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PROBING CANCER SIGNALING WITH RESONANT WAVEGUIDE GRATING BIOSENSORS

Ye Fang, Ph.D.
Senior Research Manager, Biochemical Technologies, Science and Technology Division, Corning Inc., Sullivan Park, Corning, NY 14831, Tele: 607-9747203, Fax: 919-9745957, fangy2@corning.com

Abstract

Importance of the field—Cancer is a collection of diseases that arise from the progressive accumulation of genetic alterations in somatic cells. Genomic approaches have identified a great variety of genetic abnormalities associated with tumorigenesis, and molecular imaging and quantification assays have further elucidated the complex interactions within or between pathways. It is acknowledged that it is proteins, rather than genes, to fulfill most cellular functions; and signaling proteins largely operate through a large and complex network. To this end, cancer is mostly a pathway dysregulated disease – a small number of core pathways are dominate in aberrant cell growth leading to cancer. Thus, understanding the functional consequences of dysregulated and/or mutant signaling proteins in the context of native signaling networks is the frontier in cancer research.

Areas covered in this review—This article reviews why resonant waveguide grating (RWG) biosensor cellular assays are considered to be integrative in nature, and how RWG biosensor can be used for mining the surface markers of cancer cells, and discovering core pathway(s) of cancer receptor signaling.

What the reader will gain—The reader will gain an overview of cancer biology from pathway perspective, and have a glimpse of potential implications of integrative cellular assays, as promised by RWG biosensor, in cancer research and diagnosis.

Take home message—Successful approaches for developing next-generation anti-cancer therapies and diagnostic protocols should take into account that the dysregulation of oncogenic pathways is central to tumorigenesis. The biosensor cellular assays offer unprecedented advantage in characterizing cancer biology. However, significant challenges are also presented in deconvoluting and validating cellular mechanisms identified in cancer receptor signaling using these assays.

Keywords

Cancer signaling; cellular assays; dynamic mass redistribution; oncogene addiction; resonant waveguide grating biosensor

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1. Introduction

Human cancer is considered to be a pathway dysregulated disease (1). The ability of tumor cells to outgrow their neighboring cells is often driven by constitutive activation of downstream proteins (2,3). Genetic studies over several decades have discovered a wide range of tumor-associated genes and their mutations, many of which preferentially occur in signaling proteins involved in a small number of pathways (4–10). Genetic mutations are often enriched in positive regulatory loops (gain of function), and methylated genes in

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negative regulatory loops (loss of function), leading to the disruption of the normal cooperative behavior of cells and thus promoting tumor phenotypes (11,12). A hallmark in the onset of cancer is how mutated proteins alter and govern signaling of cancer cells in the context of intracellular or intercellular signaling networks (13–15). Despite advances in the discovery of genetic and epigenetic mutations as well as in the molecular delineation of oncogenic pathways, little is known about the systems biology of cancers and how oncogenic signaling alters the propagation routes of a diverse array of receptor pathways in cancer. Therefore, means to investigate and characterize oncogenic pathways are crucial for further elucidating the molecular mechanisms of cancer, and developing next generation molecular target-based therapies.

One promising approach is resonant waveguide grating (RWG) biosensor cellular assay, which has recently emerged as a valuable technology for studying cell signaling in a variety of disease states including cancers (16–20). RWG biosensor measures non-invasively a diverse array of cellular responses, resulting in dynamic mass redistribution (DMR) signatures of cell biology, receptor signaling and drug pharmacology (21–22). Given its high sensitivity and integrative nature in measurements, RWG biosensor can be used as a platform for characterizing cancer signaling, including mining functional receptors, discovering core pathways downstream cancer receptor signaling, and characterizing cancer cells via drug responsiveness.

2. Resonant waveguide grating biosensors

RWG biosensor belongs to a family of label-free optical biosensors that is sensitive to alterations in local refractive index at or near the sensor surface (23). A RWG biosensor consists of three components: a biological component, a detector element, and a transducer associated with both components (Fig. 1).

The biological component is a live cell or a tissue cell for whole cell sensing. Anchorage dependent cell can be directly cultured onto the transducer surface to form an adherent layer of cells (16). Suspension cells (e.g., lymphoblastic leukemia cells) can be brought to closely in contact with the transducer surface via physical sedimentation or specific biochemical binding between the immobilized molecule and a cell surface molecule (17).

The RWG transducer is a nano-grating waveguide (24). For whole cell sensing, the biosensor is often considered as a three-layer waveguide configuration: a substrate with a diffractive grating, a high index of refraction waveguide coating, and a cell layer. Such a configuration supports both transverse magnetic (TM_0) and transverse electric (TE_0) modes. The TM_0 mode has higher sensitivity and longer penetration depth (i.e., larger sensing volume) but relatively lower spatial resolution (~ tens of micrometers), comparing to the TE_0 mode (21,24). Thus, most RWG biosensors use the TM_0 mode for whole cell sensing. The penetration depth is the distance from the sensor surface at which the electric field strength has decreased to $1/e$ of its initial value. The penetration depth of a biosensor can be variable, dependent on detection scheme (21,24) and the biosensor configuration (25). The electromagnetic field, termed evanescent wave, is created by the diffraction grating coupled waveguide resonance (21). This indicates that the biosensor only samples the bottom portion of the cells contacting with the sensor surface.

The RWG detector exploits resonant coupling of light into a waveguide via the diffraction grating (23). When illuminated with broadband light at a fixed and nominally normal angle of incidence, these sensors reflect only a narrow band of wavelengths (resonant wavelength) that is a sensitive function of the local index of refraction of the biosensor (17). Since the local index of refraction is directly proportional to the density and distribution of biomass (e.g., proteins, molecular complexes) in live cells (26), the RWG can non-invasively detect

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stimulus-induced DMR in native cells. The DMR defines redistribution of cellular matters within the sensing volume. Such a redistribution is often not random; instead, it is tightly regulated and is often dynamic both spatially and temporally (27,28). The biosensor simply acts a non-invasive monitor to record the DMR in real time. The DMR contains high information, and multiple parameters can be derived from a DMR signal and used for characterizing receptor signaling (24) and drug pharmacology (29). The DMR is common to almost all types of cells, and many (if not all) receptor signaling and cellular processes. This is because cell signaling often involves protein trafficking, microfilament remodeling, cell adhesion alterations and morphological changes of cells, all of which can lead to DMR. However, since cells vary in the relative stoichiometries of intracellular signaling components and the DMR assays detect such differences, the activation of a receptor may result in cellular background-dependent phenotypic responses. Therefore, it is not surprising to see in recent years that RWG biosensor cellular assays have found broad applications to a diverse array of cellular processes, including adhesion (22,30), viral infection (31), proliferation (32) and apoptosis (33) of cells. These assays are also amenable to a wide range of receptors, including G protein-coupled receptors (GPCRs) (34,35), ion channels (36), kinases (24,37), enzymes (38), and structural proteins (39). Numerous studies have found that the DMR measurements are pathway-sensitive, and often reflect the complexity of receptor biology (40–45) and drug pharmacology (29,46–48). In general, a DMR signal may contain contributions from protein trafficking, microfilament remodeling, and cell adhesion alterations (21), but different events may dominate different DMR signals. Thus it is possible to identify many critical nodes and core pathways in receptor signaling network (49) (Fig.1).

RWG biosensor systems including Epic® and BIND™ are commercially available nowadays (49). Both systems employ the wavelength interrogation configuration, in which a broadband light source is used for illumination, and the wavelength of the reflected light is recorded (17). Such a configuration is amenable to high throughput screening (HTS) since conventional HTS often uses microtiter plates having large footprint. Alternative angular interrogation configurations have also been explored, in which a single wavelength light at variable angles is used to illuminate the biosensor (21,22,24,32). For example, optical waveguide lightmode spectroscopy (OWLS) monitors the incoupling angles by continuously changing the incident angle of the light and measuring the incoupled light intensity with a photodetector placed at the edge of the waveguide. The OWLS and other angular interrogation systems often have limited capability for parallel biosensing applications (17).

The Epic® system (Corning Inc) is the first optical biosensor that is amenable to microtiter plate-based HTS for both biomolecular interaction analysis and cell-based assays (17,50). The system consists of a RWG detector, an external liquid handling accessory and a scheduler, such that it can process large numbers of microplates using end-point measurements for HTS, or using kinetic measurements for high information content screening. The detector utilizes a linear array of fiber optics to rapidly scan a whole microtiter plate, and to track changes in the central wavelength (resonant wavelength) of the biosensor resonant spectrum. However, since the system is a standard-alone reader system, full integration with external liquid handler is prerequisite for effective DMR assays. Furthermore, since the system is stationary without any CO_2 control unit and maintained at 26°C for its internal temperature, it is obviously difficult to study cell signaling at variable temperatures, particularly under physiological conditions. Epic® biosensor microplates (typically 384well format) have appropriate surface coatings for different applications.

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3. Probing cancer biology with small molecules

Advances in molecular genetics have identified numerous molecules that control living systems (51). Specific proteins can be suppressed or eliminated in living system via RNAi knockdown or gene knockout, respectively. Specific proteins can also be increased in concentration via gene expression, or altered via mutagenesis. Determining the functional consequences of these manipulations has greatly assisted our understanding of various diseases including cancer (52). Equally important is the use of small molecules for deciphering the molecular mechanisms underlying cancer development (53). Cellular interventions with small molecules are advantageous, because of the easiness in administration, the ability to temporally control the system, and the wide accessibility and coverage in biological target space of diverse small molecules. Furthermore, it is possible for small molecules to selectively perturb one of the multifaceted functions of a protein, resulting in a level of understanding of protein function that would not be possible through gene-based perturbation (54). Thus, small molecules can be used as probes to systematically map biological-activity space and to understand a cell system including cancer cells. Combining conventional molecular characterization assays (e.g., assays for monitoring alterations in quantity, location and phosphorylation state of signaling proteins) with small molecule probes will further enable mechanistic deconvolution and validation of cellular mechanisms of cancer signaling.

Judicious collections of small molecules can be made to modulating the functions of many different proteins, all in one experiment using HTS technologies. The assembly of ideal small molecule library is possible, partly because recent genomic landscape studies have identified many protein classes that are important to tumorigenesis (4–8), and partly because diverse small molecule modulators are available for many of these proteins. More important is that although cancer is highly heterogeneous, only a small number of core pathways are essential to cancer development (1,5). This means that a relatively small library may be sufficient for characterizing cancer. Libraries can be assembled from available compounds with known effects on specific proteins and phenotypes, such as modulators for GPCRs, proteases, phosphatases, ion channels and kinases.

Phenotypic screens using chemical genetics have been used to characterize cancer cell biology (55). Phenotypic readouts, such as proliferation and migration, are often used as a metrics to characterize the activity and specificity of compounds, whereas the patterns of different compounds acting on a cancer cell are indicative for the type of cancers. Weinstein *et al.* examined the impacts of distinct chemicals on the proliferation of a panel of 60 tumor cell lines, and found that compounds with similar structures or similar mechanisms of action had similar phenotypic profiles (*i.e.*, similar ability to inhibit the growth of a similar set of tumor cell lines) (56). However, these phenotypic screening assays are inherently vague, partly due to their readouts that are largely long term cellular responses (e.g., alterations in gene expression and/or growth rate), and partly due to the presence of many compensatory pathways that may lead to similar long-term responses. Since the DMR assays often measure early events in cell signaling and receptor activity is reported as a DMR signal indicative of pathways involved, combining multi-parametric biosensor cellular assays with small molecules should allow systematic mapping of biological-activity space in cancer, and thus greatly accelerate our understanding of cancer biology.

4. Mining the surface markers of cancer cells

Signal transduction originates at the cell surface membrane. Membrane bound proteins are highly abundant, comprising more than a third of all cellular proteins. These receptors are central to signal transduction, and many of them are often up-regulated or mutated in cancer

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cells (57). Mining the surface proteome of cancer cells is useful for identifying markers to classify tumors, to monitor disease progression, regression and recurrence, and to assess the responsiveness of cancer cells to therapy. Traditionally, cancer signatures are identified mostly via gene expression analysis which is inherently more detail-rich and specific than phenotypic assays. However, since DMR assays provide a functional and global readout of receptor signaling involving altered and/or mutated proteins in cancer cells, they offer a complementary view of cancer biology.

Large scale surveying functional receptors in cancer cells is feasible using RWG biosensor cellular assays (21,34,48). For example, to mine endogenous and functional GPCRs, a library of agonists for most of all known GPCRs (~220) can be assembled and used to stimulate a cancer cell. The DMR signal of each agonist can be recorded independently in real time. The patterns of DMR signals obtained can be clustered using similarity analysis – a technology that is widely used in gene expression analysis (49,58). Results showed that the human skin carcinoma cell line A431 cells respond to a quite few of GPCR agonists (33,40,48, data unpublished). Figure 2 showed representative DMR signals, each with distinct characteristics (e.g., shape, kinetics, duration, and amplitude). For a subset of agonists tested, the heat-map generated with Hierarchical Euclidean clustering indicated that beside these agonists that did not trigger any obvious DMR signals, the other known GPCR agonist-induced DMR signals can be classified into two classes at the low resolution, each several sub-clusters. Based on the heat map shown in Fig. 3, a G_s-type DMR signal (29) was observed for the three known G_s-coupled β_2-adrenergic receptor agonist epinephrine, dopamine and phenylephrine (29), and the four G_s-coupled adenosine A_{2B} receptor agonist adenosine, IB-MECA, CCPA and CGS21680 (34), the G_s-coupled VIP_1 agonist vasoactive intestinal peptide, and the G_s-coupled EP_4 agonist prostaglandin E_2 and prostaglandin D_2. On the other hand, a distinct G_q-like DMR signal (34,48) was observed for the G_q-coupled $P2Y_2$ receptor agonist ATP and UTP (34), the G_q-coupled histamine receptor H_1R agonist histamine (50), the G_q-dominant bradykinin B_2 receptor agonist bradykinin (40), the G_q-dominant S1P receptors agonist sphinosine-1-phosphate, the G_q-dominant LPA receptors agonist LPA (34), and the G_q-dominant protease activated receptor PAR_1 agonist SFLLR-amide and PAR_2 agonist SLIGKV-amide (48). These functional DMR responses are correlated well with the expression of their corresponding receptor(s). These results suggest that DMR assays faithfully report functional receptors in the cells. However, it is known that many ligand molecules display polypharmacology. Thus, the assignments of ligand DMR signals to specific receptors need to be validated via several methods. First, a relatively large set of ligands for a specific receptor should be used, and their DMR signals can be compared to determine whether the receptor is expressed and functional in the cell. Second, pharmacology characterization using antagonists can also be used to determine the specificity of the agonist-receptor pair. Third, gene expression and gene manipulation (e.g., gene transfection or RNAi knockdown) can further validate the specificity of receptor-ligand pair.

RWG biosensor is also amenable to label-free cell attachment assays for identifying the presence of specific surface markers of cancer (30). Here, probe molecules that recognize specific antigens presented at the cancer cell surface are pre-immobilized onto the sensor surface, and are used as fishing baits to detect, and enrich the antigen presenting cancer cells. For example, a RWG biosensor has been used to identify specific antigen positive cells, including carcinoembryonic antigen (CEA) expressing cells (59). CEA is a glycosyl phosphatidyl inositol-cell surface anchored glycoprotein involved in cell adhesion, and is one of the most widely used tumor markers worldwide. CEA testing is mainly used as a tumor marker to identify recurrences after surgical resection, or localize cancer spread, particularly for gastrointestinal and colorectal malignancy (60).

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5. Probing core pathway(s) in cancer receptor signaling via chemical tools and RWG biosensor readouts

Common to cancers are the hallmarks related to survival and proliferation in foreign environments (13), evading immune surveillance (14), and stress phenotypes (15). Tumorigenesis starts with a single cell, and arises through a multistage, mutagenic process that involves genetic alterations resulting in the gain-of-function mutation, amplification, and/or over-expression of key oncogenes, and the loss-of-function mutation, deletion, and/or epigenetic silencing of key tumor suppressors (11). Gain-of-function mutations in positive regulatory loops directly lead to hyperactivity and tumor aberrant growth, whereas loss-of-function mutations in negative regulatory loops result in the removal of the restraints necessary to prevent aberrant growth and survival or genomic instability, thus leading to tumorigenesis (61–64). Cancer cells also induce angiogenesis and metastasis which are important to clinical manifestation of cancer (65).

The hallmark of cancer is its complexity in genetic and epigenetic abnormalities (1). In many cancers, there are a few of frequently mutated oncogenes and tumor suppressors such as PI3K, Ras, p53, and PTEN. For example, genetic alterations in the PIK3CA gene encoding p110a PI3K and in related pathway genes are presented in >30% of colon and breast cancers (4). Deletion or epigenetic silencing of tumor suppressor genes is also evident in many cancers. An example is the deletion of the tumor suppressor and lipid phosphatase PTEN (66). PTEN normally acts to constrain PI3K signaling, and thus cancer bearing PTEN deletion is likely to be sensitive to PI3K inhibitors. Malignant carcinomas also harbor a complex combination of infrequent mutations, many of which are thought to drive the cancer phenotype (4–7). Stratton and colleagues estimate that individual mutations in as many as 20% of all kinases can play an active role in tumorigenesis (67).

Cancer is largely a pathway dysregulated disease (1,5). Some cancers apparently depend on the continued activity of certain oncogenes for maintenance of the malignant phenotype (2). Evidence for such oncogene addiction is amounting. First, in a transgenic mouse model, switching on the c-myc oncogene in the hematopoietic cells led to the development of T-cell and myeloid leukemias. However, the phenotype of leukemia cells was reversed upon myc silencing (68). Similar was found in mouse models for c-myc-driven skin papillomas, and osteosarcomas (69,70), and for BCR-ABL-induced leukemia (71). In human colorectal cancer cells bearing a K-Ras mutation, somatic knockout of this oncogene led to reversion of the transformed phenotype and abrogated the ability of these cells to form tumors in nude mice (72). Second, global surveying several tumors including breast and colorectal cancers at genomic level has found that many genetic abnormalities are mostly associated with a small set of biological processes and biochemical pathways, and different tumors share some common dysregulated pathways (4–7). Cancer signaling network mapping studies also revealed two oncogene-signaling blocks that are enriched in gene mutations and tend to collaborate in most tumor types – p53 (composed of p53, p14, Rb, BRAC1 and BRAC2, etc.) and Ras (Ras, PI3K and EGFR, etc.) blocks (73). In 592 tumors analyzed, at least 2 signaling gene mutations, one from the p53 block and the other from the Ras block, are necessary for tumorigenesis and further support the notion that both the prevention of cell death (p53 block) and the promotion of cell proliferation (Ras or other blocks) are necessary to generate most tumors. The third, and the most convincing, evidence is from some experimental research and clinical settings in which molecule targeted therapy has demonstrated therapeutic efficacy – the inactivation of a single or a few of oncogenes appears largely impair the growth and survival of cancer (15). Successful examples include the receptor tyrosine kinase HER-2 antagonizing antibody drug Herceptin for breast cancer (74), and several oncogenic protein kinase inhibitor drugs such as imatinib/Gleevec (75), gefitinib (76) and erlotinib (77). Imatinib targets the bcr-abl oncogene in chronic myeloid

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leukemia and also targets the c-kit oncogene in gastrointestinal stromal tumors. Gefitinib and erlotinib target the EGFR in non–small cell lung carcinoma, pancreatic cancer, and glioblastoma. Taken together, these studies suggest that a limited number of central molecular pathways are crucial to cancer development. Because of that as well as the clinical efficacy of molecule targeted therapy is tied to accurate identification of the state of oncogene addiction in specific cancers, RWG biosensor cellular assays provide alternative means to identify and better define these core pathways.

Epidermal growth factor receptors (EGFR) belong to a family of receptor tyrosine kinases, and are one of the most frequently mutated proto-oncogenes in many cancers (57). RWG biosensor cellular assays were used to map the signaling and its network interactions of EGFR in A431 cells (24). A431 is well-known for its over-expression of EGFR. A previous study using the EGF DMR signal in A431 cells as a readout showed that EGFR signaling is cellular status dependent – quiescent cells respond more robustly to EGF than proliferating cells. Chemical biology studies, based on the modulation profiles of an array of known modulators, also linked several targets and cellular processes to the EGFR signaling. The EGFR signaling was found to require its intrinsic tyrosine kinase activity and to be mostly originated from the internalized receptors. The EGFR signaling also led to actin remodeling, dynamin and clathrin dependent receptor internalization, and MEK pathway-mediated cell detachment (possibly via FAK). To further determine the core pathways of EGFR signaling, a judicious selection of kinase inhibitors was made to examine their impacts on the EGF DMR signal in quiescent A431 cells (data unpublished). Figure 4 shows the different sensitivity of the EGF DMR signal in quiescent A431 cells to distinct kinase modulators. As expected, the EGFR tyrosine kinase inhibitors, A1478 and BML-265, almost completely blocked the EGF signal. However, the MEK1/2 inhibitor U0126 selectively attenuated the late DMR event, and the protein kinase C (PKC) inhibitor rottlerin selectively blocked the early DMR event. These results suggest that distinct pathways preferentially occur at different time domain during signaling – the PKC pathway plays important role in the early response, but the MAPK pathways dominate in the late cellular response upon the activation of EGFR.

In another recent RWG biosensor study, Du and her colleagues found that distinct cancer cell lines responded differently to EGF – squamous cell carcinoma of the head and neck (SCCHN) cell line UPCI-37B exhibited a rapid rise in DMR signal, while lung adenocarcinoma cell line A549 showed a biphasic DMR profile (37). Pathway deconvolution using chemical biology approach revealed that the EGF-induced DMR signal in the SCCHN cell is insensitive to inhibitors targeting the Ras/Raf/MAPK pathway, but is completely blocked by the two PI3K inhibitors LY 294002 and wortmannin, indicating that PI3K is a critical mediator of the EGFR signal in this cancer cell. Moreover, they found that the EGF-induced DMR signal in SCCHN cancer cells can also be completely suppressed by the two EGFR inhibitor drugs, gefitinib and erlotinib. Both gefitinib and erlotinib have been approved by the FDA for the treatment of NSCLC and are under phase II clinical evaluation for use in SCCHN (78). These results suggest that the biosensor cellular assays are applicable to many oncogenic pathways for the discovery of novel therapeutic agents targeting various cancers. It is worthy noting that small kinase inhibitors are well-known for their polypharmacological properties. Thus, several inhibitors for a single kinase should be used, together with conventional cell biology studies, to ascertain the roles and specificity of the kinase in receptor signaling (24).

6. Conclusions

Optical biosensors including RWG biosensors have evolved from a research tool for biomolecular interaction analysis to a high throughput and high content screening platform

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for whole cell sensing. Together with chemical biology and chemical genetics, these biosensors enable systematically mining endogenous receptors in cancer cells, and to elucidate critical nodes and core pathways of cancer signaling networks. The ability to assay endogenous receptors without any manipulations makes it possible to characterize cancer cells including primary cells, and to identify responsive therapeutics for specific cancers. Next generation RWG biosensors will have higher spatial resolution (25,79–80), enabling non-invasively measure cancer signaling in single cells including rare cancer cells, and in mixed populations of cancer cells such as tissue cells, reprogrammed cells, and unpurified primary cells.

7. Expert opinion

Cancer is the collection of complex genetic diseases. The molecular target-based therapies for cancer today is primarily for preexisting diseases, typically late in their progression. Next generation anti-cancer medicine will move toward predictive and preventive modes. This requires understanding of cancer development at the levels of genome, systems biology and single cell. Advances in gene sequencing technologies will allow individuals to have their genomes sequenced, which, in turn, allow mapping the genomic landscape of potential genetic alterations. Multi-parametric molecular diagnostics via blood analysis will make early diagnosis of cancer possible and will become a routine procedure in clinical laboratories. Advances in systems biology and systems pharmacology approaches will enable the extensive correlations of genetic alterations with cancer development, determine the unlined mechanisms separating normal from pathological processes, and identify the personalized therapies. Improvements in single cell assay technologies will allow characterization of cancerous cells at early stages in the context of dysregulated pathways and drug responsiveness.

Understanding the systems biology and pharmacology is the new frontier in cancer research. Conventional genomic approaches have been very fruitful for the discovery of genetic and epigenetic mutations (1–6). Since it is proteins, but not genes, that fulfill most biological functions of cells, the functional consequences of these genetic abnormalities are still largely uncharted by these approaches. The wide adoption of recombinant DNA technologies has made molecular characterization assays possible to delineate many oncogenic pathways. However, it is the large and complex network, rather than linear pathways, in which signaling proteins mostly operate. The integrative cellular assays, as promised by RWG biosensor, open new possibility to bridge mutated/dysregulated targets, pathways and cellular phenotypic responses associated with cancer. The biosensor cellular assays are well-suited for pathway profiling, since the DMR signal is common to many cellular processes, and signaling downstream many receptors. The target/pathway specificity can be achieved by chemical biology approaches – together with increasing numbers of small molecules for expanded biological space as well as incorporation of molecular characterization assays, the wide uses of small molecules will improve the resolution and quality of DMR assays. The DMR signal also represents a novel phenotypic response of cells, and is associated with many cellular processes related to cancer biology including signaling, trafficking and metastasis. It becomes clear that these assays not only allow systematic determination of signaling proteins and core pathways pathologically relevant to cancer, but also enable detailed characterization of cancerous cells and drug responsiveness.

However, RWG biosensor cellular assays are still in infancy. A challenge in adopting the biosensor cellular assays is that RWG biosensor, label-free biosensor in general, is largely non-specific. Many cellular processes and pathways can result in detectable DMR signals. However, due to the widely existed polypharmacology of many drug molecules, and the limited numbers of possible DMR signals which are obviously much less than the rich

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biological-activity spaces, the exact cellular mechanisms that lead to detectable DMR signal often need to be deconvoluted. Thus, novel assay designs and methodologies are required to further advance biosensor cellular assays for both basic research and industrial applications.

Article highlights

- Functional genomics and gene sequencing has led to discovery of a wide array of genetic abnormalities. Functional studies have helped to sort out pathogenically relevant alterations. As our understanding of cancer development advances, it has become clear that cancer is a pathway dysregulated disease.
- The ability to study mutated and/or dysregulated signaling proteins in the native signaling networks is crucial to advance cancer research, to develop next generation therapies and diagnostic protocols.
- Resonant waveguide grating biosensor cellular assay emerged recently as a promising platform for cancer research – it allows systematically surveying functional receptors and pathways, and determining core pathways downstream cancer receptor signaling, in combination with chemical and biological tools.
- Assay methodologies in development, together with next-generation biosensor technologies, will allow the characterization of primary cancer cells and cancer stem cells at high resolution and with high information content.

This box summarizes key points contained in the article.

Abbreviations

GPCR	G protein coupled receptor
EGFR	epidermal growth factor receptor
DMR	dynamic mass redistribution
RWG	resonant waveguide grating
HTS	high throughput screening

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Declaration of interest:

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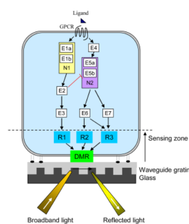

Figure 1.
Resonant waveguide grating biosensor for studying cancer signaling. The biosensor uses its intrinsic evanescent wave to characterize cancer signaling in live cells. Combining with chemical biology and molecular genetic approaches, it is possible to decode the core pathway(s) and critical nodes in receptor signaling in a cancer cell. An example is that binding of a GPCR agonist to its cognate receptor leads to two major pathways: the first one consists effectors **E1a, E1b, E2, E3**, leading to response **R1**, while another consists of **E4, E5a, E5b, E6**, and **E7**, leading to responses **R2** and **R3**. The integration of the responses **R1, R2** and **R3** when occurred within the sensing zone of the biosensor leads to the **DMR**. The effectors **E1a** and **E1b** form the critical node **N1**, while **E5a** and **E5b** form another critical node **N2**. The intervention of these critical nodes by small molecules or genetic manipulations would have greater impact on the receptor DMR signal, than other signaling components.

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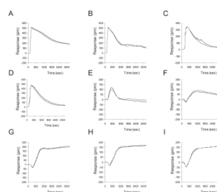

Figure 2.

Representative DMR signals of A431 cells upon stimulation with distinct GPCR agonists. The GPCR agonists include (A) ATP for the G_q-coupled $P2Y_2$ receptor; (B) bradykinin for the G_q-dominant bradykinin B_2 receptor; (C) SLIGKV-amide for the G_q-dominant protease activated receptor PAR_2; (D) SFLLR-amide for the G_q-dominant protease activated receptor PAR_1; (E) sphinosine-1-phosphate for the G_q-dominant $S1P_2$ and $S1P_5$ receptors; (F) PGE_2 for the G_s-coupled prostaglandin EP_4 receptor; (G) and (H) adenosine and CCPA, respectively, both for the G_s-coupled adenosine A_{2B}, and (I) phenylephrine for the G_s-coupled β_2-adrenergic receptor. Each agonist was assayed at 10 μM in duplicate. The A431 cells were cultured using serum medium overnight, followed by 20hr starvation with serum-free medium. After washing with assay buffer, the confluent A431 cells were directly assayed with Epic® system.

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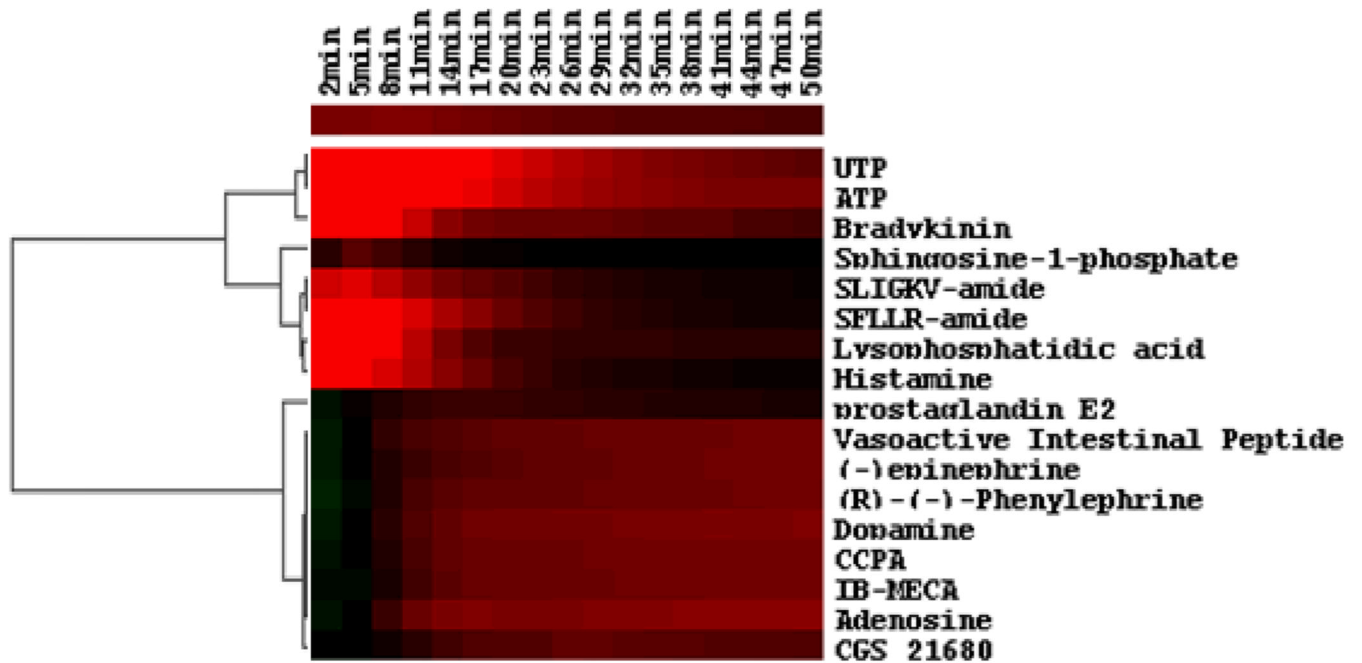

Figure 3.

The heat map classification of the DMR signals of quiescent A431 cells induced by diverse GPCR agonists. The heat map was generated using the Euclidean hierarchical cluster analysis (ref. 48–57). The real responses of all DMR signals at discrete time points, as indicated, were used as the basis for similarity analysis. The amplitude and direction is indicated by color – the red indicates a positive value, the green a negative value, and the black a value close to zero.

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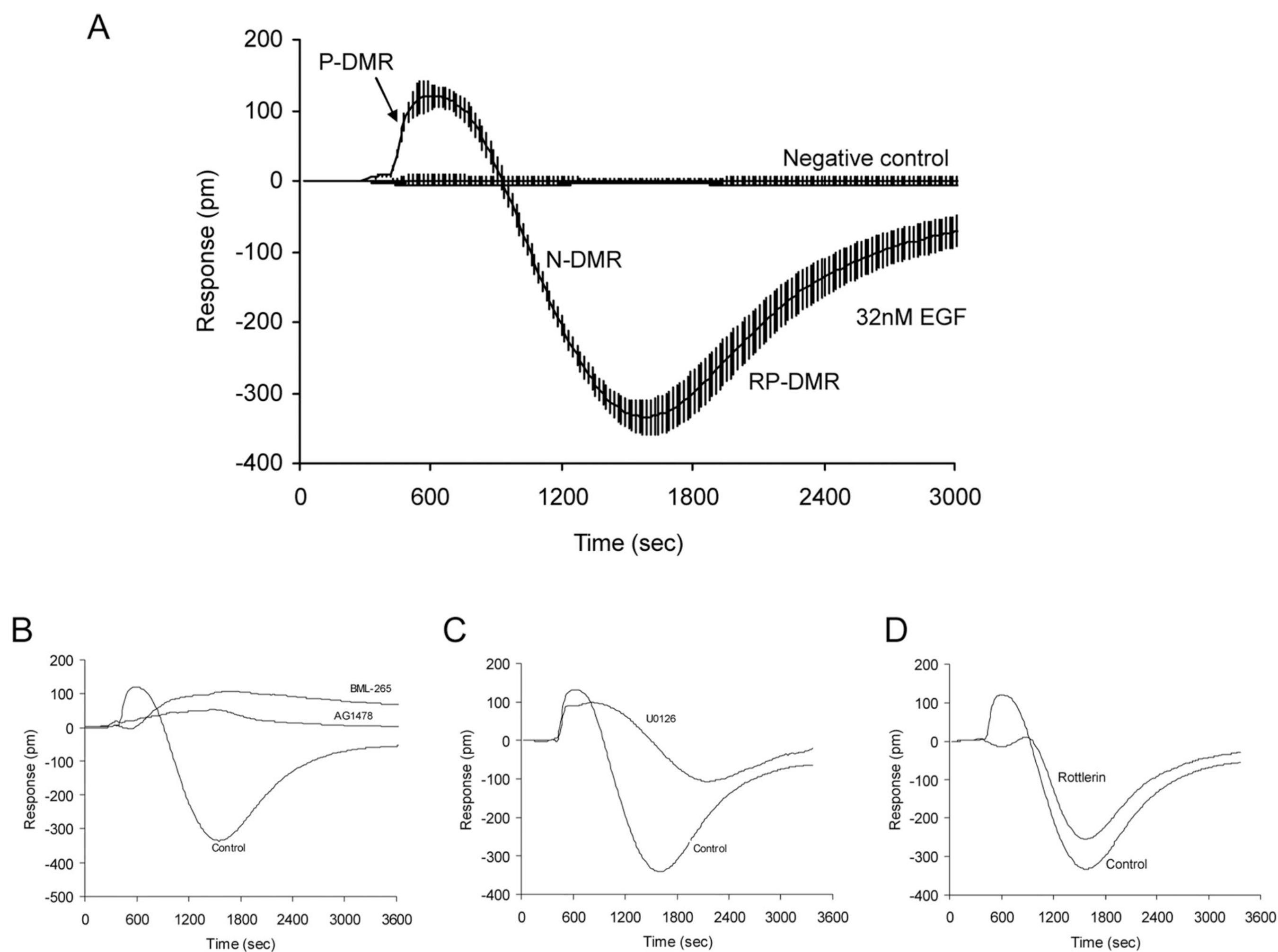

Figure 4.

The characteristics of the EGF DMR signal in quiescent A431 cells. (A) Real time EGF DMR signal in comparison with the negative control (*i.e.*, the response of cells stimulated with the assay vehicle only), each error bar representing the standard deviation of 32 replicates. The EGF DMR proceeds in three phases: an initial positive-DMR (P-DMR), followed by a negative-DMR (N-DMR) and a recovery positive DMR (RP-DMR). (B) to (D) The sensitivity of the EGF DMR signal to different modulators, AG1478 and BML-265 (B), U0126 (C) and rottlerin (D). The control is the EGF response of cells pretreated with the assay vehicle only. In all experiments, EGF was at 32nM, whereas the rest compounds used to pretreat the cells were at 10μM.