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AWARD NUMBER:
W81XWH-09-1-0710

TITLE:
The Role of IQGAP1 in Breast Carcinoma

PRINCIPAL INVESTIGATOR:
Dr. Colin D. White, PhD

CONTRACTING ORGANIZATION:
Brigham and Women's Hospital Inc

Boston
Massachusetts
02115

REPORT DATE:
October 2010

TYPE OF REPORT:
Annual Summary Report

PREPARED FOR:
U.S. Army Medical Research and Materiel Command
Fort Detrick
Maryland
21702

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-10-2010	Annual Summary Report	15 Sep 2009 - 14 Sep 2010

4. TITLE AND SUBTITLE
The Role of IQGAP1 in Breast Carcinoma

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0710

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Colin D. White, PhD

EMAIL: cwhite21@rics.bwh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Brigham and Women's Hospital Inc

Boston
Massachusetts
02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Material Command
Fort Detrick
Maryland
21702

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

HER2 is overexpressed in ~25% of breast carcinomas. Overexpression of HER2 is an adverse prognostic feature and correlates with shorter disease-free and overall survival. HER2(+) breast cancer is treated with trastuzumab but many patients do not respond. Of those who do, most become refractory to therapy and progress to metastatic disease. An insight into the molecular mechanisms underlying HER2 signaling and trastuzumab resistance is essential to reduce breast cancer morbidity and mortality.

IQGAP1 is a ubiquitously expressed scaffold protein that contains multiple protein interaction domains. Through interaction with its binding partners, IQGAP1 integrates diverse signaling pathways, several of which are relevant to breast tumorigenesis. The purpose of this proposal is to elucidate the function of selected IQGAP1 binding interactions in breast neoplasia.

During Year 1 of this fellowship, we have shown that IQGAP1 interacts with HER2 *in vitro* and in a normal cellular milieu. Furthermore, IQGAP1 and HER2 co-immunoprecipitate from SkBR3 cells. The remainder of this award will be spent evaluating the functional consequences of this interaction on HER2 signaling and trastuzumab resistance. Elucidation of the molecular mechanism underpinning this interaction could potentially lead to the development of novel and specific therapeutic agents for the treatment of patients with HER2(+) breast cancer.

15. SUBJECT TERMS
Breast Cancer, Drug Resistance, HER2, IQGAP1, Trastuzumab

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	43	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

IQGAP1 is a ubiquitously expressed scaffold protein that contains multiple protein interaction domains. By binding to numerous targets **IQGAP1 integrates diverse signaling pathways, several of which are relevant to breast tumorigenesis. The overall objective of this proposal is to delineate the role of selected IQGAP1 binding interactions in breast neoplasia. Elucidation of the molecular mechanisms underpinning these interactions could potentially lead to the development of novel and specific therapeutic agents for the treatment of patients with breast cancer.**

2. Training Accomplishments to Date

Under the supervision of my mentor, Dr. David Sacks, I have been trained in several techniques relevant to the study of the pathogenesis of breast cancer. These include analysis of intracellular signaling by Western blotting, determination of cell proliferation by sulforhodamine B staining, fluorescence-activated cell sorting (FACS) analysis, stable cell line generation, production of and transduction using retroviral and lentiviral supernatants, immunocytochemistry and confocal laser microscopy, immunohistochemistry, and analysis of chemotherapeutic responsiveness. Dr. Sacks continues to support my professional development and working in his laboratory has reaffirmed my desire to become an independent breast cancer researcher. **During the previous funding period, these accomplishments have been recognized in the form of an invited review on IQGAP proteins in cancer (1), an invited book chapter on mitogen-activated protein kinase (MAPK) signaling (2) and 2 invited lectures on the work funded by this fellowship (1 at Experimental Biology 2010 (a prestigious national conference) and 1 at an external research group meeting at Massachusetts General Hospital).**

3. Research Accomplishments to Date

3.1. Difficulties Encountered with the Original Proposal

During the previous funding period, several difficulties with cell culture impeded the studies that were designed in the original proposal. Recurrent mycoplasma and fungal contamination (as a result of suboptimal standards of cleanliness in the accessible tissue culture suites) prevented any of the stable cell lines that were described in the original submission from being made. During the past 12 months several strategies were employed in an attempt to overcome this issue. For example, tissue culture facilities in other laboratories were used. Despite a huge effort on the part of both Dr. Sacks and myself, generation of viable uninfected clones proved impossible. **It should be highlighted that this issue was not as a result of any lack of experience with cell culture on the part of the PI** (see attached letter from Dr. Sacks). Instead, it was attributable to contamination of the parental clones described in the original proposal (which are themselves stable cell lines and cannot be obtained from an external source). **As a result of the difficulties described above, the statement of work for this fellowship has been altered** (see Revised Statement of Work). [Note, however, that the overall scope of the award remains the same – this fellowship is still focused on elucidating the function of selected IQGAP1 binding interactions in breast neoplasia.] **Dr. Katherine Moore, grants manager at the congressionally-directed medical research program, has approved this change.** Appropriate background and preliminary data, **which were successfully generated during Year 1 of this award,** together with a revised Statement of Work is provided below.

3.2. Background

3.2.1. ErbB Receptors

The ErbB family of receptor tyrosine kinases comprises 4 members, namely epidermal growth factor receptor (EGFR)/ErbB1, HER2/Neu/ErbB2, ErbB3 and ErbB4 (3). Each member has an extracellular ligand binding domain, a single membrane spanning region and a tyrosine kinase-containing C-terminal tail. Ligand binding induces a conformational change in the receptor, resulting in dimerization and the phosphorylation of tyrosine residues within the kinase domain. These phosphorylated tyrosines serve as docking sites for a variety of molecules, which in turn signal through several downstream cascades. The ErbB family members are potent mediators of normal cell growth and development. **Their aberrant expression or functioning also plays a crucial role in the development and evolution of cancer (3).**

3.2.2. HER2 and HER2-Targeted Therapies

Unlike other ErbB receptors, HER2 does not bind any of the EGF-like ligands directly. Instead, it exists in a constitutively activated state (4). Active HER2 signals primarily through the phosphoinositide-3-kinase

(PI3K)/Akt and the MAPK pathways (5), **both of which have defined roles in breast cancer progression.** Activation of both pathways has frequently been reported in clinical studies of HER2(+) tumors (6, 7), and the antiproliferative effects of HER2-targeted therapies have often been attributed to inhibition of 1 or both of these cascades (5).

The data supporting the transforming potential of HER2 are irrefutable. For example, **HER2 overexpression is sufficient to stimulate tumorigenesis in human breast epithelial cells *in vitro* (8, 9). Furthermore, HER2 is known to be overexpressed in ~25% of primary breast cancers (10), and this overexpression is associated with an aggressive tumor phenotype and reduced survival rate (11).** Unlike oncogenes such as Ras and B-Raf, HER2 is rarely mutated in breast cancer (12, 13). Thus, rational therapeutic targeting of HER2 has 2 general aims: **1) to reduce HER2 expression and/or 2) to inhibit HER2 signaling (11).** Trastuzumab (herceptin), a recombinant humanized monoclonal antibody directed against the HER2 extracellular domain (5), is used to treat HER2(+) breast cancer. The mechanisms by which trastuzumab induces regression of HER2 overexpressing tumors are incompletely defined, but evidence from pre-clinical models has begun to shed light on the processes and signaling pathways that may be involved (11). For example, trastuzumab may mediate endocytosis and degradation of HER2 and consequently inhibit signaling through the PI3K/Akt and MAPK cascades (5). This induces expression of $p27^{kip1}$, a cyclin-dependent kinase inhibitor, which promotes cell cycle arrest and apoptosis (14, 15). Other mechanisms proposed for trastuzumab function include suppression of tumor angiogenesis, antibody-dependent cellular cytoxicity and/or inhibition of DNA synthesis (11).

Although trastuzumab has revolutionized the treatment of HER2(+) breast cancer, many patients are refractory to therapy (5). Of those who do respond, ~60% relapse within 1 year and become resistant (16-18). The reason for trastuzumab resistance is not understood, but proposals include altered interaction of the antibody with HER2, increases in other growth-stimulatory signaling pathways or a reduction in $p27^{kip1}$ levels (11). **An insight into the molecular mechanisms underlying HER2 signaling and trastuzumab resistance is essential to develop effective therapies for patients with HER2(+) breast neoplasms.**

3.2.3. IQGAP1

The ubiquitously expressed 189 kDa scaffold protein IQGAP1 was cloned in 1994 from metastatic human osteosarcoma tissue (19). Its name is derived from the presence of an IQ domain [with 4 tandem IQ motifs (IQ motifs bind calmodulin)] and a region with significant sequence similarity to the catalytic domain of RasGAPs (GTPase activating proteins). [Note that despite its name, IQGAP1 is not a GAP and actually inhibits the intrinsic GTPase activity of Cdc42 *in vitro*, stabilizing Cdc42 – and Rac1 – in its active GTP-bound form (20).] IQGAP1 participates in protein-protein interactions and integrates diverse signaling pathways (21). Proteins that bind IQGAP1 include the Rho GTPases Cdc42 and Rac1, actin, calmodulin, E-cadherin, β-catenin, adenomatous polyposis coli and components of the MAPK pathway, **all of which are involved in cancer (1, 21). IQGAP1 regulates the function of its binding partners.** For example, it inhibits cell-cell adhesion mediated by the E-cadherin-β-catenin complex and increases β-catenin-mediated transcriptional activation (22-24).

Accumulating evidence suggests IQGAP1 is an oncogene which is important for tumorigenesis (1). The IQGAP1 gene and protein are overexpressed in several human neoplasms (1). In breast cancer, we have shown that IQGAP1 overexpression enhances *in vitro* motility and invasion of MCF-7 and MDA-MB-231 breast epithelial cells (25). In addition, we documented that siRNA-mediated knockdown of IQGAP1 reduces anchorage-independent growth of MCF-7 cells as well as their growth and invasion in immunocompromised mice (26). Collectively, these data strongly suggest that IQGAP1 is an oncogene in breast tumorigenesis.

Data obtained during Year 1 of this fellowship indicate that IQGAP1 and HER2 interact (see Data Obtained to Date). The potential role of IQGAP1 in HER2 function and HER2(+) breast cancer has, however, not been investigated.

3.3. Data Obtained to Date

During Year 1 of this fellowship we have shown that **IQGAP1 interacts with HER2 *in vitro* and in a normal cellular milieu** (Fig. 1). Moreover, **IQGAP1 and HER2 co-immunoprecipitate** from SkBR3 cell lysates (Fig. 2).

[SkBR3 cells have high levels of endogenous HER2 due to amplification of the Her2 gene. This cell line is one of the most commonly used models to study HER2(+) breast cancer.] These data, which are discussed in more detail in the coming paragraphs, strongly suggest that **IQGAP1 and HER2 directly interact.** Given that **IQGAP1 regulates the function of its binding partners** (see Background), the remainder of this award will be spent elucidating the **functional consequences of this interaction on HER2 signaling and HER2-stimulated tumorigenesis.** HER2(+) breast cancer is treated with trastuzumab but many patients eventually become resistant to therapy (see Background). Therefore, **we shall also evaluate if manipulation of IQGAP1 expression alters trastuzumab resistance.** Pilot studies have confirmed that IQGAP1 expression can be successfully manipulated in SkBR3 cells using overexpression constructs and siRNAs (Fig. 3).

3.3.1. IQGAP1 binds HER2 *in vitro*

In vitro analysis with pure proteins was used to examine a possible interaction between IQGAP1 and HER2. GST-HER2 or GST alone was incubated with purified IQGAP1 and complexes were isolated with glutathione-Sepharose. Analysis by Western blotting revealed that **HER2 binds IQGAP1** (Fig. 1, top panel). The binding is specific as no HER2 is present in the samples incubated with GST alone (Fig. 1, top panel). IQGAP1 binds calmodulin with high affinity (27). Therefore, calmodulin-Sepharose was used as a positive control (Fig. 1, top panel). Analysis of inputs revealed that the amount of IQGAP1 in all pulldowns was equivalent (Fig. 1, top panel). To ascertain whether IQGAP1 interacts with HER2 in a normal cell milieu, SkBR3 cells were lysed and incubated with GST-HER2. **IQGAP1 in cell lysates binds to GST-HER2** (Fig. 1, bottom panel). The specificity of the binding is validated by the absence of IQGAP1 from samples that were incubated with GST alone (Fig. 1, bottom panel). IQGAP1 binds calmodulin with high affinity (27). Therefore, calmodulin-Sepharose was used as a positive control (Fig. 1, bottom panel). Analysis of lysates revealed that the amount of IQGAP1 in all pulldowns was equivalent (Fig. 1, bottom panel). Viewed collectively, **these data reveal a direct interaction between IQGAP1 and HER2.**

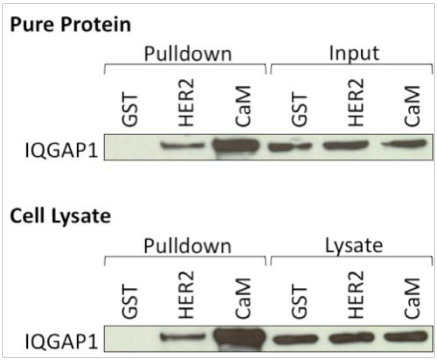

Fig. 1: IQGAP1 binds to HER2. GST-HER2 (HER2), GST alone or calmodulin-Sepharose (CaM) was incubated with equal amounts of purified IQGAP1 (top panel) or equal amounts of protein from SkBR3 cell lysates (bottom panel). Complexes were isolated and the samples were resolved by SDS-PAGE, transferred to PVDF membranes and probed with anti-IQGAP1 antibody. An aliquot of each sample (equivalent to 2% of the amount in each pulldown) was also processed by Western blotting (Input, top panel; lysate, bottom panel). The data are representative of 5 independent experiments.

3.3.2. IQGAP1 and HER2 co-immunoprecipitate

Immunoprecipitation of IQGAP1 revealed that **endogenous HER2 binds to endogenous IQGAP1** (Fig. 2, top panel). The specificity of the interaction is validated by the absence of HER2 from samples that were precipitated with non-immune rabbit serum (NIRS) (Fig. 2, top panel). Binding of endogenous HER2 to endogenous IQGAP1 was also demonstrated by immunoprecipitating HER2. IQGAP1 co-immunoprecipitates with HER2 from SkBR3 cell lysates (Fig. 2, bottom panel). The interaction is specific as no IQGAP1 is present in the samples that were precipitated with NIRS (Fig. 2, bottom panel). Note that the level of HER2 and IQGAP1 in all cell lysates was equivalent (Fig. 2). **These data indicate that IQGAP1 and HER2 associate in human breast epithelial cells.**

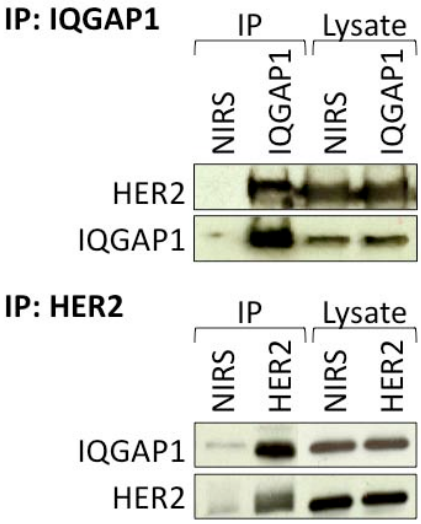

Fig. 2: IQGAP1 co-immunoprecipitates with HER2. Equal amounts of protein from SkBR3 cell lysates were immunoprecipitated (IP) with anti-IQGAP1 antibody (top panel) or anti-HER2 antibody (bottom panel). In both panels, non-immune rabbit serum (NIRS) was used as a negative control. Both unfractionated lysates (Lysate) and immune complexes (IP) were resolved by SDS-PAGE, transferred to PVDF membranes and probed with anti-IQGAP1 and anti-HER2 antibodies. The data are representative of 5 independent experiments.

3.3.3. IQGAP1 Expression Level can be Manipulated in SkBR3 Cells

We have successfully optimized protocols to manipulate IQGAP1 expression using siRNA and overexpression constructs. Before selecting siRNAs, a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search of the entire human RefSeq database was performed to exclude the possibility of off-target effects. 2 independent siRNAs (designated siIQ12 and siIQ14) directed against different regions of IQGAP1 were transfected into SkBR3 cells using Lipofectamine 2000. Transfection of siIQ12 or siIQ14 resulted in a ~40% reduction in IQGAP1 protein (Fig. 3). No significant decrease in IQGAP1 expression was observed when untransfected cells were compared to cells transfected with control siRNA against renilla luciferase (designated Sc; data not shown). The knockdown of IQGAP1 was specific as the mRNA levels of IQGAP2 and IQGAP3 (IQGAP1 homologs which have a high degree of sequence similarity) were unaffected by transfection of siIQ12 or siIQ14 (data not shown). For overexpression, myc-tagged IQGAP1 in pcDNA3 (designated IQ) was transfected into SkBR3 cells using Lipofectamine 2000. Transfection of myc-IQGAP1 resulted in a ~2-fold increase in IQGAP1 protein (Fig. 3). No significant difference in IQGAP1 expression was observed when untransfected cells were compared to cells transfected with pcDNA3 only (designated V; data not shown).

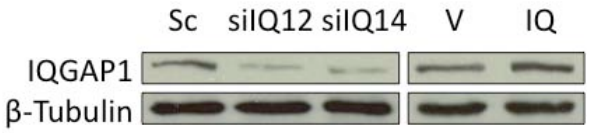

Fig. 3: IQGAP1 Expression Level can be manipulated in SkBR3 Cells. SkBR3 cells were transiently transfected with siRNA against renilla luciferase (Sc), siRNAs against IQGAP1 (siIQ12 and siIQ14), pcDNA3 (V) or myc-IQGAP1 (IQ). Equal amounts of protein from the cell lysates were resolved by SDS-PAGE, transferred to PVDF membranes and probed with anti-IQGAP1 (IQGAP1) and anti-β-Tubulin (β-Tubulin) antibodies. The data are representative of 5 independent experiments.

3.4. General Hypothesis

IQGAP1 regulates the function of its binding partners. Furthermore, our initial data indicate that IQGAP1 and HER2 directly interact. Therefore, the general hypothesis driving this proposal is that **IQGAP1 is an important component of HER2 signaling. Deregulation of the normal homeostatic interactions between IQGAP1 and HER2 may contribute to breast tumorigenesis and trastuzumab resistance. Elucidation of the molecular mechanism underpinning this interaction could potentially lead to the development of novel and specific therapeutic agents for the treatment of patients with HER2(+) breast cancer.**

3.5. Revised Statement of Work

The approved Revised Statement of Work for the duration of this project is shown below. All of the reagents and techniques necessary for the completion of the proposed experiments are available and well established in our laboratory. In addition, the SkBR3 cell line is uninfected and a large battery of frozen stocks has been accrued during the past 12 months.

Task 1: To determine the role of IQGAP1 in HER2 signaling and HER2-stimulated tumorigenesis, we propose:

- To determine whether IQGAP1 and HER2 interact *in vitro* and in a normal cellular milieu using GST-pulldown assays and co-immunoprecipitation from SkBR3 cell lysates (months 1-12). This subaim has been completed successfully.
- To overexpress and knockdown IQGAP1 in SkBR3 cells and evaluate the effect on HER2 activation and signaling using Western blotting (months 13-18).
- To overexpress and knockdown IQGAP1 in SkBR3 cells and evaluate the effect on cell proliferation using sulforhodamine B staining (months 19-24).
- To identify the region of IQGAP1 to which HER2 binds using GST-pulldown assays and selected IQGAP1 mutants and fragments (months 19-24).

Task 2: To evaluate the expression level of IQGAP1 in HER2(+) breast carcinoma, we propose:

- To immunohistochemically stain IQGAP1 in formalin-fixed paraffin-embedded HER2(+) breast tumors and normal breast tissue (see attached letter from Dr. Dillon) (months 13-36).

Task 3: To evaluate the role of IQGAP1 in trastuzumab resistance, we propose:

- To engineer trastuzumab resistant SkBR3 cells by culturing wild type cells in the presence of 4-8 μg/ml trastuzumab for 6-8 months as previously described (28) (months 20-30).
- To evaluate IQGAP1 expression in wild type and trastuzumab resistant SkBR3 cells (months 31-33).
- To overexpress and knockdown IQGAP1 in wild type and trastuzumab resistant SkBR3 cells and evaluate the effect of trastuzumab on HER2 activation and signaling using Western blotting (months 31-36).
- To overexpress and knockdown IQGAP1 in wild type and trastuzumab resistant SkBR3 cells and evaluate the effect of trastuzumab on cell proliferation using sulforhodamine B staining (months 31-36).

3.6. Summary of Experimental Procedures

The optimized protocol for transfection of siRNA against IQGAP1 (to knockdown the endogenous protein) and myc-IQGAP1 (to overexpress the endogenous protein) will be used (see Data Obtained to Date). In addition to these plasmids, a dominant negative IQGAP1 construct, termed IQGAP1ΔGRD (20), will be transfected to inhibit IQGAP1 function. Other growth factor receptors, such as EGFR, activate some of the same signaling pathways activated by HER2. Therefore, all experiments will be performed in serum-starved cells to prevent pathways other than those induced by HER2 from being activated (29). HER2 activation and signaling will be measured by Western blotting for active (phosphorylated) HER2, Akt and MAPK, and $p27^{kip1}$ (see Background). Western blotting for total (phosphorylated and unphosphorylated) HER2, Akt and MAPK will be used to quantify signaling responses by densitometry. All blots will also be probed with anti-IQGAP1 antibody to verify successful manipulation of IQGAP1 expression, and anti-β-Tubulin antibody to verify equal protein loading. HER2-stimulated cell proliferation will be quantified by sulforhodamine B staining as previously described (30). All of the reagents and techniques necessary for the completion of the proposed experiments are available and well established in our laboratory.

Immunohistochemical staining will be graded by an experienced Board-certified breast pathologist (see attached letter from Dr. Dillon). She will also provide the tissue. Dr. Dillon has developed tissue arrays with **>**200 HER2(+) tumors. Staining for both HER2 and IQGAP1 will be graded as routinely done in clinical samples: 0, no staining; 1+, faint incomplete membrane staining; 2+, weak to moderate complete membrane staining; 3+, strong complete membrane staining. For IQGAP1, 0 and 1+ will be considered negative, while 2+ and 3+ will be positive. The grading system for IQGAP1 will be modified if necessary based on an initial test set of specimens. Statistical analysis and advice will be provided by an experienced biostatistician (Dr. Hurwitz, Director of the Biostatistics Center at Brigham and Women's Hospital). Initial statistical analysis and power calculation will be performed using nQuery version 6, chi-square uncorrected. A two group test with a 0.05 two-sided significance level will have 90% power to detect the difference between a normal group proportion with positivity of 0.40, and a cancer group proportion of 0.67, when the sample size in each group is 70. Dr. Hurwitz will refine the statistics as we generate additional data enabling us to efficiently optimize time and resources in this aspect of the study.

3.7. Potential Problems and Alternative Approaches

It is important to emphasize that, despite the issues highlighted in the Difficulties Encountered with the Original Proposal section of this report, we expect no significant problems with any of the proposed experiments. As previously highlighted, **the difficulties encountered during Year 1 were attributable to contamination of the parental cell clones described in the original proposal. The cell lines described in the Revised Statement of Work are uninfected and several ampoules have been frozen to preserve the parental stocks.** In addition, all cultures are tested no less than once a month to determine whether they may be infected with mycoplasma. All of the reagents and techniques necessary for the completion of the experiments described in the Revised Statement of Work are available and well established in our laboratory. Thus, interpretable data are anticipated.

4. Key Research Accomplishments

- Demonstrated that IQGAP1 interacts with HER2 *in vitro* using GST-pulldowns and purified proteins.
- Demonstrated that IQGAP1 interacts with HER2 in a normal cellular milieu using GST-pulldowns from SkBR3 cell lysates.
- Demonstrated that IQGAP1 and HER2 co-immunoprecipitate from SkBR3 cell lysates.
- Optimized protocols to manipulate IQGAP1 expression in SkBR3 cells using overexpression constructs and siRNA.

5. Reportable Outcomes

- Trained in several techniques relevant to the study of the pathogenesis of breast cancer. These include analysis of intracellular signaling by Western blotting, determination of cell proliferation by sulforhodamine B staining, FACS analysis, stable cell line generation, production of and transduction using retroviral and lentiviral supernatants, immunocytochemistry and confocal laser microscopy, immunohistochemistry and analysis of chemotherapeutic responsiveness.
- Published an invited review on IQGAP proteins in cancer (1).
- Published an invited book chapter on mitogen-activated protein kinase (MAPK) signaling (2).
- Given 2 invited lectures on the work funded by this fellowship (1 at Experimental Biology 2010 (a prestigious national conference) and 1 at an external research group meeting at Massachusetts General Hospital).

6. Conclusions

The **important and novel** data obtained during Year 1 of this fellowship reveal that **IQGAP1 directly interacts with HER2. IQGAP1 regulates the function of its binding partners.** Therefore, the remainder of this award will be spent elucidating the **functional consequences of this interaction on HER2 signaling and HER2-stimulated tumorigenesis.** We hypothesize that **deregulation of the normal homeostatic interactions between IQGAP1 and HER2 may contribute to breast tumorigenesis and trastuzumab resistance. Elucidation of the molecular mechanism underpinning this interaction could potentially lead to the development of novel and specific therapeutic agents for the treatment of patients with HER2(+) breast cancer.**

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8. Appendices

An adjusted budget and justification for Year 2 and Year 3 of this fellowship and PDF copies of the articles and abstracts published during Year 1 are shown on the following pages. Letters of support from Dr. Sacks and Dr. Dillon (which is relevant to Task 2 in the Revised Statement of Work) are also included.

Budget Justification
Year 2 (9/15/10 – 9/14/11):

A. PERSONNEL (Role & Percentage of Effort for each and State the Escalation Rate Applied [no higher than 3%]):

Dr. Colin White, Principal Investigator. I am requesting full salary support. My responsibilities include overall management of the project and direct participation in all of the experiments. Effort = 100%. Salary has been escalated by 3% annually.

B. FRINGE RATE (State the Fringe Rate % for EACH Employee for EACH Year & Date of Fringe Rate Agreement):

The fringe benefit rate for postdoctoral fellows is in accordance with the agreement between Brigham and Women's Hospital and the Department of Health and Human Services dated December 2nd, 2009: 28% for FY10 (period ending 9/30/2010). Fringe benefits have been increased by 1% for years 2 and 3 (29%).

C. INDIRECT RATE (State the Indirect Rate % for Each Year & Date of Indirect Rate Agreement):

The indirect benefit rate is in accordance with the agreement between Brigham and Women's Hospital and the Department of Health and Human Services dated December 2nd, 2009: 8% for years 2 and 3.

D. CONSULTANT COSTS (Hours of Service and Rate per Hour):

N/A

E. MAJOR EQUIPMENT (Over $5,000 [Itemized]):

N/A

F. MATERIALS, SUPPLIES, AND CONSUMABLES (Itemized by Category & State the Institution's In-House Policy Regarding how Fair & Reasonable Pricing is Determined on Goods Purchased):

Materials and Supplies:

Tissue Culture:

Fetal calf serum (10 bottles @ $430 each):
Tissue culture plasticware and medium:
Trastuzumab:

Immunoblotting Supplies:

Gel electrophoresis and immunoblotting supplies:
Primary and labeled secondary antibodies and ECL reagents:
X-Ray film:

Immunohistochemistry Supplies

Primary antibodies:
Slide staining (70 slides @ 28.50 each):

Chemicals:

General chemicals and buffers:

General Supplies:

Glassware:
Plasticware:

All cost estimates are based on previous use. All purchases will be in accordance with Brigham and Women's Hospital procurement procedures, and will be subject to second level review by materials management to verify fair and reasonable pricing.

G. TRAVEL COSTS (Location, Dates and Purpose): _____
Travel to a breast cancer research-related meeting. Location and dates are currently unknown.

H. RESEARCH-RELATED SUBJECT COSTS (Itemized): ___
N/A

I. RESEATCH-RELATED INJURY MEDICAL COSTS (Itemized): ___
N/A

J. OTHER EXPENSES (Itemized by Category): ___
N/A

K. SUBCONTRACTOR COSTS (If Applicable): ___

PERSONNEL Role & Percentage of Effort for each and State the Escalation Rate Applied [no higher than 3%]): ___
N/A

FRINGE RATE (State the Fringe Rate % for EACH Employee for EACH Year & Date of Fringe Rate Agreement): ___
N/A

INDIRECT RATE (State the Indirect Rate % for Each Year & Date of Indirect Rate Agreement): ___
N/A

CONSULTANT COSTS (Hours of Service and Rate per Hour): ___
N/A

MAJOR EQUIPMENT (Over $5,000 [Itemized]): ___
N/A

MATERIALS, SUPPLIES, AND CONSUMABLES (Itemized by Category & State the Institution's In-House Policy Regarding how Fair & Reasonable Pricing is Determined on Goods Purchased): ___
N/A

TRAVEL COSTS (Location, Dates and Purpose): ___
N/A

RESEARCH-RELATED SUBJECT COSTS (Itemized): ___
N/A

RESEATCH-RELATED INJURY MEDICAL COSTS (Itemized): ___
N/A

OTHER EXPENSES (Itemized by Category): ___
N/A

Budget Justification
Year 3 (9/15/11 – 9/14/12):

A. PERSONNEL (Role & Percentage of Effort for each and State the Escalation Rate Applied [no higher than 3%]):

Dr. Colin White, Principal Investigator. I am requesting full salary support. My responsibilities include overall management of the project and direct participation in all of the experiments. Effort = 100%. Salary has been escalated by 3% annually.

B. FRINGE RATE (State the Fringe Rate % for EACH Employee for EACH Year & Date of Fringe Rate Agreement):

The fringe benefit rate for postdoctoral fellows is in accordance with the agreement between Brigham and Women's Hospital and the Department of Health and Human Services dated December 2nd, 2009: 28% for FY10 (period ending 9/30/2010). Fringe benefits have been increased by 1% for years 2 and 3 (29%).

C. INDIRECT RATE (State the Indirect Rate % for Each Year & Date of Indirect Rate Agreement):

The indirect benefit rate is in accordance with the agreement between Brigham and Women's Hospital and the Department of Health and Human Services dated November 19th, 2008: 8% for years 2 and 3.

D. CONSULTANT COSTS (Hours of Service and Rate per Hour):

N/A

E. MAJOR EQUIPMENT (Over $5,000 [Itemized]):

N/A

F. MATERIALS, SUPPLIES, AND CONSUMABLES (Itemized by Category & State the Institution's In-House Policy Regarding how Fair & Reasonable Pricing is Determined on Goods Purchased):

Materials and Supplies:

Tissue Culture:

Fetal calf serum (10 bottles @ $430 each):
Tissue culture plasticware and medium:
Trastuzumab:

Immunohistochemistry Supplies

Primary antibodies:
Slide staining (70 slides @ 28.50 each):

Chemicals:

General chemicals and buffers:

General Supplies:

Glassware:
Plasticware:

Publication Costs:

Publications costs:

All cost estimates are based on previous use. All purchases will be in accordance with Brigham and Women's Hospital procurement procedures, and will be subject to second level review by materials management to verify fair and reasonable pricing.

G. TRAVEL COSTS (Location, Dates and Purpose): _____
Travel to a breast cancer research-related meeting. Location and dates are currently unknown.

H. RESEARCH-RELATED SUBJECT COSTS (Itemized): ___
N/A

I. RESEATCH-RELATED INJURY MEDICAL COSTS (Itemized): ___
N/A

J. OTHER EXPENSES (Itemized by Category): ___
N/A

K. SUBCONTRACTOR COSTS (If Applicable): ___

PERSONNEL Role & Percentage of Effort for each and State the Escalation Rate Applied [no higher than 3%]): ___
N/A

FRINGE RATE (State the Fringe Rate % for EACH Employee for EACH Year & Date of Fringe Rate Agreement): ___
N/A

INDIRECT RATE (State the Indirect Rate % for Each Year & Date of Indirect Rate Agreement): ___
N/A

CONSULTANT COSTS (Hours of Service and Rate per Hour): ___
N/A

MAJOR EQUIPMENT (Over $5,000 [Itemized]): ___
N/A

MATERIALS, SUPPLIES, AND CONSUMABLES (Itemized by Category & State the Institution's In-House Policy Regarding how Fair & Reasonable Pricing is Determined on Goods Purchased): ___
N/A

TRAVEL COSTS (Location, Dates and Purpose): ___
N/A

RESEARCH-RELATED SUBJECT COSTS (Itemized): ___
N/A

RESEATCH-RELATED INJURY MEDICAL COSTS (Itemized): ___
N/A

OTHER EXPENSES (Itemized by Category):

___

N/A

FEBS Letters 583 (2009) 1817–1824

FEBS Letters

journal homepage: www.FEBSLetters.org

Minireview

IQGAPs in cancer: A family of scaffold proteins underlying tumorigenesis

Colin D. White, Matthew D. Brown, David B. Sacks *

Brigham and Women's Hospital and Harvard Medical School, Thorn 530, 75 Francis Street, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 27 March 2009
Revised 28 April 2009
Accepted 2 May 2009
Available online 9 May 2009

Edited by Lukas Huber

Keywords:
Cancer
IQGAP1
IQGAP2
IQGAP3
Metastasis
Neoplasia
Tumorigenesis

ABSTRACT

The IQGAP family comprises three proteins in humans. The best characterized is IQGAP1, which participates in protein–protein interactions and integrates diverse signaling pathways. IQGAP2 and IQGAP3 harbor all the domains identified in IQGAP1, but their biological roles are poorly defined. Proteins that bind IQGAP1 include Cdc42 and Rac1, E-cadherin, β-catenin, calmodulin and components of the mitogen-activated protein kinase pathway, all of which are involved in cancer. Here, we summarize the biological functions of IQGAPs that may contribute to neoplasia. Additionally, we review published data which implicate IQGAPs in cancer and tumorigenesis. The cumulative evidence suggests IQGAP1 is an oncogene while IQGAP2 may be a tumor suppressor.

1. Introduction

IQGAPs comprise a class of multidomain proteins, which are present in diverse organisms ranging from yeast and *Caenorhabditis elegans* to *Xenopus laevis* and mammals [1]. There are three IQGAPs in humans (Fig. 1). The first to be described was the 190-kDa protein IQGAP1, which was cloned in 1994 [2]. IQGAP2, which is 62% identical to IQGAP1, was identified 2 years later [3] and IQGAP3 was isolated in 2007 [4]. The vast majority (>85%) of the published literature focuses on IQGAP1. Less is known about IQGAP2 (20 primary papers in PubMed [http://www.ncbi.nlm.nih.gov/pubmed] at the time of writing) and there are only two primary papers on IQGAP3. The cytoskeletal [5–8] and cellular signaling [1,9] functions of IQGAP1 have been extensively reviewed in the last few years. Here, we briefly compare the characteristics of IQGAP1, IQGAP2 and IQGAP3, then focus on published data that address their involvement in neoplasia.

2. Comparison of human IQGAP proteins

The IQGAP proteins share a similar domain structure and have considerable sequence homology (Fig. 1). These domains mediate the association of IQGAPs with a diverse spectrum of proteins [9]. Binding to IQGAP1 modulates the function of the interacting proteins, resulting in the alteration of multiple cellular behaviors [5,7,9]. Despite limited information on IQGAP2 and IQGAP3, it is apparent that they differ from IQGAP1 in several respects (including tissue distribution, subcellular localization and interaction with binding proteins). These distinctions may account for some of the functional differences among the three IQGAPs that are beginning to emerge.

The tissue distribution of the IQGAP proteins varies considerably. IQGAP1 has ubiquitous expression [2]. IQGAP2 is found predominantly in liver, but can be detected in prostate, kidney, thyroid, stomach, testis, platelets and salivary glands [3,4,10,11], while IQGAP3 is reported to be present in brain, lung, testis, small intestine and colon [4,12].

IQGAPs exhibit both similarities and differences in their subcellular localization. In human epithelial cells in culture, endogenous IQGAP1 is distributed throughout the cytoplasm and accumulates at cell–cell junctions where it co-localizes with E-cadherin [13]. In quiescent human platelets IQGAP2 demonstrates diffuse cytoplasmic staining [10]. When platelets are activated, IQGAP2 is found predominantly in filopodia, with less prominent staining in

Abbreviations: APC, adenomatous polyposis coli; CK1, casein kinase 1; ECM, extracellular matrix; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GAP, GTPase-activating protein; GSK-3β, glycogen synthase kinase-3β; HCC, hepatocellular carcinoma; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; MMP, matrix metalloproteinase; VEGF, vascular endothelial-derived growth factor

* Corresponding author. Fax: +1 617 278 6921.
E-mail address: dsacks@rics.bwh.harvard.edu (D.B. Sacks).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.05.007

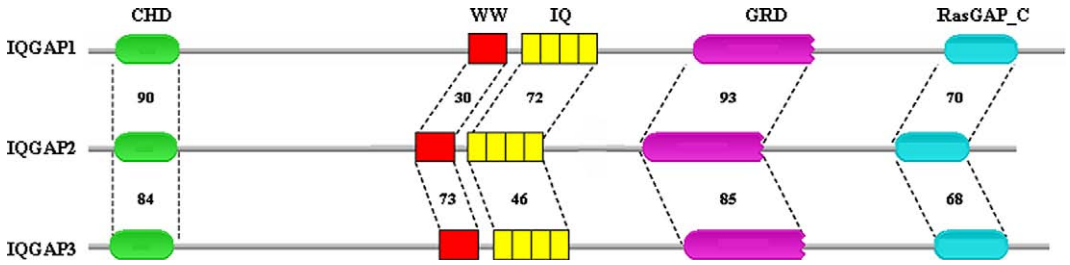

Fig. 1. A schematic diagram of human IQGAP proteins. Domain structure (adapted from SMART and Pfam databases) and percentage amino acid identity of human IQGAP1, IQGAP2 and IQGAP3 are shown. CHD, calponin homology domain; WW, poly-proline protein–protein domain; IQ, IQ domain (with four IQ motifs); GRD, RasGAP-related domain; RasGAP_C, carboxy-terminal sequence found in members of the IQGAP family.

the cell body. By contrast, IQGAP2 is predominantly localized in the nucleus and at sites of cell–cell contact in isolated rabbit gastric glands in primary culture [14]. In this study, IQGAP1 was observed to be targeted predominantly to the cortex of chief and mucous neck cells. These findings contradict those of an earlier publication where IQGAP1 and IQGAP2 were localized to the basolateral and apical membranes, respectively, in rabbit gastric parietal cells [15]. The localization of IQGAP3 in human cells has not been described. In PC12 rat phaeochromocytoma cells IQGAP3 is diffusely distributed in the cytoplasm [4], while in cultured Eph4 mouse epithelial cells it is found at cell–cell junctions [12]. IQGAP1 and IQGAP3 have similar distribution in the cell bodies, distal parts of axons and axon growth cones of rat embryo hippocampal neurons [4]. Interestingly, IQGAP3 expression is reported to be confined to proliferating cells [12]. Additional studies are necessary to clearly delineate the subcellular distribution of IQGAP2 and IQGAP3 in human tissue.

3. IQGAP binding proteins

IQGAP1 binds numerous proteins [9]. Much less is known about the binding partners of IQGAP2 and IQGAP3. Nevertheless, sufficient information is now available to permit one to begin to tease out differences. IQGAP1 binds to GTP-Cdc42 and GTP-Rac1 with substantially higher affinity than to the inactive, GDP-bound form of the GTPases [16,17]. Similarly, the interaction of IQGAP3 with Rac1 and Cdc42 appears to be GTP-dependent [4]. In contrast, although not observed in all cases [15], IQGAP2 has been reported to interact with both the GDP- and the GTP-bound forms [3,18]. Another protein that may bind differentially to IQGAPs is Ras. No interaction between H-Ras and IQGAP1 [16,18,19] or IQGAP2 [3,18] has been detected (IQGAP1 was identified in a complex with M-Ras [20], but direct binding has not been demonstrated). For IQGAP3, the evidence is contradictory. One group reported that Ras binds to IQGAP3 [12], while other investigators failed to observe an interaction between the two proteins [4]. Further work is required to reconcile these discrepant findings and provide detailed analysis of the binding partners of IQGAP2 and IQGAP3.

It is important to emphasize that, despite the presence of a domain with sequence similarity to RasGAPs, none of the IQGAPs have GTPase-activating protein (GAP) activity. As mentioned, IQGAP proteins bind to the Rho family GTPases Rac1 and Cdc42. These proteins act as molecular switches by cycling between "on" GTP-bound and "off" GDP-bound states [21]. Interaction with a GAP accelerates GTP hydrolysis leading to inactivation. By associating with GTP-bound Rac1 and Cdc42, IQGAP1 and IQGAP2 inhibit the intrinsic rate of GTP hydrolysis and thus *stabilize* the active GTP-bound state [3,16,22]. Consistent with these in vitro findings, overexpression of IQGAP1 in MCF-7 malignant human breast epithelial cells increases the amount of active Cdc42 and Rac1 [22,23].

In addition to regulating Rac1 and Cdc42 signaling, recent work has shown that IQGAPs, and IQGAP1 in particular, modulate many different signaling pathways and cellular functions [9], including mitogen-activated protein kinase (MAPK) signaling, Ca^{2+}/calmodulin signaling, cell–cell adhesion, β-catenin-mediated transcription and microbial invasion [9,24,25]. Ca^{2+} and calmodulin appear to be of great importance to the function of IQGAP1. Calmodulin binds to IQGAP1 in a Ca^{2+}-regulated manner [16,17], and association with calmodulin inhibits the ability of IQGAP1 to interact with every other binding partner studied to date [1,9].

A finding with particular relevance to the topic of this review is that many IQGAP1 binding partners have well-defined roles in tumorigenesis (Table 1). These proteins include the well-described oncogenes β-catenin and Src, the tumor suppressor E-cadherin, the Rho GTPases Cdc42 and Rac1, and components of the MAPK cascade. These observations, in conjunction with the ability of IQGAP1 to modulate fundamental cellular functions, have led to considerable attention being directed towards IQGAP1 in the field of cancer biology. Work from several laboratories suggests that IQGAP1 is an oncogene that promotes both tumorigenesis and metastasis and, to a more limited extent, implies that it may be a useful tumor marker. IQGAP2, in contrast, appears to have the opposite effect and may act as a tumor suppressor. Little can be inferred regarding the possible role of IQGAP3 in neoplasia. Here, we review the available evidence for the involvement of IQGAPs in the regulation of signaling pathways and cellular functions known to be involved in neoplastic transformation and/or tumor progression. Additionally, we discuss how, by virtue of their cellular expression and/or localization, IQGAPs are directly implicated in cancer. While most of the data presented are germane only to IQGAP1, we also discuss the possible roles of IQGAP2 and IQGAP3 where published evidence is available.

4. IQGAP functions with potential relevance to cancer

IQGAP1 regulates many different cellular processes, and changing intracellular IQGAP1 expression or function can alter these activities [1,5,7,9]. Therefore, IQGAP1 appears to be important for normal cellular function and homeostasis. The contribution of some of these cellular activities to different stages of cancer progression provides a clear link between IQGAP1 and cancer. Selected cell functions, and how the IQGAPs control them, will be described.

4.1. MAPK signaling

The MAPK pathway, which modulates multiple cellular processes, such as differentiation, proliferation and migration, is deregulated in neoplasia [26,27]. For example, mutations in Ras [28] or B-Raf [29,30] are highly prevalent in neoplasms. Activating mutations in Ras have been reported in over 15% of all human tumors and in pancreatic carcinoma this frequency may be as high as

Table 1
IQGAP1 binding proteins with identified roles in cancer.

Binding partner	Effect of IQGAP1 on target protein	Functional consequence of interaction	Potential relevance to cancer	References
Arf6	Promotes Arf6-induced Rac1 activation	Enhances cell migration	Metastasis	[70]
β-Catenin	Promotes β-catenin-mediated transcription; inhibits cell–cell adhesion	Disrupts cell–cell adhesion	Transformation and metastasis	[45,53,95]
B-Raf	MAPK scaffold; increases B-Raf activity	Enhances proliferation and angiogenesis	Transformation	[35,49]
Calmodulin	Prevents interaction of IQGAP1 with other binding proteins	Regulates IQGAP1 functions	Proliferation and metastasis	[16,17,96]
CD44	Links hyaluronan to actin cytoskeleton	Promotes migration	Metastasis	[38]
Cdc42/Rac1	Stabilizes active form	Promotes proliferation, cell motility and invasion	Proliferation, invasion and metastasis	[16,19,23,63]
E-cadherin	Disrupts E-cadherin function	Inhibits cell–cell adhesion	Invasion and metastasis	[13,53]
Exo70	Promotes correct exocyst localization	Regulates exocytosis	Invasion	[61]
ERK1/2	Regulates MAPK signaling	Promotes invasion	Invasion	[36,37]
MEK1/2	Regulates MAPK signaling	Promotes invasion	Invasion	[23,36]
Sec3/8	Promotes MMP accumulation at invadopodia	ECM degradation	Invasion	[60]
Src	Unknown	Proliferation and angiogenesis	Transformation	[49]

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase.

90% [28]. In addition, increased extracellular signal-regulated kinase (ERK) phosphorylation and expression has been found in pancreatic cancer [31], and enhanced ERK phosphorylation correlates with tumor progression in prostate cancer [32]. Increased MAPK kinase (MEK) phosphorylation has been identified in colon cancer [33] and in 74% of myeloblasts in acute myelogenous leukemia [34].

IQGAP1 is a MAPK scaffold, which binds directly to and modulates the functions of B-Raf [35], MEK [36] and ERK [37]. IQGAP1 is required for activation of B-Raf by epidermal growth factor (EGF) [35]. Similarly, IQGAP1 regulates the activation of MEK and ERK in response to both EGF [36,37] and CD44 [38]. Thus, IQGAP1 is required for efficient propagation of the MAPK cascade.

EGF differentially modulates the association of components of the MAPK pathway with IQGAP1. Knockout of IQGAP1 from cells renders B-Raf insensitive to EGF stimulation, while B-Raf associated with IQGAP1 has a much higher kinase activity compared with free B-Raf [35]. It is unclear whether interacting with IQGAP1 enhances activation of B-Raf by EGF, or whether IQGAP1 preferentially associates with B-Raf that has already been activated. Interestingly, while ERK binds constitutively to IQGAP1 and the binding is not sensitive to EGF, the interaction between IQGAP1 and MEK1 increases, while that with MEK2 decreases, following EGF treatment [36]. This raises the possibility that IQGAP1 preferentially activates the MEK1 signaling pathway. It has been suggested that MEK1 promotes proliferation, while MEK2 promotes differentiation [39]. Scaffold proteins serve as signaling nodes, influencing signal intensity, time course and the specific cellular response to an extracellular cue [40–42]. Thus, the scaffold functions of IQGAP1 may modulate the cellular response to activation of MAPK signaling, enhancing proliferation and reducing differentiation. These changes could contribute to neoplasia.

Analogous to IQGAP1, siRNA-mediated knockdown of IQGAP3 suppresses ERK phosphorylation and significantly reduces proliferation of Eph4 mammary epithelial cells [12]. Moreover, exogenous expression of IQGAP3 induces a proliferative response, which is blocked by the ERK inhibitor U0126. Thus, it appears that IQGAP3-induced ERK activation may have a role in the regulation of cellular proliferation.

4.2. β-Catenin-mediated transcription

β-Catenin, the central molecule in the Wnt signaling pathway, is integral to the control of cellular proliferation and cell–cell adhesion, both of which are deregulated in malignancy [43,44]. Under unstimulated conditions, β-catenin is held in a complex with adenomatous polyposis coli (APC) and axin, and is targeted for degradation by casein kinase 1 (CK1) and glycogen synthase kinase-3β (GSK-3β). In response to Wnt stimulation, CK1 and GSK-3β are inhibited, and β-catenin accumulates in the cytoplasm from where it translocates to the nucleus. Here, it promotes gene transcription by binding to the T cell factor/lymphoid enhancer factor family of transcription factors.

IQGAP1 binds directly to β-catenin. Overexpression of IQGAP1 enhances β-catenin nuclear localization and β-catenin-dependent transcription in SW480 colon carcinoma and human bronchial epithelial cells [45,46]. Furthermore, targeted disruption of the murine *Iqgap2* gene results in increased expression of IQGAP1 in the cytoplasm of hepatocytes, with a concomitant increase in cytoplasmic β-catenin, β-catenin activation and expression of cyclin D1 (a nuclear target of the Wnt/β-catenin pathway) [47]. Taken together, these findings suggest that IQGAP1 is an important regulator of β-catenin function.

4.3. Cellular proliferation

Uncontrolled cellular proliferation is a fundamental characteristic of neoplastic transformation. Recent studies have shown that IQGAP proteins are important regulators of the proliferative response. Overexpression of IQGAP1 increases proliferation of MCF-7 cells, an effect dependent, at least in part, on increased active Rac1 and Cdc42 [23]. Similarly, IQGAP1 is required for vascular endothelial-derived growth factor (VEGF)-stimulated proliferation as knockdown of IQGAP1 with siRNA abrogates proliferation of human umbilical vein [48] and aortic [49] endothelial cells. These observations suggest that the IQGAP1 expression level directly dictates the rate of cellular proliferation. Indeed, siRNA-induced IQGAP1 knockdown significantly reduces VEGF-stimulated angiogenesis in vivo [49]. Furthermore, quercetin, an anti-oxidative flavonoid which is known to have strong anti-proliferative properties [50], decreases IQGAP1 expression in HepG2 human hepatocellular carcinoma (HCC) cells [51]. Interestingly, research in the small intestine has shown that IQGAP3, but not IQGAP1 or IQGAP2, is exclusively expressed in proliferating cells [12].

4.4. Cell–cell adhesion

Decreased tumor cell adherence at the primary site, increased proteolytic degradation of surrounding tissue and enhanced cell motility are required for cancer cells to metastasize. Loss of cell–cell adhesion occurs as a result of reduced E-cadherin function, but the precise molecular mechanisms underlying this effect are

poorly understood [52]. E-cadherin mediates intercellular adhesion through homophilic associations with the extracellular domains of E-cadherin on a neighboring cell. Importantly, IQGAP1 binds directly to E-cadherin [13,53] and overexpression of IQGAP1 reduces E-cadherin-mediated adhesion [53]. Similarly, translocation of IQGAP1 to cell–cell junctions attenuates E-cadherin function [13].

While no published studies have investigated the possible role of mammalian IQGAP2 or IQGAP3 in cell–cell adhesion, a *X. laevis* IQGAP2 homolog (XIQGAP2) localizes at cell–cell junctions in both cultured *Xenopus* cells and embryos [54]. Suppression of XIQGAP2 expression by microinjection of morpholino antisense oligonucleotides results in ectodermal lesions in mid-neurula stage embryos due to loss of cell–cell adhesion [55]. These findings suggest that XIQGAP2 expression positively regulates cell–cell adhesion during early development. It remains to be determined whether IQGAP2 contributes to the maintenance of cell–cell adhesion in mammals.

4.5. Exocytosis

Tumor cell invasion across tissue boundaries is dependent on the capacity of neoplastic cells to breach the basement membrane and remodel the extracellular matrix (ECM), events which commonly occur by proteolytic cleavage by matrix metalloproteinases (MMPs) [56]. Active MMPs are delivered to the sites of contact between invasive tumor cells and the ECM via dynamic cellular protrusions known as invadopodia [57,58]. MMP accumulation at invadopodia is thought to rely on vesicle exocytosis which, in turn, depends on the successful targeting and tethering of vesicles to appropriate sites on the cell membrane. Here, the exocyst, a multiprotein complex consisting of eight subunits including Sec3, Sec8 and Exo70 [59], is believed to play a pivotal role. Importantly, IQGAP1 binds Sec3, Sec8 and Exo 70 [60,61], implicating IQGAP1 in the regulation of exocytosis. Moreover, interaction between IQGAP1 and the exocyst was shown to be necessary for invadopodia activity in MDA-MB-231 cells [60]. Interestingly, silencing IQGAP1 inhibits the invasion of ovarian carcinoma HO-8910PM cells in vitro [62] and MCF-7 cells in vitro and in vivo [23,63]. It is therefore tempting to speculate that IQGAP1 mediates invasion, at least in part, by regulating exocytosis and subsequent degradation of the ECM.

4.6. Cell migration

Most cancer deaths are caused by metastatic disease. The mechanism by which metastases develop remains to be fully elucidated, but it is agreed that cells must have both invasive and migratory properties [64]. Many factors which are known to increase cell migration in vitro have been shown to promote metastasis in vivo [64]. IQGAP1 was originally characterized as regulator of Rac1/Cdc42 signaling and actin dynamics [1,5–7], and much of the early work on IQGAP1 focused on its role in regulating the cytoskeleton. Consequently, IQGAP1 was observed to be an important modulator of cell migration [63,65–67]. IQGAP1 cross-links actin filaments [5,68], and localizes to the leading edge of migrating cells [63,66]. Increasing IQGAP1 expression in cells increases the amount of active Cdc42 and promotes cell migration [63], although other IQGAP1 binding partners, including actin, calmodulin [69], and APC [66], are also likely to contribute to this effect. siRNA-induced knockdown of IQGAP1 reduces the migration of several human cell lines, such as MCF-7 [63] and U87MG human glioblastoma cells [70]. In agreement with these studies, IQGAP1 is required for the induction of cell migration by fibroblast growth factor (FGF), VEGF and hyaluronan [38,48,71].

5. Role of IQGAP proteins in cancer

The work outlined above implicates the IQGAP proteins, particularly IQGAP1, in neoplasia by virtue of their cellular functions. Nevertheless, it is important to note that much of these data were obtained from cultured cell lines, and a critical reader may argue that their relevance to cancer is largely circumstantial. In the following sections we discuss evidence derived from human neoplasms and mouse models of cancer, which more directly identify the involvement of IQGAP1 and IQGAP2 in neoplastic transformation and metastasis.

5.1. Genetic studies

The level of expression of IQGAP genes and mRNAs are frequently altered in neoplasia. IQGAP1 has been proposed to be an oncogene [23]. Consistent with this postulate, comparison of the genetic profiles of tumors with those of normal tissue, and comparison of more aggressive cancers with less aggressive neoplasms, reveals that the *Iqgap1* gene and/or mRNA are overexpressed in all analyses reported (Table 2). Increased expression of *Iqgap1* has been observed in several human neoplasms, including lung [72], colorectal [73] and oligodendroglioma [72]. Analogous observations have been reported in mouse models (Table 2). *Iqgap1* is overexpressed in a genetically engineered mouse that recapitulates the stages of human prostate cancer progression [74]. While not

Table 2
Changes in Iqgap gene/mRNA expression level in neoplasms.[B]

Tissue comparison	IQGAP	Species	Method	Neoplasm	Expression change	References
Cancer vs. normal	*Iqgap1*	Human	Array	Colorectal	+	[73]
				Glioma	+	[97]
			RT-PCR	Glioma	+	[97]
				Lung	+	[72]
		Mouse	Array	Prostate	+	[74]
	Iqgap2	Human	Array	Colorectal	+	[78]
				Prostate	+	[79]
Aggressive vs. less aggressive cancer[A]	*Iqgap1*	Human	Array	Glioma	+	[88][a]
		Mouse	Array	Medulloblastoma	+	[98][b]
				Melanoma	+	[90][c]
	Iqgap2	Human	Array	Prostate	−	[77][d]
		Mouse	Array	Prostate	−	[74][e]
	Iqgap3	Human	Array	Colorectal	−	[99][f]

+, Increased expression; −, decreased expression.

[A] Aggressive vs. less aggressive cancer is defined by a decrease in long-term survival[a,f], an increase in the likelihood of metastasis[b,c,e] or a decline in tumor responsiveness to hormone therapy[d].

[B] Only published studies with primary tissue are included. Data with cultured cell lines have been omitted.

included in Table 2, similar findings have been documented in cultured cell lines. For example, the *Iqgap1* gene is amplified in HSC39 and HSC40A gastric cancer cell lines [75]. This amplification corresponds to an increase in both IQGAP1 mRNA and protein, compared with normal gastric cell lines, and an accumulation of IQGAP1 protein at the cell membrane [75].

IQGAP2 has also generated several "hits" in genetic screens comparing normal with neoplastic tissue (Table 2). The results, however, are less unequivocal than those for IQGAP1. IQGAP2 expression is lost from 5/9 gastric cancer cell lines due to aberrant methylation of the IQGAP2 promoter [76]. This abnormal methylation was also observed in 47% of primary gastric cancer tissues (compared with 0% in normal tissue), and is significantly associated with tumor invasion and a poor prognosis [76]. The inverse correlation of *Iqgap2* expression with cancer progression suggests that IQGAP2 may be a tumor suppressor. This hypothesis is supported by studies which show reduced expression of the *Iqgap2* gene in hormone-refractory prostate cancer [74,77]. However, other reports contradict these findings as they observe overexpression of *Iqgap2* in tissue from cancers of the colon [78] and prostate [79] (Table 2). Thus, while there is evidence to suggest that *Iqgap2* acts as a tumor suppressor, more thorough investigations are required in order to verify this postulate and ascertain whether it pertains only to selected neoplasms.

5.2. IQGAP protein expression and localization

The level of expression of IQGAP proteins is also altered in neoplasia (Table 3). Compared with normal tissue, IQGAP1 is overexpressed in colorectal carcinoma [80], breast cancer [23], astrocytoma [81] and squamous cell carcinoma of the head and neck [82]. Furthermore, IQGAP1 expression in aggressive ovarian adenocarcinomas is higher than in adenomas or borderline tumors [62], while a lack of IQGAP1 protein expression is associated with a favorable prognosis in gastric cancer [83]. There is little published on IQGAP2 protein expression in neoplasia. Only one paper, published recently, addresses this topic. In this study, IQGAP2 expression is lost from 61% of human gastric carcinoma tissue, but is detected in 100% of normal gastric mucosa [76]. This observation is consistent with the postulated role for IQGAP2 as a tumor suppressor.

In addition to increased expression, the subcellular localization of IQGAP1 is altered in carcinoma. IQGAP1 is localized at the invasive front of more aggressive colorectal [80] and ovarian [84] neoplasms. In the latter study, a diffuse expression pattern of IQGAP1 indicates a high histological grade and clinicopathological stage. IQGAP1 overexpression and diffuse invasion pattern were significantly associated with poor prognosis by multivariate analysis [84]. Data from other groups support the premise that peripheral localization of IQGAP1 is associated with more aggressive tumors. For example, translocation of IQGAP1 from the cytoplasm to the cell membrane correlates with dedifferentiation of gastric carcinoma [85]. IQGAP1 is also implicated in endometrial adenocarcinoma [86]. Compared with normal tissue, in well-differentiated tumors, IQGAP1 disappears from cell adhesion sites, while E-cadherin is still present around cell boundaries [86]. However, in poorly differentiated tumors, IQGAP1 and E-cadherin accumulate in aggregates [86], suggesting that IQGAP1 and E-cadherin function is disrupted in advanced, poorly differentiated tumors.

The connection between IQGAP1 and E-cadherin is also seen in gastric cancers. In normal epithelium, IQGAP1 and E-cadherin are localized to the cell–cell boundary [85]. However, IQGAP1 localizes to the cytoplasm in intestinal-type tumors and to the cell periphery in diffuse-type tumors [85]. These results are further supported by fractionation data showing that in differentiated tumors IQGAP1 is present in the soluble fraction and E-cadherin in the insoluble fraction, but both are insoluble in undifferentiated tumors [85]. Consequently, it is thought that as tumor cells de-differentiate, IQGAP1 moves from the cytoplasm to the cell periphery where it disrupts E-cadherin function [85].

There is strong evidence to suggest that IQGAP1 expression can serve as a biomarker for diagnosis of glioblastomas. In a rat model of glioma, IQGAP1 expression is restricted to a subpopulation of nestin-positive amplifying tumor cells in glioblastoma-like tumors, but not oligodendroglioma-like tumors [87]. In human glioblastoma samples, IQGAP1 is a marker for nestin-positive cancer cells, which appear to represent stem-like cancer progenitors [87]. In a study to identify biomarkers of aggressive gliomas, IQGAP1 expression, along with insulin-like growth factor binding protein-2, identifies a subgroup of patients with grade III gliomas with poor prognosis [88]. While normal glial tissue does not express IQGAP1, cytoplasmic IQGAP1 staining is seen in 64% of gliomas [88].

Finally, outcome studies in other cancers are also beginning to emerge. For example, increased *Iqgap1* expression constitutes part of a genetic signature that significantly predicts the likelihood of recurrence of colon cancer [89]. While it remains to be elucidated if these findings are relevant to other tumors, these data suggest that IQGAP1 may be useful in evaluation of patient prognosis.

5.3. IQGAP1 in metastasis

Genetic evidence using an in vivo scheme indicates a role for *Iqgap1* in metastasis. A screen for genes exhibiting altered expression in a mouse model of metastatic melanoma identified *Iqgap1*

Table 3
Changes in IQGAP protein expression level in neoplasms.[B]

Tissue comparison	IQGAP	Species	Method	Neoplasm	Expression change	References
Cancer vs. normal	IQGAP1	Human	IHC	Colorectal	+	[80]
			MS	Squamous cell (Head and Neck)	+	[82]
			WB	Astrocytoma	+	[81]
			WB	Breast	+	[23]
	IQGAP2	Human	IHC	Gastric	−	[76]
		Mouse	IHC/WB	Liver	−	[47]
Aggressive vs. less aggressive cancer[A]	IQGAP1	Human	IHC	Gastric	+	[83][a]
				Glioma	+	[88][b]
				Lung	+	[100][c]
				Ovary	+	[84][d]
			IHC/WB	Glioma	+	[87][e]

+, Increased expression; −, decreased expression.
Abbreviations: IHC, immunohistochemistry; WB, western blot; MS, mass spectrometry.
[A] Aggressive vs. less aggressive cancer is defined by a decrease in long-term survival[a,b,d] or an increase in the likelihood of metastasis[c,e].
[B] Only published studies with primary tissue are included. Data with cultured cell lines have been omitted.

and its binding proteins calmodulin and ERK as 3 of only 32 genes (from ~10500 arrayed genes) that showed a >2.5-fold increase in expression in metastatic cells [90]. The small number of genes identified implies that increased expression of IQGAP1 and calmodulin are likely to be important in metastasis, rather than an indirect result of the altered phenotype.

6. IQGAPs in tumorigenesis

As we have highlighted, accumulating evidence reveals that IQGAP1 expression, both RNA and protein, is increased in several human malignancies. IQGAP2 concentration is also altered, but the changes are less consistent and not as well-documented. These studies are observational and do not indicate whether the reported changes are a cause or simply a consequence of neoplastic transformation. This question has been addressed in two recent publications, both of which provide strong evidence to suggest that IQGAPs contribute to tumorigenesis. In the first study, Jadeski et al. [23] manipulated intracellular concentrations of IQGAP1 in malignant MCF-7 cells. Specific knockdown of IQGAP1 by siRNA significantly reduces both serum-dependent proliferation and anchorage-independent growth in soft agar. These in vitro data strongly suggest that IQGAP1 is required for the transformed phenotype of MCF-7 cells, a postulate validated by in vivo analysis. Subcutaneous injection into immunocompromised mice of MCF-7 cells overexpressing IQGAP1 gives rise to the formation of tumors in 100% of mice and these tumors extensively invade skeletal muscle [23]. Control MCF-7 cells form tumors in 60% of animals and do not invade host tissue, whereas stable knockdown of IQGAP1 results in tumors in only 20% of mice and the complete abrogation of invasion. Collectively these data strongly support the hypothesis that IQGAP1 is an important component of breast cancer. Additional work is necessary to ascertain whether IQGAP1 functions as an oncogene and is required for neoplastic transformation of breast epithelial cells.

IQGAP2 is expressed predominantly in liver. Consistent with its putative role as a tumor suppressor, targeted disruption of the murine *Iqgap2* gene results in the development of HCC [47]. The neoplasia is restricted to the liver; non-hepatic tissue exhibits no defects. Congruent with the evidence that it is an oncogene, IQGAP1 is necessary for *Iqgap2*$^{-/-}$ mice to develop HCC; interbreeding *Iqgap2*$^{-/-}$ mice into an *Iqgap1*-null background significantly decreases the incidence, size and aggressiveness of the tumors. IQGAP1 expression is increased ~9-fold in liver, but is not altered in other organs [47]. While these findings need to be validated in human HCC, they strongly suggest that deregulation of IQGAP1 and IQGAP2 may underlie the pathogenesis of this disease.

7. Perspectives

IQGAP1 is frequently overexpressed in cancer while IQGAP2 expression is reduced in some neoplasms. The association of IQGAP1 with its binding partners Cdc42, Rac1, E-cadherin, β-catenin, components of the MAPK cascade and others may have a role in transformation and metastasis. However, the specific interactions that directly contribute to tumorigenesis have not been fully elucidated. Moreover, it is not known at which of the multiple stages that occur during the conversion of normal cells into malignant derivatives IQGAP1 participates. The published data, albeit limited, on IQGAP2 and IQGAP3 reveal that substantial differences exist among the IQGAP family members with respect to tissue distribution, subcellular localization and binding partners. Additional work is necessary to dissect out the biological implications of these differences and determine the molecular mechanisms by which IQGAP2 and IQGAP3 are likely to influence tumorigenesis.

A fundamental question which remains to be answered is what triggers overexpression of IQGAP1 and, in some neoplasms, loss of IQGAP2? Several oncogenes and tumor suppressor genes, for example Ras, B-Raf, p53 and APC [91,92], are mutated. Other oncogenes, like the Rho GTPases, are not mutated, but are deregulated during tumor progression [93]. A preponderance of evidence suggests that, analogous to the Rho family, the *Iqgap1* gene is amplified in cancer and there is little indication of IQGAP1 mutation. Genomic sequence analysis of *Iqgap1* in 38 gastric cancers reveals a missense nucleotide change at an allelic frequency of only 2.6% (although other silent nucleotide changes were also observed) [94]. There are no published reports describing mutation of the *Iqgap2* or *Iqgap3* genes in tumors. Further work is needed to ascertain the molecular mechanisms by which *Iqgap1* and *Iqgap2* (and perhaps *Iqgap3*) expression is altered in neoplasia.

The cumulative evidence strongly implicates the IQGAP proteins in cancer. In addition to their altered levels in human neoplasms, IQGAPs appear to contribute to tumorigenesis. The documented roles for many IQGAP binding proteins in multiple stages of neoplastic transformation and metastasis, coupled with the participation of IQGAPs in diverse signaling pathways that are deregulated in cancer, combine to make IQGAP proteins conceptually appealing chemotherapeutic targets.

Acknowledgements

We apologize to all those whose primary work could not be cited due to lack of space. We thank Rob Krikorian for help preparing the manuscript and members of the Sacks laboratory, past and present, for insightful discussions. Work in the authors' laboratory is funded by the National Institutes of Health.

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Chapter 9

Regulation of MAP Kinase Signaling by Calcium

Colin D. White and David B. Sacks

Abstract

Mitogen-activated protein kinase (MAPK) signaling influences a variety of cellular responses, ranging from stimulation of cell proliferation to induction of senescence and/or apoptosis. Ca^{2+} is a ubiquitous intracellular signaling molecule that controls multiple processes in cells. Published evidence has identified both direct and indirect interactions between the Ca^{2+} and MAPK signaling pathways. Here, we describe assays to accurately determine the effect of changes in intracellular Ca^{2+} concentration on MAPK activation.

Key words: A23187, BAPTA-AM, Ca^{2+}, Confocal microscopy, MAPK signaling, Western blotting

1. Introduction

Mitogen-activated protein kinases (MAPKs) are ubiquitously expressed enzymes that regulate a wide variety of functions in virtually all cell types (1). The term "MAPK" usually refers to the terminal kinase in a three-tier cascade, in which MAPKs are phosphorylated and activated by MAPK kinases (MAPKK or MEK), which themselves are phosphorylated and activated by MAPK kinase kinases (MAPKKK or MEKK). Of the major MAPK pathways, the Ras/Raf/MEK/ERK cascade is the most widely studied and is the focus of this chapter. Engagement of cell-surface receptors by extracellular signaling molecules, such as growth factors, results in activation of the intracellular small G-protein Ras. The resultant change in Ras conformation facilitates its direct interaction with Raf isoforms, namely A-Raf, B-Raf, and C-Raf (also termed Raf-1) (2). The Raf proteins are serine/threonine kinases, which phosphorylate and activate MEK1 and MEK2. In turn, MEK1 and MEK2 catalyze the phosphorylation of the extracellular signal-regulated kinases, ERK1 and ERK2.

Rony Seger (ed.), *MAP Kinase Signaling Protocols: Second Edition*, Methods in Molecular Biology, vol. 661,
DOI 10.1007/978-1-60761-795-2_9,

Once active, ERKs either dimerize and remain in the cytosol where they catalyze the phosphorylation of a variety of substrates, or, as monomers, translocate to the nucleus where they phosphorylate transcription factors (3).

MAPK function is influenced by several pathways, including Ca^{2+} (4). For example, an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) positively regulates Ras signaling in PC12 cells leading to increased ERK phosphorylation (5). Conversely, treating keratinocytes with Ca^{2+} inhibits activation of ERK by epidermal growth factor (EGF) (6). The reasons for these discrepant data are not known, but differences between the cell types are likely to contribute. The ability to manipulate $[Ca^{2+}]_i$ and accurately measure active MAPK is a welcome addition to the researchers' toolbox. In this chapter, we describe straightforward assays for evaluating the effect of Ca^{2+} on growth factor-induced MAPK signaling using Western blotting and confocal immunofluorescence.

1.1. Manipulation of $[Ca^{2+}]_i$

The protocol described in this chapter represents probably the most widely used methods to manipulate $[Ca^{2+}]_i$. A23187 is a Ca^{2+} ionophore that causes a rapid and sustained increase in $[Ca^{2+}]_i$ by permitting entry into the cell of extracellular Ca^{2+}. BAPTA-AM enters cells where it chelates intracellular Ca^{2+}, markedly reducing $[Ca^{2+}]_i$. These reagents therefore allow the investigator to elucidate the regulatory effect of Ca^{2+} on intracellular signaling.

In order to identify the source of the Ca^{2+} responsible for a specific effect, pharmacological compounds are available (which selectively modulate individual Ca^{2+} channels or pumps (Table 1)). Each compound can be broadly characterized as inducing either an "on" or an "off" signal (Fig. 1). On signals increase $[Ca^{2+}]_i$, while off signals reduce it. These are discussed in more detail below.

1.1.1. "On" Signals

Certain extracellular stimuli induce a rise in $[Ca^{2+}]_i$. The increase in $[Ca^{2+}]_i$ is mediated either by Ca^{2+} entering the cell from the outside (across the plasma membrane) or by release from intracellular stores. There are three classes of channels in the plasma membrane which facilitate Ca^{2+} influx from the outside (7). Voltage-activated Ca^{2+} channels respond to changes in the membrane potential of the cell, while ligand-activated Ca^{2+} channels are opened in response to the binding of a specific ligand. Store-activated Ca^{2+} channel opening is stimulated by the emptying of intracellular Ca^{2+} stores.

Ca^{2+} is released from the endoplasmic reticulum, an organelle that acts as an intracellular Ca^{2+} store. The mechanism underlying this release is similar to that of ligand-activated Ca^{2+} channels, but the activating ligands differ. The best studied examples are the inositol triphosphate (IP_3) and ryanodine receptors, which may be modulated by binding of their cognate ligands, IP_3 and ryanodine, respectively. Interestingly, the most important regulator of Ca^{2+}

Table 1
Pharmacological agents used to selectively manipulate $[Ca^{2+}]_i$

Site of manipulation	Compound	Effect on $[Ca^{2+}]_i$	Mode of action	Solubility	Notes
Voltage-activated Ca^{2+} channels	ω-Agatoxin	–	Inhibits P-type Ca^{2+} channels	H_2O	
	(±)-Bay K 8644	+	Activates L-type Ca^{2+} channels	$EtOH/H_2O$	Induces p44/42 MAPK activation in Jurkat cells
	ω-Conotoxin	–	Inhibits N-type Ca^{2+} channels	H_2O	
	(+)-*cis*-Diltiazem hydrochloride	–	Inhibits L-type Ca^{2+} channels	$MeOH/H_2O$	Causes Ca^{2+} release from intracellular stores in neutrophils
	Mibefradil dihydrochloride	–	Inhibits T-type Ca^{2+} channels	H_2O	Inhibits L-type Ca^{2+} channels at high concentrations
	Neomycin trisulfate	–	Inhibits voltage-activated Ca^{2+} channels	H_2O	No effect on Na^+/Ca^{2+} antiporter in neurons
	Nifedipine	–	L-type Ca^{2+} channel antagonist	DMSO/EtOH	Induces apoptosis in human glioblastoma cells
	Nimodipine	–	L-type Ca^{2+} channel antagonist	MeOH	Photosensitive
Receptor-activated Ca^{2+} channels	Ruthenium red	–	Inhibits RyR-activated Ca^{2+} channels	H_2O	May also inhibit voltage-activated Ca^{2+} channels
	Ryanodine	+/–	Locks RyR-activated Ca^{2+} channels in a half open state at nM concentrations. Fully closes them in the μM range	DMSO/EtOH	RyRs are expressed primarily in skeletal and cardiac muscle, and the brain
	Xestospongin C	–	Inhibits IP_3 receptor-activated Ca^{2+} channels	DMSO/EtOH	May also inhibit voltage-activated Ca^{2+} channels
Ca^{2+}-ATPases	Cyclopiazonic acid	+	Inhibits ER/SR Ca^{2+}-ATPase	DMSO	Toxic at high concentrations
	Thapsigargin	–	Inhibits ER/SR Ca^{2+}-ATPase	DMSO/EtOH	Widely used, potent, cell-permeable inhibitor

+, increases $[Ca^{2+}]_i$; –, decreases $[Ca^{2+}]_i$
SR sarcoplasmic reticulum; *ER* endoplasmic reticulum; *RyR* ryanodine receptor

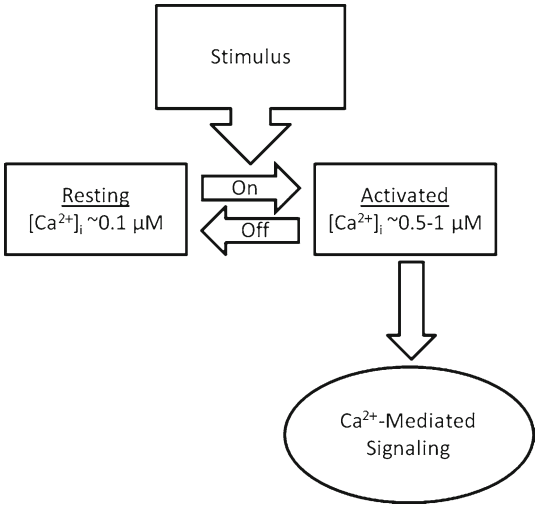

Fig. 1. The basic Ca^{2+} signaling network. A stimulus activates various "on" or "off" signals. "On" signals trigger an increase in $[Ca^{2+}]_i$ which, in turn, induces Ca^{2+}-mediated signaling events. "Off" signals restore $[Ca^{2+}]_i$ to its resting level.

channels on intracellular stores is Ca^{2+} itself. This observation forms the basis of the concept of Ca^{2+}-induced Ca^{2+} release (8, 9).

1.1.2. "Off" Signals

Off signals involve the rapid removal of intracellular free Ca^{2+} from the cytoplasm by a variety of pumps and exchangers. Ca^{2+} can be pumped out of the cell by Ca^{2+}-ATPases or Na^+/Ca^{2+} exchangers located on the plasma membrane. Alternatively, Ca^{2+} can be moved into intracellular storage compartments by Ca^{2+}-ATPases on the endoplasmic reticulum or through Ca^{2+} uniporters on the inner mitochondrial membrane.

2. Materials

2.1. Cell Culture, Treatment, and Lysis

Unless otherwise stated, all reagents are stored at room temperature (~22°C).

1. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/glutamine (PSG). Store at 4°C.
2. DMEM supplemented with 1% PSG and 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Store at 4°C.
3. 0.05% Trypsin/ethylenediamine tetraacetic acid (EDTA). Store for up to 1 month at 4°C.
4. Sterile phosphate-buffered saline (PBS).

5. A23187 (Sigma, St. Louis, MO) (50 mg/ml in dimethyl sulfoxide (DMSO)). Store in single use aliquots at –80°C. See Notes 1 and 2.
6. 1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (tetra(acetoxymethyl) ester) (BAPTA-AM) (Sigma, St. Louis, MO) (15 mg/ml in DMSO). Store in single use aliquots at –80°C. See Notes 3 and 4.
7. EGF (Gibco, Carlsbad, CA) (1 mg/ml in sterile PBS). Store in single use aliquots at –80°C. See Note 5.
8. PBS: 150 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 (pH 7.4). Store at 4°C.
9. Lysis buffer: 50 mM Tris–HCl, 150 mM NaCl, 1% Triton X-100. Store at 4°C. Prior to use, add 1% 0.1 M phenylmethanesulfonyl fluoride (PMSF), 0.1% Protease Inhibitor Cocktail (Sigma, St. Louis, MO), 10 μg/ml leupeptin, 1% Phosphatase Inhibitor Cocktail 1 (Sigma, St. Louis, MO) and 1% Phosphatase Inhibitor Cocktail 2 (Sigma, St. Louis, MO).
10. Disposable cell lifters.
11. 6× Sample buffer: 180 mM Tris (pH 6.8), 12% (w/v) sodium dodecyl sulfate (SDS), 50% glycerol, 10% (w/v) dithiothreitol, 0.006% (w/v) bromophenol blue. Store at 4°C.

2.2. SDS-Polyacrylamide Gel Electrophoresis

1. 4× Protogel Separating Buffer (National Diagnostics, Atlanta, GA).
2. 4× Protogel Stacking Buffer (National Diagnostics, Atlanta, GA).
3. Protogel (National Diagnostics, Atlanta, GA).
4. 10% (w/v) Ammonium persulfate (APS).
5. *N*,*N*,*N*′,*N*′-tetra-methyl-ethylenediamine (TEMED).
6. Isobutanol: decant 25 ml into a 500 ml spray bottle and use vapor.
7. Running buffer: 50 mM Tris, 0.4 M glycine, 0.1% (w/v) SDS.
8. All Blue Precision Plus Protein Standards (Bio-Rad, Hercules, CA). Store at –20°C.

2.3. Western Blotting for Active MAPK

1. Transfer buffer: 30 mM Tris, 0.25 M glycine. Prior to use, to 800 ml transfer buffer add 200 ml MeOH and 2 ml 10% (w/v) SDS.
2. Prefrozen ice container.
3. Immobilon-P Transfer Membrane (0.45 μm pore) (Millipore, Bedford, MA).
4. MeOH.

5. Tris-buffered saline with Tween (TBS-T): 10 mM Tris, 150 mM NaCl, 0.2% Tween-20 (pH 8.0).
6. Blocking buffer: 4% (w/v) bovine serum albumin (BSA) in TBS-T. Store at 4°C.
7. 10% (w/v) sodium azide.
8. Primary antibody: Anti-phospho-p44/42 MAPK rabbit monoclonal antibody (Cell Signaling Technology, Danvers, MA (catalog no. 4370)). Store at –20°C.
9. Secondary antibody: Horseradish peroxidase (HRP)-linked anti-rabbit immunoglobulin G (GE Healthcare, Buckinghamshire, UK). Store at 4°C.
10. Immobilon Western Chemiluminescent HRP Substrate Kit (Millipore, Bedford, MA). Store at 4°C.
11. Kodak BioMax XAR X-ray Film (Carestream Health, Rochester, NY).

2.4. Stripping Blots and Reprobing for Total MAPK

1. Stripping buffer: 62.5 mM Tris–HCl (pH 6.8), 2% (w/v) SDS, 0.7% β-mercaptoethanol. Make fresh as required.
2. Primary antibody: Anti-p44/42 MAPK mouse monoclonal antibody (Cell Signaling Technology, Danvers, MA (catalog no. 4696)). Store at –20°C.
3. Secondary antibody: HRP-linked anti-mouse immunoglobulin G (GE Healthcare, Buckinghamshire, UK). Store at 4°C.
4. Immobilon Western Chemiluminescent HRP Substrate Kit. Store at 4°C.
5. Kodak BioMax XAR X-ray Film.

2.5. Confocal Immunofluorescence for Active and Total MAPK

1. Microscope Cover Glass.
2. Lab-Tek four-well Glass Chamber Slides.
3. PBS. Store at 4°C.
4. 4% (w/v) Paraformaldehyde (PFA) in PBS. Store at 4°C. See Note 6.
5. Blocking and permeabilization buffer: 0.2% Triton X-100, 3% BSA in PBS. Store at 4°C.
6. Antibody diluent: 0.2% Triton X-100, 1% BSA in PBS. Store at 4°C.
7. Primary antibodies: Anti-phospho-p44/42 MAPK rabbit monoclonal antibody and anti-p44/42 MAPK mouse monoclonal antibody. Store at –20°C.
8. Secondary antibodies: Alexa-Fluor 488-labeled anti-rabbit immunoglobulin G (Molecular Probes, Carlsbad, CA) and Alexa-Fluor 488-labeled anti-mouse immunoglobulin G (Molecular Probes, Carlsbad, CA). Store both in light-protected single use aliquots at –20°C.

9. Nuclear stain: 4,6-diamidino-2-phenylindole (DAPI) (Molecular Probes, Carlsbad, CA). Store in light-protected single use aliquots at –20°C. See Notes 7 and 8.
10. Mounting medium: PermaFluor Aqueous Mounting Medium (Fisher, Pittsburgh, PA). Store at 4°C.

3. Methods

3.1. Cell Culture, Treatment, and Lysis

1. Passage cells when approaching confluence by washing with sterile PBS and detaching with prewarmed 0.05% trypsin/EDTA. One 100 mm dish is required for each data point (each dish holds a volume of ~5–10 ml). Allow cells to attach and approach confluence in prewarmed DMEM supplemented with 10% FBS and 1% PSG.
2. At 90–100% confluence, rinse cells twice with sterile PBS. Starve cells of serum by incubating in prewarmed (37°C) DMEM supplemented with 1% PSG and 1 mM HEPES for 16 h at 37°C.
3. Prepare all materials for cell treatment and lysis (see Notes 1–5). Other materials also required at this stage include three prechilled and labeled microcentrifuge tubes per dish, PBS and lysis buffer (both at 4°C), disposable cell lifters, 70% EtOH and 6× sample buffer.
4. Aspirate growth medium from each 100 mm dish and replace with medium containing either vehicle (DMSO), A23187 or BAPTA-AM as appropriate. Incubate for 20 min at 37°C. See Notes 1–4 and 9.
5. Treat each experimental culture with either vehicle (0.01% BSA) or 100 ng/ml EGF as appropriate. Incubate for 5 min at 37°C. See Note 10.
6. Immediately place all 100 mm dishes on ice and aspirate growth medium. Wash rapidly with cold PBS. Aspirate and add 500 µl cold lysis buffer to each dish. Swirl dishes gently to ensure even coverage. See Note 11.
7. Using a disposable cell lifter, scrape the contents of each 100 mm dish into an appropriately labeled prechilled microcentrifuge tube. Rinse the disposable cell lifter in 70% EtOH between samples. Sonicate twice at high power for 5–10 s and clarify by high speed centrifugation (~15,000 × *g*) for 10 min at 4°C. See Note 12.
8. Carefully aspirate supernatant and transfer into a separate appropriately labeled prechilled microcentrifuge tube. Discard pellet. If desired, protein concentration in an aliquot of the supernatant may be measured using the Modified Bradford Assay (Bio-Rad, Hercules, CA).

9. To 10 µl 6× sample buffer, add 40 µl lysate (or an appropriate normalized volume). Mix well and boil at 100°C for 5 min. Centrifuge briefly, cool to 22°C and proceed to Subheading 3.2. See Notes 13–15.

3.2. SDS-PAGE

This protocol describes the use of the Bio-Rad Mini-Blot Gel System (Bio-Rad, Hercules, CA). Nevertheless, it is easily adaptable to other formats.

1. Prior to (and following) each use, clean each glass plate with 70% EtOH and rinse well with ddH_2O.
2. Prepare a 1.5 mm thick separating gel of the appropriate percentage (Table 2). After addition of TEMED, proceed immediately to step 3.
3. Pour the gel ensuring that ~1.5 cm of space is left at the top for the stacking gel. Use isobutanol vapor to remove any air bubbles. Polymerization should take place in 30–45 min.
4. Prepare the stacking gel by mixing 330 µl Protogel, 630 µl 4× Protogel Stacking Buffer, 1.53 ml ddH_2O, 12.5 µl 10% (w/v) APS and 2.5 µl TEMED. Pour the stack, use isobutanol vapor to remove any air bubbles and carefully insert the comb. Polymerization should take place in 30–45 min. See Note 16.
5. Once the stacking gel has set, carefully remove the comb and use a 5 ml syringe fitted with a 22-gauge needle to wash the wells with running buffer.
6. Assemble the gel unit and fill each chamber with running buffer. Load 10 µl All Blue Precision Plus Protein Standard in well 1. Each sample should be added carefully to a separate well.

Table 2
Separating gel components for different % acrylamide gels

Component	6%	8%	10%	12%	15%
Protogel (ml)	2.0	2.7	3.4	4.0	5.0
4× Protogel separating buffer (ml)	2.5	2.5	2.5	2.5	2.5
ddH_2O (ml)	5.4	4.7	4.0	3.4	2.4
10% (w/v) APS (µl)	100	100	100	100	100
TEMED (µl)	10	10	10	10	10
Typical protein size resolved (kDa)[a]	60–300	40–300	20–300	20–200	10–150

[a]The range of proteins resolved using different % gels is based on our experience using the reagents in this protocol. The use of other reagents may substantially alter these values

7. Complete the assembly of the gel unit and connect to a power supply. Run at 50 mA for ~60 min or until the dye front reaches the bottom of the gel.

3.3. Western Blotting for Active MAPK

1. At this stage it is necessary to prepare for gel transfer. This protocol assumes the use of a "wet" transfer system but is easily adaptable to the "semi-dry" equivalent. Cut a piece of Immobilon-P Transfer Membrane approximately 7 cm × 5 cm in size and soak thoroughly for ~5 min in MeOH. After soaking, rinse thoroughly with ddH_2O and soak in transfer buffer until SDS-PAGE (SDS-Polyacrylamide Gel Electrophoresis) is complete. Both foam pads of the transfer cassette should also be soaked thoroughly in transfer buffer for at least 30 min prior to use (see Note 17).
2. Disconnect the gel unit from the power supply and disassemble. Using a clean razor blade, cut away the stacking gel and discard. Similarly, if still present, cut away anything below the dye front on the separating gel. Carefully submerge the remaining separating gel in transfer buffer.
3. Assemble the transfer cassette as follows: open the cassette and place one soaked foam pad on each side. Place the separating gel on a foam pad and carefully lay the Immobilon-P Transfer Membrane on top. Gently remove any air bubbles in the stack by rolling with a clean test tube, then place the other foam pad on top. Gently remove any air bubbles again and close the transfer cassette (see Note 18).
4. Place the transfer cassette into the transfer tank such that the separating gel is closest to the negative cathode and the Immobilon-P Transfer Membrane to the positive anode. This orientation is critical or the proteins will be lost. Fill the transfer tank with transfer buffer and drop in a small magnetic stir bar. Slot a prefrozen ice container into place.
5. Put the lid on the transfer tank and connect to a power supply. Place the apparatus on a magnetic stirrer and switch on. Transfer at 100 V for 1 h (see Notes 19 and 20).
6. Disconnect the transfer tank from the power supply and remove the transfer cassette. Discard the separating gel and place the Immobilon-P Transfer Membrane in a clean plastic container. If the transfer was successful, the All Blue Precision Plus Protein Standards should be clearly visible.
7. Incubate the Immobilon-P Transfer Membrane in 10 ml blocking buffer for 1 h at 22°C or overnight at 4°C.
8. Prepare the primary antibody solution as follows: To 10 ml blocking buffer add 100 µl 10% sodium azide and 10 µl anti-phospho-p44/42 MAPK. Store at 4°C.

9. Remove the blocking buffer and incubate the Immobilon-P Transfer Membrane in 10 ml primary antibody solution for 1 h at 22°C or overnight at 4°C (see Note 21).
10. Remove the primary antibody solution and wash the Immobilon-P Transfer Membrane three times for 10 min each with 20 ml TBS-T.
11. The secondary antibody is freshly prepared for each experiment. To 10 ml blocking buffer add 2 µl HRP-linked anti-rabbit immunoglobulin G. After washing is complete, add the secondary antibody and incubate for 1 h at 22°C.
12. Remove the secondary antibody and wash the Immobilon-P Transfer Membrane three times for 10 min each with 20 ml TBS-T.
13. Once the final wash is finished, mix together 1 ml of each reagent in the Immobilon Western Chemiluminescent HRP Substrate Kit and pour on to the Immobilon-P Transfer Membrane. Rotate using forceps for 1.5 min to ensure even coverage and place between the leaves of a lightweight sheet protector that has been pre-cut to the same size as an X-ray film cassette.
14. Place the sheet protector in the X-ray film cassette and proceed immediately to a dark room. Delays at this stage in the protocol will result in loss of the chemiluminescent signal.
15. Under safe light conditions, place a sheet of Kodak BioMax XAR X-ray film into the cassette and expose for a suitable time. For most proteins, including phospho-p44/42 MAPK, typical exposure times range between 1 s and 1 min.

3.4. Stripping Blots and Reprobing for Total MAPK

1. Upon satisfactory exposure of active phosphorylated MAPK, it is necessary to strip the Immobilon-P Transfer Membrane and reprobe with an antibody that recognizes both phosphorylated and nonphosphorylated MAPK. This provides a loading control and allows quantification of the various EGF-stimulated responses (see Note 22).
2. Using a preheated waterbath, incubate the Immobilon-P Transfer Membrane in 50 ml stripping buffer for 30 min at 55°C (see Note 23).
3. Remove the stripping buffer and wash the Immobilon-P Transfer Membrane six times for 5 min each with 20 ml TBS-T.
4. Incubate the Immobilon-P Transfer Membrane in 10 ml blocking buffer for 1 h at 22°C or overnight at 4°C.
5. Prepare the primary antibody solution as follows: To 10 ml blocking buffer add 100 µl 10% sodium azide and 10 µl anti-p44/42 MAPK. Store at 4°C.

6. Remove the blocking buffer and incubate the Immobilon-P Transfer Membrane in 10 ml primary antibody solution for 1 h at 22°C or overnight at 4°C (see Note 21).
7. Remove the primary antibody solution and wash the Immobilon-P Transfer Membrane three times for 10 min each with 20 ml TBS-T.
8. As before, the secondary antibody is freshly prepared for each experiment. To 10 ml blocking buffer add 2 μl HRP-linked anti-mouse immunoglobulin G. After washing is complete, add the secondary antibody and incubate for 1 h at 22°C.
9. Remove the secondary antibody and wash the Immobilon-P Transfer Membrane three times for 10 min each with 20 ml TBS-T.
10. Repeat steps 13–15 in Subheading 3.3. Typical exposure times for p44/42 MAPK range from between 1 and 20 s.

3.5. Confocal Immunofluorescence for Active and Total MAPK

1. Passage cells when approaching confluence by washing with sterile PBS and detaching with prewarmed 0.05% trypsin/EDTA. One well of a Lab-Tek 4-well Glass Chamber Slide is required for each data point (each well holds a volume of ~500 μl). Allow cells to attach and approach confluence in prewarmed DMEM supplemented with 10% FBS and 1% PSG.
2. At 70–80% confluence, rinse cells twice with sterile PBS. Starve cells of serum by incubating in prewarmed (37°C) DMEM supplemented with 1% PSG and 1 mM HEPES for 16 h at 37°C (see Note 24).
3. Prepare all materials for cell treatment and permeabilization (see Notes 1–5). Other materials also required at this stage include PBS, PFA, and blocking and permeabilization buffer (all at 4°C).
4. Aspirate growth medium from each well and replace with medium containing either vehicle (DMSO), A23187 or BAPTA-AM as appropriate. Incubate for 20 min at 37°C (see Notes 1–4 and 9).
5. Treat each experimental culture with either vehicle (0.01% BSA) or 100 ng/ml EGF as appropriate. Incubate for 5 min at 37°C (see Note 10).
6. Immediately place all Lab-Tek four-well Glass Chamber Slides on ice and aspirate growth medium. Wash rapidly with cold PBS. Aspirate and add 500 μl cold PFA to each well. Leave for 20 min at 22°C.
7. Wash twice with cold PBS. Aspirate and add 500 μl cold blocking and permeabilization buffer to each well. Leave for 1 h at 22°C.

8. The primary antibody solution is prepared freshly for each experiment. To 500 µl antibody diluent add 5 µl anti-phospho-p44/42 MAPK or 5 µl anti-p44/42 MAPK.
9. Remove the blocking and permeabilization buffer and incubate the experimental cultures in 500 µl primary antibody solution overnight at 4°C.
10. Remove the primary antibody solution and wash three times with cold PBS. The experimental cultures are protected from light for all subsequent steps.
11. The secondary antibody is freshly prepared for each experiment. To 500 µl antibody diluent add 1 µl Alexa-Fluor 488-labeled anti-rabbit immunoglobulin G or 1 µl Alexa-Fluor 488-labeled anti-mouse immunoglobulin G. Add the secondary antibody and incubate for 1 h at 22°C.
12. Remove the secondary antibody and wash three times with cold PBS. Incubate the experimental cultures in 500 µl DAPI for 5 min at 22°C (see Notes 7 and 8).
13. Remove the DAPI and wash three times with cold PBS. Aspirate all the liquid and carefully remove the wells using the supplied tool. Apply ~2–3 ml PermaFluor Aqueous Mounting Medium and a Microscope Cover Glass. Leave in light-protected conditions for 24 h at 4°C.
14. View the slides using phase-contrast microscopy to locate the cells and identify the focal plane. Under confocal conditions, excitation at 488 nm induces green fluorescence for either phospho-p44/42 MAPK or p44/42 MAPK. Excitation at 364 nm induces blue fluorescence for DAPI (see Note 25).

4. Notes

1. Working solutions of A23187 are prepared by diluting to 50 µg/ml in DMSO and subsequent dilution to 5 ng/ml in DMEM supplemented with 1% PSG and 1 mM HEPES.
2. A23187 is a selective Ca^{2+} ionophore (10). It greatly increases the ability of divalent ions to cross biological membranes by forming stable 2:1 complexes with them, thus rendering them cell-permeable. A23187 is commonly used to increase $[Ca^{2+}]_i$ in intact cells. A less Ca^{2+}-selective alternative is Ionomycin (Sigma, St. Louis, MO).
3. Working solutions of BAPTA-AM are prepared by diluting to 30 µg/ml in DMSO and subsequent dilution to 30 ng/ml in DMEM supplemented with 1% PSG and 1 mM HEPES.

4. BAPTA-AM is a Ca^{2+} chelator with 105-fold greater affinity for Ca^{2+} than for Mg^{2+} (10). Once inside the cell, the acetoxymethyl moiety is hydrolyzed by cytosolic esterases and BAPTA, which is unable to cross the plasma membrane, is trapped intracellularly.
5. Working solutions of EGF are prepared by diluting to 100 μg/ml in 0.01% BSA.
6. To dissolve PFA, heat to ~50°C with constant stirring. Precipitation after long term storage indicates that the solution should be discarded.
7. Working solutions of DAPI are prepared by dilution to 200 ng/ml in PBS.
8. DAPI is a known carcinogen. Always wear gloves.
9. It is our experience that the concentrations and incubation times of A23187 and BAPTA-AM we have suggested are sufficient to elicit effects on EGF-induced MAPK activation. Nevertheless, incubation of each reagent at different concentrations for different times should be performed in order to optimize the protocol for each cell type.
10. EGF typically induces maximal p44/42 MAPK activation ~2–5 min poststimulation. Nevertheless, stimulation at different concentrations for different times should be performed to optimize the protocol for each cell type.
11. 500 μl is the recommended initial lysis volume. It can be decreased in order to concentrate protein should a satisfactory phospho-p44/42 MAPK signal not be obtained.
12. Ear protection should be worn when using a sonicator.
13. 6× Sample buffer should be warmed to 22°C before use to allow accurate pipetting.
14. Microcentrifuge tube caps should be "locked" shut in order to prevent them springing open during boiling which may result in loss of some of the sample. If using conventional 1.5 ml microcentrifuge tubes, Microtube Lid Locks (Fisher, Pittsburgh, PA) provide an inexpensive way to achieve this.
15. If required, the protocol may be stopped at this point and the samples stored at –80°C.
16. We use 1.5 mm thick 10-well combs. Both 12- and 15-well models are also available, but limit the sample volume that may be loaded in each well to ~30 μl and ~10 μl, respectively.
17. The Immobilon-P Transfer Membrane is extremely hydrophobic and will not wet in aqueous solutions unless prewet in methanol. After prewetting, do not let the membrane dry. In the event it does dry, it should again be wet in MeOH.

18. Air bubbles should be carefully rolled out to avoid disturbing the flow of current from the negative cathode to the positive anode and thus the transfer of proteins from the separating gel to the Immobilon-P Transfer Membrane.
19. Stir at low speed to avoid the introduction of air bubbles.
20. Coomassie Blue staining can be used to evaluate transfer efficiency. After gel transfer, remove the Immobilon-P Transfer Membrane and incubate the separating gel in Coomassie Blue stain (50% MeOH, 10% acetic acid, 40% ddH_2O, 0.2% (w/v) Coomassie Blue) for 1 h at 22°C. After staining, wash with ddH_2O and incubate in Gel Destain Buffer (10% MeOH, 10% acetic acid, 80% ddH_2O) for ~16 h at 22°C.
21. It is our experience that both anti-phospho-p44/42 MAPK and anti-p44/42 MAPK may be reused ~20 times after which fresh primary antibody solutions should be prepared.
22. Densitometry should be performed to quantify the effect of EGF on MAPK activation. Scan the exposed Kodak BioMax XAR X-ray film into a computer and analyze using a suitable quantification program. Several software packages are available. We recommend ImageJ (available free from http://rsb.info.nih.gov/ij/index.html) as it is both accurate and easy to use. The densitometrical value of each sample when probed with anti-phospho-p44/42 MAPK should be corrected for the value of the same sample when probed with anti-p44/42 MAPK.
23. Incubation of the Immobilon-P Transfer Membrane in stripping buffer may not remove all of the protein-bound primary antibody. When reprobing for a protein of size similar to that already imaged, it is advisable to verify that all of the primary antibody has been removed. After completing step 4 in Subheading 3.4, add the secondary antibody and incubate for 1 h at 22°C without first adding the primary antibody solution. Remove the secondary antibody and wash the Immobilon-P Transfer Membrane three times for 10 min each with 20 ml TBS-T. Repeat steps 13–15 in Subheading 3.3. A positive signal indicates that not all of the primary antibody has been removed during the stripping process. Stripping again, or increasing the temperature at which the Immobilon-P Transfer Membrane is incubated in stripping buffer to 80°C, may solve this problem.
24. Seventy to eighty percent confluence is recommended for microscopy studies in order to ensure that individual cells are clearly visible under the microscope.
25. Confocal laser scanning microscopy allows high-resolution optical images to be obtained. The defining feature is the ability to optically section a sample and thus effectively produce a

three-dimensional image. Confocal microscopes are commonly used in immunofluorescence studies as they generally obtain much higher quality images than would be afforded by a fluorescent microscope.

Acknowledgments

We thank Zhigang Li for critically reviewing the text prior to submission and other members of the Sacks laboratory, past and present, for insightful discussions. Work in the authors' laboratory is funded by the National Institutes of Health (to D.B.S) and the Department of Defense Breast Cancer Research Program (to C.D.W).

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IQGAP1 is a Novel HER2 Binding Partner and Regulates HER2-Mediated Cell Proliferation

Colin David White[1], Zhigang Li[1], Mario Niepel[2] and David Barry Sacks[1]

[1] Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[2] Department of Systems Biology, Harvard Medical School, Boston, MA

ABSTRACT

The receptor tyrosine kinase HER2 is overexpressed in 25–30% of breast carcinomas. These tumors often have an increased rate of proliferation and give rise to more frequent metastases than HER2(–) neoplasms. HER2(+) breast cancer is treated with Trastuzumab, but ~60% of patients do not respond. Of those who do, ~15% subsequently develop metastatic disease. An understanding of the molecular mechanisms of HER2 signaling is necessary to develop novel therapeutics. IQGAP1 is a ubiquitously expressed 190 kDa scaffold protein. Through interaction with multiple binding partners, IQGAP1 regulates processes such as cell proliferation and migration, cell-cell adhesion and cytoskeletal remodeling. IQGAP1 plays defined roles in several human malignancies, including those of the breast. Here, we investigate the interaction between IQGAP1 and HER2. *In vitro* assays using purified IQGAP1 and the GST-tagged intracellular domain of HER2 (GST-HER2) revealed that IQGAP1 binds HER2. GST-HER2 binds IQGAP1 in Sk-BR-3 cells, and endogenous HER2 co-immunoprecipitates with endogenous IQGAP1 from cell lysates. Analogous to other IQGAP1 binding partners, calmodulin abrogates the association of IQGAP1 with HER2. Overexpression of IQGAP1 significantly increases Sk-BR-3 cell proliferation, while siRNA-mediated knockdown of IQGAP1 significantly reduces proliferation. These data suggest that IQGAP1 may be a potential target for HER2(+) breast cancer therapy.

Work in the authors' laboratory is funded by the NIH (to D.B.S.) and the DoD BCRP (to C.D.W).

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Harvard Medical School **Brigham and Women's Hospital**

Departments of Pathology

David B. Sacks, M.B., Ch.B., F.A.C.P., FRCPath
Associate Professor of Pathology

Tel: (617) 732-6627
Fax: (617) 278-6921
dsacks@rics.bwh.harvard.edu

Medical Director, Clinical Chemistry
Director, Clinical Pathology
Training Program
Brigham and Women's Hospital
75 Francis Street
Boston, Massachusetts 02115

September 22, 2010

Progress Review Committee
Congressionally-Directed Medical Research Program:

Re: BC087504 - The Role of IQGAP1 in Breast Carcinoma

Dear Committee Members:

I am writing this letter of support for Dr. Colin White, the named PI on BCRP grant number BC087504 - The Role of IQGAP1 in Breast Carcinoma. Dr. White encountered considerable problems during year 1 of this award with the studies proposed. Stable cell lines were necessary to perform most of the planned experiments. Unfortunately, Dr. White was plagued with repeated infections of his cell cultures. The problem was caused by others who use the shared cell culture facilities made available to him within the Department of Pathology, Brigham and Women's Hospital. Although we attempted several strategies to overcome these issues, none of the measures we employed was successful. As a result, the Statement of Work for the remainder of this award has been changed. Please note, the overall boundaries of the original proposal remain the same. Dr. Katherine Moore, grants manager at the CDMRP, has approved this revision.

In my opinion Dr. White has made very satisfactory progress with the revised project. He has a good work ethic and has been very productive during his time in my laboratory. I have no hesitation in recommending that his funding continue through years 2 and 3.

Yours sincerely,

David B. Sacks, M.B., Ch.B., FRCPath

BRIGHAM AND
WOMEN'S HOSPITAL

HARVARD
MEDICAL SCHOOL

Department of Pathology
Breast Pathology Services
75 Francis Street, TH613
Boston, Massachusetts 02115

Tel: 617 525-7496; Fax: 617 264-5169
Pager: 617 732-5656 # 33432
Email: ddillon@partners.org

Deborah A. Dillon, M.D.
Assistant Professor of Pathology

September 21, 2010

Dear Colin,

I am writing to indicate my enthusiastic support for your proposed work "The Role of IQGAP1 in Breast Carcinoma". Your recent preliminary results implying a possible role for IQGAP1 in HER2 signaling are exciting and clearly merit further investigation. As you know, a significant number of patients whose breast tumors are HER2 positive fail initially to respond to trastuzumab. Furthermore, of those who do initially respond, many eventually develop resistance. Thus, there is a pressing need for the discovery and validation of potential alternative targets in this pathway.

As a breast pathologist at Brigham and Women's Hospital/Dana Farber Cancer Institute with a particular interest in the development of novel markers of potential diagnostic/therapeutic relevance for breast cancer patients, I have considerable experience in the molecular and immunohistochemical analysis of formalin-fixed paraffin embedded breast cancer tissues. Tissues that we may access for the purposes of the proposed study include anonymous excess tumor tissue of patients undergoing resection at Brigham and Women's Hospital. In addition, I have recently assembled into tissue microarrays the tissue blocks of >600 patients consented for linkage of molecular markers with treatment and outcome. Of particular importance to this proposal, both the anonymous and the linked tissues are of known estrogen receptor, progesterone receptor and HER2 status, allowing us to determine if IQGAP1 expression might be linked to one of the important clinical treatment tumor phenotypes.

In summary, I can provide you with the tissues you need to accomplish the goals of the proposed research and look forward to assisting in the analysis of IQGAP1 in these tissues. This is an exciting project with the potential to broaden the number of specific molecular targets available for breast cancer therapy.

Best regards,

Deborah

Deborah Dillon, MD
Assistant Professor of Pathology
Breast Pathologist, Brigham and Women's Hospital/Dana-Farber Cancer Institute