



Met receptor tyrosine kinase: enhanced signaling through adapter proteins

Kyle A Furge¹, Yu-Wen Zhang¹ and George F Vande Woude^{*1}

¹Van Andel Research Institute, 333 Bostwick, N.E., Grand Rapids, Michigan, MI 49503, USA

The Met receptor tyrosine kinase is the prototypic member of a small subfamily of growth factor receptors that when activated induce mitogenic, motogenic, and morphogenic cellular responses. The ligand for Met is hepatocyte growth factor/scatter factor (HGF/SF) and while normal HGF/SF-Met signaling is required for embryonic development, abnormal Met signaling has been strongly implicated in tumorigenesis, particularly in the development of invasive and metastatic phenotypes. Following ligand binding and autophosphorylation, Met transmits intercellular signals using a unique multi-substrate docking site present within the C-terminal end of the receptor. The multisubstrate docking site mediates the binding of several adapter proteins such as Grb2, SHC, Crk/CRKL, and the large adapter protein Gab1. These adapter proteins in turn recruit several signal transducing proteins to form an intricate signaling complex. Analysis of how these adapter proteins bind to the Met receptor and what signal transducers they recruit have led to more substantial models of HGF/SF-Met signal transduction and have uncovered new potential pathways that may be involved into Met mediated tumor cell invasion and metastasis. *Oncogene* (2000) 19, 5582–5589.

Keywords: receptor tyrosine kinase; signal transduction; c-Met; hepatocyte growth factor/scatter factor; HGF/SF

Introduction

Induction of several biological activities critical for development and maintenance of normal cellular functions such as proliferation, motility, survival, and differentiation are mediated by protein growth factors and their associated receptor tyrosine kinases. The Met receptor tyrosine kinase (RTK) was discovered as an activated oncogene (Cooper *et al.*, 1984; Park *et al.*, 1986). The growth factor that binds and activates Met is commonly named HGF/SF as it was identified independently as both a growth factor for hepatocytes (HGF) and a fibroblast-derived cell motility factor, scatter factor (SF) (Bottaro *et al.*, 1991; Nakamura *et al.*, 1984; Stoker and Perryman, 1985). Consistent with the idea that this receptor-growth factor pair can induce different cellular responses depending on the cellular context, activation of Met by HGF/SF *in vitro* leads to increased hepatocyte, renal tubule cell, and endothelial cell proliferation (Bussolino *et al.*, 1992; Kan *et al.*, 1991), stimulation of both cell dissociation and motility ('scattering') (Rosen *et al.*, 1990), and stimulation of cell movement through the extracellular

matrix ('invasion') (Jeffers *et al.*, 1996c; Matsumoto *et al.*, 1994; Rong *et al.*, 1994; Weidner *et al.*, 1990). In addition, HGF/SF-Met signaling can induce several different epithelial and mesenchymal cell types to undergo an involved differentiation program termed branching morphogenesis when the cells are grown in a three dimensional matrix (Brinkmann *et al.*, 1995; Jeffers *et al.*, 1996c; Montesano *et al.*, 1991a; Niemann *et al.*, 1998). During branching morphogenesis, groups of cells proliferate, migrate, and differentiate to form a connected series of tubules arranged like branches from a tree. However, even in the absence of a three dimensional matrix, signaling through the Met receptor can induce morphogenesis and lumen formation in certain cell types (Jeffers *et al.*, 1996a; Tsarfaty *et al.*, 1992).

In vivo, Met expression is predominately found in cells of epithelial origin while HGF/SF expression is usually restricted to fibroblasts and stromal cells in the surrounding mesenchyme (Sonnenberg *et al.*, 1993). Several aspects of organogenesis such as tissue growth and morphogenic differentiation are regulated by interactions between the organ epithelia and the surrounding mesenchyme. As such, paracrine signaling between HGF/SF and Met is believed play an important role in regulating these epithelial-mesenchyme interactions (Tsarfaty *et al.*, 1994). *In vivo*, Met and HGF/SF likely play a key role in regulating many aspects of embryonic development including kidney and mammary gland formation (Santos *et al.*, 1994; Soriano *et al.*, 1995; Woolf *et al.*, 1995; Yang *et al.*, 1995), migration/development of muscle and neuronal precursors (Bladt *et al.*, 1995; Streit *et al.*, 1995), and liver and placenta organogenesis (Schmidt *et al.*, 1995; Uehara *et al.*, 1995). HGF/SF-Met signaling also promotes angiogenesis (Bussolino *et al.*, 1992; Grant *et al.*, 1993) and has been described to facilitate wound healing (Nusrat *et al.*, 1994) and tissue regeneration (Matsumoto and Nakamura, 1993).

While normal HGF/SF-Met signaling is involved in many aspects of embryogenesis, abnormal HGF/SF-Met signaling has been implicated in both tumor development and progression (Jeffers *et al.*, 1996d). In particular, HGF/SF-Met signaling was shown to play a significant role in promoting tumor cell invasion and metastasis (Rong *et al.*, 1994). Recently, Met mutations have been identified in patients with papillary renal carcinoma, metastatic head and neck squamous cell carcinomas, and isolated cases of ovarian cancer and early-onset hepatocellular carcinoma (Schmidt *et al.*, 1997, 1998; Olivero *et al.*, 1999; Di Renzo *et al.*, 2000; Tanyi *et al.*, 1999; Park *et al.*, 1999). Point mutations within Met, primarily mutations within the activation loop of the tyrosine kinase domain, can lead to constitutive Met activation. Constitutive Met activation can also arise from inappropriate expression of

*Correspondence: GF Vande Woude

HGF/SF in cells that have already expression Met (or vice-versa) forming an autocrine stimulatory loop (Bellusci *et al.*, 1994; Jeffers *et al.*, 1996b; Rahimi *et al.*, 1998; Rong *et al.*, 1992, 1994). Cell lines generated that express mutated Met receptors that mimic those found in human cancers or cell lines that express both Met and HGF/SF form tumors that are both highly invasive and metastatic (Jeffers *et al.*, 1997, 1998; Rong *et al.*, 1992, 1994). Additionally, several groups have reported that Met and/or HGF/SF expression is increased in a variety of human tumors and often is associated with high tumor grade and poor prognosis (reviewed in Jeffers *et al.*, 1996d). Met likely activates similar signal transduction pathways to promote invasive growth both during embryonic development and tumor progression. Therefore, insights into the signaling requirements for either one of these events will likely be applicable to both. As it is now well established that receptor tyrosine kinases stimulate various signal transduction pathways by organizing large signaling complexes proximal to the receptor following growth factor stimulation, this review will focus on some of the critical components of the Met signaling complex(s) and how these components are recruited to the activated receptor.

c-Met and related RTKs

The Met tyrosine kinase is the prototypic member of a small subfamily of RTKs that can induce proliferation, cell movement, and morphogenic differentiation in several different cell types (Brinkmann *et al.*, 1995; Jeffers *et al.*, 1996b,c; Medico *et al.*, 1996; Montesano *et al.*, 1991b; Santoro *et al.*, 1996). The growth factors that bind and activate members of the Met receptor subfamily are sometimes called plasminogen-related growth factors (PRGFs) since they all are large proteins (~100 kD) that have a domain structure similar to the blood protease plasminogen (Gherardi *et al.*, 1997). HGF/SF is the prototypic PRGF and is sometimes referred to as PRGF-1. Similar to the production of active plasminogen, biologically active HGF/SF results from cleavage of a single chain HGF/SF precursor (proHGF) by the urokinase protease or other proteases to produce a mature, disulfide-linked, α - β heterodimer (Figure 2) (Nakamura *et al.*, 1987; Naldini *et al.*, 1992; Shimomura *et al.*, 1992, 1995). However unlike plasminogen, HGF/SF and its relatives lack two active site residues that are critical for proteolysis and have no detectable protease activity (Rosen *et al.*, 1990).

Other members of the Met subfamily include the mammalian *ron* and the avian *sea* receptors (Huff *et al.*, 1996; Ronsin *et al.*, 1993). The ligand for Ron is macrophage stimulating protein (MSP) and a chicken homolog of MSP (chMSP) has recently been identified as a ligand for Sea (Gaudino *et al.*, 1994; Wahl *et al.*, 1999). Analogous to HGF/SF-Met signaling, stimulation of Ron with MSP or stimulation of a chimeric Trk-Sea receptor with nerve growth factor can induce mitogenic responses and stimulate cell motility and invasive growth in epithelial cells suggesting that members of the Met receptor subfamily have a conserved biological function (Medico *et al.*, 1996; Santoro *et al.*, 1996). Interestingly, while *met* homologs have been found in several vertebrates including mice

(Chan *et al.*, 1988), rat (Wallenius *et al.*, 1997), chickens (Thery *et al.*, 1995), frogs (Aberger *et al.*, 1997; Aoki *et al.*, 1996) and puffer fish (Cottage *et al.*, 1999), *met* family members are notably absent from the genomes of the worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* ((Rubin *et al.*, 2000) and K Furge and G Vande Woude, unpublished observations). The absence of Met homologs in worms and flies curtails using these powerful genetic systems to examine HGF/SF-Met signaling. Whether this absence indicates Met and its related receptor tyrosine kinases are exclusive to vertebrates awaits the sequencing efforts from additional species.

Met signal transduction

Met was originally identified following the isolation of a transforming cDNA fragment from a chemically treated human osteosarcoma cell line (Cooper *et al.*, 1984; Park *et al.*, 1986). In these cells, a chromosome rearrangement fused a region of chromosome 1 called *tpr* (for translocated promoter region) encoding a leucine-zipper dimerization motif with the C-terminal tyrosine kinase domain of *met* found on chromosome 7 (Dean *et al.*, 1987; Rodrigues and Park, 1993). The resulting oncoprotein, Tpr-Met, has high constitutive tyrosine kinase activity and can potently transform cells *in vitro*. Isolation of the *tpr-met* cDNA led to the identification of the full-length *met* receptor (Park *et al.*, 1986). In wild-type cells, the primary *met* transcript produces a 150 kD polypeptide that undergoes partial glycosylation to produce a 170 kD precursor protein (Faletto *et al.*, 1992; Gonzatti-Haces *et al.*, 1986). p170^{Met} is further glycosylated and cleaved into a 50 kD α -chain and a 140 kD β -chain. The α -subunit of the mature, di-sulfide linked, α - β Met heterodimer is highly glycosylated and entirely extracellular while the β -subunit contains a large extracellular region, a membrane spanning segment, and an intracellular tyrosine kinase domain (Figure 2) (Chan *et al.*, 1988; Giordano *et al.*, 1989; Park *et al.*, 1987). Activation of signal transduction pathways in response to HGF/SF stimulation is mediated in part by autophosphorylation of specific tyrosine residues within intracellular region of Met. Phosphorylation of two tyrosines (Tyr¹²³⁴ and Tyr¹²³⁵) located within the activation loop of the tyrosine kinase domain activate the intrinsic kinase activity of the receptor (Naldini *et al.*, 1991; Rodrigues and Park, 1994) while phosphorylation of two tyrosine residues (Tyr¹³⁴⁹ and Tyr¹³⁵⁶) in a cluster of amino acids in the C-terminus of Met activates a multisubstrate docking site that is conserved among Met family members (Ponzetto *et al.*, 1994). Sequences surrounding tyrosines 1349 and 1356 (Y¹³⁴⁹VHVNATY¹³⁵⁶VNV) in mouse Met form the docking site that binds Src homology-2 (SH2) domain, phosphotyrosine binding (PTB) domain, and Met binding domain (MBD) containing signal transducers (Pelicci *et al.*, 1995; Ponzetto *et al.*, 1994; Weidner *et al.*, 1996). Chimeric receptors that contain this amino-acid sequence can induce mitogenic, motogenic, and morphogenic responses similar to those observed with the wild-type Met receptor suggesting the multisubstrate binding site is primarily responsible for Met-mediated signal transduction (Komada and Kitamura, 1993; Weidner *et al.*, 1993; Zhu *et al.*, 1994).

Components of the signaling complex that are recruited to activated Met include the adapter proteins Grb2 (Ponzetto *et al.*, 1994), SHC (Pelicci *et al.*, 1995), Gab1 (Weidner *et al.*, 1996), and Crk/CRKL (Garcia-Guzman *et al.*, 1999; Sakkab *et al.*, 2000) along with several other well established signal transducers including phosphatidylinositol-3-OH kinase (PI3K) (Graziani *et al.*, 1991), the signal transducer and activator of transcription-3 (Stat3) (Boccaccio *et al.*, 1998), phospholipase C- γ (PLC- γ) (Ponzetto *et al.*, 1994), the Ras guanine nucleotide exchange factor son-of-sevenless (SOS) (Graziani *et al.*, 1993), the Src kinase (Ponzetto *et al.*, 1994), and the SHP2 phosphatase (Fixman *et al.*, 1996). Mutational analysis of the multisubstrate docking site suggest that together Y1349 and Y1356 mediate the interactions with SHC, Src, and Gab1 while Y1356 is primarily responsible for recruitment of Grb2, PI3K, PLC- γ , and SHP2 to the Met signaling complex (*vide infra*).

Grb2/SHC and Ras-MAPK signaling

The adapter protein Grb2 consists of an SH2 domain sandwiched between two SH3 domains (Figure 1) and is well known for recruiting SOS to activated RTKs to induce Ras-MAP-kinase signaling (Schlessinger, 1993). In mouse Met, the amino-acids surrounding phosphorylated Y1356 (pY¹³⁵⁶VNV) form a consensus SH2 binding site for Grb2 (pYXN) (Songyang *et al.*, 1994) and several groups have shown that Grb2 can associate with the activated receptor (Fixman *et al.*, 1995, 1997; Ponzetto *et al.*, 1994; Tulasne *et al.*, 1999). Mutations in Met adjacent to the upstream tyrosine Y1349 do not seem to significantly affect Grb2 binding; however, mutations of Y1356 or N1358 disrupt the Met-Grb2 interaction (Ponzetto *et al.*, 1994; Fixman *et al.*, 1996). It is worth noting that while the Y1356F mutation in Met disrupts association of several signal transducers (Grb2, PI3K, PLC- γ , and SHP2), the N1358H mutation seems to specifically disrupt the Met-Grb2 association (Fixman *et al.*, 1996). Grb2, in addition to a direct interaction with activated Met, may also be recruited to Met indirectly via the SHC adapter protein (Figure 1). The PTB of SHC can associate with the Met receptor via phosphotyrosines Y1349 and Y1356 (Pelicci *et al.*, 1995). Subsequent to Met activation, SHC is tyrosine phosphorylated and forms a Grb2

binding site (pY³¹⁷VNV) that is similar to the Grb2 binding site of Met (pY¹³⁵⁶VNV) (Pelicci *et al.*, 1995). Therefore, activation of the Ras pathway in response to HGF/SF-Met stimulation may be the result of a SHC-Grb2-Sos interaction.

While the Grb2-Sos/SHC-Grb2-Sos models of Ras activation are well established in other RTK systems, it is not completely clear whether the (Met/Met-SHC)-Grb2-Sos complex(s) plays an identical role in activating the Ras-MAP kinase pathway following HGF/SF stimulation. In Madin-Darby canine kidney (MDCK) cells, scattering is likely dependent on Ras signaling as overexpression of dominant-negative Ras mutants can disrupt this process (Hartmann *et al.*, 1994). A chimeric CSF-1-Met receptor mutant (N1358H) that has a disrupted Met-Grb2 interaction still can induce scattering (Fournier *et al.*, 1996; Royal *et al.*, 1997). Overexpression of SHC, but not SHC mutants that are no longer able to bind Grb2, enhances HGF/SF mediated proliferation and cell migration consistent with the recruitment of a SHC-Grb2-SOS complex to activated Met (Pelicci *et al.*, 1995). In addition, selective inhibitors of the Grb2 SH2 domain that likely disrupt both Met-Grb2 and SHC-Grb2 interactions can inhibit Met mediated Ras activation and cell migration (Gay *et al.*, 1999). All of this data support a role of a SHC-Grb2-SOS in Ras activation. In contrast, Trk-Met chimeric receptors that have both Y1349F and Y1356F mutations and no longer bind either Grb2 or SHC can still induce Ras-dependent phosphorylation of the downstream kinases ERK1 and ERK2, generate transcriptional responses, and induce some cell scattering (Tulasne *et al.*, 1999; Weidner *et al.*, 1995). Also, activation of the Ras-MAP pathway by Tpr-Met is not blocked by dominant-negative Grb2 mutants that should disrupt either Met-Grb2 or SHC-Grb2 interactions (Rodrigues *et al.*, 1997). While chimeric receptors and Tpr-Met do not signal exactly as their wild-type counterparts (Jeffers *et al.*, 1998), it nevertheless raises the possibility that either only residual amounts of Met activity can induce Ras-MAPK activation or Ras activation can occur independent of a (Met/Met-SHC)-Grb2-Sos complex.

Met and the large docking protein Gab1

While significant effort has focused on Grb2 and the Ras-MAPK pathway, recently Grb2 has also been implicated in recruiting the large adapter protein Gab1 to the Met signaling complex. As its name implies, Gab1 (Grb2-associated binding protein-1) was identified as a protein produced from a glial tumor expression cDNA library that could bind Grb2 (Holgado-Madruga *et al.*, 1996). Gab1 is a member of a family of adapter proteins that have similarity to the insulin receptor substrate-1 (reviewed in White, 1998) and contains an N-terminal pleckstrin homology (PH) domain that binds the membrane lipid phosphatidylinositol 3,4,5-trisphosphate (PIP3), a central region that contains sixteen potential tyrosine phosphorylation sites that other signal transduction proteins can bind, and a C-terminal proline-rich MBD domain (Figure 1) (Holgado-Madruga *et al.*, 1996; Maroun *et al.*, 1999b; Rodrigues *et al.*, 2000; Weidner *et al.*, 1996). While the MBD domain within Gab1 can mediate a direct interaction with the multisubstrate binding site

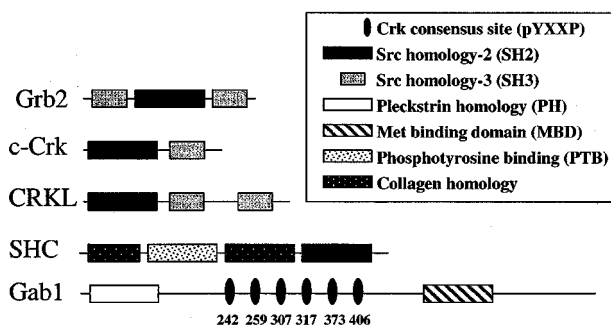


Figure 1 Domain architecture of adapter proteins that associate with the Met receptor. Depicted are schematic representations of several important adapter proteins that are part of the Met signaling complex. The numbers beneath Gab1 refer to tyrosine amino acid residues present within potential Crk/CRKL binding sites

of Met, within the MDB domain are two optimal sites for binding the SH3 domains of Grb2 suggesting Gab1 may also bind Met indirectly via a Grb2 linkage (Nguyen *et al.*, 1997; Weidner *et al.*, 1996). Met mutants that contain both Y1349 and Y1356 mutations are unable to associate with Gab1 while Met Y1356F mutants, in which Grb2 binding is eliminated, show significantly decreased, but not complete loss of, Met-Gab1 association (Bardelli *et al.*, 1997; Maroun *et al.*, 1999a; Nguyen *et al.*, 1997). Therefore, while Gab1 may prefer to bind Met via a Grb2 linkage, the ability of Gab1 to bind Met both directly using Y1349 and indirectly via a Grb2 linkage at Y1356 may allow for both a more specific and a higher affinity interaction (Figure 2). Regardless of the exact nature of the interaction between Gab1 and Met, following HGF/SF stimulation Gab1 becomes strongly tyrosine phosphorylated and overexpression of Gab1 is sufficient to induce branching morphogenesis at least in some cell types suggesting that Gab1 and the signal transducers that it recruits to the Met signaling complex are major effectors of Met signaling (Bardelli *et al.*, 1997; Nguyen *et al.*, 1997; Niemann *et al.*, 1998; Weidner *et al.*, 1996).

A number of growth factors including HGF/SF, EGF, NGF, insulin, and various cytokines can induce Gab1 tyrosine phosphorylation and its direct associa-

tion with several signal transducers including PI3K, PLC- γ , and the SHP2 phosphatase (Bardelli *et al.*, 1997; Holgado-Madruga *et al.*, 1996; Weidner *et al.*, 1996; Nishida *et al.*, 1999). One reasonable hypothesis is that following stimulation by each of these factors, Gab1 becomes uniquely phosphorylated and recruits distinct sets of signal transducers to induce different cellular responses (i.e. branching morphogenesis by HGF/SF stimulation vs proliferation by EGF stimulation). However, phosphopeptide maps of Gab1 following stimulation by either EGF or HGF are virtually identical suggesting mechanisms other than differential phosphorylation contribute to HGF/SF mediated branching morphogenesis (Gual *et al.*, 2000). The one significant difference between EGF and HGF/SF induced Gab1 phosphorylation is the duration in which Gab1 remains phosphorylated following growth factor addition. In HGF/SF stimulated cells, Gab1 remains phosphorylated approximately three to four times longer than EGF stimulated cells (Gual *et al.*, 2000; Maroun *et al.*, 1999a). Sustained activation of signal transduction pathways is one way to regulate differential signaling responses and may be a major mechanism that mediates HGF/SF induced branching morphogenesis (Marshall, 1995).

Some insight has been gained as to which Gab1 signaling pathways may require sustained activation for HGF/SF-Met mediated branching/invasive growth. As mentioned earlier, Gab1 binds PI3K, PLC- γ , and the SHP2 phosphatase. A triple mutation in Gab1 (Y307F, Y373F, Y407F) that disrupts the Gab1-PLC- γ interaction also blocks HGF/SF induced branching when overexpressed, implicating PLC- γ in this process. However, as selective inhibitors of PLC- γ only partially reduce Gab1 mediated branching, this particular Gab1 mutant may disrupt interactions with other signal transducers in addition to PLC- γ that are important for invasive growth (Gual *et al.*, 2000). Also, while PLC- γ has been reported to weakly bind Met directly (Ponzetto *et al.*, 1994), the association of PLC- γ with Gab1 combined with the disruption of branching morphogenesis by Gab1-PLC- γ mutants, suggests that PLC- γ may prefer to associate with the Met signaling complex via the Gab1 adapter (Figure 2).

PI3K also may play a significant role in Gab1 mediated signal transduction as inhibitors of PI3K activity (wortmannin and LY294002) prevent Gab1 mediated branching and Gab1 mutants that are unable to bind PI3K have a diminished ability to induce branching (Royal and Park, 1995; Niemann *et al.*, 1998; Maroun *et al.*, 1999b). While the p85 regulatory subunit of PI3K can associate directly with Met via the multisubstrate binding site (Ponzetto *et al.*, 1994), more PI3K activity co-precipitates with Gab1 than co-precipitates with Met suggesting PI3K, like PLC- γ , associates primarily with Gab1 (Bardelli *et al.*, 1997; Maroun *et al.*, 1999a). Supporting a requirement of PI3K in Gab1 mediated signaling, Gab1 PH domain mutants that have reduced affinity for PIP3 are impaired in inducing branching morphogenesis and do not localize properly to the membrane (Maroun *et al.*, 1999a,b). Relocation to the membrane (and subsequent cell branching) is also disrupted by inhibitors of PI3K demonstrating that while PI3K binds Gab1 following Met activation, PI3K is required for effective Gab1 signaling (Figure 2). This situation

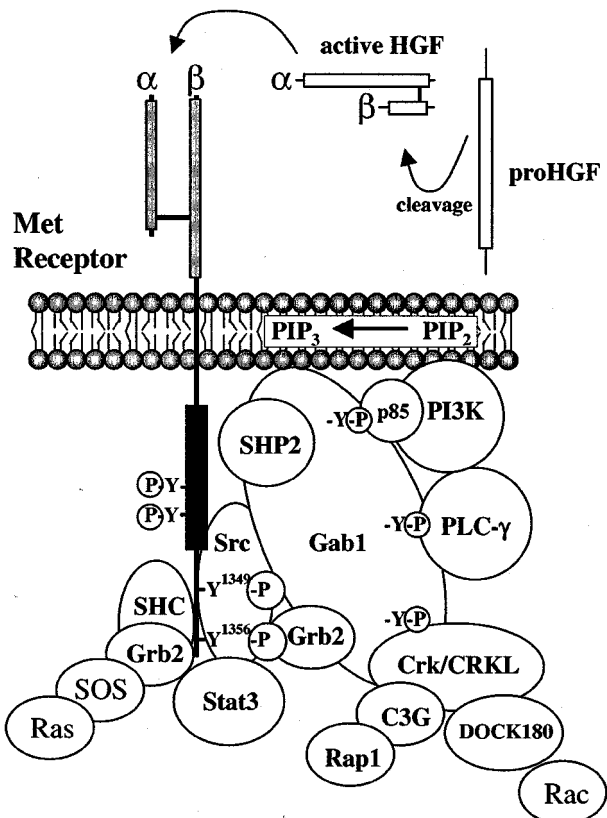


Figure 2 A model of the signaling complex recruited to activated Met. Shown are the α and β chains of both HGF/SF and Met. The dark gray box highlights the intracellular tyrosine kinase domain and the numbered phosphotyrosines indicate the position of the multisubstrate binding site of mouse Met. The multisubstrate docking site mediates prolonged interactions with signal transducing and adapter proteins that likely are important for HGF/SF-Met mediated growth, motility, and morphogenic differentiation

sets up a positive feedback loop between Gab1 and PI3K that is important for regulating Gab1 signaling and branching morphogenesis (Maroun *et al.*, 1999a,b; Rodrigues *et al.*, 2000).

The Crk family of adapters

Gab1, in addition to binding SHP2, PI3K, and PLC- γ , contains multiple Tyr-X-X-Pro (YXXP) sequences that represent potential Crk family member SH2 binding sites (Figure 1) (Knudsen *et al.*, 1995). The Crk family of adapter proteins are homologs of the v-Crk oncogene that induces sarcomas in chickens (Mayer *et al.*, 1988) and are composed primarily of SH2 and SH3 domain(s) (Figure 1). Crk family members have been implicated in number of signal transduction events including neuronal differentiation of PC12 cells, T-cell receptor signaling, integrin signaling, and signal transduction of the CML-causing Bcr-Abl oncoprotein (reviewed in Feller *et al.*, 1998). Crk signaling has also been implicated in regulating cell migration and protecting cells from apoptosis during invasive growth (Cho and Klemke, 2000; Feller *et al.*, 1998). As predicted by sequence analysis, both Crk and the related Crk-like (CRKL) proteins bind to phosphorylated Gab1 and add an new array of signal transducers that become activated upon HGF/SF stimulation (Garcia-Guzman *et al.*, 1999; Sakkab *et al.*, 2000). The SH3 domain of Crk family members binds to proline rich regions found in C3G, a guanine nucleotide exchange factor that activates the Ras-superfamily GTPase Rap1 (Gotoh *et al.*, 1995). Crk family members also bind Dock180 which in turn binds and activates, via an undefined mechanism, the Rac GTPase (Kiyokawa *et al.*, 1998). In response to HGF/SF, Met activates both Rap1 (Sakkab *et al.*, 2000) and Rac (Ridley and Hall, 1992; Ridley *et al.*, 1992; Royal *et al.*, 2000). While the functions of the signals emanating from Rap1 have not been identified, the involvement of Rac in cell spreading, dissociation, and migration following HGF/SF stimulation is well characterized (Hall, 1998; Ridley *et al.*, 1992).

Other downstream effectors of Crk include the c-Jun N-terminal kinase (JNK). Activation of Met either by HGF/SF stimulation or by expression of the constitutively active Tpr-Met oncoprotein leads to JNK activation and has been implicated in cellular transformation (Garcia-Guzman *et al.*, 1999; Rodrigues *et al.*, 1997). Crk is likely a mediator of JNK activation since (i) HGF/SF-Met mediated activation of JNK can be blocked by expression of loss-of-function mutants of Crk and (ii) Crk mediates JNK activation in response to other stimuli such as epidermal growth factor receptor or integrin receptor signaling (Dolfi *et al.*, 1998; Garcia-Guzman *et al.*, 1999). Crk family members can signal through Rac to activate JNK and dominant-negative forms of Rac block Met mediated JNK activation raising the possibility of a Met-Gab1-Crk-Rac-JNK signaling pathway (Feller *et al.*, 1998; Rodrigues *et al.*, 1997). Interestingly, the Gab1 triple mutant that has impaired PLC- γ binding also contains a mutated Crk family binding site (Y307, Figure 1) (Gual *et al.*, 2000). As overexpression of this Gab1 mutant completely disrupts Met mediated branching while PLC- γ inhibitors only partially disrupt branching morphogenesis, it is possible that signaling

pathways downstream of Crk may be involved in branching. Thus far, Crk has only been shown to bind Gab1 in response to Met activation as opposed to activation of other tyrosine kinases. Whether this is a unique event in HGF/SF-Met signal transduction and whether this event is involved in branching morphogenesis and/or tumor cell invasiveness remains to be determined.

Src and Stat3

Two other important signal transducers, the Src kinase and the Stat3 transcription factor, also associate with Met following receptor activation (Ponzetto *et al.*, 1994; Rahimi *et al.*, 1998; Boccaccio *et al.*, 1998). Src can associate directly with Met using Y1349 and Y1356 and, similar to other receptor systems, Src activation is important for HGF/SF-Met mediated cell migration and cell transformation (Ponzetto *et al.*, 1994; Rahimi *et al.*, 1998). Activated Met also recruits the Stat3 transcription factor. While Stat3 can bind directly to Met, a small amount of Stat3 co-precipitates with Gab1 (Boccaccio *et al.*, 1998). When activated by tyrosine phosphorylation, STATs migrate to the nucleus where they promote cell proliferation and survival by regulating gene transcription (Bowman *et al.*, 2000). In response to HGF/SF-Met stimulation, Stat3 activation and subsequent nuclear translocation/gene transcription is required for branching morphogenesis (Boccaccio *et al.*, 1998). Interestingly, while Stat3 can be phosphorylated by RTKs (including by Met *in vitro*), Stat3 can also be activated by Src family kinases and dominant-negative mutants of Stat3 can block v-Src induced cellular transformation (Bromberg *et al.*, 1998; Turkson *et al.*, 1998; Bowman *et al.*, 2000).

Conclusions

While the importance of the multidocking site in Met mediated proliferation, cell movement/invasion, and branching morphogenesis has been appreciated for several years, only recently have more complete models been developed for how this site organizes a signaling complex following HGF/SF stimulation. Disruption of the signaling complex by either mutating tyrosines Y1349 and Y1356 or introducing peptide mimetics of the C-terminal end of Met leads to loss of cell invasion, transformation, and branching morphogenesis (Jeffers *et al.*, 1998; Bardelli *et al.*, 1999). Adapter proteins play a critical role in assembling signal complexes, not only by recruiting additional signal transducers, but also by recruiting other adapter proteins. While this interplay may lead to essential cross-talk between receptor signaling pathways, it also has made isolation of critical pathways that are responsive to HGF/SF-Met signaling more difficult. For example, mutations in Gab1 (such as those that involved in PLC- γ binding) may also effect Crk mediated signal transduction and Met mutations, such as Y1356, disrupt at least in part the binding of the adapters Grb2, Gab1, and SHC. Disruption of these adapters in turn can affect PI3K, PLC- γ , Rap1, Rac, and Ras signaling (Figure 2). Even more selective mutations such as the N1358H mutation, in which Grb2 binding is selectively lost still may lead to subtle perturbations in many of these pathways do to the multiplicity of the interactions. However,

careful examination of signaling pathways invoked by these mutant receptors coupled with the use of dominant negative proteins and selective small molecule inhibitors suggest that cell motility is dependent on at least Ras-MAPK activation (Hartmann *et al.*, 1994), cytoskeletal remodeling mediated by the Rac, Rho, and Cdc42 GTPases (Ridley and Hall, 1992; Ridley *et al.*, 1992; Royal *et al.*, 2000), Src kinase (Rahimi *et al.*, 1998) and PI3K (Royal *et al.*, 1995). Induction of invasion/branching morphogenesis likely requires these pathways in addition to PLC- γ (Gual *et al.*, 2000) and Stat3 (Boccaccio *et al.*, 1998). Based on the many different cellular responses that are invoked by HGF/SF-Met signaling the list of signal transducers

and adapter proteins that associate with activated Met will likely continue to grow and generate more involved models of HGF/SF-Met signaling transduction. In addition, it is likely that many of these pathways will be important for Met mediated tumorigenicity and tumor cell invasion/metastasis.

Acknowledgments

Thanks to LL Furge for a critical reading of the manuscript and to Lynn Ritsema for help in the manuscript preparation.

References

- Aberger F, Weidinger G and Richter K. (1997). *Biochem. Biophys. Res. Commun.*, **231**, 191–195.
- Aoki S, Takahashi K, Matsumoto K and Nakamura T. (1996). *J. Biochem. (Tokyo)*, **120**, 961–968.
- Bardelli A, Longati P, Gramaglia D, Stella MC and Comoglio PM. (1997). *Oncogene*, **15**, 3103–3111.
- Bardelli A, Longati P, William TA, Benvenuti S and Comoglio PM. (1999). *J. Biol. Chem.*, **274**, 29274–29281.
- Bellusci S, Moens G, Gaudino G, Comoglio P, Nakamura T, Thiery JP and Jouanneau J. (1994). *Oncogene*, **9**, 1091–1099.
- Bladt F, Riethmacher D, Isenmann S, Aguzzi A and Birchmeier C. (1995). *Nature*, **376**, 768–771.
- Boccaccio C, Ando M, Tamagnone L, Bardelli A, Michieli P, Battistini C and Comoglio PM. (1998). *Nature*, **391**, 285–288.
- Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmieciak TE, Vande Woude GF and Aaronson SA. (1991). *Science*, **251**, 802–804.
- Bowman T, Garcia R, Turkson J and Jove R. (2000). *Oncogene*, **19**, 2474–2488.
- Brinkmann V, Foroutan H, Sachs M, Weidner KM and Birchmeier W. (1995). *J. Cell Biol.*, **131**, 1573–1586.
- Bromberg JF, Horvath CM, Besser D, Lathem VW, and Darnell JE. (1998). *Mol. Cell. Biol.*, **18**, 2553–2558.
- Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, Gaudino G, Tamagnone L, Coffer A and Comoglio PM. (1992). *J. Cell Biol.*, **119**, 629–641.
- Chan AM, King HW, Deakin EA, Tempest PR, Hilkens J, Kroezen V, Edwards DR, Wills AJ, Brookes P and Cooper CS. (1988). *Oncogene*, **2**, 593–599.
- Cho SY and Klemke RL. (2000). *J. Cell Biol.*, **149**, 223–236.
- Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM and Vande Woude GF. (1984). *Nature (London)*, **311**, 29–33.
- Cottage A, Clark M, Hawker K, Umrana Y, Wheller D, Bishop M and Elgar G. (1999). *FEBS Lett.*, **443**, 370–374.
- Dean M, Park M and Vande WG. (1987). *Mol. Cell. Biol.*, **7**, 921–924.
- Di Renzo MF, Olivero M, Martone T, Maffe A, Maggiora P, De Stefani A, Valente G, Giordano S, Cortesina G and Comoglio PM. (2000). *Oncogene*, **19**, 1547–1555.
- Dolfi F, Garcia-Guzman M, Ojaniemi M, Nakamura H, Matsuda M and Vuori K. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 15394–15399.
- Faletto DL, Tsarfaty I, Kmieciak TE, Gonzatti M, Suzuki T and Vande Woude GF. (1992). *Oncogene*, **7**, 1149–1157.
- Feller S, Posern G, Voss J, Kardinal C, Sakkab D, Zheng J and Knudsen B. (1998). *J. Cell Physiol.*, **177**, 535–552.
- Fixman ED, Naujokas MA, Rodrigues GA, Moran MF and Park M. (1995). *Oncogene*, **10**, 237–249.
- Fixman ED, Fournier TM, Kamikura DM, Naujokas MA and Park M. (1996). *J. Biol. Chem.*, **271**, 13116–13122.
- Fixman ED, Holgado-Madruga M, Nguyen L, Kamikura DM, Fournier TM, Wong AJ and Park M. (1997). *J. Biol. Chem.*, **272**, 20167–20172.
- Fournier TM, Kamikura D, Teng K and Park M. (1996). *J. Biol. Chem.*, **271**, 22211–22217.
- Garcia-Guzman M, Dolfi F, Zeh K and Vuori K. (1999). *Oncogene*, **18**, 7775–7786.
- Gaudino G, Follenzi A, Naldini A, Collesi C, Santoro M, Gallo KA, Godowski PJ and Comoglio PM. (1994). *EMBO J.*, **13**, 3524–3532.
- Gay B, Suarez S, Weber C, Rahuel J, Fabbro D, Furet P, Caravatti G and Schoepfer J. (1999). *J. Biol. Chem.*, **274**, 23311–23315.
- Gherardi E, Hartmann J, Hepple J, Chirgadze D, Srinivasan N and Blundell T. (1997). *Ciba Found. Sym.*, **212**, 84–98.
- Giordano S, Ponzetto C, Di Renzo MF, Cooper CS and Comoglio PM. (1989). *Nature*, **339**, 155–156.
- Gonzatti-Haces M, Park M, Dean M, Blair DG and Vande Woude GF. (1986). *Princess Takamatsu Symp.*, **17**, 221–232.
- Gotoh T, Hattori S, Nakamura S, Kitayama H, Noda M, Takai Y, Kaibuchi K, Matsui H, Hatase O and Takahashi H. (1995). *Mol. Cell. Biol.*, **15**, 6746–6753.
- Grant DS, Kleinman HK, Goldberg ID, Bhargava MM, Nickloff BJ, Kinsella JL, Polverini P and Rosen EM. (1993). *Proc. Natl. Acad. Sci. USA*, **90**, 1937–1941.
- Graziani A, Gramaglia D, Cantley LC and Comoglio PM. (1991). *J. Biol. Chem.*, **266**, 22087–22090.
- Graziani A, Gramaglia D, dalla Zonca P and Comoglio PM. (1993). *J. Biol. Chem.*, **268**, 9165–9168.
- Gual P, Giordano S, Williams TA, Rocchi S, Obberghen EV and Comoglio PM. (2000). *Oncogene*, **19**, 1509–1518.
- Hall A. (1998). *Science*, **279**, 509–514.
- Hartmann G, Weidner KM, Schwartz H and Birchmeier W. (1994). *J. Biol. Chem.*, **269**, 21936–21939.
- Holgado-Madruga M, Emler DR, Moscatello DK, Godwin AK and Wong AJ. (1996). *Nature*, **379**, 560–564.
- Huff JL, Jelinek MA, Jamieson TA and Parsons JT. (1996). *Oncogene*, **12**, 299–307.
- Jeffers M, Koochekpour S, Fiscella M, Sathyanarayana BK and Vande Woude GF. (1998). *Oncogene*, **17**, 2691–2700.
- Jeffers M, Rao MS, Rulong S, Reddy JK, Subbarao V, Hudson E, Vande Woude GF and Resau JH. (1996a). *Cell Growth Differ.*, **7**, 1805–1813.
- Jeffers M, Rong S, Oskarsson M, Anver M and Vande Woude GF. (1996b). *Oncogene*, **13**, 853–861.
- Jeffers M, Rong S and Vande Woude GF. (1996c). *Mol. Cell. Biol.*, **16**, 1115–1125.
- Jeffers M, Rong S and Vande Woude GF. (1996d). *J. Mol. Med.*, **74**, 505–513.
- Jeffers M, Schmidt L, Nakaigawa N, Webb CP, Weirich G, Kishida T, Zbar B and Vande Woude GF. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 11445–11450.

- Kan M, Zhang G, Zarnegar R, Michalopoulos G, Myoken Y, McKeehan WL and Stevens JI. (1991). *Biochem. Biophys. Res. Commun.*, **174**, 331–337.
- Kiyokawa K, Hashimoto Y, Kobayashi S, Sugimura H, Kurata Y and Matsuda M. (1998). *Genes Dev.*, **12**, 3331–3336.
- Knudsen BS, Zheng J, Feller SM, Mayer JP, Burrell SK, Cowburn D and Hanafusa H. (1995). *EMBO J.*, **14**, 2191–2198.
- Komada M and Kitamura N. (1993). *Oncogene*, **8**, 2381–2390.
- Maroun CR, Holgado-Madruga M, Royal I, Naujokas MA, Fournier TM, Wong AJ and Park M. (1999a). *Mol. Cell. Biol.*, **19**, 1784–1799.
- Maroun CR, Moscatello DK, Naujokas MA, Holgado-Madruga M, Wong AJ and Park M. (1999b). *J. Biol. Chem.*, **274**, 31719–31726.
- Marshall CJ. (1995). *Cell*, **80**, 179–185.
- Matsumoto K, Matsumoto K, Nakamura T and Kramer RH. (1994). *J. Biol. Chem.*, **269**, 31807–31813.
- Matsumoto K and Nakamura T. (1993). *Hepatocyte Growth Factor-Scatter Factor and the Met Receptor*, Vol. 65. Goldberg ID and Rosen EM. (eds). Birkhauser-Verlag: Basel, Switzerland, pp 225–250.
- Mayer BJ, Hamaguchi M and Hanafusa H. (1988). *Nature*, **332**, 272–275.
- Medico E, Mongiovi AM, Huff J, Jelinek MA, Follenzi A, Gaudino G, Parsons JT and Comoglio PM. (1996). *Mol. Biol. Cell.*, **7**, 495–504.
- Montesano R, Matsumoto K, Nakamura T and Orci L. (1991a). *Cell*, **67**, 901–908.
- Montesano R, Schaller G and Orci L. (1991b). *Cell*, **66**, 697–711.
- Nakamura T, Nawa K and Ichihara A. (1984). *Biochem. Biophys. Res. Commun.*, **122**, 1450–1459.
- Nakamura T, Nawa K, Ichihara A, Kaise N and Nishino T. (1987). *FEBS Lett.*, **224**, 311–316.
- Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F and Comoglio PM. (1992). *EMBO J.*, **11**, 4825–4833.
- Naldini L, Vigna E, Ferracini R, Longati P, Gandino L, Prat M and Comoglio PM. (1991). *Mol. Cell. Biol.*, **11**, 1793–1803.
- Nguyen L, Holgado-Madruga M, Maroun C, Fixman ED, Kamikura D, Fournier T, Charest A, Tremblay M, Wong AJ and Park M. (1997). *J. Biol. Chem.*, **272**, 20811–20819.
- Niemann C, Brinkmann V, Spitzer E, Hartman G, Sachs M, Naudorf H and Birchmeier W. (1998). *J. Cell Biol.*, **143**, 533–545.
- Nishida K, Yoshida Y, Itoh M, Fukada T, Ohtani T, Shirogane T, Atsumi T, Marika T, Katsuhika I, Masahiko H and Hirano T. (1999). *Blood*, **93**, 1809–1816.
- Nusrat A, Parkos CA, Bacarra AE, Godowski PJ, Delp-Archer C, Rosen EM and Madara JL. (1994). *J. Clin. Invest.*, **93**, 2056–2065.
- Olivero M, Valente G, Bardelli A, Longati P, Ferrero N, Cracco C, Terrone C, Rocca-Rossetti S, Comoglio P and Di Renzo M. (1999). *Int. J. Cancer*, **82**, 640–643.
- Park M, Dean M, Cooper CS, Schmidt M, O'Brien SJ, Blair DG and Vande Woude GF. (1986). *Cell*, **45**, 895–904.
- Park M, Dean M, Kaul K, Braun MJ, Gonda MA and Vande Woude GF. (1987). *Proc. Natl. Acad. Sci. USA*, **84**, 6379–6383.
- Park W, Dong S, Kim S, Na E, Shin M, Pi J, Kim B, Bae J, Hong Y, Lee K, Lee S, Yoo N, Jang J, Pack S, Zhuang Z, Schmidt L, Zbar B and Lee J. (1999). *Cancer Res.*, **59**, 307–310.
- Pellicci G, Giordano S, Zhen Z, Salcini AE, Lanfrancone L, Bardelli A, Panayotou G, Waterfield MD, Ponzetto C, Pellicci PG and Comoglio PM. (1995). *Oncogene*, **10**, 1631–1638.
- Ponzetto C, Bardelli A, Zhen Z, Maina F, dalla Zonca P, Giordano S, Graziani A, Panayotou G and Comoglio PM. (1994). *Cell*, **77**, 261–271.
- Rahimi N, Wesley H, Tremblay E, Saulnier R and Elliot B. (1998). *J. Biol. Chem.*, **273**, 33714–33721.
- Ridley AJ and Hall A. (1992). *Cell*, **70**, 389–399.
- Ridley AJ, Paterson HF, Johnston CL, Diekmann D and Hall A. (1992). *Cell*, **70**, 401–410.
- Rodrigues GA, Falasca M, Zhang Z, Ong SH and Schlessinger J. (2000). *Mol. Cell. Biol.*, **20**, 1448–1459.
- Rodrigues GA and Park M. (1993). *Mol. Cell. Biol.*, **13**, 6711–6722.
- Rodrigues GA and Park M. (1994). *Oncogene*, **9**, 2019–2027.
- Rodrigues GA, Park M and Schlessinger J. (1997). *EMBO J.*, **16**, 2634–2645.
- Rong S, Bodescot M, Blair D, Dunn J, Nakamura T, Mizuno K, Park M, Chan A, Aaronson S and Vande Woude G. (1992). *Mol. Cell. Biol.*, **12**, 5152–5158.
- Rong S, Segal S, Anver M, Resau JH and Vande Woude GF. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 4731–4735.
- Ronsin C, Muscatelli F, Mattei MG and Breathnach R. (1993). *Oncogene*, **8**, 1195–1202.
- Rosen EM, Meromsky L, Setter E, Vinter DW and Goldberg ID. (1990). *Proc. Soc. Exp. Biol. Med.*, **195**, 34–43.
- Royal I, Lamarche-Vane N, Lamorte L, Kozo K and Morag P. (2000). *Mol. Cell. Biol.*, **11**, 1709–1725.
- Royal I, Fournier TM and Park M. (1997). *J. Cell Physiol.*, **173**, 196–201.
- Royal I and Park M. (1995). *J. Biol. Chem.*, **270**, 27780–27787.
- Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein LS, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJ, Kuehl PM, Lemaitre B, Littleton JT, Morrison, DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vossball LB, Zhang J, Zhao Q, Zheng XH, Zhong F, Zhong W, Gibbs R, Venter JC, Adams MD and Lewis S. (2000). *Science*, **287**, 204–214.
- Sakkab D, Lewitzky M, Posern G, Schaeper U, Sachs M, Birchmeier W and Feller S. (2000). *J. Biol. Chem.*, **275**, 10772–10788.
- Santoro MM, Collesi C, Grisendi S, Gaudino G and Comoglio PM. (1996). *Mol. Cell. Biol.*, **16**, 7072–7083.
- Santos OFP, Barros EJG, Yang X-M, Matsumoto K, Nakamura T, Park M and Nigam SK. (1994). *Dev. Biol.*, **163**, 525–529.
- Schlessinger J. (1993). *Trends Biochem. Sci.*, **18**, 273–275.
- Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi E and Birchmeier C. (1995). *Nature (London)*, **373**, 699–702.
- Schmidt L, Duh F-M, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allilmetts R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJM, Walter MM, Tsui L-C, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI, Linehan WM and Zbar B. (1997). *Nat. Genet.*, **16**, 68–73.
- Schmidt L, Junker K, Weirich G, Glenn G, Choyke P, Lubensky I, Zhuang Z, Jeffers M, Vande Woude GF, Neumann H, Walther M, Linehan WM and Zbar B. (1998). *Cancer Res.*, **58**, 1719–1722.
- Shimomura T, Miyazawa K, Komiyama Y, Hiraoka H, Naka D, Morimoto Y and Kitamura N. (1995). *Eur. J. Biochem.*, **229**, 257–261.

- Shimomura T, Ochiai M, Kondo J and Morimoto Y. (1992). *Cytotechnology*, **8**, 219–229.
- Songyang Z, Shoelson SE, McGlade J, Olivier P, Pawson T, Bustelo XR, Barbacid M, Sabe H, Hanafusa H, Yi T, Ren R, Baltimore D, Ratnofsky S, Feldman RA and Cantley LC. (1994). *Mol. Cell. Biol.*, **14**, 2777–2785.
- Sonnenberg E, Weidner KM and Birchmeier C. (1993). *Exs.*, **65**, 381–394.
- Soriano JV, Pepper MS, Nakamura T, Orci L and Montesano R. (1995). *J. Cell Sci.*, **108**, 413–430.
- Stoker M and Perryman M. (1985). *J. Cell. Sci.*, **77**, 209–223.
- Streit A, Stern CD, Thery C, Ireland GW, Aparicio S, Sharpe MJ and Gherardi E. (1995). *Development*, **121**, 813–824.
- Tanyi J, Tory K, Rigo JJ, Nagy B and Papp Z. (1999). *Pathol. Oncol. Res.*, **5**, 187–191.
- Thery C, Sharpe MJ, Batley SJ, Stern CD and Gherardi E. (1995). *Dev. Genet.*, **17**, 90–101.
- Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL and Vande Woude GF. (1992). *Science*, **257**, 1258–1261.
- Tsarfaty I, Rong S, Resau JH, Rulong S, Pinto da Silva P and Vande Woude GF. (1994). *Science*, **263**, 98–101.
- Tulasne D, Paumelle R, Weidner KM, Vandenbunder B and Fafeur V. (1999). *Mol. Biol. Cell*, **10**, 551–565.
- Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP and Jove R. (1998). *Mol. Cell. Biol.*, **18**, 2545–2552.
- Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T and Kitamura N. (1995). *Nature (London)*, **373**, 702–705.
- Wahl RC, Hsu R, Huff J, Jelinek MA, Chen K, Courchesne P, Patterson SD, Parson JT and Welcher AA. (1999). *J. Biol. Chem.*, **274**, 26361–26368.
- Wallenius VR, Rawet H, Skrtic S, Helou K, Qiu Y, Levan G, Ekberg S, Carlsson B, Isaksson OG, Nakamura T and Jansson JO. (1997). *Mamm. Genome*, **8**, 661–667.
- Weidner KM, Behrens J, Vandekerckhove J and Birchmeier W. (1990). *J. Cell Biol.*, **111**, 2097–2108.
- Weidner KM, Di Cesare S, Sachs M, Brinkmann V, Behrens J and Birchmeier W. (1996). *Nature*, **384**, 173–176.
- Weidner KM, Sachs M and Birchmeier W. (1993). *J. Cell Biol.*, **121**, 145–154.
- Weidner KM, Sachs M, Riethmacher D and Birchmeier W. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 2597–2601.
- White MF. (1998). *Mol. Cell Biochem.*, **182**, 3–11.
- Woolf AS, Kolatsi-Joannou M, Hardman P, Andermarcher E, Moorby C, Fine LG, Jat PS, Noble MD and Gherardi E. (1995). *J. Cell Biol.*, **128**, 171–184.
- Yang Y, Spitzer E, Meyer D, Sachs M, Niemann C, Hartmann G, Weidner KM, Birchmeier C and Birchmeier W. (1995). *J. Cell Biol.*, **131**, 1–12.
- Zhu H, Naujokas MA and Park M. (1994). *Cell Growth Differ.*, **5**, 359–366.