium sulfide nanoparticles (CdS-NPs) to release the NPs to the solution. The electrochemical signal was measured using anodic stripping voltammetry technique. This doubly-amplified genosensor has shown an ultra-high sensitivity and selectivity with LOD of 0.48 fM (Fig. 3). The use of DSN nuclease poses, however, certain problems when attempting to utilize the method *in vivo* since the presence of intracellular nucleases and double-stranded DNA may interfere in cleaving the duplexes of the mRNA with capture probes. Also, the up-regulated glutathione in cancer cells may interfere or even block the electrode surface in ASV transduction process. Due to the high sensitivity of this genosensor, an extra effort for the sample pretreatment, for instance in a microfluidic framework may be worth trying.

Recently, an optical biosensor for Sur mRNA detection based on a fiber nanotip coupled with molecular beacons was developed by Giannetti et al., (2015). The Authors have reached the limit of detection of 0.57 nM for a complementary target. This genosensor offers an advantage of a high sensitivity and design simplicity. It is simple to operate and therefore may be utilized without highly trained personnel.

2.2. *Microfluidic and gated systems based on biosensors for the detection of Sur protein and Sur mRNA*

Microfluidic lab-on-a-chip biosensing devices integrate and miniaturize multiple stages of analytical procedures, thereby enabling high throughput and highly reproducible and inexpensive testing. Furthermore, lateral flow paper-based microfluidic biosensors offer versatility and simplicity, making them very useful in points-of-care and doctor offices.

Li et al., (2013) have described the detection of intracellular Sur mRNA biomarker using antibody-based microfluidic microchannel devices. The microchannel was first modified with prostate stem cell antigen (PSCA) monoclonal antibody for prostate cancer cells detection. Then, using a graphene-oxide-poly(ethylene glycol) bis(amine) as the nanocarrier of fluorescein isothiocyanate-labeled oligonucleotide, encoded for recognition of Sur mRNA, the biomarker detection was carried out. Here, the advantage of microfluidic detection platform clearly

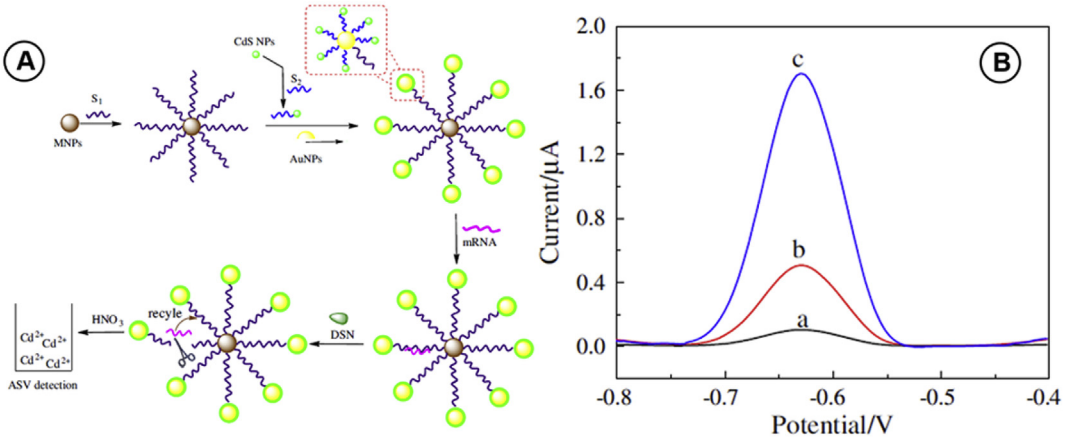


Fig. 3. (A) Schematic representation of the sensing interface for the label-free electrochemical detection of mRNA with dual-amplification of duplex-specific nuclease and bio-bar-code. Anodic stripping voltammetry response of (a) baseline, (b) DNA and (c) mRNA. Reprinted with permission from (Li et al., 2015).

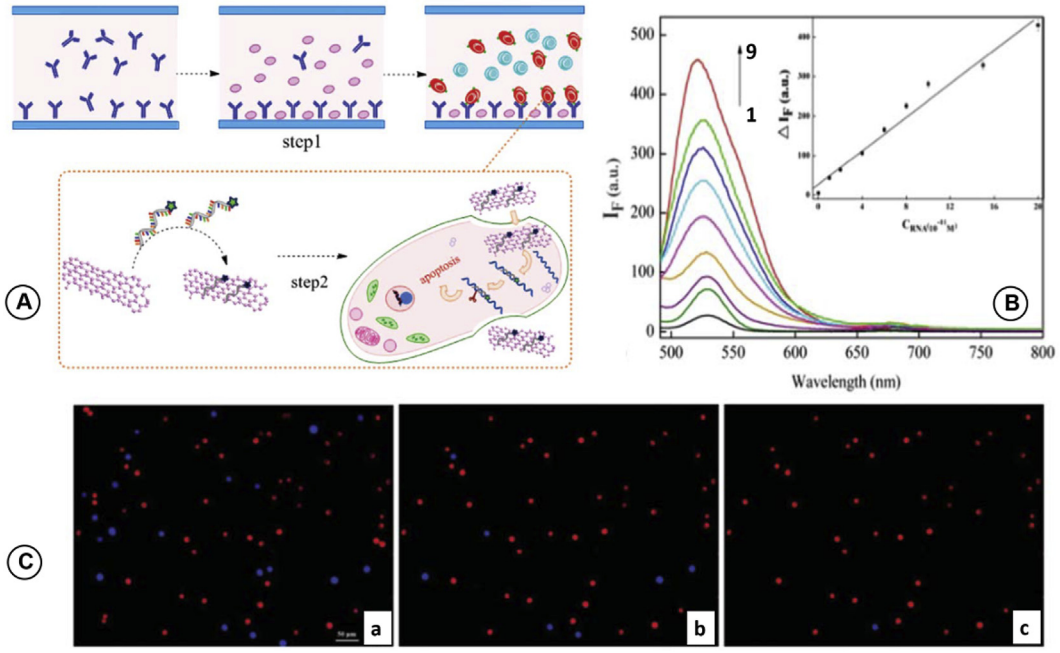


Fig. 4. (A) Schematic illustration the capture of target cells in an anti-PSCA modified microchannel device (step 1) and the fluorescence assay of survivin mRNA (step 2). (B) Fluorescence intensity of survivin target S2 detection from (1) 0 to (9) 2×10^{-10} M; INSET: Dependence of I_F vs. C_{S2}; (C) Microscope images of mixed cell samples of flow at rates (a) 0, (b) 10 and (c) 20 μL/min. Adopted with permission from (Li et al., 2013).

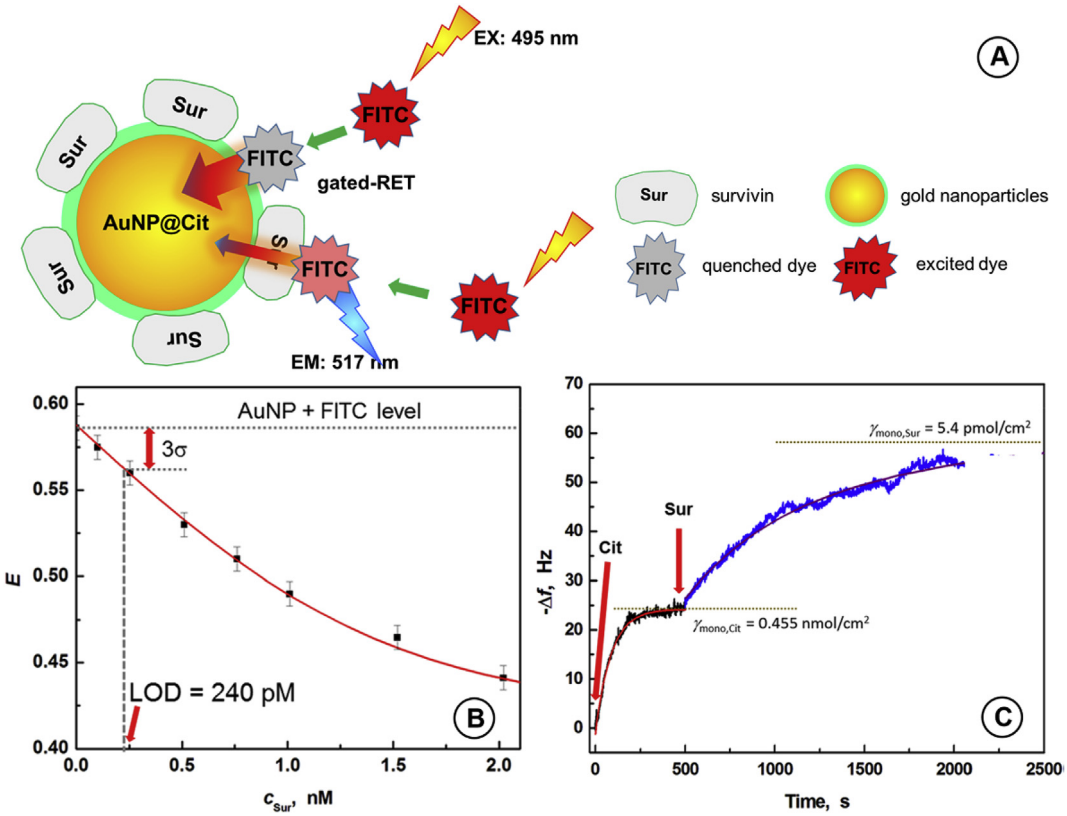
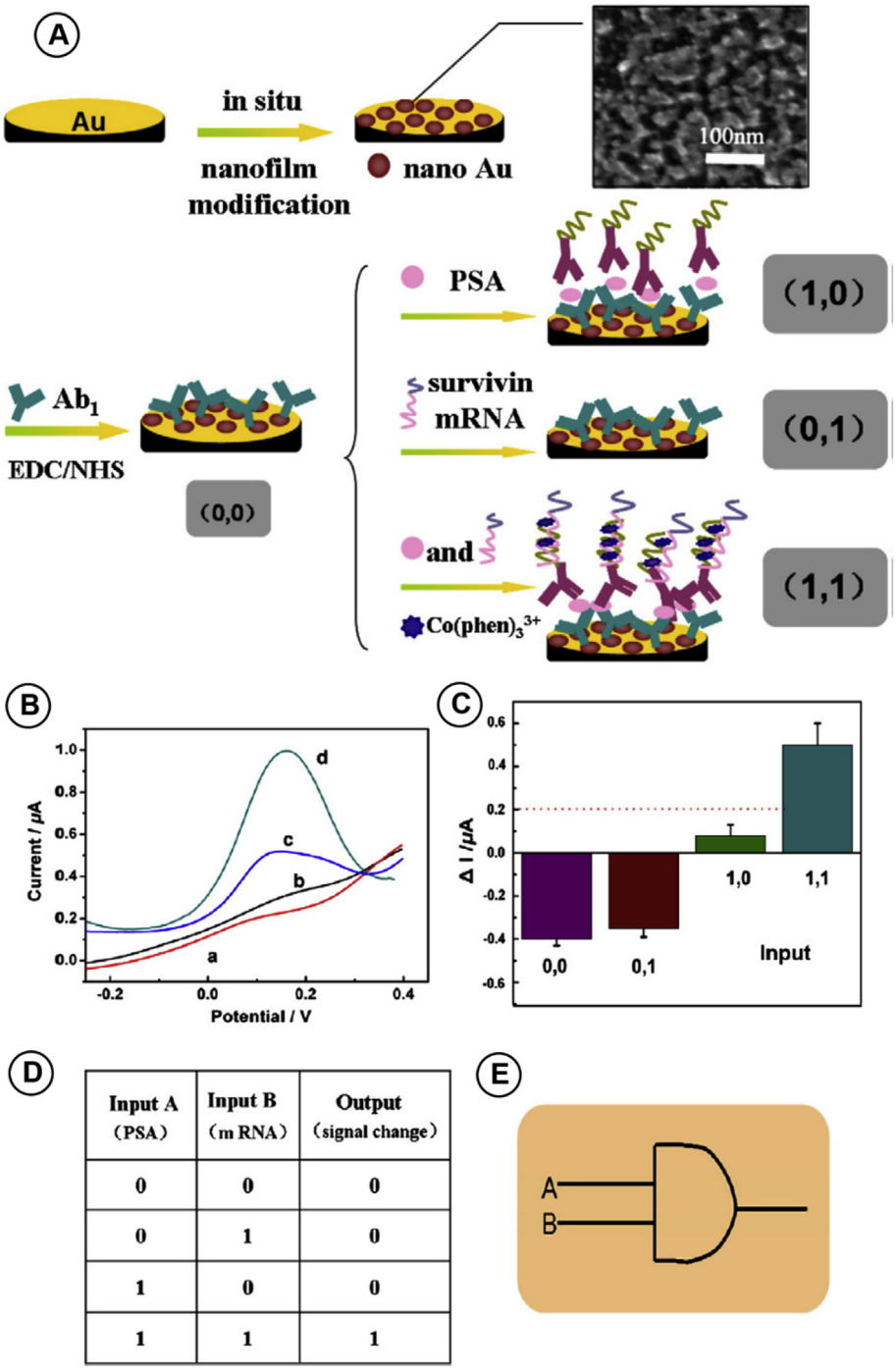


Fig. 5. (A) Principle of gated fluorescence resonance energy transfer (gRET); (B) Dependence of the gRET efficiency on survivin concentration; (C) Nanogravimetric transients of citrate (Cit) adsorption on Au piezoelectrode followed by a survivin (Sur) film formation. Adopted with permission from (Stobiecka and Chalupa, 2015).

lies in the ability to selectively capture cancer cells in a microfluidic channel for further internalization of genosensor nanoprobes in the collected cells. The Authors have estimated the survivin mRNA content in a single cell as $(4.8 \pm 1.8) \times 10^6$ copies (Fig. 4).

A comprehensive method for the isolation of distinct cancer cell populations from body fluids using a microfluidic device has been developed by Abreu 2016a, 2016b. The *in situ* immunocytochemical evaluation using an optimized panel of five biomarkers, including DAPI, pan-CK, vimentin, survivin, and CD45, was performed. The cell capture efficiency of the microfluidic platform was determined by



Input A (PSA)	Input B (m RNA)	Output (signal change)
0	0	0
0	1	0
1	0	0
1	1	1

Fig. 6. (A) The schematic presentation of the logic-controlled "AND" system; (B) Differential pulse voltammograms upon different input signals: (a) (0,0), (b) (0,1), (c) (1,0), (d) (1,1); (C) Bar diagrams for $Co(phen)_3^{3+}$ peak current changes; (D) Table for the AND logic gate; (E) Circuit for the AND logic gate. Reprinted with permission from (Liu et al., 2013b).

counting the number of the entrapped fluorescent cells, followed by staining with the cell-permeable dye Calcein AM. This work demonstrates that an inexpensive microfluidic detection platform employing multiple biomarkers can serve as the efficient cancer screening method based only on non-invasive body fluids testing.

Wang et al., (2015) have shown that the target Sur mRNA after hybridization with ssDNA probe can trigger the release of luminophore $Ru(bpy)_3^{2+}$ encapsulated in the positively charged mesoporous silica nanoparticles functionalized with 3-aminopropyltriethoxysilane and the negatively charged ssDNA. The reaction of luminophore with the coreactant tripropylamine in the solution has given an electrochemiluminescence (ECL) signal proportional to the concentration of Sur mRNA. The Authors have found Sur mRNA level to be in the range 0.5–50 fM with a limit of detection of 0.1 fM. The ECL biosensor based on DNA bio-gate with the mesoporous silica nanoparticles system provided high signal amplification owing to the duplex-specific nuclease used in the investigations for specific digestion of the ssDNA in the DNA-RNA heteroduplexes. The proposed assay offers the advantage of a very high sensitivity to Sur mRNA due to the applied amplification paradigm. The complexity introduced with the amplification scheme may however lead to some interference from the biological matrix.

Another kind of the gate system, the gated plasmon-enhanced

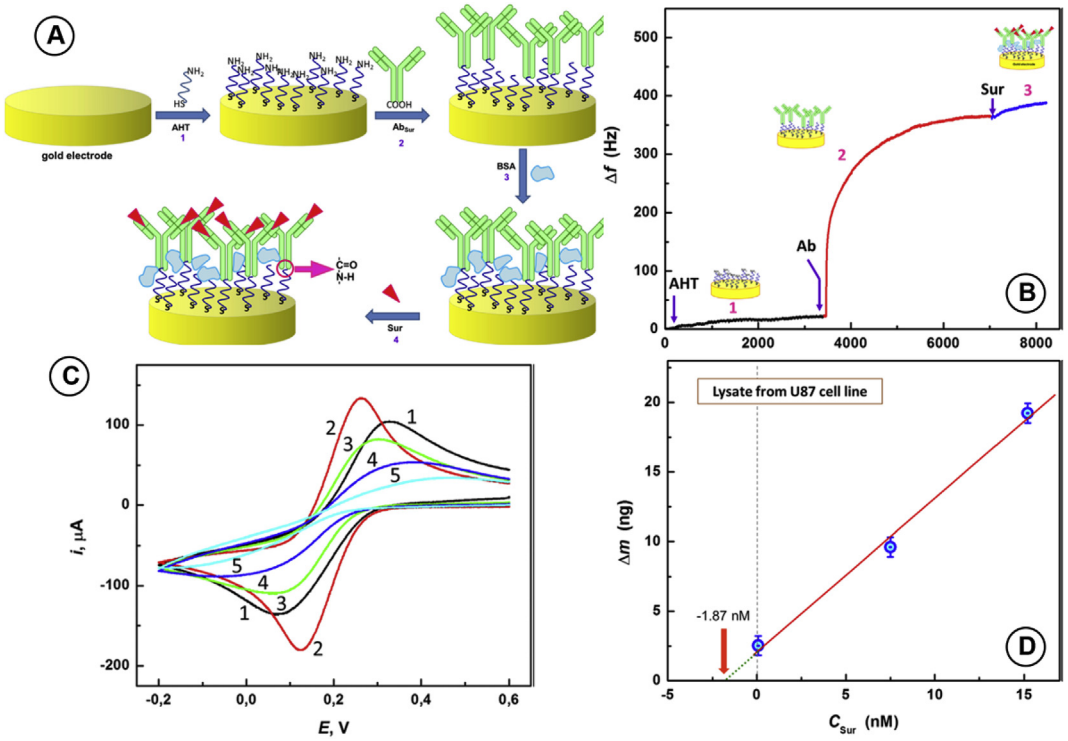


Fig. 7. (A) Schematic illustration of the construction of immunosensors; (B) Frequency shift transients: (1) aminohexanethiol (AHT), (2) anti-survivin antibody (Ab), (3) survivin; (C) Cyclic voltammograms: (1) bare gold piezoelectrode and (2–5) after immobilization of successive layers, v = 100 mV/s; electrolyte: 3.33 mM $K_3Fe(CN)_6$ + 66 mM PBS. (D) Determination of Sur in a lysate solution from U-87MG cell line. Reprinted with permission from (Stobiecka et al., 2016a). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

resonance energy transfer (gRET) for the detection of survivin protein was developed by Stobiecka and Chalupa, (2015). The Authors have demonstrated that the resonance energy transfer occurring between a fluorescent dye and gold nanoparticles can be modulated by the width of gates (channels) in the shell of protein surrounding the nanoparticle core. In the experiments, citrate–capped gold nanoparticles were used in conjunction with a fluorescein isothiocyanate dye and Sur protein forming sub-monolayer films on AuNPs. Despite of the electrostatic repulsive forces between these components, the method was ultrasensitive to survivin with LOD = 240 pM (Fig. 5).

A logic-controlled "AND" mode biosensor for detection of two different cancer biomarkers: prostate-specific antigen and Sur mRNA was designed by Liu et al., (2013b). The biosensor was based on the immobilization of primary capture antibody Ab_1 and secondary detection antibody Ab_2 - DNA complexes onto the mercaptopropionic acid-functionalized gold nanostructured film formed on the Au electrode surface. The electrochemical signal obtained after intercalation of $Co(phen)_3^{3+}$ ions into DNA duplex, formed upon hybridization of the Sur antisense oligonucleotides with Sur mRNA strands, was measured using differential pulse voltammetry technique. The Authors have demonstrated that faradaic current generated by the intercalation agent increases only when both biomarkers are detected in the sample (Fig. 6), thus resembling a logic AND gate operation principle. The advantage of the logic gate system is in the simple YES/NO answer to the diagnosis of cancer. However, when the input signals are close to the threshold values, any change in background level or noise in the electrochemical output reading may lead to false positive or false negative results. In the former case, further testing using methods with analog output may solve the problem.

2.3. Nanogravimetric and chemiluminescence based biosensors for the detection of Sur protein

Recently, Stobiecka et al., (2016a) have developed a piezoimmunosensor for direct detection of Sur protein using electrochemical quartz crystal nanobalance. The gold piezoelectrode was modified with a self-assembled monolayer of aminohexanethiol molecules and covalently bound monoclonal anti-survivin antibodies. The proposed sensor was constructed layer-by-layer with monitoring of the resonance frequency of the piezoresonator and cyclic voltammetry characteristics for each layer. The Authors were able to detect Sur protein with LOD = 1.7 nM. The assessment of piezoimmunosensor applicability for the analysis of U87 MG cell line lysate was also presented (Fig. 7). The advantage of the combined piezometric and electrochemical testing is the assurance of the sensor responses which is equivalent to embedding of an internal control to the sensory system.

Yang et al., (2018) have used magnetic particles-based chemiluminescence enzyme immunoassay for the detection of Sur protein and evaluation of the diagnostic value of urinary Sur for bladder cancer and renal cell carcinoma. The method was based on a double-antibody sandwich immunoreaction and luminol-H_2O_2 chemiluminescence system. One kind of monoclonal antibodies against Sur was coupled to magnetic beads and the second one was labeled with horseradish peroxidase (HRP). After interaction with Sur, the luminescence signal was measured. The achieved limit of detection for Sur was LOD = 57 pM (0.949 ng/mL), competing well with ELISA, as tested with 90 clinical urine samples. Thus, this highly sensitive amplified method has a potential for non-invasive cancer screening.

2.4. Molecular beacon (MB) probes-based fluorescence biosensing systems for the detection of Sur mRNA

Recently, extensive studies have been performed with molecular beacon (MB) probes consisting of an oligonucleotide strand labeled with a fluorophore and a quencher. The sequence of this loop-stem structured probe is complementary to a part of the Sur mRNA sequence. The use of oligonucleotide molecular beacons encoded for recognition of Sur mRNA is becoming one of the leading cancer diagnostic tools.

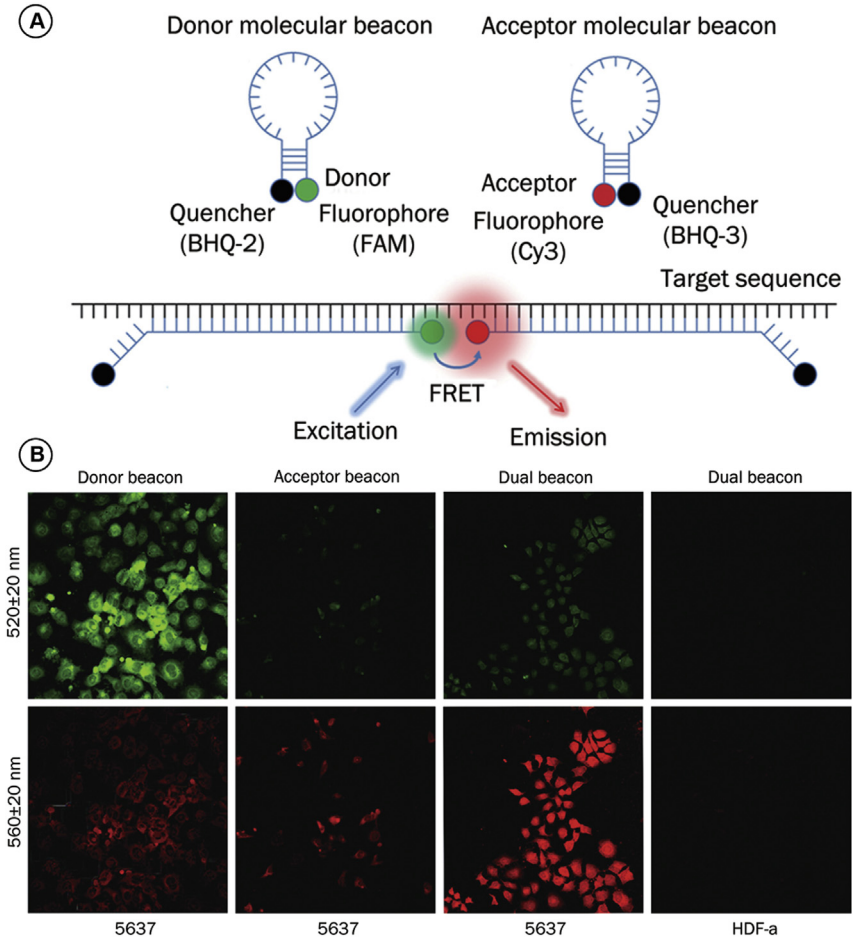


Fig. 8. (A) Scheme of mechanism of a dual FRET MB. Hybridization of donor and acceptor MBs to mRNA target upon donor excitation (490 nm); (B) Laser scanning confocal fluorescence microscopy images of 5637 and HDF-a cells incubated with MBs for survivin mRNA detection. Reprinted with permission from (Wang et al., 2011).

The different kinds of molecular beacon probes are described in this and the next sections. The advantage of these biosensing systems is that they enable direct monitoring of the expression of Sur mRNA *in situ*.

Wang and coworkers (Wang et al., 2011) have detected survivin mRNA in bladder cancer (BCa) cell lines 5637, 253 J, and T2, as well as in exfoliated cells in urine samples from patients with BCa using survivin-targeting dual fluorescence resonance energy transfer molecular beacon. The Authors have used a pair of molecular beacons with sequences complementary to survivin mRNA. One MB with FAM or Cy3 fluorophore and BHQ-2 quencher was acting as the donor and second MB with BHQ-3 quencher and Cy3 or Cy5 fluorophore was acting as the acceptor. After hybridization with target oligonucleotide, the fluorescence resonance energy transfer (FRET) between the donor and acceptor fluorophores has occurred upon the donor excitation, showing clearly the fluorescence signal. The Authors used also the human dermal fibroblasts-adult cells as a negative control which has shown almost no fluorescence signal in contrast to BCa cells (Fig. 8).

Molecular beacons with different fluorophores (FITC and Texas Red) and Dabcyl quencher have been designed for simultaneous detection of mRNA of survivin and cyclin D1 tumor markers in breast cancer cell lines: SKBr-3, MDA-MB-231, MCF-7 and MDA-MB-435 by Peng et al., (2005). The expression of these genes was also confirmed by real-time RT-PCR and survivin protein by Western Blot analysis. The Authors have also studied the Sur gene expression *in situ* in frozen breast cancer tissue sections, as well as in viable cells and in cells treated with EGF, docetaxel, and *p53*. The fluorescent signal was very low for both molecular beacons in a normal immortalized human mammary epithelial cell line.

The survivin mRNA was also detected in colorectal cancer cells SW480 using molecular beacon probe modified with Joe fluorescence dye and Dabcyl quencher by Ratajczak et al., (2018a). The Authors have proposed a new model of interactions of MB with complementary oligonucleotides based on the hairpin – hairpin interactions of oligonucleotides. They have shown an efficient transfection of malignant SW480 cells with molecular beacon probes using liposomal nanocarriers, which resulted in a strong green emission from internalized Sur mRNA corroborating the overexpression of survivin mRNA. Similar experiments with healthy human-colon epithelial cells CCD 841 CoN have shown only negligible expression of survivin mRNA. A single nucleotide polymorphism sensitivity and a low detection limit of 26 nM for complementary targets have been achieved (Fig. 9).

Recently, Xue et al., (2011) have used MBs that target a wild-type survivin mRNA for the diagnosis of cervical cancer. The molecular beacon probes were covalently linked with FITC or Cy3 fluorescence dyes and Dabcyl quencher. The Authors have shown, that both MB probes were able to detect the expression of survivin and generated strong fluorescent signals corresponding to the survivin mRNA in cytoplasm and nucleus of the cervical cancer cell lines HeLa and SiHa. They have also found that the expression of Sur mRNA in the normal human dermatological fibroblast cells was negligible. The fluorescent signal generated by MB was consistent with results obtained using Western blotting, RT-PCR, and immunocytochemical staining method. Survivin expression was also detected in clinical smear samples obtained from patients with cervical cancer using MB with 61.4% (27/44)

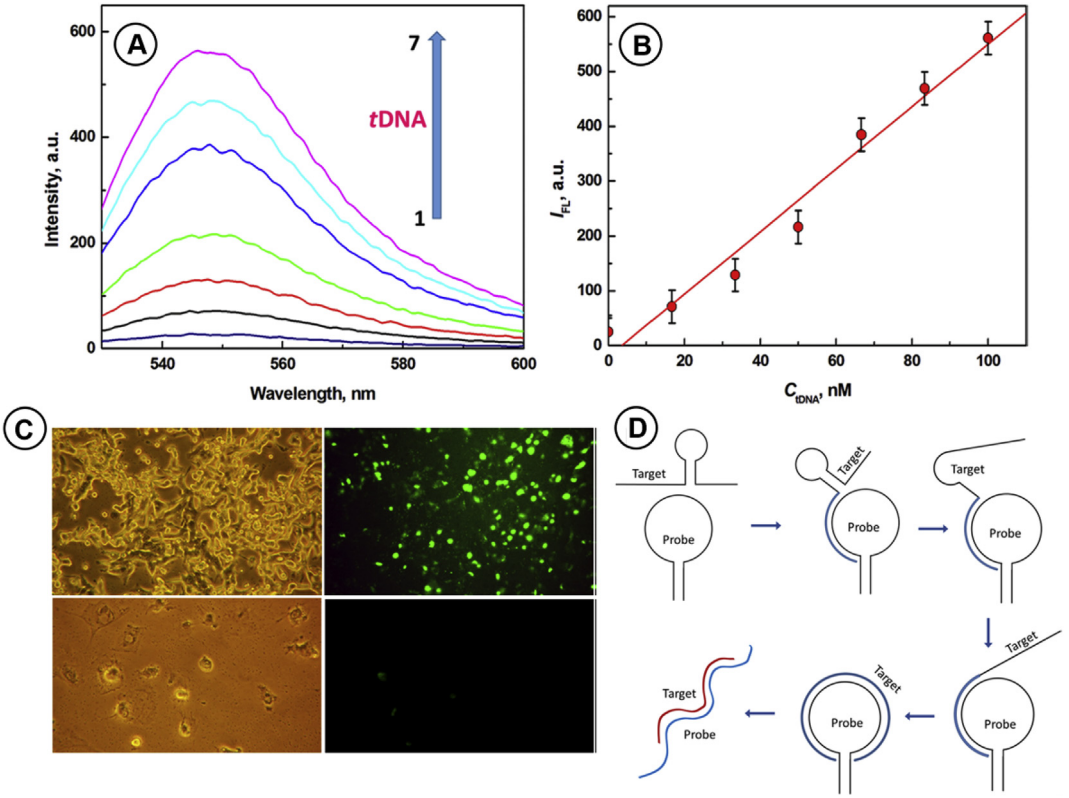


Fig. 9. (A) Fluorescence emission spectra of SurMB-Joe recorded with complementary tDNA; (B) Dependence of $I_{FL,max}$ vs C_{tDNA}. Conditions: $C_{SurMB\text{-}Joe}$ = 100 nM, buffer: 10 mM PBS, pH 7.4; λ_{ex} = 520 nm. C_{tDNA} (nM): from 0 to 100; (C) Optical (left) and fluorescence (right) microscopic images of SW480 cells (top panel) and CCD 841 CoN cells (bottom panel) transfected with SurMB-Joe; (D) Mechanism of hairpin – hairpin interactions for SurMB-Joe with target. Reprinted with permission from (Ratajczak et al., 2018a).

sensitivity and 72.7% specificity (34/44).

Carpi et al., (2014) have found that treatment of human malignant melanoma A375 cell line and human metastatic melanoma 501 Mel cell line with MBs encoded for recognition of Sur mRNA, have induced apoptosis of these cells. This process was investigated by measuring the dissipation of mitochondrial membrane potential, internucleosomal DNA fragmentation, and changes in nuclear morphology, revealed by DAPI staining. The effect of MB on survivin gene expression and protein expression was investigated using real-time PCR and Western blot experiments.

According to Piao et al., (2012), the MB with graphite nanoparticles is a robust molecular probe for the detection of survivin mRNA in human breast cancer MCF-7 cells. It can also be utilized for real-time monitoring of gene expression and survivin mRNA level quantification. The Authors have shown that the intensity of fluorescence signal of MB was increased after the addition of docetaxel which can up-regulate the survivin gene expression.

2.5. Probes attached to membrane-penetrable nanocarriers of graphene oxide, polymers and plasmonic nanoparticles based biosensing system for the detection of Sur mRNA

Recently, the Stobiecka's group has shown that a graphene oxide nanosheet (GON) can be used as the nanocarrier of MB (GON@MB) for survivin mRNA detection in human malignant glioma cell 87 MG (Stobiecka et al., 2016b) and colorectal cancer cell line SW480 (Ratajczak et al., 2018b). The Authors have achieved the limits of detection of 24 nM and 16 nM, for these cell lines, respectively. The GONs were characterized using resonance elastic light scattering, Raman spectroscopy, SEM and TEM, and their efficacy was evaluated by MTT cell viability testing (Fig. 10).

Adinolfi et al. 2014, 2017 have used the polymethylmethacrylate nanoparticles (PMMA-NPs) with adsorbed molecular beacons for the detection of Sur mRNA in A549 human lung adenocarcinoma epithelial cells. A molecular beacon equipped with an ATTO 647N fluorescence dye and a Blackberry Quencher was utilized for Sur mRNA monitoring. Authors have shown that the MB loaded onto PMMA-NP carriers were effectively internalized into A549 cells generating fluorescent signal in the presence of survivin mRNA expression.

Recently, Klymchenko's group (Melnychuk and Klymchenko, 2018) proposed a highly sensitive nanoantenna nanoprobes for the detection of Sur mRNA. The nanoprobe consists of a rhodamine-loaded poly (methylmethacrylate-co-methacrylic acid) nanoparticle functionalized with Cys5 dye-modified short oligonucleotides. The Authors have shown, that the rhodamine dyes donate the energy to Cy5 acceptors via FRET process resulting in the strong red-light emission at 670 nm. Upon hybridization with a target nucleic acid, the FRET process is blocked and restoration of the green emission signal from the nanoantenna is observed. The Authors have achieved an excellent LOD = 5 and 0.25 pM, for the operation in solution and on the surface of a single particle, respectively.

The group of Tang has assembled the gold nanoparticles (AuNPs) with a bi-molecular beacon molecules (bi-MBs) for mRNA detection in breast cancer cells (Qiao et al., 2011). The molecular beacons for identification of Sur mRNA and cyclin D1 mRNA were labeled with FITC and Cy5, respectively. The tests have shown that the AuNP/bi-MB probes can be used for simultaneous detection of the expression of cancer genes in SK-BR-3 cells.

2.6. Detection of Sur in exosomes and circulating tumor cells (CTCs)

Survivin localizes in cytoplasm, mitochondria and the nucleus.

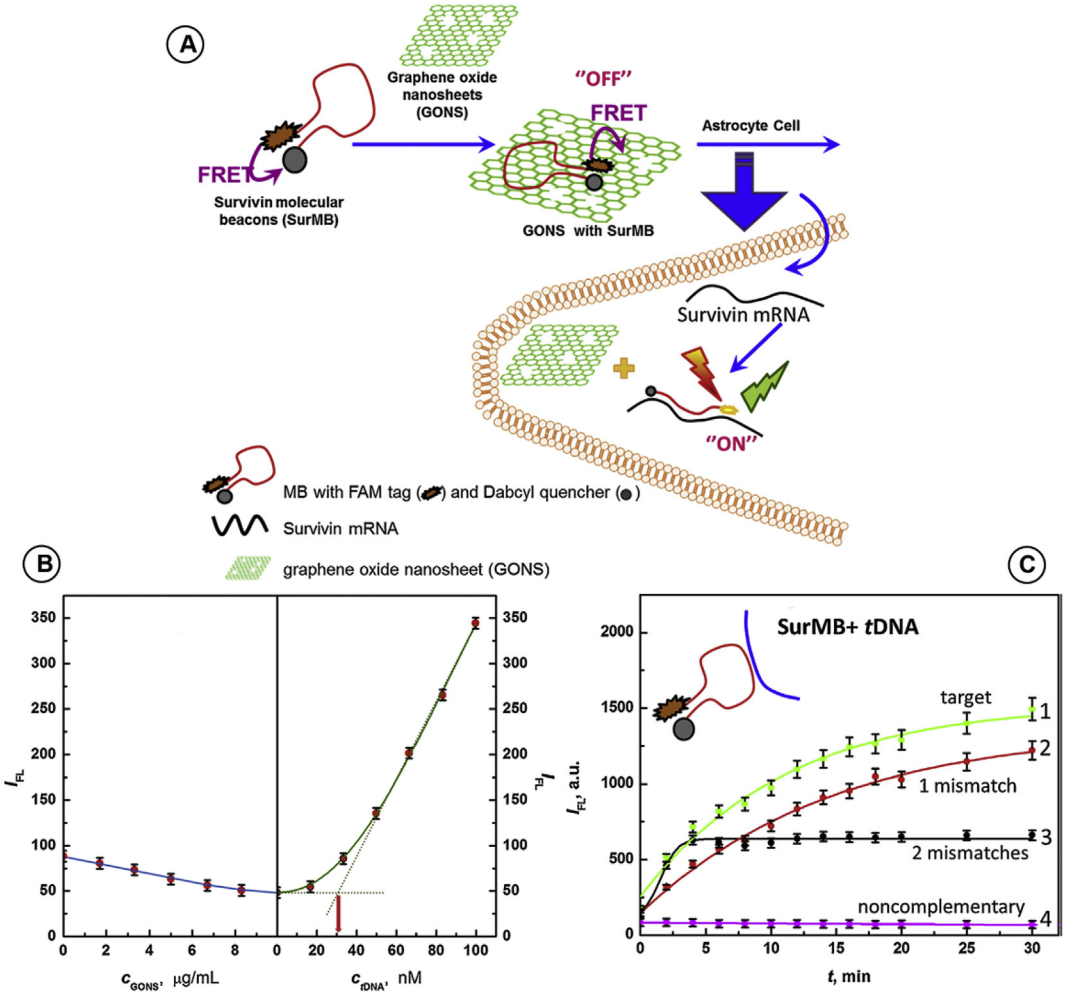


Fig. 10. (A) Schematic view of formation and delivery of GONS@SurMB to the astrocyte cells to detect survivin mRNA. (B) GONS quenching and target binding characteristics; (C) Isothermal fluorescence emission transients: (1) S_t perfect complement to SurMB loop, (2) S_{t-1} strand with a single mismatch, (3) S_{t-2} strand with a double mismatch; (4) S_n non-complementary strand; C_{tDNA}[nM]: from 0 to 100; Adopted with permission from (Stobiecka et al., 2016b).

Recently, the Wall's group has shown that survivin is released from cancer cells, such as HeLa (Khan et al., 2011) and prostate cancer patient plasma (Khan et al., 2012) via exosomes. Kreger et al. have demonstrated that the treatment of highly aggressive MDAMB231 breast cancer cells with paclitaxel induced the formation of exosomes enriched with survivin (Kreger et al., 2016). Also, the flow cytometry images indicate that the serum-derived exosomes from patients with malignant gliomas contain Sur protein on their surface (Galbo et al., 2017) (Fig. 11).

In addition to exosomes, survivin was also detected in circulating tumor cells (CTCs). Therefore, the analysis of CTCs can provide valuable information for the prediction of metastasis and recurrence of cancers, such as the gastric and colorectal cancer (Yie et al., 2008), non-small cell lung cancer (Yie et al., 2009) and breast cancer (Yie et al., 2006). These results confirm that the exosomes and CTCs offer a great potential for clinical diagnostics and therapy by investigations of cancer biomarkers without invasive biopsies.

3. Conclusions

A remarkable progress in the development of various types of biosensors and biosensing systems for the detection of survivin protein and survivin mRNA observed recently has become the basis for a new potential approach for advancing our ability to a widespread early cancer screening. Since the Sur biomarkers are so ubiquitous and are expressed in all types of cancers, the ability to inexpensively perform an early cancer screening is a promising direction enabling the selection of an appropriate therapeutic intervention for the increased survivability rate and reduced overall mortality. Since the expressed survivin has also been found in CTCs and exosomes, the biosensing platforms may become, in not too distant future, the leading technology for the non-invasive early cancer detection. The importance of detecting Sur biomarkers in exosomes and circulating tumor cells is associated with metastasis of tumors which is the key challenge to be dealt with and the main cause of the high mortality rate.

In the development of future biosensing platforms for the detection of Sur protein and Sur mRNA, special attention has to be paid to the high sensitivity, robustness, and biocompatibility. Among these, the sensitivity can further be increased by employing some of the recently developed amplifying multi-tier paradigms in which the basic detection process generates a product that is then detected by a higher-tier amplifying analytical detection process which generates in turn a new product with concentration related to the original analyte, and so on. In case of *in vivo* analysis, the biocompatibility of sensing process is of utmost importance, as the immunoresponse may be detrimental to the analysis, especially when longer term monitoring of analytes is required. Finally, the robustness is necessary due to the analysis of complex biological samples and in the absence of extensive separation procedures (which is usually the case in biosensor technologies), the minimized impact of the matrix is required. Novel detection modalities

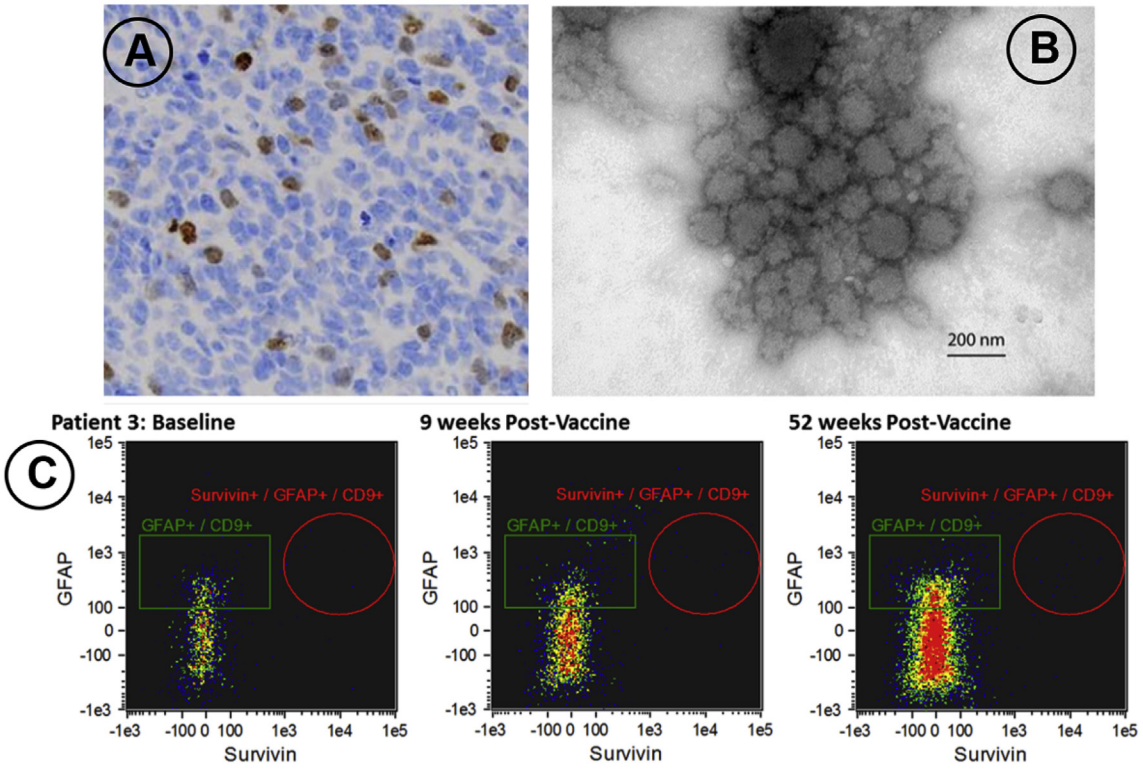


Fig. 11. (A) Immunohistochemical stains for survivin; (B) Electron microscopic image of exosomes isolated from the baseline serum sample; (C) Imaging flow cytometry plots of CD9 + /GFAP + /Sur + exosomes in patient with late tumor progression after the doses of survivin vaccine. Adopted with permission from (Galbo et al., 2017).

aiming at the enhancement of sensitivity and rapid response, such as those employing bio-gates, sub-monolayer protein gates, or logic gates, as well as the microfluidic-based and multi-sensor array-based detection systems offer an expanded field of biosensor technology for further development and utilization in early cancer screening.

In summary, the biosensors and biosensor arrays, self-standing or in form of microfluidic for the detection of survivin and other cancer markers are becoming a promising platform capable to advance our ability to diagnose cancer and help in selecting appropriate types of therapy. With early cancer screening, the survivin detection technology should see in near future a decrease in mortality rates for a range of cancer types.

Declaration of interests

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.

Conceptualization-MS, SJ; Funding acquisition -MS; Investigation-MS, KR, SJ; Project administration –MS, SJ; Supervision MS, SJ; Writing - original draft: MS, KR, SJ; Writing - review & editing MS, KR, SJ.

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