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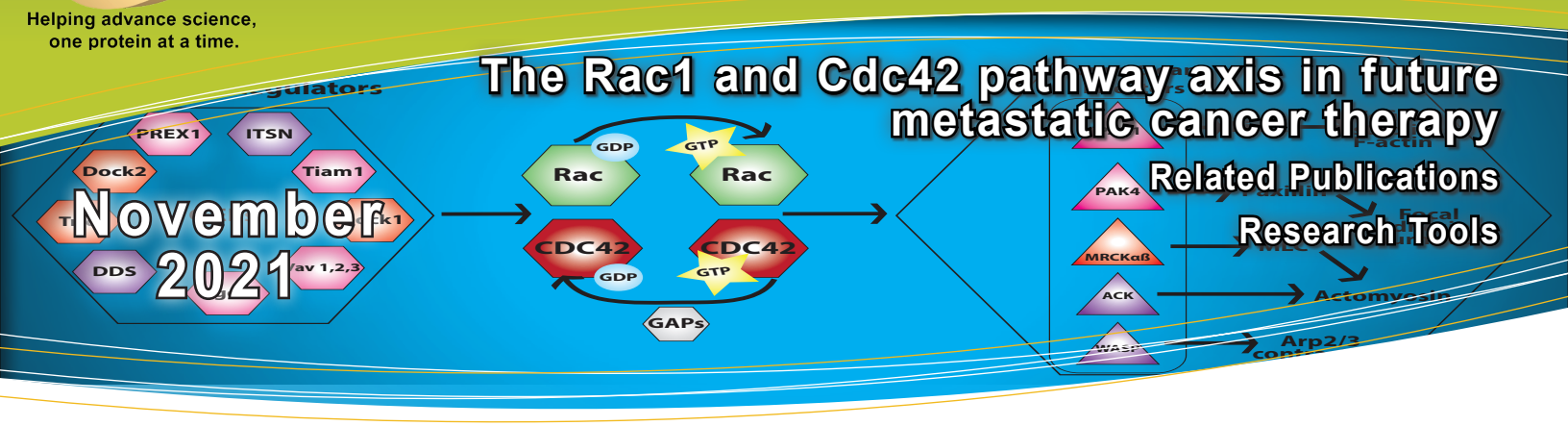


## The Rac1 and Cdc42 pathway axis in future metastatic cancer therapy

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## Rac1 and Cdc42 pathways as pivotal axis in future metastatic cancer therapy

The appearance of Rac and Cdc42 proteins on the radar of the medical scientific community started in the 1990's with seminal papers by Professor Alan Hall's laboratory at MRC, UK. They identified Rac and Cdc42 small G-proteins (R&C) as central regulators of cell motility, shape and protrusions (lamellipodia and filopodia)[1,2]. Now several decades later, the concept has been fully proven in a multitude of publications (see refs. in these reviews 3 & 4), and the realization that these proteins are central to cancer cell motility and invasion has led the field to explore them as candidates for therapeutic intervention. Targeting metastatic cancer has become mainstream due to an average of 58% of cancer-related deaths, 10-90% depending on cancer type, are due to consumption by metastasis[5,6]. It must be noted, therapies based on the R&C axis are cell stasis interventions rather than the more typical cytotoxic cancer therapies such as cisplatin and paclitaxel; however, these different mechanisms could be more interdependent than previously thought.

### The Rac & Cdc42 central axis

As shown in Figure 1, the Rho family small G-proteins hold a central regulator focal point between upstream receptor signaling pathways and down stream effector pathways. In some instances, this centrality is highlighted with mutated isoforms or altered levels of protein which directly link to cancer development and prognosis. For example, the human Rac1 mutation P29S/L is present in melanomas[7] where haptotaxis (directionality) and velocity of cells are reduced[8], and also Programmed Cell Death Ligand (PD-L1) is up-regulated[9]. In contrast, elevated levels of Cdc42 expression in mammary gland epithelial cells increased motility and invasion and poor prognosis of metastatic breast cancer[10]. These effects are highly dominant, but when considering other genes, which also dominate a metastatic phenotype, the frequency of their occurrence is very low i.e. mutations occur at 0-1%, and altered levels at 0.5-4.0%[11,12]. On the other hand, these proteins are ubiquitous in all mammalian cell types, so therapeutic intervention has to circumvent the normal cell's functional requirements. As a result, most of the early R&C direct binding drug candidates have faltered due to toxic side effects (reviewed in 13). In an effort to find a good therapeutic window, the field is turning its focus to the upstream and downstream regulators of the R&C axis as shown in Fig1.

### Upstream Activators

Upstream activators are dominated by GTP Exchange Factors (GEFs), which number almost 100 different genes in the human genome. GEFs are activated by cell surface receptors e.g. GPCRs, RTKs, integrins. In turn, GEFs activate small G-proteins by increasing GTP/GDP exchange rate at the nucleotide-binding site. e.g. Tiam, Vav1 and Vav2 are GEFs of Rac, and Dbl, Dbs, and ITSN are GEFs of Cdc42 and some, Tiam1, Vav1,2 activate both GTPases (Fig.1). Due to Mg2+:GTP having a higher binding affinity than GDP, and a ten-fold higher level than GDP in cells, the exchange of nucleotides favors GTP in a homeostatic cancer cell. GEFs can be upregulated by mutations that increase binding affinity to the small G-protein, or mutations that simulate its phosphorylated form, or simply increased expression via demethylation of their promoter regions (reviewed in 14).

Only in the last 10 years, has the prevalence of GEF oncogenes in metastatic cancer been found to be broad

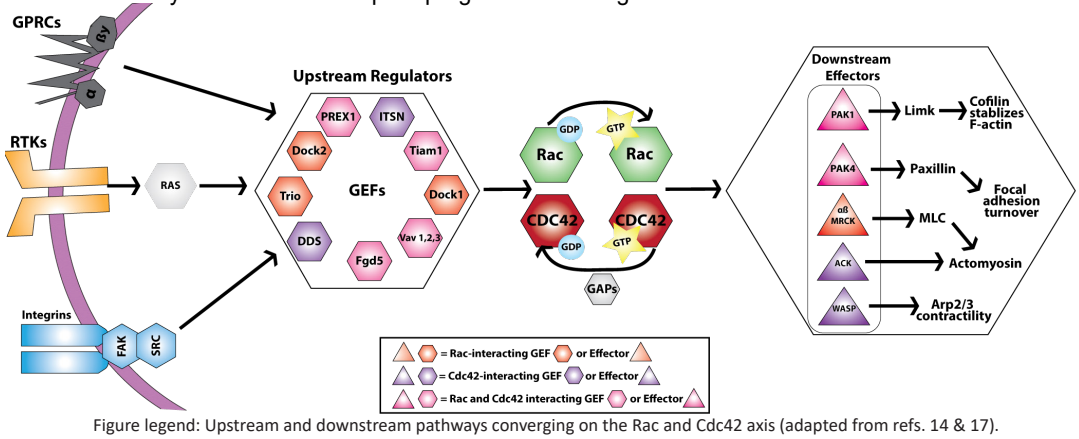


Figure legend: Upstream and downstream pathways converging on the Rac and Cdc42 axis (adapted from refs. 14 & 17).

## Sponsored Conferences

Society for Neuroscience 2021
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Journal of Cell Science Meeting 2022
Wotton House, Surrey, UK
May 8th-11th 2022

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# Rac1 and Cdc42 Detection PRODUCTS

## Continued from Page 1

due to the advent of genomic databases[15]. Although GEFs are broadly represented as oncogenes, individually mutated GEF genes make up a small portion of the total pool of players. For example, in melanoma metastasis, Tiam GEF mutations have a 9.0% prevalence while Vav2 mutations have a 3.6% prevalence, whereas all mutated RC GEFs are present in 80.7% of melanomas[14]. Under these circumstances, many GEF targets are necessary for a comprehensive drug development effort, and tumor screening is necessary to delineate causative GEF genes in individual patients. There are many options for drug development in R&C GEFs, for example small molecules could bind the protein interaction site, or an allosteric site, or mask the phosphorylation site thus inhibiting its activity[16].

**Downstream Activators**

Fewer players are currently known on the downstream side of the R&C axis. Most are kinases that are activated by GTP-bound forms of R&C. In particular, p21-activated kinase (PAK), activated Cdc42-associated kinase (ACK), and more recently myotonic dystrophy-related Cdc42 binding kinase (MRCK) have been the focus of drug development efforts[17]. For example, Unbekandt et al. (2018) present a tour de force of MRCK inhibitor development for melanoma[18].

As a side note, many of the oncogenic GEFs' promotors are demethylated during transition to metastasis[14], which could conceivably be targeted with demethylase inhibitors, but the question of specificity comes into the picture when using those enzymes with a broad, sometimes undefined, substrate range.

**Interdependence of the Ras and R&C axes in metastatic cancer**

Critically, it has been reported that primary cancer oncogenes, such as K-Ras and N-Ras, work in concert with the R&C pathway when transitioning from primary to metastatic phenotype[19,20], which makes primary cancer progression to metastasis highly dependent on R&C axis activation/dysfunction. Some GEFs, e.g. P-Rex, have been shown to contribute at a later stage metastatic cancer progression[21,22], which opens the possibility to develop later stage-specific therapeutics. The multitude of RC axis modulators make it essential to develop diagnostics to determine which GEFs and effectors are differentially expressed or mutated, this information will allow oncologists to pick the matched drug for a particular patient.

Although metastatic cancer is destined to kill on average 58% of cancer patients[5,6], this realization is not equated to the NIH & FDA's focus to encourage therapeutics in this area. In fact, only 16% of active cancer clinical trials are for metastatic disease[23]. Clinical trials targeting prevention and treatment of metastasis are a difficult sell when evidence of metastasis is not apparent early in the disease. And hence, its imperative for scientists and clinical oncologists to communicate this aspect at every opportunity so the barriers are decreased as early as possible. Cytoskeleton is proud to provide highly dependable and accurate Pulldown and GLISA Activation Assay Kits which have been used by researchers in this field over the past 20 years.

## Bead pull-down Activation Assays

| Product | Assays | Cat. # |
|---|---|---|
| **Cdc42 Activation Assay Biochem Kit** | **50** | **BK034** |
| **Rac1 Activation Assay Biochem Kit** | **50** | **BK035** |
| **Ras Activation Assay Biochem Kit** | **50** | **BK008** |
| **RhoA Activation Assay Biochem Kit** | **80** | **BK036** |
| **RhoA/Rac1/Cdc42 Activation Assay Combo Biochem Kit** | **3 x 10** | **BK030** |

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23 Clinicaltrials.gov as of 2021-09-16, and search for "cancer" and limit to "active, not recruiting". Then calculate percent of titles containing the word "metastatic". Result: 1271 active, not recruiting, cancer trials, wherein 203 contained the word "metastatic" in the title.

## G-LISA Activation Assay Kits

| Product | Assays | Cat. # |
|---|---|---|
| **Cdc42 G-LISA™ Activation Assay Kit** (Colorimetric format) | **96** | **BK127** |
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