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A novel reporter system for cyclic AMP mediated gene expression in mammalian cells based on synthetic transgene expression system

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ABSTRACT

Cyclic AMP (cAMP) is an important second messenger that mediates various biological functions in both prokaryotes and eukaryotes. Due to the ever increasing significance in studying the function and modulation of cAMP-based signaling, it is important to develop a protein-based biosensor that reports the cAMP mediated gene expression. Based on a synthetic transgene approach, an artificial mammalian transactivator was developed by fusing a transcriptional regulatory element cAMP receptor protein (CRP) of *Escherichia coli* to the VP16 transactivation domain of *Herpes simplex* virus in a mammalian expression vector (pLA1) that activates CRP specific operator site present in a chimeric promoter (O_{CRP}- $PhCMV_{min}$- Luciferase) in a concentration dependent manner in mammalian cells. Our results reveal that the engineered transactivator report the gene expression mediated by cAMP directly in mammalian cells and this cAMP reporter system works irrespective of Protein kinase A (PKA) - cyclic AMP response element binding protein (CREB) - cyclic AMP response element (CRE) signaling since the luciferase activity mediated by synthetic gene construct is seen even in the presence of PKA inhibitor H-89 (derived from H-8 (N-[2-(methylamino) ethyl]-5-isoquinoline-sulfonamide). Furthermore this synthetic transcription factor plays a significant role in reporting and mediating cAMP signaling in tumorigenic cells which possess an aberrant cAMP signaling due to PKA and CREB mutations.

1. Introduction

Cyclic AMP (cAMP), the second messenger of many hormones, is one of the most common signaling molecules involved in intracellular signal transduction in many different organisms from bacteria to humans (Beavo and Brunton, 2002), (Bai et al., 2009). In bacteria, cAMP controls metabolism, phototaxis, protein secretion and virulence by transcriptional regulator CRP which is also called catabolite activator protein (CAP) (Jens et al., 2018). CRP (45 kDa) acts bi-functionally as a cAMP binding domain and as a transcription factor. Upon binding with cAMP, CRP binds to a conserved DNA sequence from which it can either activate or repress transcription initiation from various promoters in a simple mechanism (Lawson et al., 2004), (Busby and Ebright, 1999). cAMP has a high binding affinity with *E.coli* CRP (K_d Value = 13–16 μM) that enhances the DNA binding approximately 1000 fold in 1:1 cAMP/CRP interaction (Green et al., 2014). In mammalian system, cAMP is produced by adenylyl cyclase which is activated by binding of agonists to G protein coupled receptors and it is degraded by the action of phoshodiesterases (Schindler, Brand, 2016). When the intracellular cAMP level surges, various effector proteins such as Protein kinase A (PKA), Exchange protein activated by cAMP (EPAC), Cyclic Nucleotide-gated ion channels (CNG) and Popeye domain containing (Popdc) proteins, get activated (Tanwar et al., 2017), (Fimia and Sassone-Corsi, 2001), (Schindler, Brand, 2016). Of these, PKA is the primary mediator of cAMP signaling in mammals which gets activated in presence of cAMP and phosphorylates CREB. The phosphorylated CREB then binds to certain DNA sequences called CRE, thereby regulating the transcription of the downstream genes (Skalhegg, 2000), (Seino, 2005). The bacterial CRP and mammalian PKA regulatory subunits share a common cAMP binding domain wherein cAMP binding to their functional domain activates signal transduction (Weber et al., 1982). Many hormones like epinephrine, adrenocorticotropin, vasopressin and glucagon use cAMP as a second messenger to mediate a wide variety of physiological functions including metabolism, ion channel activation, neuronal signaling, muscle relaxation and many other cellular functions (Thomsen et al., 2005; Hill et al., 2010). Hence development of an orthogonal cell based assay to monitor the cAMP signaling would be a useful tool to assess the actions of endocrine

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hormones, adenylyl cyclases and phosphodiesterases.

Synthetic biology is an emerging field that aims to design and build functioning biological circuits to rewire endogenous gene regulatory systems, including gene expression systems (Kis et al., 2015; Kolar et al., 2015; Deans et al., 2007; Hörner and Weber, 2012). In recent years, a variety of transgene expression systems have been developed to regulate several genes in mammalian cells using several prokaryotic transcriptional regulatory elements including TetR, LacI, CymR, GAL4 and GyrB for a broad range of biotechnological applications (Gossen et al., 1995; Hu and Davidson, 1987; Ornitz et al., 1991; Hui-Fen Zhao et al., 2004; Deans et al., 2007; Wang et al., 2008). These are considered to be a great advancement in synthetic biology which controls the gene expression at the transcriptional level. The current study shows application of a transgene approach to report the gene expression mediated by cAMP signaling in mammalian cells using bacterial transcriptional regulator CRP. By converting this *E.coli* CRP in to a mammalian transcription factor which in turn binds to chimeric promoter containing transcription factor-specific operator site, we designed a synthetic cAMP-sensitive gene switch that enables to report the cAMP signaling exclusively and the presence of intracellular cAMP in a concentration dependent manner.

2. Materials and methods

2.1. Plasmid design

pLA1: Constitutive expression vector P_{SV40}-CRP-VP16-PA as a mammalian transactivator for cAMP. **pLA2:** This plasmid contains a single CRP specific operator site (O_{CRP}) 5′ AAATGTGATCTAGATCACA TTT-3' that is fused upstream of $PhCMV_{min}$ and luciferase gene. **pGL4.29:** A constitutive human CRE expression vector. **pGK**: This plasmid constitutively expresses the human β2-adrenergic receptor in mammalian cells. **pLD2:** This plasmid contains two CRP specific operator sites (O_{CRP}).

2.2. Reagents

Forskolin, H-89 (derivative of H-8 (N-[2-(methylamino)ethyl]-5-isoquinoline-sulfonamide), 3-isobutyl-1-methylxanthine (IBMX) and Isoprenaline were purchased from Sigma Aldrich. Versene, D-luciferin and coelentrazine were purchased from Invitrogen. Polyethyleneimine (PEI), #23966–2, MW 25,000, was procured from Polysciences, USA. LANCE cAMP kit was purchased from PerkinElmer, USA.

2.3. Cell lines and culture

Human embryonic kidney (HEK 293, HEK 293T), Chinese hamster Ovarian (CHO), human non-small cell lung cancer (A549), breast cancer (MCF-7) and Osteosarcoma (KHOS) cell lines were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% (v/v) fetal calf serum and 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich, Munich, Germany). All the cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4. Transfection

Transient transfection was performed by PEI method for all the plasmids in both cancer and non-cancerous cell lines. Cells were seeded in a 12-well plate and DNA-PEI mixture (1:3 ratios) was added next day in serum-starved cells followed by treatment of cAMP agonist, isoprenaline/forskolin and PKA inhibitor H-89.

2.5. Luciferase assay

Cells were co-transfected with plasmids pLA1 and pLA2/pLD1. The medium was changed next day, and cells were treated with appropriate cAMP agonist/Forskolin/H-89. After 48 h, cells were lysed using passive lysis buffer and dual luciferase assay was performed using luciferase assay buffer containing 25 mM glycylglycine, 15 mM phosphate buffer at pH 8.0, 4 mM EGTA, 2 mM ATP, 1 mM dithiothreitol, 15 mM magnesium sulfate and 75 μM luciferin and internal control assay buffer containing 1.1 M NaCl, 2.2 mM disodium EDTA, 0.72 M KH2PO4, 0.44 mg/mL bovine serum albumin (BSA), 1.3 mM sodium azide and 1.43 μM coelenterazine. The luciferase activity was measured in a luminescence plate reader (Molecular Devices Inc., USA), with renilla luciferase as the internal control.

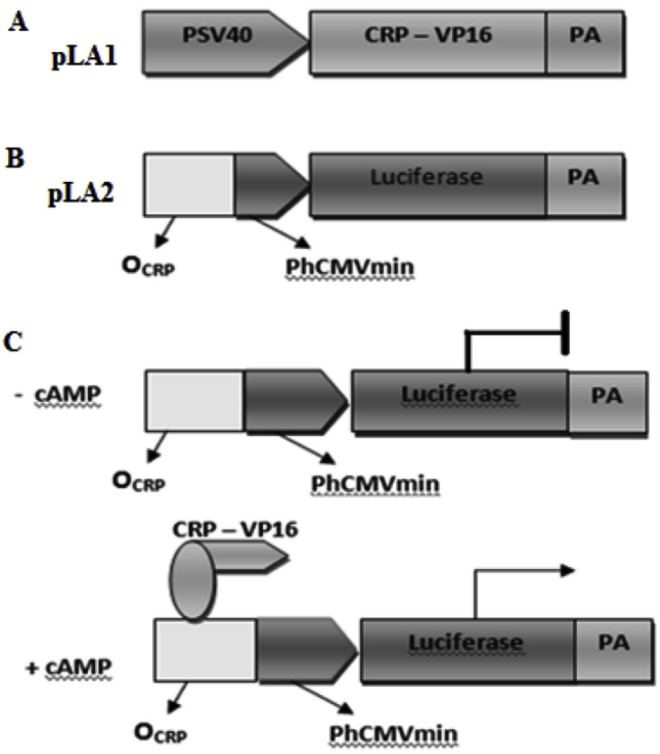

Fig. 1. Construction of cAMP responsive transgene control system in mammalian cells. (A) pLA1 plasmid was constructed by fusing cAMP receptor protein (CRP) from E.coli to the VP16 transactivation domain of Herpes simplex virus that results in a synthetic cAMP dependent transcription factor. (B) pLA2 was constructed by fusing a single CRP-specific operator site (O_{CRP}) 5′ AAAT GTGATCTAGATCACATTT-3′ to the minimal version of the cytomegalo virus immediate early promoter ($PhCMV_{min}$). (C) At basal cAMP levels, CRP-VP16 cannot bind to its operator and suppresses cAMP mediated gene expression whereas an increase in intracellular cAMP level leads to binding of cAMP to CRP-VP16 which in turn interacts with its operator and transactivates cAMP mediated gene expression.

2.6. Measurement of intracellular cAMP assay

Intracellular cAMP concentration was measured by using the Lance cAMP kit (PerkinElmer Corp., Waltham, MA) as per the instructions of the manufacturer. Cells were seeded in 6 well plates, transfected with engineered constructs and next day the assay was performed by dissociating them with versene and by suspending in assay buffer (HBSS, 5 mM HEPES, 0.5 mM IBMX, and 0.1% BSA) at 2.0×10^6 cells/ml. Cells were added in white OptiPlate-96 microplate from PerkinElmer and the protocol was continued as per manufacturer's instructions. Samples were measured in triplicates using a PerkinElmer multiplate reader and the results were compared with a cAMP standard curve.

2.7. Statistical analysis

Each experiment was repeated at least three times. Concentration-

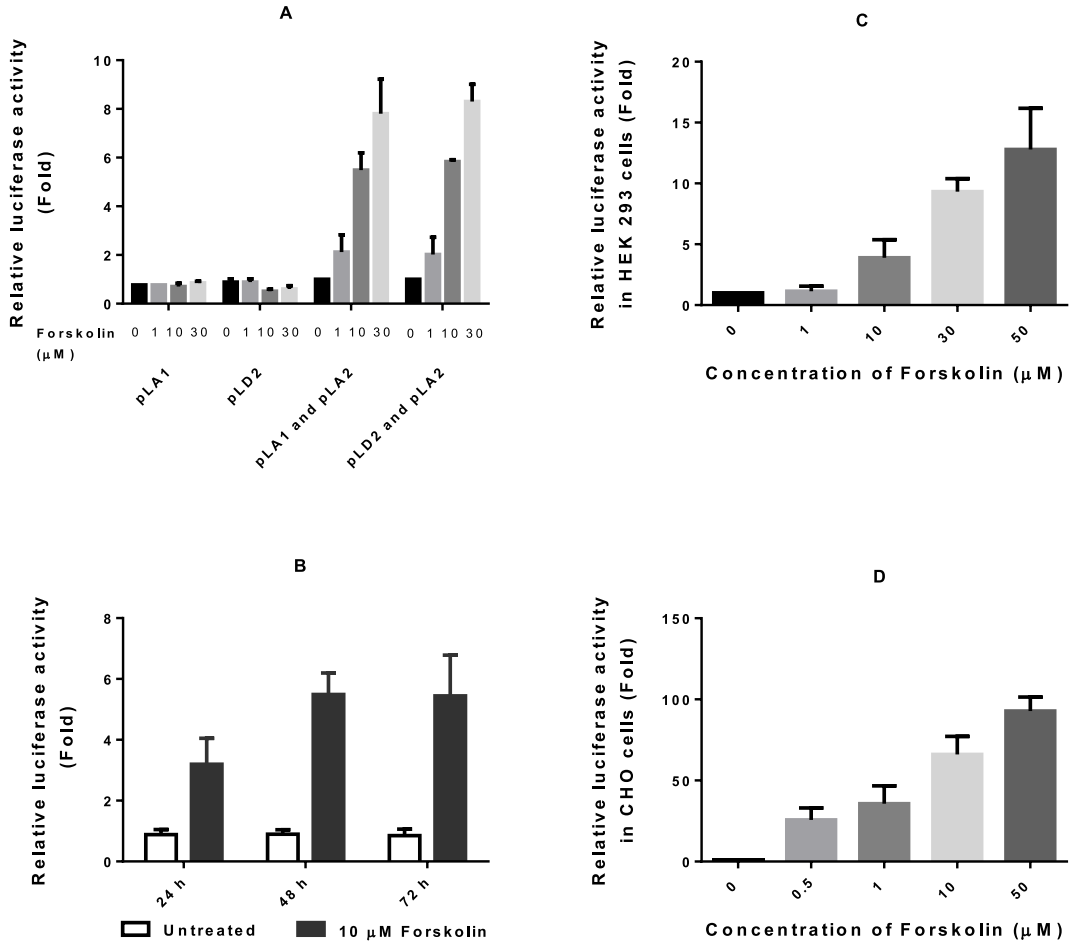

Fig. 2. Functionality of cAMP responsive transgene control system. (A) HEK 293 cells were transfected with pLA1 or pLD2 or were co-transfected with pLA1 and pLA2 or pLD2 and pLA2, treated with or without forskolin and measured the luciferase activity after 48 h of treatment. (B) HEK 293 cells co-transfected with pLA1 and pLA2 were treated with or without forskolin and measured the luciferase activity after 24 h, 48 h and 72 h of treatment. (C) HEK 293 cells and (D) CHO cells were co-transfected with pLA1 and pLA2, treated without or with increasing concentration of forskolin and measured the luciferase activity after 48 h of treatment.

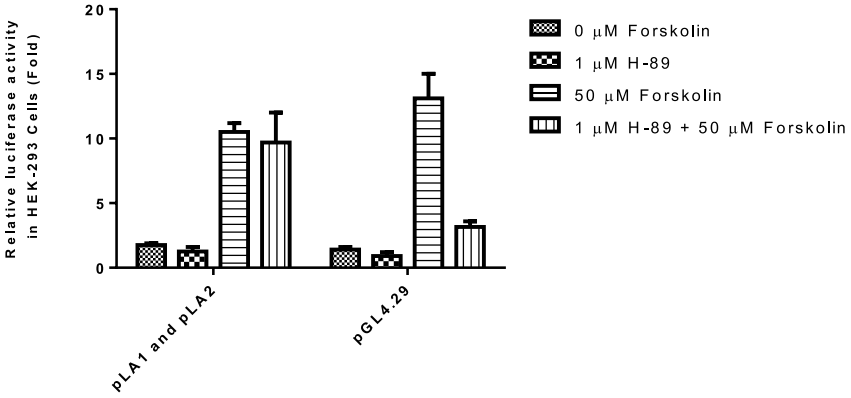

Fig. 3. PKA independent reporter system for cAMP signaling in mammalian cells. HEK 293 cells co-transfected with pLA1 and pLA2 were untreated or treated either with forskolin or H-89 (PKA inhibitor) or their combination at the indicated concentrations and measured the luciferase activity after 48 h. HEK 293 cells transfected with pGL4.29 (CRE reporter), treated either with forskolin or H-89 (PKA inhibitor) or their combination at the indicated concentrations and measured the luciferase activity after 48 h of treatment.

dependent curves and statistical analyses were done with GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA). Analyses included mean ± S.E.M, one- or two-way ANOVA, and Bonferroni post-test wherever appropriate (*, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$).

3. Results

3.1. Design and functionality of a cAMP-responsive transgene mammalian transcription tool

cAMP - dependent mammalian transgene expression system was developed by constructing two plasmids as shown in Fig. 1. pLA1 is generated by fusing the cAMP receptor protein of *E.coli* to the VP16 transactivation domain of the Herpes simplex virus that results in a synthetic cAMP-dependent transcription factor. pLA2 is constructed by fusing a single CRP specific operator site O_{CRP} linked to a minimal

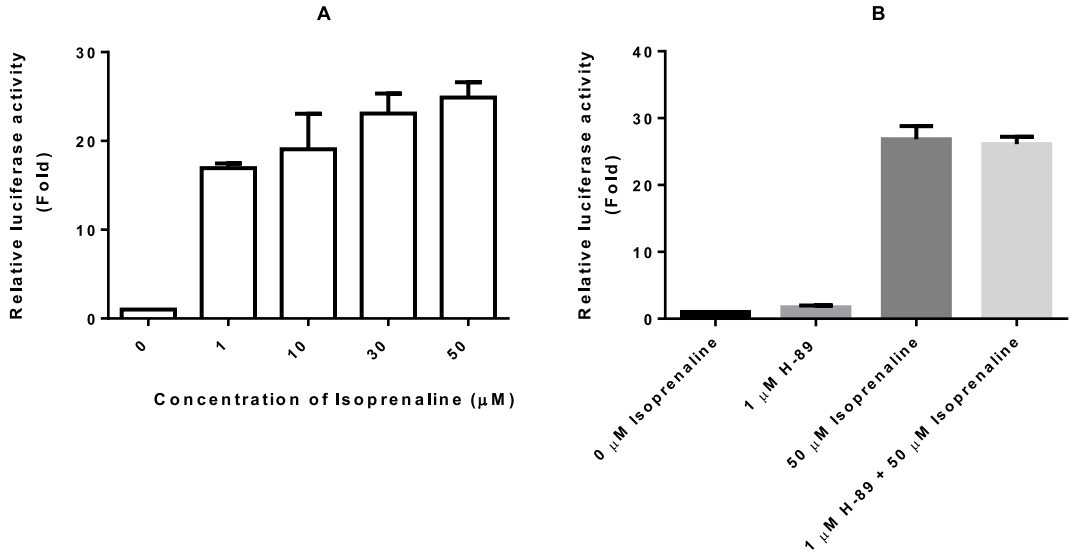

Fig. 4. Synthetic reporter system integrates mammalian β2-adrenergic receptor and mediates cAMP signaling. (A) HEK 293 cells were co-transfected with pGK (β-adrenergic receptor), pLA1 and pLA2 constructs and untreated or treated with increasing concentration of isoprenaline. (B) pGK, pLA1 and pLA2 transfected HEK 293 cells were treated either with forskolin or H-89 (PKA inhibitor) or their combination at the indicated concentrations and measured the luciferase activity after 48 h treated with either isoprenaline or H-89 or their combination and measured the luciferase activity after 48 h.

version of the cytomegalovirus immediate early promoter (PhCMVmin) to the luciferase gene that results in a chimeric promoter. These constructs were used for subsequent experiments to transactivate and report cAMP-induced gene expression. The engineered transcription tool mediates the gene expression in mammalian cells by binding to its operator in presence of cAMP which was measured by luciferase activity. HEK 293 cells were co-transfected with pLA1 and pLA2, treated with or without different concentrations of Forskolin and the luciferase activity was measured to assess the cAMP mediated gene expression after 48 h. The results show that luciferase activities were lower in the absence of forskolin but were progressively induced by increase in the concentration of forskolin (Fig. 2A). Further we analysed the cAMP mediated gene expression by designing two operator sites for CRP in a plasmid (pLD2) and transfected it along with pLA1, the cAMP-mediated gene expression was not enhanced further as shown in Fig. 2A. This might be due to that CRP from *E.coli* is 2-fold symmetric i.e one CRP-subunit interacts with one half of operator site and other subunit interacts with other half of subunit and sharply bends a operator site (Busby and Ebright, 1999) which could prevent the binding of additional operators. Hence we performed further experiments with pLA2 containing a single operator site. When the cells were treated with 10 μM forskolin, the gene expression reported by the transgene constructs increased at 24 h and 48 h but did not increase after 48 h as shown in Fig. 2B. When pLA1 and pLA2 were co-transfected in mammalian cell lines (HEK 293 and CHO) and treated with different concentrations of forskolin, the cells responded positively in a concentration dependent manner and the results show 10 fold induction in HEK 293 cells (Fig. 2C) and 90 fold induction in CHO cells over the control (Fig. 2D). These results demonstrate that the synthetic transgene constructs (CRP-VP16/O_{CRP} tool) report the presence of cAMP at the cellular level and mediate cAMP induced gene expression. A negative control is shown in Fig. 2A, where the promoter containing operator (pLA1) alone was transfected and checked the luciferase activity after inducing with forskolin. Neither the promoter nor the inducer transactivates the gene expression. It also shows that the system (pLA1) was not influenced by endogenous cAMP signalling in the absence of CRP-VP16 (present in pLA2).

3.2. A cAMP-dependent transgene system works independent of PKA/CREB signaling

In mammalian cells, cAMP-induced gene expression is mediated by PKA activation followed by phosphorylation of CREB and transcription of CRE (Kopperud et al., 2003). In order to determine whether the CRP-VP16/O_{CRP} tool works independent of mammalian PKA signaling, pLA1 and pLA2 transfected HEK 293 cells were pretreated with H-89 (a potent and selective inhibitor of PKA) followed by addition of forskolin. The luciferase activity measured after 48 h was unaffected by PKA inhibitor and showed the same 10-fold induction over the control (Fig. 3). On the other hand, a positive control was examined by transfecting pGL4.29 (CRE reporting plasmid: P_{SV40}-CRE-luciferase) in HEK 293 cells. The luciferase gene expression was considerably inhibited by H-89 treatment (Fig. 3). These results demonstrate that the CRP-VP16/O_{CRP} reporter system reports cAMP mediated gene expression irrespective of cAMP-PKA cascade.

3.3. Mammalian GPCR signaling can be mediated by synthetic mammalian transcription factor

To further validate the cAMP-dependent synthetic transcription tool, pGK harbouring β2-adrenergic receptor was co-transfected along with plasmids pLA1 and pLA2 in HEK 293 cells and treated with increasing concentration of isoprenaline. Isoprenaline is a direct adrenergic agonist that predominantly stimulates beta receptors which in turn increases intracellular cAMP level. The luciferase activity driven by transgenic tool was measured after 48 h and the results showed up to 25 fold induction over control (Fig. 4A). Further to determine that the reporter system can integrate and report mammalian cAMP signaling independent of cAMP-PKA cascade, the luciferase activity measured after 48 h in transfected HEK 293 cells which was pretreated with H-89, prior to the addition of isoprenaline were unaltered as shown in Fig. 4B. These results demonstrate that the synthetic reporter system works independently and thus it is an orthogonal reporter system to transactivate and mediate cAMP induced gene expression.

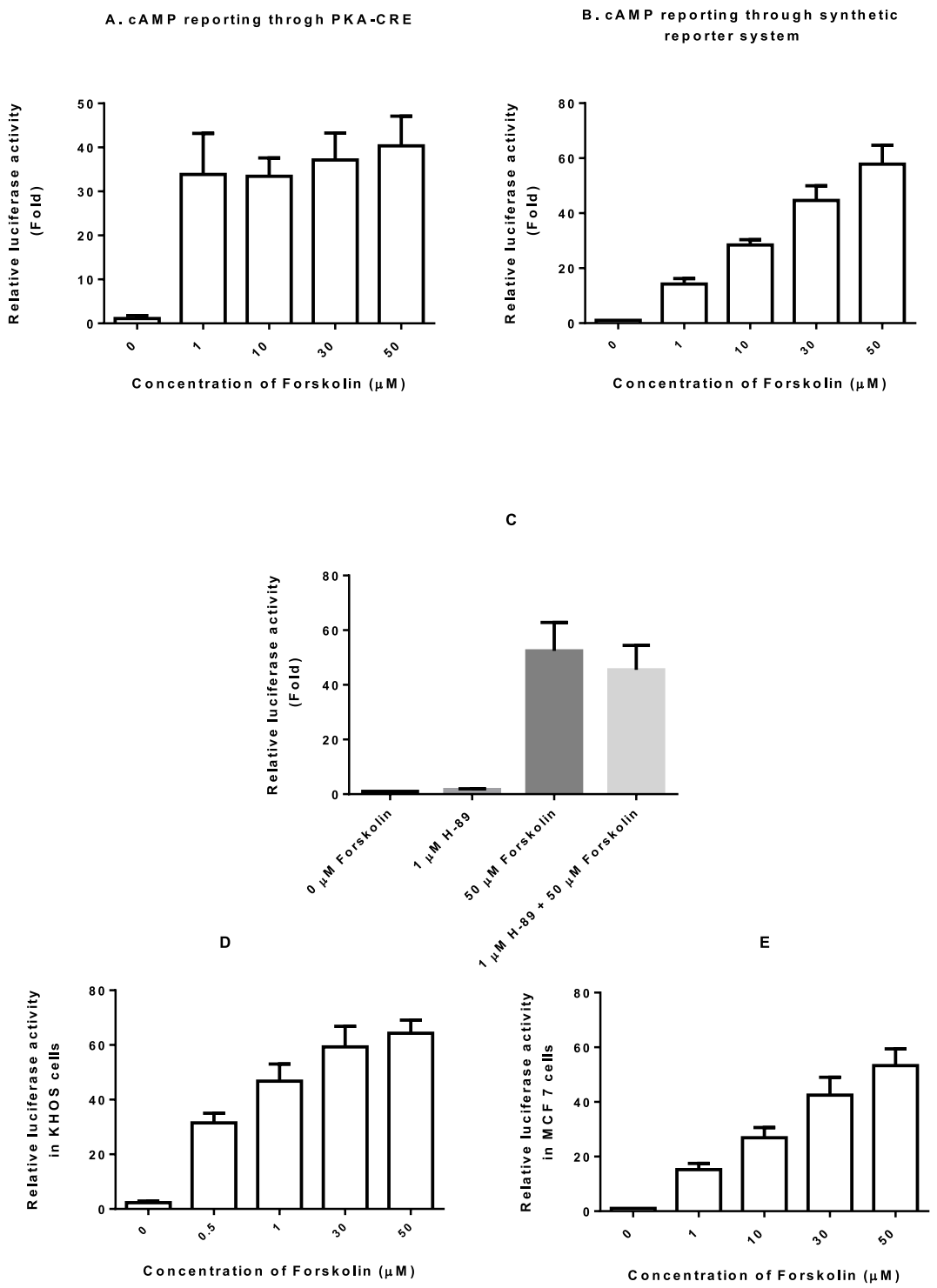

Fig. 5. Concentration dependent transactivation of cAMP mediated gene expression in Cancer cell lines. (A) Non-small cell lung cancer cell line (A549) cells transfected with pGL4.29, were treated with or without forskolin and measured the luciferase activity after 48 h. (B) Cells transfected with pLA1 and pLA2 plasmids, were treated with or without forskolin and measured the luciferase activity after 48 h. (C) Cells transfected with pLA1 and pLA2 plasmids were treated either with forskolin or H-89 (PKA inhibitor) or their combination at the indicated concentrations and measured the luciferase activity after 48 h. (D) Osteosarcoma cells (KHOS) and (E) Breast cancer cells (MCF-7) were co-transfected with pLA1 and pLA2 plasmids treated with or without forskolin and measured the luciferase activity after 48 h.

3.4. A synthetic transcriptional tool mediates cAMP signaling in a concentration dependent manner in deregulated cAMP signaling cancer cell lines

Deregulation of cAMP signaling and its aberrant downstream effector pathways occur during cancer development and progression. It appears that certain parts of the cAMP response system altered in some cancer cells. In such cases, determining the cAMP signaling through CRE reporter assay may not be appropriate as other signaling pathways crosstalk and hyperphosphorylates CREB which in turn results in the uncontrolled activation of CRE. CRE reporter plasmid pGL4.29 transfected in A549 cells (Non-small cell lung cancer) were treated with

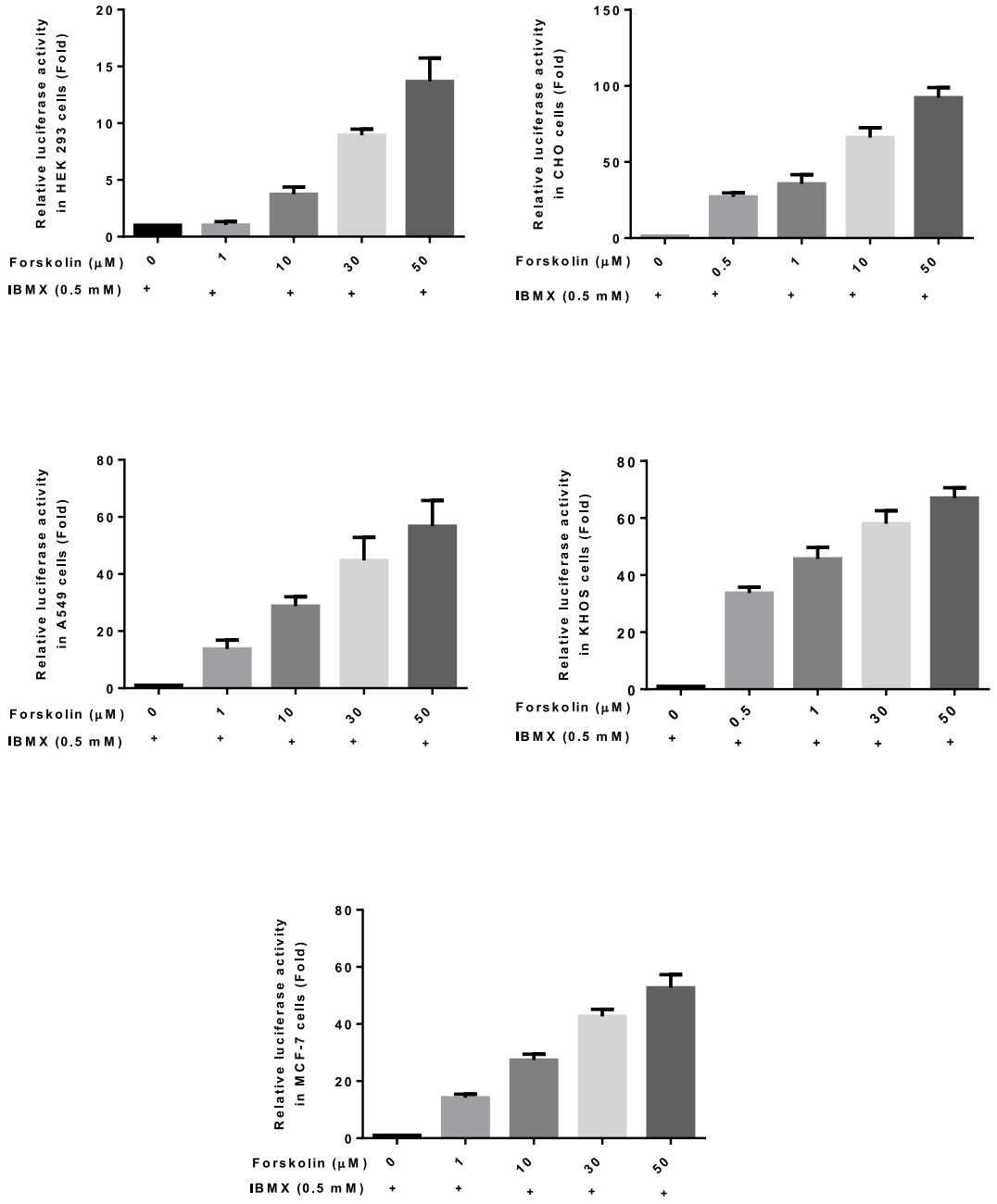

Fig. 6. Effect of adenylyl cyclase activator (Forskolin) and phosphodiesterases inhibitor (IBMX) on synthetic inducible gene expression system. Tumorigenic and non-tumorigenic cells transfected with synthetic constructs pLA1 and pLA2, were treated with 0.5 mM of IBMX and without or with increasing concentration of forskolin and measured the luciferase activity after 48 h of treatment.

increasing concentration of forskolin and the luciferase activity was measured after 48 h. The result imply that gene expression did not correlate with concentration dependencies of cAMP as shown in Fig. 5A, whereas pLA1 and pLA2 transfected cancer cell lines A549, KHOS and MCF-7 when treated with different concentrations of forskolin, detected the presence of intracellular cAMP level and the gene expression in a concentration dependent manner as shown in (Fig. 5B, D, E). Furthermore, this CRP-VP16/O_{CRP} tool mediate PKA/CREB independent cAMP signaling in A549 cells as shown in Fig. 5C.

3.5. Determination of intracellular cAMP concentration in non-tumorigenic and tumorigenic cells

In order to determine that the tool reports the presence of cAMP level and cAMP mediated gene expression in a concentration dependent manner, an intracellular cAMP concentration was measured in non-tumorigenic cell lines HEK 293, CHO and in tumorigenic cell lines including A549, MCF-7, and KHOS as per the protocol mentioned by PerkinElmer LANCE cAMP assay. Prior to the assay, all the cell lines mentioned were grown in 6-well plate and transfected with the engineered plasmids. Firstly, an assay was performed in 96 well microplates by using different concentrations of cAMP (provided by PerkinElmer kit) and the lance signal (TR-FRET) was measured at 615 nm on PerkinElmer EnVision and determined a cAMP standard curve. It has been shown that TR-FRET signal increased with increase in cAMP concentration (Fig. 7A). Following that, intracellular cAMP levels in different cell lines were measured by performing forskolin dose-responsive curve. For each cell lines, 3000 cells were plated per well in 96 well plates and incubated with different concentration of forskolin from 1 μM to 50 μM for 2 h with 0.5 mM IBMX, a phosphodiesterase inhibitor

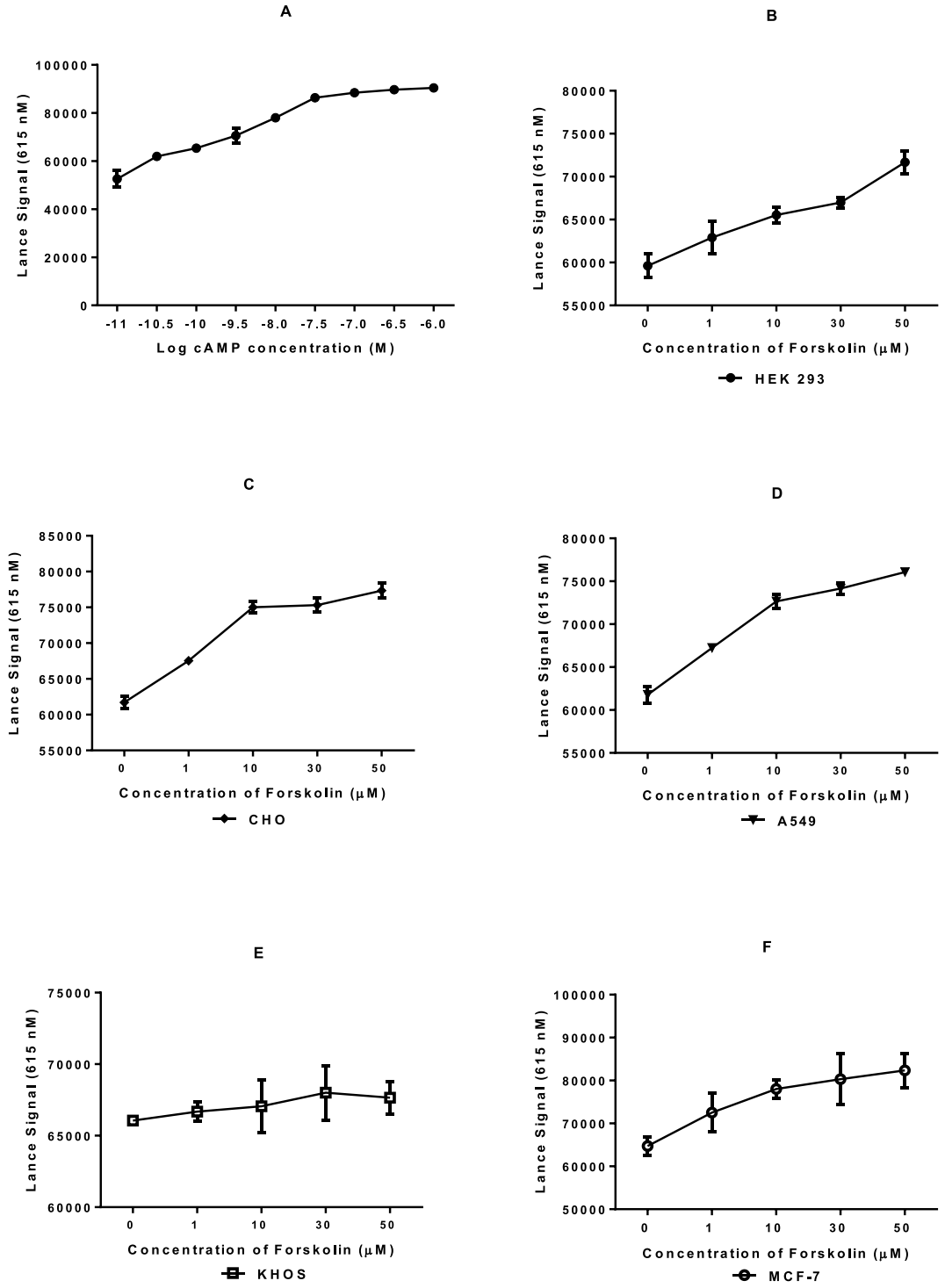

Fig. 7. Measurement of intracellular cAMP concentration. (A) A standard curve for cAMP was performed by using different concentrations of cAMP diluted from the cAMP standard stock and measured the fluorescence intensity (Lance signal) at 615 nm. (B), (C), (D), (E), (F) Forskolin dose responsive curves of various cell lines. Cells were harvested with a non-enzymatic cell dissociation solution versene and then the assays were performed as per Manufacturer's protocol by testing 3000 cells per well in 96 wells optiplate with different concentrations of forskolin. Then the Lance signal was measured at 615 nm on PerkinElmer EnVision instrument after 2 h of forskolin and IBMX treatment.

in order to prevent the reduction of LANCE signal produced by cAMP. The fluorescence intensities were then measured at 615 nm. The results shown in Fig. 7 B, C, D, F demonstrate that TR-FRET signals were increased with increasing concentration of forskolin. However in Fig. 7E, the KHOS cells did not increase the amount of cAMP when treated with forskolin for 2 h. This might be due to that during cancer pathogenesis, normal cell cAMP activity is imbalanced in some of the cancers due to the alteration in rate of cAMP synthesis/degradation (Antonio and Carla, 2001). Hence it has been presumed that the intracellular cAMP level would have been increased after some hours (more than 2 h) of forskolin treatment and hence could not see significant changes in Lance signal points. Increase in intracellular cAMP level after some hours mediate its signaling was measured by the synthetic reporter system after 48 h of Forskolin treatment as shown in Fig. 6 and Fig. 5D.

Table 1
Intracellular cAMP concentration in non-tumorigenic and tumorigenic cell lines.

Forskolin (μM)	cAMP (nM)				
	HEK 293	CHO	A549	KHOS	MCF7
0	0.05	0.05	0.06	0.04	0.08
1	0.05	0.4	0.32	0.06	0.63
10	0.1	1.58	1.0	0.1	1.5
30	0.16	1.6	1.58	0.16	1.8
50	0.5	10	10	0.2	11

The Lance cAMP assay that we performed has a limitation of forskolin treatment for 30 min - 2 h. The resulting signals produced in different cell lines were correlated with cAMP standard curve lance signals as they are directly proportional to the concentration of cAMP and calculated the corresponding intracellular cAMP level as shown in Table 1. Simultaneously, a synthetic cell based assay was performed for each cell line, which was treated with 0.5 mM IBMX and different concentration of Forskolin and measured the luciferase activity after 48 h of treatment (Fig. 6). These results were correlated with Fig. 2 (C, D); 5 (B, E). Based on this assay, we found that the synthetic reporter system reports the intracellular cAMP level and mediates the gene expression in a concentration dependent manner. It has been found that cAMP levels were comparatively higher in cancer cell lines when compared to HEK 293 cells however CHO cells also showed higher concentration of cAMP (Table 1).

4. Discussion

Precise analysis and reporting of gene expression is essential for gene function analysis, genomic studies and clinical applications in mammalian system. Recent advances in developing gene circuits have developed the ability to explore and program mammalian cell behavior that lead to the discovery of synthetic biology research tools with potential therapeutic applications. Several synthetic signaling networks have been engineered to set up novel control systems or to switch the information flow (Lim, 2010; Lienert et al., 2014). Synthetic gene switches have been successfully applied for gene-function analysis (Windbichler et al., 2011) and for design of cell-based assays in mammalian system (Burbelo et al., 2010). cAMP signaling is involved in a wide variety of biochemical to cellular processes and most of the therapeutic drugs elicit their biological functions via cAMP signaling. However, the precise monitoring of cAMP signaling in mammalian cell populations has remained challenging (Reimann and Gribble, 2016; Zaccolo, 2011). Though there are methods (fluorescence and radio-ligand binding assays) (Zhang and Xie, 2012; Chen et al., 2015) available to report and measure the intracellular cAMP level, techniques to determine cAMP mediated gene expression is limited to CRE reporter method. This CRE reporting plasmid (P_{SV40}-CRE-luciferase) which has been widely used as a reporter system in most of the research studies may not exclusively report the cAMP/PKA/CREB signaling since the CRE in mammalian cells is also activated by other intracellular signaling pathways like calcium signaling, MAPK signaling and Rho signaling (Dumaz and Marais, 2005; Stork and Schmitt, 2002; Siso-Nadal et al., 2009). In contrast, the present orthogonal engineered transcription tool can report the cAMP mediated gene expression directly. This simple reporter system works by introducing the engineered gene constructs in to mammalian cells and when the intracellular cAMP level surges, the cAMP binds to the engineered construct and transactivates the gene expression in a concentration-dependent manner in response to compounds that trigger cAMP level. Thus, our present synthetic transcription factor can be widely used as a cell based assay to assess cAMP signaling which is inexpensive and easy to set up. Furthermore, in recent years, it has been found that most of the cancers have deregulated cAMP signaling. For instance, over-expression of R1α subunit of PKA was reported in several malignancies including breast, colon and lung cancers (Khor et al., 2008; Shi et al., 2004; Bradbury et al., 1994; Fajardo et al., 2014). CRE reporter cell based assay which reports cAMP signaling via PKA/CRE pathway failed to detect the gene expression mediated by cAMP in a concentration dependent manner in cancer cell lines with an aberrant PKA/CREB/CRE signaling whereas our assay transactivates and reports intracellular cAMP level and cAMP mediated gene expression orthogonally independent of PKA/CREB signaling in a concentration dependent manner. Hence this synthetic cell based assay would be exclusively useful even under conditions in which there are alterations in intracellular cAMP levels or cAMP binding domain of PKA or the nuclear uptake of PKA. Overall, the reporter system can be used to study cAMP signaling, as a basic inducible gene switch to fine tune cAMP mediated transgene expression and to identify agonists of GPCR triggered cAMP signaling, to screen the inhibitors of cAMP production and to determine the effect of phosphodiesterases.

Conflicts of interest

Authors declare that they have no conflict of interest.

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