Chapter 1
Cell-Based Microarrays: Recent Advances for Gene Function Analyses

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Abstract Reverse transfected cell microarrays (RTCM) are a powerful tool for the systematic analyses of gene functions. With this technology more than a thousand different nucleic acids can be transfected into eukaryotic cells in parallel on a single glass slide. This allows high-throughput analyses of gene functions using gain-of-function, loss-of-function, and mutation approaches. RTCM paved the way for genome-wide gene function analyses in order to determine gene functions involved in the molecular regulation of cell phenotypes in physiologic and pathophysiologic processes. Since RTCM was first introduced in 2001, the technique has been well established. The method was successfully used in several genome-wide and large-scale screenings, and novel analysis methods to detect gene functions have been developed. This chapter will summarize the most recent technological developments in the usage of RTCM, including optimization of (1) transfection efficiency, (2) reporter systems and automated data acquisition, (3) spotting density with decreased cross-contamination, and (4) the new development of assays to screen for paracrine gene effects.

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Abbreviations

A549	Human alveolar basal epithelial cells
cDNA	Complementary DNA
CFP	Cyan fluorescent protein
COPI/COPII	Coat protein I/Coat protein II
COX-2	Cyclooxygenase 2
cPARP	Cleaved poly ADP ribose polymerase
CRE	cAMP response element
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded ribonucleic acid
ECM	Extracellular matrix protein
EdU	5-ethynyl-2′-deoxyuridine
ER	Endoplasmic reticulum
GBP-1	Guanylate-binding protein 1
(E)GFP	(Enhanced) green fluorescent protein
GPCR	G protein-coupled receptor
GPR160	G protein-coupled receptor 160
HCT116	Colon carcinoma cell line
HEK293	Human embryonic kidney cell line
HEK239T	Human embryonic kidney cells expressing SV40 large T antigen
HeLa	Cervix carcinoma cell line
HFIB	Human fibroblasts
HIV	Human immunodeficiency virus
HT29	Colon adenocarcinoma cell line
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule 1
IFN-α	Interferon-α
IFN-γ	Interferon-γ
JNK	c-Jun N-terminal kinase
K562	Human erythroleukemia cell line
KPL-4	Breast cancer cell line
LICM	Lentivirus-infected cell microarray
LMP	Low melting point
MAPK	Mitogen-activated protein kinase
MCF-7	Breast cancer cell line
MicroSCALE	Microarrays of spatially confined adhesive lentiviral features
miRNA	Micro ribonucleic acid
NF-κB	Nuclear factor-kappa B
NLS	Nuclear localization signal
NPY	Neuropeptide Y
PC3	Prostate cancer cell line
PEST	Proline–glutamic acid–serine–threonine–protein sequence
PLL	Poly-L-lysine

RFP	Red fluorescent protein
RNAi	Ribonucleic acid interference
RTCM	Reverse transfected cell microarray
S2R+	Schneider S2 embryonic drosophila cell line
SHARPIN	SHANK-associated RH domain interactor
shRNA	Short hairpin ribonucleic acid
siRNA	Small interfering ribonucleic acid
SW480	Colon adenocarcinoma cell line
TNF-α	Tumor necrosis factor-α
TORC1	Target of rapamycin complex 1
tsO45G	Temperature-sensitive CFP-coupled viral membrane protein
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end label
U2OS	Human osteosarcoma cell line
VCAM-1	Vascular cell adhesion molecule 1
WiDr	Colon adenocarcinoma cell line
YFP	Yellow fluorescent protein

1.1 Introduction

Nowadays, genome-wide sequence analyses can be accomplished rapidly and within an affordable cost range by next-generation sequencing approaches, based on the highly parallel sequencing of DNA fragments [1]. However, nucleotide sequences provide only very limited insights into the functions of the respective genes. Gene functions are commonly studied within gain-of-function experiments by overexpression of genes or by loss-of-function experiments via silencing of genes with RNA interference (RNAi) technology [2].

Whole-genome gene function analyses require several thousands of transfections as well as time-consuming procedures. Applying standard technology, this is associated with high costs for plastic ware and reagents. The costs increased exponentially with not only single gene effects, but combinatorial effects with two or more genes, in different cell types and under different types of external stimulation, in the focus of the study. This demanded the development of new techniques allowing high-throughput gene function analyses within an affordable time and cost range.

1.1.1 Reverse Transfected Cell Microarray

Ziauddin and Sabatini introduced the method of gene function analysis at the microarray level [3]. They printed the DNA of respective gene expression plasmids together with gelatin on glass slides. Subsequently, the slides were dried, exposed to a transfection reagent, and overlaid with adherent mammalian cells. After 40 h of incubation, the cells were fixed and expression and/or function of the respective gene

product was analyzed using immunofluorescence. This procedure was called reverse transfected cell microarray (RTCM), because, in contrast to conventional transfection approaches, the DNA was seeded first and afterwards the cells were added.

As an alternative to the method described above, the transfection reagent was mixed with DNA and gelatin, before the mixture was printed on the slide [3, 4]. The latter approach is nowadays most commonly used. The dried slides can be stored for up to 15 months [5]. In the first decade of introduction, the RTCM technology has been used successfully by researchers worldwide and has been adapted to several different screening goals as reviewed previously (for review, see: [2, 4]) and shortly summarized below.

The optimization of transfection efficiency for various cell types was achieved by the use of different slide coatings, for example fibronectin for the transfection of primary human mesenchymal stem cells or collagen IV for the transfection of pheochromocytoma cells [6, 7].

Overexpression of genes on the cell microarray level lead to the identification of pro-apoptotic genes by analyzing the yellow fluorescent protein (YFP)-contrasted phenotypic changes associated with the late stage of apoptosis, such as apoptotic body formation, DNA cleavage, and fragmentation of the nucleus [8]. Apoptosis-regulating genes were also analyzed with the RTCM technology using a terminal deoxynucleotide transferase dUTP nick end label (TUNEL) assay [9].

Gene effects on different signaling pathways were analyzed by transfection of reporter constructs containing an indicator gene such as green fluorescent protein (GFP) or red fluorescent protein (RFP) under the control of a promoter activated by the signaling pathway of interest. In this way, functional screens for genes activating NF-κB [10], MAPK, JNK [11], or the CRE pathway [12] were performed.

Moreover, gene-silencing approaches were accomplished using the RTCM. Silva and colleagues successfully applied short hairpin RNAs (shRNAs) on a chip to identify genes involved in cytokinesis and proteasomal degradation [13]. In order to introduce shRNA or complementary DNA (cDNA) into hard-to-transfect primary cells and nondividing cells, Bailey and colleagues developed a lentivirus-infected cell microarray (LICM), where pseudotyped lentiviruses encoding either shRNA or cDNA were printed on a slide [14].

RTCM was also combined with time-lapse microscopy and computational phenotyping as it was demonstrated by a genome-wide RNAi approach studying genes involved in the regulation of the cell cycle [15, 16].

Furthermore, RTCM was adapted to the use with non-adherent cells. Kato and colleagues immobilized human erythroleukemia cells (K562) to a glass slide with a biocompatible anchor for membranes [17]. The immobilized cells were reverse transfected with either GFP- or RFP-encoding plasmids or siRNA and showed no interspot cross-contamination.

Recently, different research groups introduced further technological improvements of the RTCM technology. Most relevant developments will be summarized here and include (1) the increase of transfection efficiencies in many different cell types, (2) the optimization of reporter systems and automated data acquisition, (3) the increase of spotting density with reduced cross-contamination, and (4) the development of assays to screen for paracrine gene effects (Table 1.1).

Table 1.1 Recent advances in RTCM

Goal	Approach	Cell type	Reporter system	Coating	Gene transfer	Ref.
Transfection efficiency						
Optimization of gene/substrate uptake	Generation of a silica film composed of an upright nanosheet network, adsorbing DNA/transfection-mix	HEK293	GFP expression	Silica-film composed of upright nanosheet	LyoVec	[18]
Transgene expression in a broad range of adherent cell types	Rapid and parallel approach for the purification of lentiviruses, which are spotted onto the slide and are used for cell transduction	27 of 32 different cell types transduced	GFP expression and immunofluorescence staining	Hydrogel-coated glass slides	Transduction with lentiviruses	[19]
Reporter systems						
Identification of human host cell factors required for *Trypanosoma cruzi* infection	Human genome siRNA library targeting 21,127 human genes impairing entrance and/or proliferation of *T. cruzi*	U2OS	Automated quantification of cell nuclei and number of parasites per cell (cell and parasite nuclei labeled with DRAQ5 and differentiated by size)	Glass coverslips	Lipofectamine 2000	[20]
Identification of host genes involved in the early steps of HIV-1 infection without affecting cellular growth or viability	Human genome siRNA library targeting 21,127 human genes screened for HIV-1 infection with 15 imaging parameters	Hela-CD4+ LTR-GFP	Automated acquisition of 15 different imaging parameters (HIV-1 infection monitored by GFP expression via LTR-activation and syncytia formation)	Glass coverslips	Effectene	[21]
Identification of proteins regulating the early secretory pathway	Screen of 51,000 siRNAs for interference with ER-to-plasma membrane transport of fluorescently labeled cargo protein of the secretory pathway (tsO45G)	HeLa	Automated detection of immunostained intracellular and cell surface tsO45G; in addition COPI, COPII, and GM130 were detected as indicators for the early secretory pathway	Chambered cover glass slides	Lipofectamine 2000	[23]
Identification of novel regulators of the TORC1-signaling pathway	Screen of 13,618 dsRNAs to identify regulators of the TORC-1-signaling pathway in *Drosophila* cells	S2R+ cell expressing human S6 ribosomal protein (S6_S2R+)	Immunofluorescent staining of human pS6 (target of mTOR-activated S6 kinase, involved in cell size, cell growth, cell survival, and glucose homeostasis)	Poly-lysine glass slides	Not given	[25]

(continued)

Table 1.1 (continued)

Goal	Approach	Cell type	Reporter system	Coating	Gene transfer	Ref.
Time-lapse approaches						
Construction of a reporter to monitor live dynamics of transcriptional activity	Newly generated fluorophore reporter (Venus-NLS-PEST) with high signal intensity, fast maturation and degradation. and signal restriction to the nucleus	HEK293T	Fluorophore reporter downstream of inducible promoter	Chambered cover glass slides	Lipofectamine 2000	[26]
Increase of spotting density and reduced cross-contamination						
Increase of spotting density, optimization of signal-to background ratio, and minimization of interspot contamination in different screening approaches	Use of hydrophobic microplates, spotting of ECM proteins in tranfection mixtures, nonenzymatic cell dissociation prior to seeding, restricted cell adhesion time, and careful washings in order to cause exclusive attachment of cells onto transfection spots. 92 different cell types were analyzed. Screenings with different siRNA and miRNA libraries for regulators of proliferation [27, 29–31], apoptosis [27, 31], and β1-integrin activation [32]	92 different cell lines; 85 were successfully seeded and cultured on the transfection spots	Immunofluorescent staining of proliferation markers EdU and Ki-67, [27, 29–31], apoptosis marker-cleaved PARP [27, 31], and activated β1-Integrin [32]	Untreated polystyrene microplates [27, 29–32] or chambered cover glasses [28]	siLentFect [27, 29–32] or Lipofectamine 2000 [28]	[27–32]
Paracrine cell interactions						
Evaluation of paracrine cell interactions	Establishment of selective transfection of only one of the two different cell types simultaneously spotted and restriction of the diffusion of paracrine effectors	HEK293T as effector cell; human fibroblasts, colorectal carcinoma cell lines (WiDR, HT29), and endothelial cells (HUVEC) as indicator cells	Immunofluorescent staining of paracrine induced expression of GBP-1, COX-2, VCAM-1, ICAM-1	Glass slides	Lipofectamine 2000	[33]

1.2 Recent Technological Improvements of the RTCM Technology

1.2.1 Optimization of Reverse Transfection Efficiencies

Glass slides and microplates were frequently coated with organic materials such as gelatin, collagen, or poly-L-lysine in order to improve transfection efficiency [2]. Recently, also inorganic substrates such as silicon and titanium films were used for reverse transfections. Newly developed wafers with surfaces composed of vertically oriented parallel silica sheets in the nanometer range, which increase the surface, were shown to result in high reverse transfection efficiencies in HEK293 cells as monitored by GFP expression [18]. Cell adherence and morphology were not impaired on the silica film. However, the authors did not show how different cell types may behave and whether parallel transfection of different genes on one film may be feasible. Microarray spotting of different plasmids on these surfaces has to be proven.

For hard-to-transfect cells, transduction with lentiviruses can be used. However, the use of LICM for cells which are difficult to transfect harbors the disadvantage that large libraries of purified lentiviruses are essential, which requires ultracentrifugation and cannot be carried out in a highly parallel mode [14]. Due to this limitation, Wood and colleagues established a simple procedure for lentivirus purification, which can be performed in parallel in a multiwell range. Lentiviruses are precipitated and purified by the addition of a polymer complex (polyelectrolyte complexation) and printed onto coated slides. The respective procedure was called microarrays of spatially confined adhesive lentiviral features (MicroSCALE) [19]. This method allowed stable and selectable transgene expression for up to 14 days. The authors showed that 27 different cell lines of 32 analyzed in total were suitable for MicroSCALE. Focusing on 618 lentivirally overexpressed kinases, the authors screened for modifiers of the sensitivity of melanoma cells to clinically relevant therapies [19].

1.2.2 Optimization of Reporter Systems and Automated Data Acquisition

In the last decade, loss-of-function analyses have emerged as a potent tool also for RTCM, which became specifically accessible with the availability of commercially available siRNA libraries against the whole genome.

Using whole-genome siRNA libraries, Genovesio and colleagues were able to identify human host genes involved in infections with *Trypanosoma cruzi* and human immunodeficiency virus-1 (HIV-1) [20, 21]. In both studies, a human genome siRNA library targeting more than 21,000 human genes was spotted, followed by seeding of U2OS cells (infection by *T. cruzi*) or HeLa-CD4+ LTR-GFP cells (infection by HIV-1). Infection with *T. cruzi* was quantitatively determined by automated fluorescence microscopy counting the nuclei of infected cells and the numbers of parasites on individual transfection spots. Transfection spots were labeled by

co-transfection of a fluorescent oligonucleotide. Using this approach, 162 candidate genes which affected *T. cruzi* infection were identified. Of note, 15 genes were validated in secondary screening approaches using classic methodology [20].

The screen for genes involved in HIV-1 replication was carried out as described above. HeLa-CD4+ LTR-GFP cells were used which indicate HIV-1 infection via GFP expression driven by the LTR. In this work the authors increased the stringency of the screening by including a higher number of replicas (seven) and inclusion of a high number of different parameters in the automated data acquisition [21]. These parameters included, among others, cell number, cell distribution, syncytia formation, GFP reporter intensity and local signal background. Using this setup, they identified 56 cellular genes, of which 45 were novel genes involved in HIV-1 infection [21].

In another genome-wide siRNA screen, Simpson and colleagues expanded earlier approaches [22] in order to identify genes involved in endoplasmatic reticulum (ER)-to-plasma membrane transport. As an indicator, they used a cargo protein of the secretory pathway, namely the temperature-sensitive viral membrane protein (tsO45G), coupled to cyan fluorescent protein (CFP) [23]. Using automated microscopy, they quantified the level of the fluorescently labeled protein inside the cell and at the plasma membrane at the single cell level. They identified 554 siRNAs which repressed the amount of membrane associated tsO45G and consequently may be involved in the secretion pathway. In a second RNAi-based screening, they monitored phenotypic effects on the coating proteins I and II (COPI, COPII) and the Golgi matrix protein GM130 as indicators of the early secretory pathway. By this approach, 331 of 554 identified genes were classified to the early secretory pathway [23].

Moreover, large-scale siRNA screens were performed in *Drosophila melanogaster* cells [24]. Lindquist and colleagues attempted to identify regulators of the TORC1-S6K-signaling pathway in a genome-wide screening using 13,618 dsRNAs [25]. A *Drosopophila* cell line stably expressing human S6 ribosomal protein, which is phosphorylated by the kinase S6K was generated. The phosphorylation of hS6 was monitored with immunofluorescence using a specific antibody, whereas no antibodies were available for the respective *Drosophila* protein. The screening revealed 70 novel regulators of p-S6 or S6K. Among those were genes involved in the MAPK pathway, lipid metabolism, cell cycle, and the proteasome [25].

1.2.3 Time-Lapse Approaches

Most RTCM cell microarray screenings were combined with endpoint measurements. Only recently, Neumann and colleagues combined RTCM technology with time-lapse microscopy [15, 16]. On the basis of this technique, Rajan and colleagues aimed to improve the reporter molecule for time-lapse approaches [26]. They were specifically interested in measuring live dynamic changes of the transcriptional activity of different promoters. Optimal reporter molecules should (1) exhibit a high

signal intensity, (2) appear rapidly upon activation of the promoter, (3) disappear after promoter activity is ceased, and (4) be restricted to a regularly shaped cell compartment which can be easily detected by automatic microscopy. To this goal, they constructed a cDNA encoding a recombinant Venus-NLS-PEST reporter protein. The Venus polypeptide matures 15 times faster and is 30 times brighter than enhanced yellow fluorescent protein (EYFP), the PEST domain regulates rapid degradation of the protein in case transcription has stopped, and the nuclear localization signal (NLS) restricts the signal to the cell nucleus. The function of this reporter construct could be successfully demonstrated in pilot time-lapse experiments using three test promoters and co-transfecting different activators with the RTCM method and HEK293T cells [26].

1.2.4 Increase in Spotting Density and Reduced Cross-Contamination

Protocols which restrict cell adhesion to the transfection spots have been established in order to increase the spotting density and to reduce cross-contamination between neighboring transfection areas. This was achieved by spotting extracellular matrix (ECM) proteins on untreated polystyrene-coated microplates where the cells were seeded [27]. Polystyrene microplates are more hydrophobic compared to glass slides, thus prohibiting cell attachment. Moreover, nonenzymatic cell dissociation methods prior to cell seeding, reduced cell adhesion times, and careful washing steps were found to be necessary for the cells to grow exclusively on the transfection spots. It was shown that spot-to-spot cross-contamination was basically absent using this approach [28].

Rantala and colleagues tested 92 cell lines by using this protocol and demonstrated that 85 cell lines were successfully seeded and cultured on the arrays. Five of these cell lines [prostate epithelial cells (RWPE-1), prostate cancer cells (LAPC-4, PC3, VCaP), osteosarcoma cells (U2OS)] were efficiently transfected under these conditions [27]. Using this methodological setup, the expression of 492 different G protein-coupled receptor (GPCR) molecules was inhibited with siRNA. Subsequently, the effects on cell growth and survival were analyzed by immunofluorescent staining of Ki-67 (proliferation marker) and cleaved poly ADP ribose polymerase (cPARP, apoptosis marker) [27]. Neuropeptide Y (NPY) and GPR160 siRNAs were found to be the strongest inhibitors of prostate cancer cell growth indicating that both genes are potent activators of cell growth [27].

This new protocol was used successfully in four other screens. (1) Fredlund and colleagues validated previously classified cell cycle genes in breast cancer as involved in either mitotic checkpoint or mitotic progression. To this end, they used an siRNA library, targeting 5,760 selected genes in breast cancer cells (KPL-4) and stained for the proliferation marker Ki-67 [29]. (2) Cepeda and colleagues identified

novel F-box proteins involved in tumor cell proliferation in four different types of tumor cells by spotting an F-box siRNA library and measuring 5-ethynyl-2′-deoxyuridine (EdU)-incorporation [30]. In a second siRNA screen, targeting 6,135 genes including 53F-box genes, the authors validated the first screening results measuring Ki-67 fluorescence intensity. In both screens, FBXO28, an ubiquitin ligase, was identified as a regulator of tumor cell proliferation [30]. (3) In a further screen, an miRNA precursor library containing 319 chemically modified double-stranded RNA molecules was used to mimic endogenous mature human miRNAs [31]. In the colorectal carcinoma cell lines HT29, HCT116, and SW480, the effects on cell proliferation (EdU-incorporation) and apoptosis (immunofluorescent staining of cleaved PARP) were analyzed [31]. Firty-three miRNAs were identified as oncogene-like miRNAs and 93 miRNAs as tumor-suppression-like miRNAs. (4) Integrin-inactivating kinases and/or phosphatases were investigated in PC3 prostate cancer cells using a commercially available kinase-phosphatase siRNA library ($n=897$) [32]. A total of 2.5 % of all siRNAs increased the active integrin expression as determined by immunofluorescent staining for active β-integrins. Of these, SHANK-associated RH domain interactor (SHARPIN) was identified as a novel integrin inhibitor, which keeps integrins in an inactive state and prevents recruitment of integrin activators [32].

1.2.5 Paracrine Cell Interactions

The RTCM technique is commonly used to study intra- or autocrine effects. However, in the past few years, paracrine mechanisms became increasingly relevant, as indicated by the numerous publications addressing the role of the microenvironment in human diseases.

In order to investigate paracrine interactions between different cell types, either transwell or conditioned medium approaches are required as yet, which are both costly and time-consuming. To allow a high-throughput screening for putative paracrine gene activities, the RTCM procedure was modified for this purpose (Fig. 1.1) [33]. Genes of interest were spotted as in the original procedure, however, subsequently, two different cell types with low and high permissiveness for transfection were seeded onto the slide: (1) an effector cell which is transfected with the genes of interest and (2) an indicator cell, which is not transfected under the conditions used, in order to detect specific paracrine effects exerted from the transfected effector cells (Fig. 1.2a, b). Spot-to-spot diffusion of the paracrine mediators was prevented by a matrix overlay [2 % low melting point (LMP) agarose, Fig. 1.2c], ultimately allowing 192 parallel tests for paracrine gene activations on a single chip. As a read-out, typically an immunofluorescent staining of the indicator protein was performed. Alternatively, activation/inhibition of signaling pathways in the indicator cell can be monitored by using cells expressing a reporter protein under the control of a

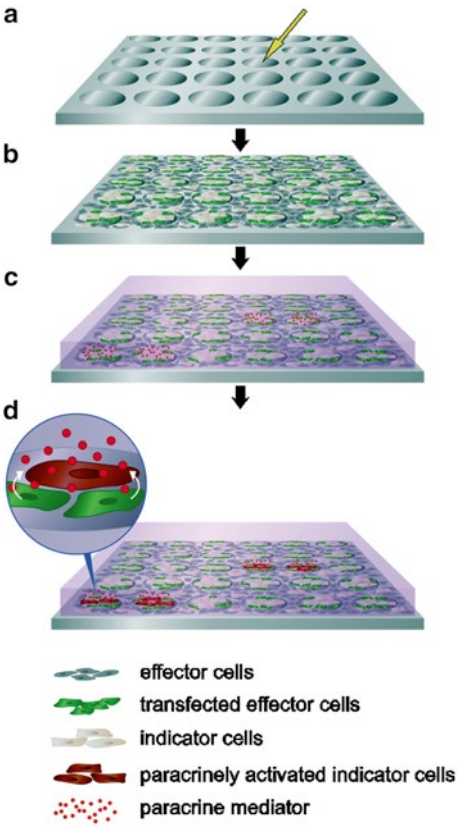

Fig. 1.1 Scheme of the *Parachip*. (**a**) Slide with transfection spots (indicated by *yellow arrow*). (**b**) Overlay with effector cells (*grey*) and indicator cells (*light brown*). Indicator cells are selectively transfected (selective reverse transfection, *green*). (**c**) Overlay of a matrix (*pink*), which locally restricts diffusion of paracrine mediators (*red*) released from transfected effector cells. (**d**) Activation of indicator cells (*dark brown*) by the paracrine mediator. The transfected gene may code directly for the secreted paracrine mediator or may modify/activate a cellular factor in the effector cell, which is then secreted and functions as a paracrine mediator (*white arrows*). [Detailed protocol published in Kuhn et al. (2012)]

promoter, which is activated/inhibited by the respective signal transduction pathway. The broad applicability and robustness of this technique was demonstrated using (1) HEK293T cells as indicator cells in combination with various responder cell types (fibroblasts, colorectal cancer cell lines and endothelial cells), (2) various paracrine inducers [tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), and interferon-γ (IFN-γ)], and (3) various indicator genes [guanylate-binding protein 1 (GBP-1), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and cyclooxygenase 2 (COX-2)] [33]. This approach allowed for the first time a highly parallel analysis of paracrine gene functions and thus may facilitate the characterization of genes involved in heterotypic cell communication in a broad range of research areas. Of note, this technology allows identification of genes involved in paracrine interactions in multiple ways, including that the gene introduced into the effector cells (1) may code directly for a secreted product which activates the indicator cells or (2) may induce or modify in the effector cell another factor which subsequently is secreted and activates the indicator cells [33].

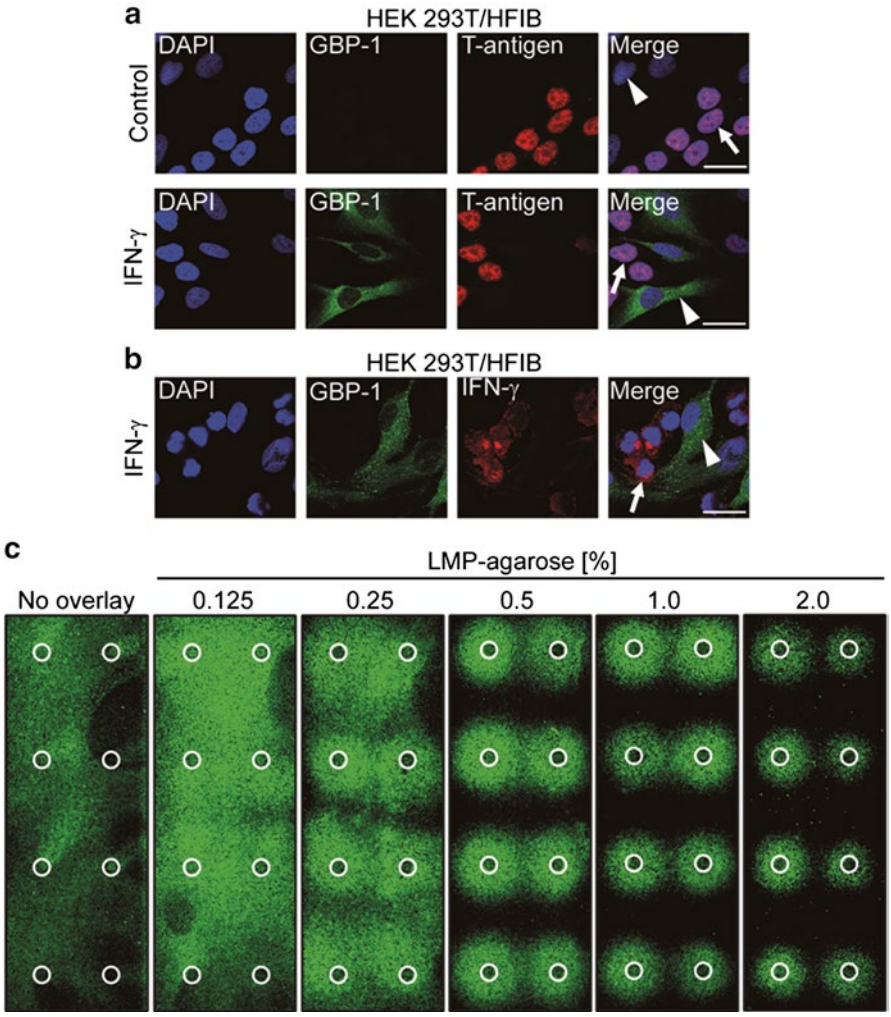

Fig. 1.2 Paracrine induction of the large GTPase GBP-1 by IFN-γ on a chip. An IFN-γ expression plasmid (IFN-γ) or empty vector (control) was spotted onto the chip. Subsequently, a mixture of HEK293T cells (effector cells, *arrow*) and human fibroblasts (indicator cells, *arrowhead*) were seeded. Selectively, HEK293T cells (**a**, T-antigen positive, *red*) expressed IFN-γ (**b**, *red*), whereas expression of the IFN-γ-induced GBP-1 was observed only in human fibroblasts (**a**, **b**, *green*). Panels show DAPI staining (*blue*) and immunofluorescence staining of GBP-1 (*green*), T-antigen (**a**, *red*), or IFN-γ (**b**, *red*). Scale bars = 25 μm. (**c**) Identical approach as in (**a**) and (**b**), but the transfected cells were overlaid with low melting point (LMP) agarose in increasing concentrations to restrict the diffusion of IFN-γ secreted by HEK293T cells. Paracrine induction of GBP-1 (*green*) was restricted to the transfection spot (*indicated by circles*) when an LMP agarose overlay of 1.5 or 2 % was used. [Kuhn et al. (2012) A novel chip-based parallel transfection assay to evaluate paracrine cell interactions. Lab Chip 12: 1363–1372]. Reproduced by permission of The Royal Society of Chemistry (RSC). [Kuhn et al. (2012)]

1.3 Summary and Outlook

RTCM has been well established in the past years and has been successfully used for several gene function screens on a genome-wide level, both within gain-of-function and loss-of-function applications. Meanwhile, a high variety of different cell types can be subjected to this method and signal detection has been significantly optimized to allow automated data acquisition and processing. Innovative new screening methods for gene activities have been reported. Most approaches are presently focusing on autocrine gene activities, but in a first report, the application of RTCM in the analysis of paracrine gene functions within heterotypic cell–cell interaction, suggests that there may be perspectives for the development of novel screening methods analyzing multicellular interactions. RTCM is a miniaturization approach of cell biological methodology. This allows a highly parallel screening approach under identical conditions and at a clearly reduced cost basis as compared to classical approaches of cell biology. In the future, this may result in a similar "supernova" of new data in the field of gene function analyses as presently observed in the areas of gene sequencing and transcriptome analyses.

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