DOI 10.1002/biot.200800020 Biotechnol. J. 2008, 3, , 484–495

Review

The application of cell-based label-free technology in drug discovery

Biao Xi, Naichen Yu, Xiaobo Wang, Xiao Xu and Yama A. Abassi

ACEA Biosciences, San Diego, CA, USA

Cell-based assays are an important part of the drug discovery process allowing for interrogation of targets and pathways in a more physiological setting compared to biochemical assays. One of the main hurdles in the cell-based assay field is to design sufficiently robust assays with adequate signal to noise parameters while maintaining the inherent physiology of the pathway or target being investigated. Conventional label and reporter-based cell assays may be more prone to artifacts due to considerable manipulation of the cell either by the label or over-expression of targets or reporter proteins. Cell-based label-free technologies preclude the need for cellular labeling or over-expression of reporter proteins, utilizing the inherent morphological and adhesive characteristics of the cell as a physiologically relevant and quantitative readout for various cellular assays. Furthermore, these technologies utilize non-invasive measurements allowing for time resolution and kinetics in the assay. In this article, we have reviewed the various label-free technologies that are being used in drug discovery settings and have focused our discussion on impedance-based label-free technologies and its main applications in drug discovery.

Received 23 January 2008
Revised 2 March 2008
Accepted 3 March 2008

Keywords: Cell-based · Drug discovery · Impedance · Kinetic · Label-free

1 Introduction

The current status of drug discovery and development can probably be best summed up by two contradictory views. On the one hand, the mapping and sequencing of the human genome, the advances in combinatorial chemistry, functional genomics, proteomics and high-throughput technologies has created an unprecedented paradigm shift in which drug discovery is conducted. This endeavor has led to the assessment of new and better targets and generation of numerous lead drug candidates. On the other hand, drug discovery continues to be a risky endeavor and thus far, these advances have not yet translated into blockbuster drugs that were once envisioned [1]. High attrition rates, particularly at the preclinical and clinical stages continue to plague the drug development process. While there may be a multitude of reasons for the high attrition rate, at least one important reason is that drug candidate leads are advanced through the early stages of the drug development process without full interrogation of the underlying biochemical mechanism(s), toxicological profile, off-target interactions and pharmacokinetic and pharmacodynamic properties of candidate lead compounds [2, 3]. What is urgently needed in the field are technologies that can provide incisive and predictive information about the lead candidate drugs early on in the process in order to reduce the attrition rate and save the enormous late development costs of candidate compounds.

Cell-based label-free technologies have recently received considerable attention in preclinical drug development processes [4–10]. In this review, we will discuss the general attributes of cell-based label-free technologies, which make them attractive for implementation in the drug discovery are-

Correspondence: Dr. Yama Abassi, ACEA Biosciences, Cell Biology and Assay Development, 6779 Mesa Ridge Rd. #100, San Diego, CA 92121, USA
E-mail: yabassi@aceabio.com
Fax: +1-858-724-0927

Abbreviations: FN, fibronectin; **GPCR**, G-protein-coupled receptors

na. In addition, we will briefly discuss the different label-free technology platforms and finally focus our discussion on impedance-based label-free technologies.

2 Cell-based label-free technologies

Even though some of the label-free technologies discussed in this review have existed for at least a couple of decades, the essence of label-free technology as applied to cell biology and drug discovery is increasingly being realized. As the name implies, the preclusion of labels allows for assessment of cells in their native and physiologically relevant environment circumventing the potential negative impact of labels on cellular processes. The inclusion of certain labels and reporters, particularly labels for live cells has been shown to affect various aspects of cellular behavior. For example, it has been shown that that the live cell fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorescein (BCECF-AM) and rhodamine 6G (R6G) can dose-dependently block the migration of macrophage and mononuclear cells, respectively, urging caution when utilizing these dyes for cell-based assays [11, 12].

Label-free technologies have the added advantage of being non-invasive and therefore live cells present in tissue culture wells can be continuously investigated. This feature directly leads to one of the main advantages of label-free technologies, which is real-time kinetic measurement [4, 5, 9, 13–15]. Real-time monitoring of cellular processes offers distinct and important advantages over traditional end-point assays. First, comprehensive representation of the entire length of the assay is possible, allowing the user to make informed decisions regarding timing of manipulations or treatments. Second, the actual kinetic response of cells provides important information regarding the biological status such as cell growth, arrest, morphological changes, and apoptosis. There are a number of optical and non-optical technologies available that allow for label-free assessment of cell-based experiments [4, 5]. We will review the main label-free technologies described for cell-based assays in drug discovery and will limit our detailed discussion to impedance-based technologies due to space limitations.

3 Label-free technologies for cell-based assays

3.1 Impedance-based label-free technologies

The utilization of impedance as a cellular readout was first described about two decades ago by Giaever and Keese [13, 16]. The basic workflow of the electric cell-substrate impedance system (ECIS) they describe entails cultivating adherent cells on gold electrodes deposited in the bottom of tissue culture vessels by lithographic techniques [16]. The gold electrodes are composed of a small 250 μm sensing electrode and a larger counter electrode [13, 16, 17]. Application of a small AC signal of 1 μA in the presence of media electrolytes leads to the generation of an electric field between the electrodes, which are impeded by the presence of cells. The applied AC signal is harmless to the cell and does not adversely affect the physiology of the cells. The cellular plasma membrane acts as an insulating agent forcing the current to flow between or beneath the cells, leading to large and measurable changes in impedance. The degree of impedance change is primarily determined by the number of cells seeded in the wells, cell-cell interaction, the quality of interaction of the cells with the electrodes and the overall morphology of the cells [13]. ACEA Biosciences in partnership with Roche, MDS Sciex and Bionas have also developed products for *in vitro* monitoring of cells that utilize cell-substrate impedance as readout [7, 15, 18]. The main distinguishing feature of these technologies is the design and geometry of the electrodes in the bottom of the wells, the material composition of the electrodes, the electrode coverage area, the magnitude of the AC signal used to generate impedance response, the range and number of frequencies used to measure impedance and throughput [7, 15, 18]. For the specific details of each technology, the reader is referred to the references outlined in Table 1. The specific drug discovery application for impedance-based technologies will be discussed in the ensuing section.

3.2 Refractive index-based technologies

Technologies such as the Corning Epic system, Owls technology from Micro Vacuum and Bind technology from SRU, utilize evanescent waves to characterize the cellular processes that are taking place at or near the sensor surface [9, 19–25]. The cells are placed on the optical biosensors that are composed of a material with high refractive index deposited on a glass substrate. The evanescent wave is created by total internal reflection of light

Table 1. Label-free cell-based technologies and applications

Company	Technology	Product	Cell-based assays	Selected References
Corning	Resonant waveguide grating	Epic	Receptor-mediated signaling	[9], [19], [20]
SRU Biosystems	Resonant waveguide grating	BIND	Receptor-mediated signaling	[21]
Q-Sense	Quartz Crystal Microbalance	Q-Sense E4	Cell adhesion and proliferation	[26-30]
MicroVacuum	Optical Waveguide grating	Owls	Cell adhesion and proliferation	[22-25]
Essen Instruments	Automated microscopy	IncuCyte	Cell Proliferation, cytotoxicity	
Chipman Technologies	Automated microscopy	Cell-IQ	Cell proliferation; cytotoxicity	
Bionas	Impedance	Bionas 2500	Cytotoxicity	[18]
Seahorse Biosciences	Fiber-optic waveguide together with fluorescent biosensors	XF-24	Fatty acid oxidation and mitochondrial function	
Applied Biophysics	Impedance	ECIS	Cell adhesion, proliferation, barrier function, receptor-mediated signaling and wound healing	[13], [16], [17], [59], [64]
ACEA Biosciences and Roche[a)]	Impedance	xCELLigence	Cell proliferation, drug and cell-mediated cytotoxicity, cell adhesion, receptor-mediated signaling, barrier function, immune-cell signaling, cell migration and invasion	[8], [14], [15], [42], [48-50], [61]
MDS Sciex	Impedance	Cell Key	Receptor-mediated signaling	[6], [44]

[a)] The xCELLigence system is the new and improved version of ACEA Biosciences RT-CES technology.

at a solution-surface interface, which can have a characteristic penetration depth depending on the physical properties of the sensors [9, 10]. With respect to cells, the penetration depth can extend only a short distance (100-200 nm) into cells from the sensor surface allowing for monitoring mass redistribution of proteins and organelles due to different kinds of treatments. Local changes in the refractive index can convey important information regarding the cellular status. The major drug discovery application of this technology has been described for functional monitoring of receptors in cell-based assays, cell adhesion and proliferation [19–21, 23, 24].

3.3 Acoustic technology

Q-sense offers a unique label-free technology (QCM) for cellular monitoring that is based on measuring the resonant frequency of piezoelectric quartz crystals [26–30]. QCM consists of thin quartz discs that are sandwiched between two gold electrodes. Application of an AC signal across electrodes leads to the excitation and oscillation of the crystal, which acts as a sensitive oscillator circuit. By measuring the resonance frequency as well as the decaying or dissipative frequency of cells associated with the crystals, important biological information such as cell adhesion, cell proliferation and cytotoxicity can be obtained [29, 30].

3.4 Fiber optic waveguide measurement of extracellular flux

The XF24 Analyzer from Seahorse Biosciences measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of cells growing in tissue culture plates in response to various treatments. OCR is an indicator of mitochondrial respiration while ECAR is predominately the result of glycolysis. Dissolved oxygen and pH levels in the media are measured by inert optical biosensors that reside approximately 300 μm above the cell monolayer. Two fluorophores for analyte detection are embedded in a polymer spotted on the end of each of the 24-sensor sleeves. The OCR is determined by the amount of fluorescence of a fluorescine complex quenched by the dissolved oxygen. The ECAR sensor responds to changes in proton concentration as they diffuse across the fluorophore based polymetric substrate. The negative log of proton concentration is equal to the pH. The XF Analyzer's fiber optic waveguides are inserted into the sleeves of the sensor cartridge while light is pumped through the fiber optic bundles, exciting the fluorophores and the resultant emission is detected and measured. The major applications of this technology are in the areas of cancer, immunology and diabetes and include bioenergetic measurements of disease relevant cells in response to different stimuli [31–33].

3.5 Automated microscopy

The IncuCyte instrument from Essen Instruments and the Cell-IQ system from Chipman Technologies utilize automated microscopy for online real-time monitoring of cells under label-free conditions. The Essen instrument fits inside a CO_2 incubator and obtains phase contrast images of cells at predetermined time intervals, while the Cell-IQ system is a continuous and integrated cell culture platform with temperature and CO_2 controls. A number of applications have been developed around these technologies including cell proliferation, cytotoxicity and wound healing.

4 Biological basis for label-free measurement of cellular status

In addition to the preclusion of label, the other unifying principle of cell-based label-free technologies entails utilizing various aspects of cell adhesion and morphology as a biologically relevant readout [6, 8, 9, 13]. One of the main determinants of cell morphology and adhesion is the actin cytoskeleton. The actin cytoskeleton provides the architectural framework of mammalian cells, affording the cell with either rigidity and or plasticity depending on the specific requirements of the cellular milieu [34]. In addition, the cytoskeleton serves as a scaffold for the organization and appropriate localization of the various signaling components of the cell including membrane receptors and their downstream effectors [35]. It is important to note that these two functions are not necessarily mutually exclusive; the architectural specification of the cell can certainly influence the signaling cascades while different signaling pathways can dictate the dynamic structure of the actin cytoskeleton and cell substrate adhesion [36]. Therefore, at any given point the characteristics of cell substrate adhesion and actin cytoskeletal dynamics can be an extension or manifestation of the signaling pathways within the cell. Thus, cellular processes including proliferation, migration, adhesion and apoptosis are associated with very specific and well-defined changes in cell morphology and adhesion.

In the next section, we will explore how the impedance cell-based technologies have taken advantage of this inherent characteristic of the cell and have used cell adhesion and morphological dynamics as a quantitative functional output for various cellular outcomes including cytotoxicity, receptor-mediated signaling, cell migration and cell adhesion. We will discuss the molecular basis of how different treatments can influence cell adhesion and/or morphological changes ultimately affecting the quantitative nature of the label-free readout.

5 Applications of impedance technologies in drug discovery: G-protein-coupled receptors (GPCR) and receptor tyrosine kinase (RTK) signaling

G-protein-coupled receptors (GPCR) constitute one of the most sought after targets in drug discovery because they are associated with conditions ranging from cardiovascular diseases, autoimmune diseases, inflammation, cancer and diseases of the nervous system [37]. Moreover, they are one of the most amenable targets in drug discovery because they can be modulated by small molecule, peptides, proteins and antibodies and therefore it may not come as a surprise that close to 50% of the drugs that are currently on the market are targeting GPCR [37]. GPCR include a large and diverse class of seven helical transmembrane receptors that interact with the hetero-trimeric G-protein complex, consisting of the Gα subunit as well as the Gβγ complex [38]. Upon stimulation of GPCR the Gα subunit dissociates from the complex and interacts with downstream effectors such as PLCβ, adenylate cyclase or the Rho GTPase-activating protein (GAP), depending on the specific GPCR being activated [38] (Fig. 1A). Gs-coupled receptors activate the adenylate cyclase pathway leading to transient rise in cAMP levels within the cell. cAMP has numerous downstream targets including protein kinase A (PKA). PKA has been shown to phosphorylate and modulate the activity of p21-activated kinase (PAK) which is an upstream modulator of actin cytoskeleton [39]. In addition, some GPCRs, upon stimulation lead to phosphorylation and activation of FAK, paxillin, CAS and CRK, which are well-characterized mediators of actin cytoskeletal dynamics [40–44]. The Gβγ subunit which is common to all GPCR signaling pathways can also modulate the actin cytoskeleton through several different mechanisms including direct association with PH-domain containing signaling mediators and direct interaction with tyrosine kinases such as Csk [38] (Fig. 1A). Collectively, the evidence highlighted here demonstrates that GPCR stimulation is linked to actin cytoskeletal dynamics and cell shape/adhesion changes.

Impedance-based technologies offers several distinct and important advantages for GPCR research and drug discovery that warrants careful

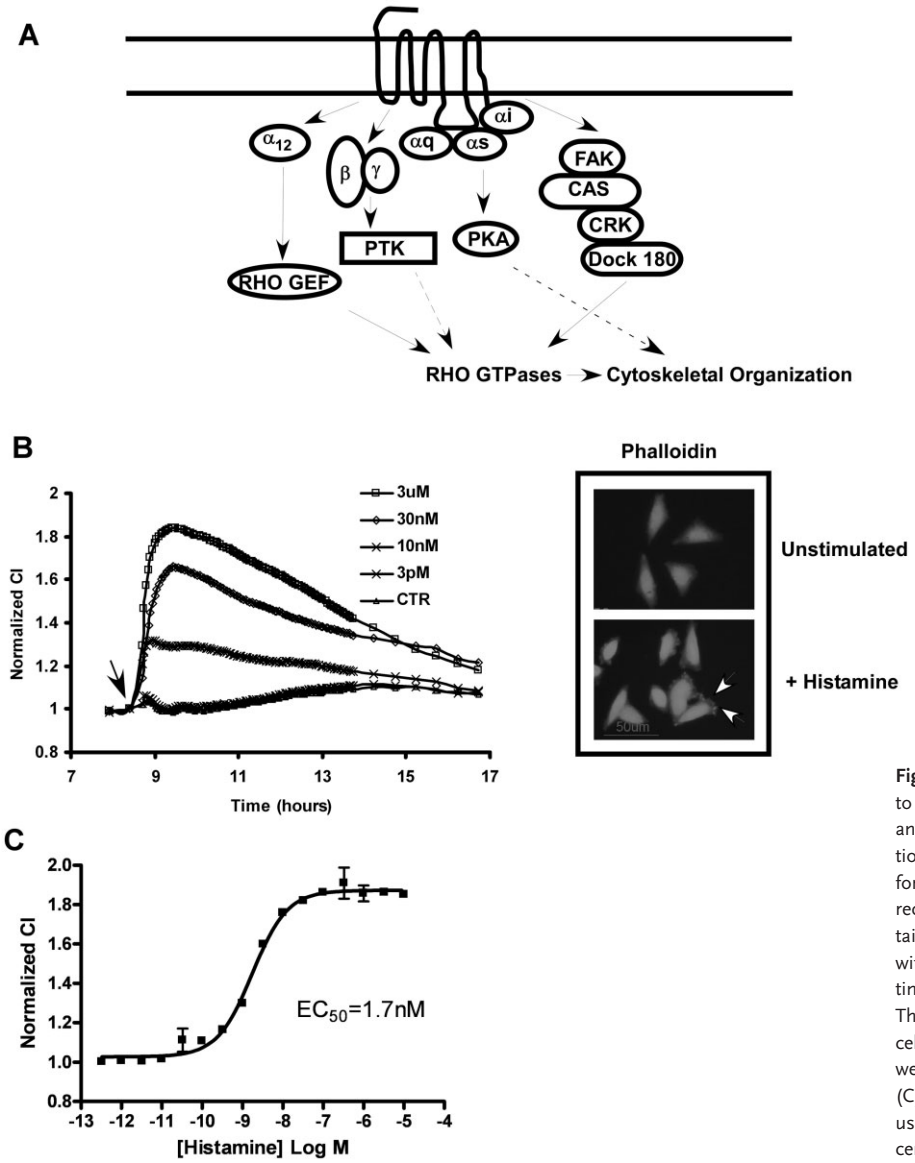

Figure 1. (A) GPCR signaling pathways leading to cytoskeletal rearrangement and cell adhesion and morphology changes. (B) Dynamic functional monitoring of GPCR on the RT-CES platform. CHO cells expressing the histamine H1 receptor were seeded in microtiter plates containing microelectronic sensors and stimulated with or without histamine. The cells were continuously monitored using the RT-CES system. The inset shows phalloidin staining of CHO cells expressing the histamine receptor that were stimulated with or without histamine [45]. (C) Dose-response curve of histamine graphed using the peak impedance value versus the concentration of histamine used.

consideration; First, GPCR linked to different pathways, Gi, Gs and Gq can all be monitored using impedance technology [7, 45] and the sensitivity of impedance readout is comparable to traditional assays such as inositol tris-phosphate, calcium and cAMP measurements. Traditionally, several different instrumentations, including those measuring calcium and cAMP levels would be required to carry out assays for GPCR coupled to different signaling pathways. Secondly, both Yu *et al.* [45] and Verdonk *et al*. [7] have demonstrated the utility of impedance-based technologies for measuring endogenous GPCR, precluding the need for overexpression of heterologuous GPCR or engineering the cells to express promiscuous G-proteins [6, 7, 45]. The GPCR of interest can therefore be assayed in appropriate cell lines under more pharmacologically relevant conditions. An additional benefit of impedance technology for functional monitoring of GPCR is that stimulation of receptors by cognate ligands or agonists can result in very specific kinetic profiles that can be predictive of the signaling pathway being triggered [7]. This feature can allow for determination of a specific pathway being activated upon engagement of GPCR by specific agonists. However, it remains to be determined if these profiles can distinguish GPCR which couple to multiple pathways. Finally, impedance-based technologies such as the Roche-ACEA xCELLigence system can also be used to identify and characterize inverse agonists of GPCR, which is much more formidable by tradi-

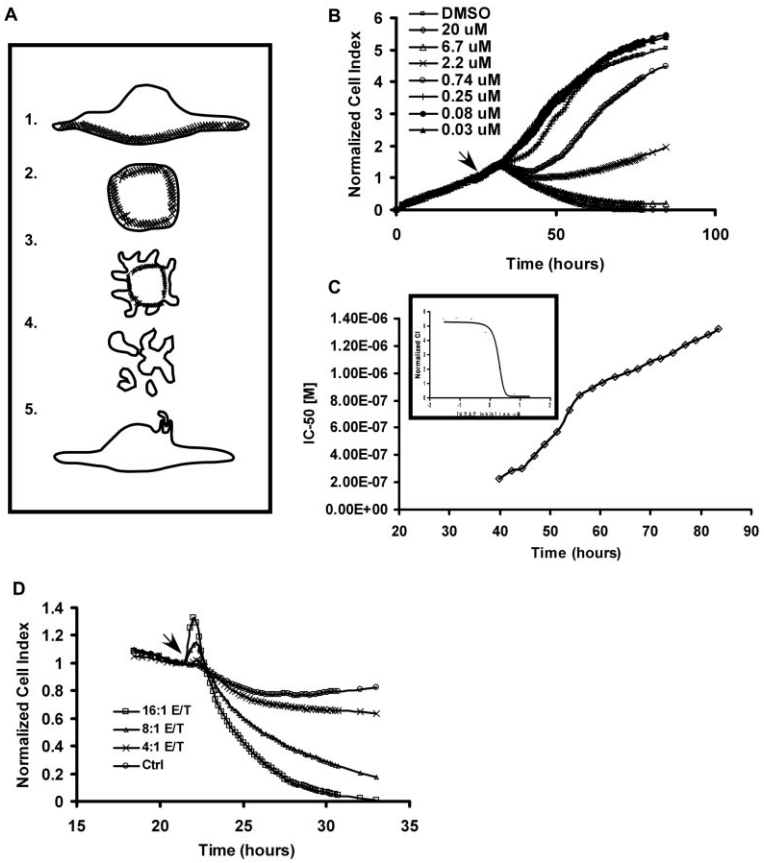

Figure 2. (A) Morphological changes associated with apoptosis. The apoptotic response leads to cell rounding [46] (2) followed by membrane blebbing, which occurs due to weak interaction between the membrane and actin cytoskeleton (3), formation of apoptotic bodies (4) and ultimately actin-mediated engulfment of apoptotic bodies by neighboring cells (5). (B) Dynamic monitoring of cytotoxicity using the RT-CES system. A549 cells were seeded in microtiter plates containing interdigitated microelectrodes and treated with the HDAC inhibitor, Scriptaid at the indicated doses. The cells were continuously monitored using the RT-CES system. (C) Real-time data from RT-CES system were used to generate real-time IC-50 values for Scriptaid. The inset shows dose-response of Scriptaid at a single time point. (D) Dynamic monitoring of cell mediated cytotoxicity using the RT-CES system. A549 cells were seeded in ACEA microelectronic plates followed by addition of NK-92 cells at the indicated effector to target (E/T) ratios. The cytotoxic activity of NK-92 cells towards A549 target cells was continuously monitored using the RT-CES system.

tional assays [45]. In addition, since the readout is non-invasive, the cells can be stimulated multiple times in order to assess desensitization or cross-talk with other receptor types [45].

Figure 1B shows the dose-dependent stimulation of CHO cells expressing the histamine H1 receptor [45]. Plotting the peak response against histamine concentration allows for the calculation of the EC-50 value for histamine (Fig. 1C). Moreover, Fig. 1B shows CHO cells expressing the histamine receptor labeled with fluorescent phalloidin in the absence or presence of histamine. Phalloidin labeling of the actin cytoskeleton reveals dramatic changes in actin cytoskeleton and cell morphology after histamine stimulation.

Similarly, the different impedance-based technologies have also been utilized to study pathways linked to receptor tyrosine kinases [7, 8]. These studies have shown that impedance-based readout is both specific and quantitative and can be used to screen and identify selective inhibitors of receptor tyrosine kinases [8]. The Epic system, which utilizes refractive index measurements, has also been shown to be able to monitor both GPCR activation as well as activation of pathways linked to stimulation of receptor tyrosine kinases [20].

The current limitation of impedance systems for GPCR drug discovery is that they are mainly suitable for low to moderate throughput and are probably better positioned as systems for secondary screening assays, mechanism of action studies and compound profiling. It will be interesting to see in the coming years if this technology can be adapted to higher throughput versions (384- and 1536-well formats).

6 Cytotoxicity

One of the hallmarks of the apoptotic response to various stimuli such as treatment with chemotherapeutic drugs, activation of the death receptors or deprivation of survival factors is the dramatic changes in cell adhesion and morphology in the form of loss of adhesion and cell rounding, membrane protrusions or blebbing, formation of apoptotic bodies and ultimately engulfment of apoptotic bodies by phagocytosis [46] (Fig. 2A). It has been demonstrated that actin cytoskeletal dynamics plays a central role in mediating both the apoptotic and phagocytic response [46]. For example, evidence has been provided that membrane blebbing

is a direct result of filamentous actin formation due to activation of Rho activated kinase, ROCK. ROCK activation by direct interaction with GTP-bound Rho or by cleavage of its inhibitory domain by Caspase-3 leads to phosphorylation of a number of downstream effectors culminating in actin polymerization and cell contraction [47]. The initial phase of actin polymerization is followed by caspase-mediated cleavage of key cytoskeletal regulatory proteins ultimately leading to actin depolymerization and formation of apoptotic bodies. In terms of impedance measurements, the apoptotic response will ultimately lead to a loss of cell-substrate impedance.

6.1 Drug-induced cytotoxicity

The ECIS, Bionas and the Roche-ACEA xCELLigence systems have been used to assess compound-mediated cytotoxicity [14, 48–50]. Figure 2B shows impedance-based monitoring of the cytotoxic effect of the histone deacetylase inhibitor (HDAC), Scriptaid on A549 cells. The kinetic response clearly shows that the time and dose-dependent cytotoxic effect of Scriptaid on the cells growing on the sensors. Several important parameters can be obtained from real-time cytotoxic data such as rate and onset of cytotoxicity as well as real-time dose-response of the compound. Figure 2C shows that the dose-response of Scriptaid can vary with time and this has to be taken into account whenever end-point assays are used for assessment of cytotoxicity. In addition to compound-mediated cytotoxicity, the xCELLigence system has also been used in conjunction with siRNA-mediated knockdown of specific chemotherapeutic targets to assess cell proliferation and cytotoxicity [51].

6.2 Cell-mediated cytotoxicity

Immune cells such as cytotoxic T lymphocytes (CTL) and natural killer (NK) cells are an integral part of the adaptive and innate immune responses, respectively. These cells have been endowed with the ability to recognize and annihilate cells with extreme stress load such as virus-infected cells as well as tumor cells [52]. NK cells express specific receptors on their surface that can recognize pathogen-infected and tumor cells by their lack of expression or low expression of the major histocompatibility complex (MHC) at the membrane [52]. These receptors can be both inhibitory and stimulatory and the combined action of these receptors determines the extent of the dual nature of NK cell response to target cells; namely cytotoxicity and/or cytokine production [52]. When NK inhibitory receptors bind to MHC class I molecules, their effector functions are blocked and therefore normal healthy cells that express adequate levels of these receptors are spared from the NK cell attack. On the other hand activating receptors such as NKG2D are engaged by ligands that are MHC-like and expressed by pathogen infected and transformed cells as well other stressed cells [53]. The immediate effector function of NK cells results in the release of secretory granules, which contain perforin and members of the granzyme family [54]. Perforin binds to the cell membrane and disrupts the integrity of the plasma membrane whereas granzymes are a special class of serine proteases with various substrate specificities, including caspases [55]. The combined interplay of these proteins and enzymes ultimately result in target cell cytotoxicity and destruction. Figure 2D shows the density-dependent cytotoxic effect of NK cell line (NK-92) towards A549 cells that had been growing on microelectronic sensors using the xCELLigence system. This system can also be used to analyze other forms of cell-mediated cytotoxicity, such as antibody-dependent cell-mediated cytotoxicity (ADCC). Glamann and Hansen [56] have shown herceptin-mediated ADCC of target cells by NK cells using impedance as readout.

7 Cell adhesion

The ability of mammalian cells to recognize, interact and respond to the extracellular matrix proteins is central to many biological processes including organogenesis, wound healing and inflammation. Cellular interaction with the extracellular matrix (ECM) is mainly mediated by the integrin class of cell surface receptors, which are heterodimeric single pass transmembrane receptors [35]. The engagement of integrins by ECM proteins leads to a conformational shift in the three-dimensional structure of integrin heterodimer resulting in the interaction of the cytoplasmic domain of integrins with signaling proteins and or structural proteins which are coupled to the actin cytoskeleton [57]. These signaling proteins including focal adhesion kinase (FAK), Src, Cas and Paxillin act in concert with structural proteins such as Talin and α-actinin to modulate the activity of RHO family GTPases (RHO, RAC, CDC42) to regulate the dynamics of actin polymerization/depolymerization particularly at the periphery of the cell membrane [35] (Fig. 3A). Adhesions through integrin receptors are necessary for a number of cellular events including growth factor-mediated cell proliferation and pro-

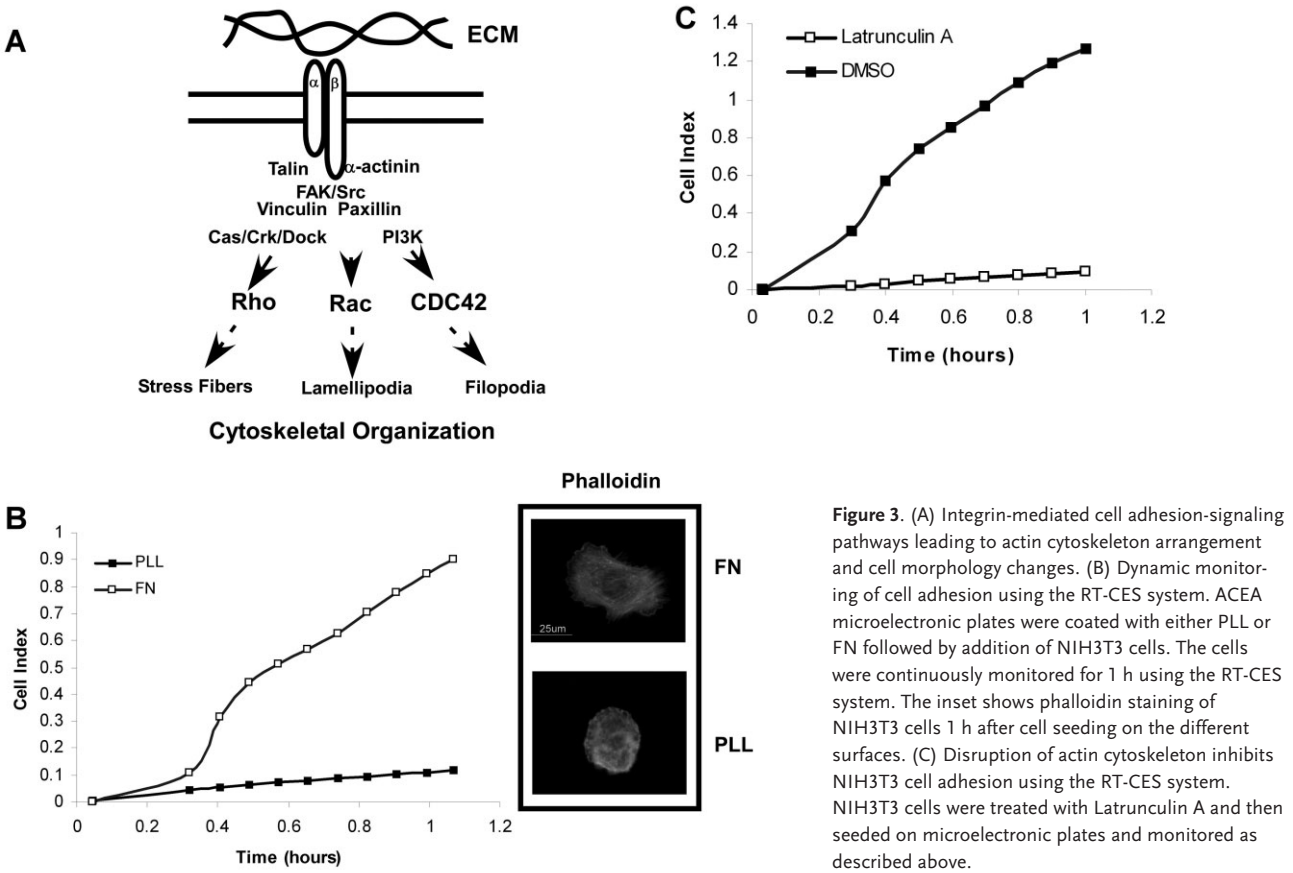

Figure 3. (A) Integrin-mediated cell adhesion-signaling pathways leading to actin cytoskeleton arrangement and cell morphology changes. (B) Dynamic monitoring of cell adhesion using the RT-CES system. ACEA microelectronic plates were coated with either PLL or FN followed by addition of NIH3T3 cells. The cells were continuously monitored for 1 h using the RT-CES system. The inset shows phalloidin staining of NIH3T3 cells 1 h after cell seeding on the different surfaces. (C) Disruption of actin cytoskeleton inhibits NIH3T3 cell adhesion using the RT-CES system. NIH3T3 cells were treated with Latrunculin A and then seeded on microelectronic plates and monitored as described above.

viding survival advantage by blocking apoptotic signaling [58]. In the past decade, it has become very clear that integrins are involved in different aspects of neoplasmic progression as well as inflammation and immune response, which has made integrins a very important therapeutic target for drug discovery [59].

Impedance-based technologies are particularly well suited for quantitative assessment of cellular interaction with the ECM because the impedance readout is highly sensitive to both cell-substrate interaction as well as morphological dynamics [60–62]. Wegener *et al*. [60] initially demonstrated the utility of impedance readout in monitoring of cell adhesion with ECM-coated electrode surfaces. The extent of electrode coverage area by the cell as well as local interaction sites through the focal complexes and adhesion will influence the degree of impedance readout. Therefore, cell-substrate impedance is a reflection of both adhesion quality as well as cell morphology [60, 62]. Figure 3B shows the impedance-based kinetic profile of NIH3T3 adhesion to the electrode surfaces that have been coated with either poly-L-Lysine or fibronectin (FN), as monitored by the xCELLigence system. PLL-mediated cell adhesion primarily through charge-charge interaction with the plasma membrane glycoproteins whereas FN interaction with integrin receptors initiates a signaling cascade that involves both adhesion and spreading and is reflected by the impedance readout. Treatment of the cells with the actin cytoskeleton disrupting agent lantrunculin A blocks FN-mediated cell adhesion as shown in Fig. 3C. Impedance-based cell adhesion can be utilized to study and measure adhesion events at the receptor level, intracellular signaling machinery and at the level of the actin cytoskeleton [56, 61].

8 Cell migration and invasion

Cell migration and invasion are dynamic cellular processes, which are central components of normal physiology such as embryogenesis, wound healing, inflammation and immunity as well as pathophysiological events such as cancer cell metastasis [63]. These multi-step processes are influenced by the extracellular microenvironment, including extracellular matrix components, growth factors,

chemotactic agents, the stroma as well as the presence of other specialized cells. The dynamic nature of cell migration and invasion involves cell adhesion, morphological changes due to rearrangement of the actin cytoskeleton and the emergence of protrusive structures at the membrane followed by contraction of the cell body and detachment of the uropod [63] (Fig. 4A). The same signaling pathways that mediate cell adhesion also play a central role in cell migration [64]. The membrane protrusions in the form of lamellipodia at the leading edge are stabilized by the interaction of integrins with the cell substratum (Fig. 4A). The main signaling proteins that regulate actin cytoskeletal dynamics such as RAC and its downstream effectors are localized to the site of these protrusions. Similarly, RHO family GTPases are also involved in the contraction and turnover of adhesion at the cell rear [64]. The inhibition of receptors and signaling proteins that participate in various aspects of cell migration can potentially offer attractive drug development targets.

Impedance-based technology has been utilized in two different ways to assess cell migration and invasion. The ECIS system has been used primarily to monitor a form of cell migration termed wound healing [65]. The workflow entails seeding a confluent layer of cells followed by application of a strong current, which leads to destruction, or "wounding" of the cells on the sensing electrode. Once the wound has been induced, the cells in the periphery of the sensing electrode well migrate onto the sensing electrode which is monitored in real-time. Alternatively, the ACEA real-time cell invasion and migration (RT-CIM) system utilized a Boyden-chamber format, in which directional cell migration in response to chemotactic agents are monitored in real time [15]. In this format, the sensing electrodes are placed on the underside of the porous membrane, which is attached to the top chamber, and the chemoattractant is placed in the bottom chamber. The migration of cells from the top chamber to the bottom chamber is then monitored in real time. In addition, application of a layer of matrigel can allow dynamic monitoring of cell invasion in this format. Figure 4B shows the migration of HT 1080 cells in response to 5% serum using the RT-CIM system. The advantage of the ECIS and RT-CIM systems for monitoring of cell migration and invasion is that it precludes that need to fix, stain and count the cells and can significantly reduce the amount of labor involved in performing these assays by traditional means.

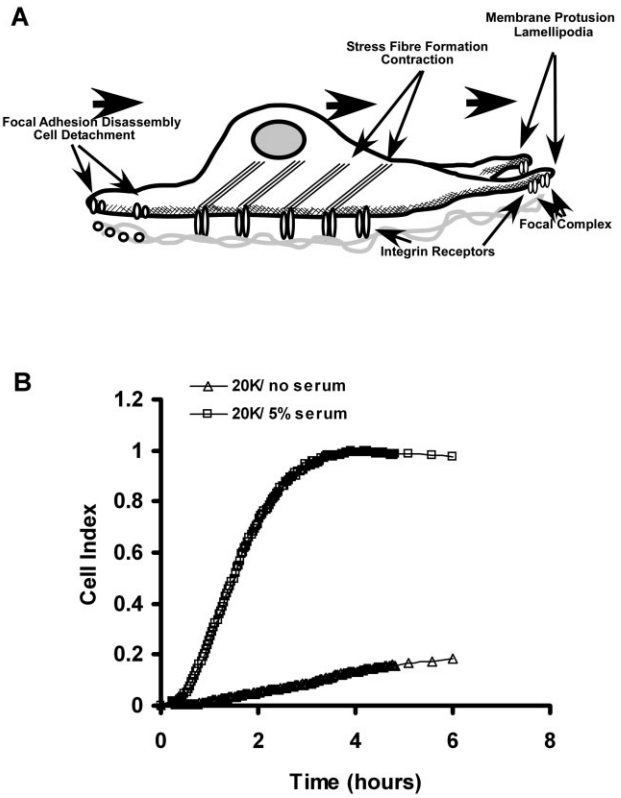

Figure 4. (A) Cell migration is a stepwise process including actin-mediated membrane protrusions, which is stabilized by integrins at nascent focal complexes. The next step is contraction of the cell body due to formation of actin stress fibers followed by detachment of the cell rear. The actin cytoskeleton and signaling pathways regulating actin cytoskeletal dynamics play a pivotal role in these processes [63]. (B) Dynamic monitoring of directional cell migration using the RT-CIM system. The modified ACEA microelectronic Boyden chamber was used in conjunction with the RT-CIM system to monitor the migration of HT1080 cells in the presence or absence of serum.

9 Conclusions

Cell-based assays have become an integral part of the pre-clinical drug discovery process, allowing for interrogation of protein targets and biochemical pathways under native conditions. A significant number of cell-based assays rely on over-expression of the protein target or reporter proteins for both primary screening purposes as well as secondary assays. While this approach can be very powerful in identifying and characterizing potential lead compounds and biologics, one of the obvious concerns is that overexpression of target proteins could potentially change or offset the stoichiometry of the target or pathways, such as GPCR, being investigated.

One of the main attributes of label-free technologies for cell-based assays is that it allows for label-free and non-invasive measurements of cells. Therefore, various targets such as GPCR can be assayed in primary cells as well as other disease-relevant cell types circumventing over-expression of the target or engineering of the cells. Utilization of such technologies could potentially reduce the number of false positives or false negative leads. An additional key aspect of label-free technologies is that they allow for dynamic monitoring of cells and depending on the specific technology being utilized, this can range from seconds to minutes and days. Dynamic monitoring of cellular events can lead to signature patterns or profiles, which can prove to be predictive of the events being studied. For example, impedance-based monitoring of GPCR responses leads to pathway-specific profiles [6, 7]. These signature profiles could potentially be very useful in characterizing orphan GPCR and dual or multi-pathway coupling. Using impedance, we have also observed signature profiles for compounds with similar mechanisms in long-term cytotoxicity assays (Abassi *et al.*, manuscript in preparation). In addition to these advantages, label-free technologies can be fairly flexible and therefore allow for rapid assay optimization and development. These technologies can be used in combination with standard end point assays to better define the time window of treatment or assay termination point for obtaining reproducible results [66].

In summary, in this review article, we have summarized the major cell-based label-free technologies and discussed their applications in both drug discovery and basic research settings. As increasingly more users begin to incorporate these technologies in their workflow, label-free technologies will continue to become an integral part of the drug discovery process. It is very likely that we will see in the near future the emergence of label-free technologies with higher rates of throughput and better analysis tools for interpreting the high content information obtained from the kinetic profiles.

Conflict of interest statement: The authors are employed by ACEA Biosciences, which is one of the companies highlighted in this review.

10 References

[1] Kola, I., Landis, J., Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 2004, *3*, 711–715.

[2] Horrobin, D. F., Modern biomedical research: an internally self-consistent universe with little contact with medical reality? *Nat. Rev. Drug Discov.* 2003, *2*, 151–154.

[3] Drews, J., Strategic trends in the drug industry. *Drug Discov. Today* 2003, *8*, 411–420.

[4] Cooper, M. A., Non-optical screening platforms: the next wave in label-free screening? *Drug Discov. Today* 2006, *11*, 1068–1074.

[5] Cooper, M. A., Optical biosensors: where next and how soon? *Drug Discov. Today* 2006, *11*, 1061–1067.

[6] McGuinness, R., Impedance-based cellular assay technologies: recent advances, future promise. *Curr. Opin. Pharmacol.* 2007, *7*, 535–540.

[7] Verdonk, E., Johnson, K., McGuinness, R., Leung, G. *et al.*, Cellular dielectric spectroscopy: a label-free comprehensive platform for functional evaluation of endogenous receptors. *Assay Drug Dev. Technol.* 2006, *4*, 609–619.

[8] Atienza, J. M., Yu, N., Wang, X., Xu, X. *et al.*, Label-free and real-time cell-based kinase assay for screening selective and potent receptor tyrosine kinase inhibitors using microelectronic sensor array. *J. Biomol. Screen.* 2006, *11*, 634–643.

[9] Fang, Y., Label-free cell-based assays with optical biosensors in drug discovery. *Assay Drug Dev. Technol.* 2006, *4*, 583–595.

[10] Hug, T. S., Biophysical methods for monitoring cell-substrate interactions in drug discovery. *Assay Drug Dev. Technol.* 2003, *1*, 479–488.

[11] Denholm, E. M., Stankus, G. P., Differential effects of two fluorescent probes on macrophage migration as assessed by manual and automated methods. *Cytometry* 1995, *19*, 366–369.

[12] Abbitt, K. B., Rainger, G. E., Nash, G. B., Effects of fluorescent dyes on selectin and integrin-mediated stages of adhesion and migration of flowing leukocytes. *J. Immunol. Methods* 2000, 239, 109–119.

[13] Giaever, I., Keese, C. R., A morphological biosensor for mammalian cells. *Nature* 1993, *366*, 591–592.

[14] Solly, K., Wang, X., Xu, X., Strulovici, B. *et al.* Application of real-time cell electronic sensing (RT-CES) technology to cell-based assays. *Assay Drug Dev. Technol.* 2004, *2*, 363–372.

[15] Atienza, J. M., Yu, N., Kirstein, S. L. Xi, B. *et al.*, Dynamic and label-free cell-based assays using the real-time cell electronic sensing system. *Assay Drug Dev. Technol.* 2006, *4,* 597–607.

[16] Giaever, I., Keese, C. R., Monitoring fibroblast behavior in tissue culture with an applied electric field. *Proc. Natl. Acad. Sci. USA* 1984, *81,* 3761–3764.

[17] Giaever, I., Keese, C. R., Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture. *IEEE Trans. Biomed. Eng.* 1986, *33,* 242–247.

[18] Thedinga, E., Kob, A., Holst, H., Keuer, A. *et al.*, Online monitoring of cell metabolism for studying pharmacodynamic effects. *Toxicol. Appl. Pharmacol.* 2007, *220,* 33–44.

[19] Fang, Y., Li, G. G., Peng, J., Optical biosensor provides insights for bradykinin B(2) receptor signaling in A431 cells. *FEBS Lett.* 2005, *579,* 6365–6374.

[20] Fang, Y., Ferrie, A. M., Fontaine, N. H., Yuen, P. K., Characteristics of dynamic mass redistribution of epidermal growth factor receptor signaling in living cells measured

with label-free optical biosensors. *Anal. Chem.* 2005, *77*, 5720–5725.

[21] Cunningham, B. T., Li, P., Schulz, S., Lin, B. *et al.*, Label-free assays on the BIND system. *J. Biomol. Screen.* 2004, *9,* 481–490.

[22] Hug, T. .S, Prenosil, J. E., Maier, P., Morbidelli, M., Optical waveguide lightmode spectroscopy (OWLS) to monitor cell proliferation quantitatively. *Biotechnol. Bioeng.* 2002, *80,* 213–221.

[23] Hug, T. S., Prenosil, J. E., Morbidelli, M., Optical waveguide lightmode spectroscopy as a new method to study adhesion of anchorage-dependent cells as an indicator of metabolic state. *Biosens. Bioelectron.* 2001, *16*, 865–874.

[24] Hug, T. S., Prenosil, J. E., Maier, P., Morbidelli, M., On-line monitoring of adhesion and proliferation of cultured hepatoma cells using optical waveguide lightmode spectroscopy (OWLS). *Biotechnol. Prog.* 2002, *18,* 1408–1413.

[25] Voros, J., Graf, R., Kenausis, G. L., Mayer, J. *et al.*, Feasibility study of an online toxicological sensor based on the optical waveguide technique. *Biosens. Bioelectron.* 2000, *15,* 423–429.

[26] Marx, K. A., Zhou, T., Montrone, A. Schulze, H. *et al.*, A quartz crystal microbalance cell biosensor: detection of microtubule alterations in living cells at nM nocodazole concentrations. *Biosens. Bioelectron.* 2001, *16,* 773–782.

[27] Marx, K. A., Zhou, T., Warren, M., Braunhut, S. J., Quartz crystal microbalance study of endothelial cell number dependent differences in initial adhesion and steady-state behavior: evidence for cell-cell cooperativity in initial adhesion and spreading. *Biotechnol. Prog.* 2003, *19,* 987–999.

[28] Marx, K. A., Zhou, T., Montrone, A., McIntosh, D. *et al.*, Quartz crystal microbalance biosensor study of endothelial cells and their extracellular matrix following cell removal: Evidence for transient cellular stress and viscoelastic changes during detachment and the elastic behavior of the pure matrix. *Anal. Biochem.* 2005, *343,* 23–34.

[29] Marx, K. A., Zhou, T., Montrone, A., McIntosh, D. *et al.*, A comparative study of the cytoskeleton binding drugs nocodazole and taxol with a mammalian cell quartz crystal microbalance biosensor: different dynamic responses and energy dissipation effects. *Anal. Biochem.* 2007, *361*, 77–92.

[30] Zhou, T., Marx, K. A., Warren, M., Schulze, H. *et al.*, The quartz crystal microbalance as a continuous monitoring tool for the study of endothelial cell surface attachment and growth. *Biotechnol. Prog.* 2000, *16,* 268–277.

[31] Wu, M., Neilson, A., Swift, A. L., Tamagnine, J. *et al.*, Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. *Am. J. Physiol. Cell Physiol.* 2007, *292,* C125–136.

[32] Sridharan, V., Guichard, J., Bailey, R. M., Kasiganesan, H. *et al.*, The prolyl hydroxylase oxygen-sensing pathway is cytoprotective and allows maintenance of mitochondrial membrane potential during metabolic inhibition. *Am. J. Physiol. Cell Physiol.* 2007, *292,* C719–728.

[33] Crunkhorn, S., Dearie, F., Mantzoros, C., Gami, H. *et al.*, Peroxisome proliferator activator receptor gamma coactivator-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 mitogen-activated protein kinase activation. *J. Biol. Chem.* 2007, *282,* 15439–15450.

[34] Disanza, A., Steffen, A., Hertzog, M., Frittoli, E. *et al.*, Actin polymerization machinery: the finish line of signaling networks, the starting point of cellular movement. *Cell. Mol. Life Sci.* 2005, *62,* 955–970.

[35] Juliano, R. L., Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. *Annu. Rev. Pharmacol. Toxicol.* 2002, *42*, 283–323.

[36] Calderwood, D. A., Shattil, S. J., Ginsberg, M. H., Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J. Biol. Chem.* 2000, *275,* 22607–22610.

[37] Nambi, P., Aiyar, N., G protein-coupled receptors in drug discovery. *Assay Drug Dev. Technol.* 2003, *1,* 305–310.

[38] Perez, D. M., Karnik, S. S., Multiple signaling states of G-protein-coupled receptors. *Pharmacol. Rev.* 2005, *57,* 147–161.

[39] Howe, A. K., Juliano, R. L., Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. *Nat. Cell. Biol.* 2000, *2,* 593–600.

[40] Garcia, L. J., Rosado, J. A., Gonzalez, A., Jensen, R. T., Cholecystokinin-stimulated tyrosine phosphorylation of p125FAK and paxillin is mediated by phospholipase C-dependent and -independent mechanisms and requires the integrity of the actin cytoskeleton and participation of p21rho. *Biochem. J.* 1997, *327,* 461–472.

[41] Rosado, J. A., Salido, G. M., Garcia, L. J., Activation of m3 muscarinic receptors induces rapid tyrosine phosphorylation of p125(FAK), p130(cas), and paxillin in rat pancreatic acini. *Arch. Biochem. Biophys.* 2000, *377,* 85–94.

[42] Zhang, Z., Neff, L., Bothwell, A. L., Baron, R. *et al.*, Calcitonin induces dephosphorylation of Pyk2 and phosphorylation of focal adhesion kinase in osteoclasts. *Bone* 2002, *31*, 359–365.

[43] Zhang, Z., Baron, R., Horne, W. C., Integrin engagement, the actin cytoskeleton, and c-Src are required for the calcitonin-induced tyrosine phosphorylation of paxillin and HEF1, but not for calcitonin-induced Erk1/2 phosphorylation. *J. Biol. Chem.* 2000, *275*, 37219–37223.

[44] Zhang, Z., Hernandez-Lagunas, L., Horne, W. C., Baron, R., Cytoskeleton-dependent tyrosine phosphorylation of the p130(Cas) family member HEF1 downstream of the G protein-coupled calcitonin receptor. Calcitonin induces the association of HEF1, paxillin, and focal adhesion kinase. *J. Biol. Chem.* 1999, *274,* 25093–25098.

[45] Yu, N., Atienza, J. M., Bernard, J., Blanc, S. *et al.*, Real-time monitoring of morphological changes in living cells by

Yama A. Abassi obtained his undergraduate degree in biochemistry from the State University of New York at Stony Brook in 1992 and his graduate degree in molecular, cell and developmental biology from the University of California at Santa Barbara in 1999. He has performed post-doctoral training in cancer biology at the Burnham Institute in La Jolla California investigating integrin-mediated signaling and its role in neoplasm. Dr. Abassi joined ACEA Biosciences in 2003 and is currently Senior Director of Cell Biology and Assay Development at ACEA.

electronic cell sensor arrays: an approach to study G protein-coupled receptors. *Anal. Chem.* 2006, *78,* 35–43.

[46] Coleman, M. L., Olson, M. F., Rho GTPase signalling pathways in the morphological changes associated with apoptosis. *Cell Death Differ.* 2002, *9,* 493–504.

[47] Coleman, M. L., Sahai, E. A., Yeo, M., Bosch, M. *et al*., Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat. Cell Biol.* 2001, *3,* 339–345.

[48] Arndt, S., Seebach, J., Psathaki, K., Galla, H. *et al*., Bioelectrical impedance assay to monitor changes in cell shape during apoptosis. *Biosens. Bioelectron.* 2004, *19*, 583–594.

[49] Xing, J. Z., Zhu, L., Gabos, S., Xie, L., Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. *Toxicol. In Vitro* 2006, *20,* 995–1004.

[50] Xing, J. Z., Zhu, L., Jackson, J. A., Gabos, S. *et al*., Dynamic monitoring of cytotoxicity on microelectronic sensors. *Chem. Res. Toxicol.* 2005, *18,* 154–161.

[51] Heidel, J. D., Liu, J. Y., Yen, Y., Zhou, B. *et al*., Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation *in vitro* and *in vivo*. *Clin. Cancer Res.* 2007, *13,* 2207–2215.

[52] Lanier, L. L,. NK cell recognition. *Annu. Rev. Immunol.* 2005, *23*, 225–274.

[53] Cerwenka, A., Lanier, L. L., Ligands for natural killer cell receptors: redundancy or specificity. *Immunol. Rev.* 2001, *181*, 158–169.

[54] Smyth, M. J., Cretney, E., Kelly, J. M., Westwood, J.A. *et al*., Activation of NK cell cytotoxicity. *Mol. Immunol.* 2005, *42,* 501–510.

[55] Trapani, J. A., Sutton, V. R., Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Curr. Opin. Immunol.* 2003, *15,* 533–543.

[56] Glamann, J., Hansen, A. J., Dynamic detection of natural killer cell-mediated cytotoxicity and cell adhesion by electrical impedance measurements. *Assay Drug Dev. Technol.* 2006, *4,* 555–563.

[57] Hynes, R. O., Integrins: bidirectional, allosteric signaling machines. *Cell* 2002, *110,* 673–687.

[58] Stupack, D. G., Cheresh, D. A., Get a ligand, get a life: integrins, signaling and cell survival. *J. Cell Sci.* 2002, *115*, 3729–3738.

[59] Mousa, S. A., Anti-integrin as novel drug-discovery targets: potential therapeutic and diagnostic implications. *Curr. Opin. Chem. Biol.* 2002, *6,* 534–541.

[60] Wegener, J., Keese, C. R., Giaever, I., Electric cell-substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. *Exp. Cell Res.* 2000, *259,* 158–166.

[61] Xiao, C., Lachance, B., Sunahara, G., Luong, J. H., An in-depth analysis of electric cell-substrate impedance sensing to study the attachment and spreading of mammalian cells. *Anal. Chem.* 2002, *74,* 1333–1339.

[62] Atienza, J. M., Zhu, J., Wang, X., Xu, X. *et al*., Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays. *J. Biomol. Screen.* 2005, *10,* 795–805.

[63] Lauffenburger, D. A, Horwitz, A. F., Cell migration: a physically integrated molecular process. *Cell* 1996, *84,* 359–369.

[64] Webb, D. J., Parsons, J. T., Horwitz, A. F., Adhesion assembly, disassembly and turnover in migrating cells — over and over and over again. *Nat. Cell Biol.* 2002, *4,* E97–100.

[65] Keese, C. R., Wegener, J., Walker, S. R., Giaever, I., Electrical wound-healing assay for cells *in vitro*. *Proc. Natl. Acad. Sci. USA* 2004, *101,* 1554–1559.

[66] Kirstein, S. L., Atienza, J. M., Xi, B., Zhu, J. *et al*., Live cell quality control and utility of real-time cell electronic sensing for assay development. *Assay Drug Dev. Technol.* 2006, *4*, 545–553.

