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3-phosphoinositide-dependent protein kinase 1 (PDK1) mediates crosstalk between Src and Akt pathways in MET receptor signaling

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Abstract: The high-affinity tyrosine kinase receptor MET plays a pivotal role in several facets of cell regulation. Although its mitogenic effect is well documented, some aspects of connection patterns between signaling pathways involved in cell cycle progression remain to be deciphered. We have used a tractable heterologous expression system, the *Xenopus* oocyte, to detect connections between distinct MET signaling

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cascades involved in G2/M progression. Our results reveal that Src acts as an adapter via its SH2 domain to recruit 3-phosphoinositide-dependent protein kinase 1 (PDK1) to the MET signaling complex leading to Akt phosphorylation. These data define an original crosstalk between Src and Akt signaling pathways that contributes to MET-induced entry into the M phase, and deserves further investigation in pathologies harboring deregulation of this receptor.

Keywords: Hepatocyte growth factor receptor; MET signaling; PDK1; Src; Akt; G2/M transition; *Xenopus* oocyte.

Abbreviations: Akt/PKB, Protein Kinase B; Erk2, Extracellular signal-regulated kinase 2; Gab1, Grb2-associated binder 1; Grb2, Growth factor receptor-bound protein 2; HGF, Hepatocyte Growth Factor; MAPK, Mitogen Activated Protein Kinase; MBD, Met-Binding Domain; MET, Hepatocyte growth factor receptor; MDS, Multi-substrate Docking Site; MS222, ethyl 3-aminobenzoate methanesulfonate; ND96, Nathan Dascal 96 mM, P, phosphorylation; PDK1, 3-Phosphoinositide-dependent protein kinase 1; PH, Pleckstrin Homology domain ; PI3-Kinase, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; Ras, Rat sarcoma virus GTPase ; SDS PAGE, Sodium DodecylSulfate PolyAcrylamide Gel Electrophoresis ; SH2, Src Homology 2; SOS, Son of Sevenless; Src, Sarcoma; STAT3, Signal Transducer and Activator of Transcription 3; TK, Tyrosine Kinase.

Introduction

The receptor for the hepatocyte growth factor/scatter factor (HGF/SF) MET is a multifunctional tyrosine kinase receptor involved in several cellular and developmental processes [1]. MET undergoes deregulations in many types of human cancers where it contributes to several aspects of the neoplastic phenotype including cell cycle progression, invasion, metastasis, and chemoresistance [2, 3, 4]. The deregulation of MET mitogenic activity affects the control of cell cycle progression and contributes to aberrant cell divisions [5, 6, 7]. To fight these pathologies, it is necessary to gain new insights into the contribution of proximal MET binding partners in the activation of downstream signaling pathways that regulate MET-induced entry in the M phase of the cell cycle.

A number of cytoplasmic signaling proteins have been shown to be involved in MET-dependent mitogenic pathways, including growth factor receptor-bound protein 2 (Grb2) for the Ras-MAPKinase mitogenic pathway, Src for the STAT3 pathway involved in enhancement of anchorage independence and tumorigenicity, and phosphatidylinositol 3-kinase (PI3-Kinase) for the 3-phosphoinositide-dependent protein kinase 1 (PDK1)-Akt/protein kinase B (PKB) pathway that inhibits apoptosis and therefore contributes to MET activity as a survival factor [1,3]. However, the mechanisms underlying the selective proximal recruitment and the subsequent activation of the various partners of these three signaling cascades remain elusive. The initial MET-multi-substrate docking site is composed of two tyrosine residues that upon their phosphorylation are capable of anchoring and activating proteins [8, 9]. Phosphorylation of tyrosine 1349 within the C-terminus is involved in the direct recruitment of the docking protein Grb2-associated binder 1 (Gab1) that couples the MET receptor to PI3-Kinase [10, 11]. Grb2 is recruited by the phosphorylated tyrosine 1356 of the activated MET receptor [1,9]. However, besides MET, other effectors have been reported to phosphorylate Gab1 such as the cytoplasmic tyrosine kinase Src [9, 10, 12]. Furthermore, through both its SH2 and SH3 domains, Src could be recruited indirectly on MET *via* Gab1 [11].

Src and MET are deregulated in many transformed cells [13, 14]. Src activation by MET mediates metastatic properties of carcinoma cells [14, 15]. It is established that Src phosphorylates STAT3 and promotes tumorigenesis [16-18]. Furthermore, Src activates Akt in response to ionizing radiation and generates resistance in human cancers [16, 19, 20]. For all these reasons, Src is on focus as a potential target to develop anticancer therapies associated with MET deregulation.

In order to determine specific molecular connections established between the signaling cascades initiated by MET and their respective involvement in cell division, we have used *Xenopus* oocytes as a model system. It is a heterologous expression system [21, 22] amenable to biochemical analysis of mitogenic transduction cascades because it is devoid of natural tyrosine kinase receptors except for the insulin receptor.

Our results reveal a possible new crosstalk between Src and Akt signaling pathways that contribute to MET activity as a factor favouring progression in the M phase of the cell cycle. Independently of its tyrosine kinase activity, Src acts as an adapter and is required to recruit PDK1 in the MET signaling complex before cell cycle progression.

Materials and methods

***Xenopus*, oocytes handling, and G2/M checkpoint assay**

Adult unprimed *Xenopus laevis* females were anesthetized with MS 222 (1 g/L Sandoz). Ovarian lobes were surgically removed and placed in ND96 medium (NaCl 96 mM, KCl 2 mM, CaCl₂ 1.8 mM, MgCl₂ 1 mM, HEPES 5 mM, adjusted to pH 7.4 with NaOH) supplemented with streptomycin/penicillin (50 µg/mL, Eurobio), sodium pyruvate (225 µg/mL, Sigma) and soybean trypsin inhibitor (30 µg/mL, Sigma). Experiments were performed according to the guidelines of the European Community Council (86/609/EEC) and protocols were approved by the institutional local "Comité d'Ethique et d'Expérimentation Animale, Région Hauts-de-France, F59-00913".

Full-grown stage VI *Xenopus* oocytes were obtained by manual dissection of follicular cells after 1h of collagenase A (1 mg/mL Boehringer Mannheim) treatment. Oocytes were kept at 19°C in ND96 medium. Stage VI oocytes expressing MET were analyzed for their state of progression in the cell cycle. The detection at the animal pole of a white spot (also called germinal vesicle breakdown, GVBD) attests to the G2/M transition. Each experiment is the mean of batches of 20 oocytes that originate from 3 different animals.

cRNA and fusion protein preparations

To transcribe capped cRNA, MET pCS2 [22, 23], was linearized with KpnI, and processed using the SP6 mMMESSAGE mMACHINE kit (Ambion) as specified by the manufacturer. The N-SH2 and C-SH2 domains of p85 PI3-Kinase, the Src-SH2, the Src-SH3, and the Grb2-SH2 domains were expressed as GST fusions as described [24, 25].

Microinjections and drug treatments

Microinjections were performed in the equatorial region of the oocytes with the MET cRNAs (60 ng). Stimulation by the hepatocyte growth factor (HGF, 50 ng/mL, R and D Systems) was performed on 48 h expressing oocytes. The SH2 constructs (20 ng) and the various drugs (wortmannin 50 nM, and PP1 10 µM, Sigma) were respectively microinjected or added 1 h before the addition of the hepatocyte growth factor to the extracellular medium.

Immunoprecipitations and Western blot analysis

For experiments on the cytosol fraction, twenty oocytes expressing MET for 48 h treated or not with inhibitors and stimulated or not were lysed in 200 µL of buffer A (25 mM MOPS at pH 7.2, 60 mM β glycerophosphate, 15 mM paranitrophenyl phosphate, 15 mM EDTA, 15 mM MgCl₂, 2 mM DTT, 1 mM sodium orthovanadate, 1 mM NaF, 1 mM phenylphosphate, 10 µg/mL leupeptin, 10 µg/mL aprotinin, 10

μg/mL soybean trypsin inhibitor, 10 μM benzamidine), and centrifuged for 10 min at 10,000 g (4°C). For studies on the membrane fraction, the pellets were vigorously resuspended in 200 μl of buffer A added with 1% Triton X-100. After a 15 min centrifugation at 10,000 g and 4°C, supernatants were precleared with protein A-agarose (Sigma) for 1 h at 4°C. Immunoprecipitations were performed using anti-MET or anti-Src antibodies (Santa Cruz) for 3 h, and protein A-agarose (Sigma) was added for 1 h at 4°C. Immunocomplexes were collected by centrifugation, rinsed three times, resuspended in Laemmli sample buffer, and subjected to SDS-PAGE analysis. Experiments were realized on three different animals. For Western blot analysis, oocyte lysates were electrophoresed, and proteins were transferred as described [24, 25]. Briefly, proteins were electrophoresed on SDS-PAGE gels (Biorad gradient from 4 to 20%), and transferred to a Hybond ECL membrane (Amersham life Sciences) in TTBS (15 mM Tris HCl, 150 mM NaCl, 0.1% tween, 10% BSA (Sigma) at pH 8). The membranes were treated with the following antibodies: anti-Erk2 (1:1500), anti-phospho-Akt1 T308 (1:1500), anti-Stat3 (1:1500) from Upstate Biotechnology, anti-phospho-Stat3 (Tyr 705) (1:2000) from Sigma Aldrich, anti-phospho-MET Y1234 and Y1235 (1:2000) from Cell signaling, and anti-Akt1 (1:2500), anti-Gab1 (1:1000), anti-Grb2 (1:1500), anti-MET (1:1000), anti-PDK1 (1:1500), anti-phospho-Erk2 (Tyr 204) (1:2500), anti-PI3-Kinase (1:1500), anti-Src (1:1500) from Santa Cruz. Primary antibodies were washed three times 10 min in TTBS, and incubated 1 h with peroxidase-labeled secondary antibody either with anti-mouse, anti-rabbit (Invitrogen Frederick Maryland USA, 1:30 000) or anti-goat (Santa Cruz, 1: 30 000) for Western blots samples or anti-mouse and anti-rabbit (True Blot, eBioscience) for immunoprecipitated samples. After three washes in TTBS for 10 min, detections of the immunocomplexes were performed with an enhanced chemiluminescence system (Advanced ECL Detection system Amersham) on hyperfilms (Amersham).

Results and discussion

MET signaling pathways induce progression in the cell cycle

Xenopus oocyte is a biological system that allows overcoming the signaling networks activated by various types of growth factors and oncoproteins in the process of transformation. These cells are naturally arrested at the G2 border of the first meiotic division and divide synchronously under stimulation of their endogenous IGF-insulin receptors [26] or stimulation of exogenously expressed tyrosine kinase receptors for EGF, NGF, HGF/SF, and FGF [27-30]. Activation of these tyrosine kinase receptors triggers classical biochemical transduction effectors shared with somatic cells such as Grb2, PI3-Kinase, and MAPK leading to a G2/M transition easily followed and detected [22, 24, 25, 28, 31].

MET receptor used in this study was shown to mediate specific signals in epithelial cells in response to HGF [23, 32]. The expression of MET in *Xenopus* oocyte successfully triggers a signal transduction 48 h after expression and appropriate treatment with HGF. The MET receptor expressed in oocytes is phosphorylated upon HGF addition (Fig. 1A, lane 4). No responses are obtained upon addition of HGF on non-expressing oocytes or when HGF was boiled before addition on MET expressing oocytes (Fig. 1A, lane 2 and 3). The serine/threonine kinases Erk2, Akt1, and the transcription factor STAT3 are phosphorylated 15 h after treatment (Fig. 1B, lane 1). These phosphorylations are associated with a progression in the cell cycle characterized by a G2/M transition (86.9 % $\pm$ 7, Fig 1C, lane 1). Untreated oocytes never undergo biochemical phosphorylation nor an M phase entry (Fig. 1C, lane 7). These results are in agreement with previous works done on Tpr-MET dimerized oncogene form of MET and MET oncogene construct in the paradigm of *Xenopus* oocytes [7, 22, 28].

Src activity mediates G2/M transition

PP1, a potent specific blocker of Src tyrosine kinase activity [32, 33], blocks STAT3 and Erk2 phosphorylations, and the G2/M transition but remains without effect on Akt1 phosphorylation (Fig. 1B and 1C, lane 2). Src is involved in STAT3 phosphorylation in MET-induced transduction and is deregulated in various cancers [17, 18, 35], but evidence of a link between Src and Erk2 in MET signaling remains sparse [36-38] and requires further studies.

Signaling crosstalk between Src and Akt pathways

A Src-SH2 domain (GST-fusion protein) that competes for the recruitment of Src by MET intracellular domain [11, 24, 39] is without effect on STAT3 phosphorylation while Akt1, Erk2 phosphorylations, and the M phase entry are suppressed (Fig. 1B, lane 3). The same inhibitory effect is observed using the N- or the C-terminal SH2 domains of the p85 PI3-Kinase and the PI3-Kinase inhibitor wortmannin suggesting a possible link between Src and Akt pathways (respectively 4.4% $\pm$ 7.1, 1.1% $\pm$ 3.3, and 1.1% $\pm$ 3 of G2/M transition, in Fig. 1B and 1C, lanes 4, 5, and 6). Inhibition of the PI3-Kinase pathway by LY294002 was already reported after MET expression in the oocyte to inhibit M Phase entry [7, 22], but the fact that the Src-SH2 domain could interact with the Akt pathway was uncovered for MET. Microinjection of a competitor for the N terminal domain of the p85 PI3-Kinase has been mentioned to block G2/M transition and Erk2 phosphorylation for another member of the tyrosine kinase receptor family the fibroblast growth factor receptor FGFR [24, 30, 40]. However, the C-terminal domain of the p85 subunit of PI3-Kinase remains a specific inhibitor of the MET-induced PI3-Kinase activity. Altogether, these results point to a

possible effect of Src through its SH2 domain but independently of its tyrosine kinase activity in the activation of the Akt pathway by MET.

Src is required for PDK1 recruitment, Akt phosphorylation and G2/M transition

To further decipher the molecular mechanism involved in the crosstalk between Src and Akt pathways, co-immunoprecipitation experiments are performed to determine whether particular binding partners of the MET signaling complex are displaced in our different conditions. Immunoprecipitations of MET in the membrane fraction of the lysate show that Src, PDK1 (3-phosphoinositide-dependent protein kinase 1), p85 PI3-Kinase, Gab1, and Grb2 are recruited by the activated receptor (Fig. 2A, lanes 1 and 2). Several downstream partners such as p85 PI3-Kinase, Gab1, Grb2, and Src have been detected by previous studies [3, 7, 9, 12, 41] as part of MET multidocking site recruited complex, but this is the first evidence of PDK1 as a binding partner of MET. PDK1 is the downstream effector of PI3-Kinase that is involved in Akt activation [42]. PDK1 recruitment to the plasmic membrane follows PI3-Kinase activation and allows subsequent Akt activation by phosphorylation on T308 [43]. Experiments performed to determine the efficiency of the inhibitory effect of Src, p85 PI3-Kinase, and Grb2 SH2-GST fusion proteins show that a dose of 20 ng is sufficient to totally inhibit the GVBD (Fig. 2E). The microinjection of the SH2 domain of Src abolishes the recruitment of Src and PDK1 to MET as seen in anti-MET and anti-Src immunoprecipitates (Fig. 2A, lanes 3 and 7), while their binding remains unaffected under PP1 the inhibitor of Src kinase activity (Fig. 2A, lane 4). Immunoprecipitations of Src after MET activation show that PDK1, p85 subunit of PI3-Kinase, Gab1, Grb2, and MET are associated in a membrane multicomplex (Fig. 2A, lanes 5 and 6). PDK1 is found in the cytosolic fraction bound to the Src-SH2 GST domain, suggesting a displacement of PDK1 from MET membrane complex to the cytosol by Src-SH2 (Fig. 2B, lane 3). Controls performed with the N-terminal-SH2 domain of p85 PI3-Kinase and the SH2 domain of Grb2 specifically break respectively the binding of p85 PI3-Kinase and Grb2 to the Src-MET multicomplex (Fig. 2A, lanes 8 and 9). Src is known to interact with cortical cytoskeleton proteins [44]. Once activated by MET, Src interacts with cortical actin in *Xenopus* oocytes [45], explaining why Src is detected in the membrane fraction (Fig. 2C, lanes 2 and 6). Our results reveal that in both MET and Src immunoprecipitations, Src-SH2 domain affects the anchorage of PDK1 to further block Akt1 activation and the G2/M transition.

Src-SH2 domain allows PDK1 recruitment and the Akt pathway activation

At this point, the molecular mechanism involved in the Src and Akt pathways connection through PDK1 remains. Gab1 and PDK1 both display phosphorylated tyrosine sites capable of binding SH2 domains, and polyproline domains in their amino acid sequence that could be engaged with the SH3

domain of Src. Two hypotheses can be raised to explain the recruitment of PDK1. In a first hypothesis, PDK1 could be recruited to the membrane and phosphorylated by the tyrosine kinase activity of the receptor as it was described for the insulin receptor [46]. PDK1 phosphorylation could generate a docking site for the SH2 domain of Src to form a PDK1-Src-MET complex where Src could be indirectly bound to MET *via* the adapter Gab1. The Src-Gab1 binding could occur through the SH3 domain of Src when the Src-SH2 domain is bound to PDK1. An association between Src and Gab1 through the SH3 and the SH2 domains of Src has been reported [11]. In this hypothesis, Gab1 could maintain its classical interactions with MET directly and indirectly at both phospho-tyrosines 1349 and 1356 [10]. In a second hypothesis, Src could bind MET or Gab1 using its SH2 domain and then anchor PDK1 through its SH3 domain. Src-SH2 domain immunoprecipitations are performed *via* an anti-GST antibody to determine a possible binding of this Src domain to PDK1 and their localization in the membrane and cytosolic fractions. The membrane fractions are separated from the cytosolic fraction and submitted to immunoprecipitations by MET or Src antibodies. The results in Fig. 2B (lanes 3 and 7) show that PDK1 lost in the MET signaling membrane complex is specifically bound to the GST-SH2 domain of Src. On the other hand, SH2 domains of PI3-Kinase or Grb2 do not displace PDK1 nor Src from the signaling complex under HGF activation (Fig. 2A, lanes 8 and 9). SH2 domains of PI3-Kinase and Grb2 specifically compete and remove respectively PI3K and Grb2 from the MET complex (Fig. 2A, lanes 8 and 9) compared to the control (Fig. 2A, lane 6). GST immunoprecipitations (in membrane and cytosol fractions) of Src, p85PI3K-N, or the Grb2 SH2-GST fusion proteins further ascertain the binding specificity of the SH2 domains respectively to PDK1, the MET complex without PI3K, or the MET complex without Grb2 (Fig. 2D, lanes 13, 14, 15). The fact that the Src-SH2 domain specifically binds to PDK1 but not to activated MET signaling complex (Fig. 2B, lanes 3 and 7) is in favour of the first hypothesis. Moreover, the GST-Src-SH3 domain could block the G2/M transition from 87% $\pm$ 7.4 to 3.3% $\pm$ 7 (Fig. 3A, lane 3). The anchorage of Src and PDK1 to the activated MET receptor is suppressed by the GST-Src-SH3 domain that is detected bound to the immunoprecipitated-MET receptor (Fig. 3B, lanes 3). PDK1 is found in the cytosolic lysates fraction while Src is present in the membrane fraction after competition by the GST-Src-SH3 domain (respectively Fig. 3C, and 3D). Moreover, immunoprecipitation of the GST-SH3 domain of Src further confirms the binding of Src-SH3 domain to the Gab1-MET complex at the membrane (Fig. 3E, and 3G) and PDK1 in the cytosolic fraction (Fig. 3F). The Src-SH2 domain allows PDK1 recruitment and Akt1 phosphorylation while the Src-SH3 domain could stabilize Src in the MET signaling complex to activate Gab1 and PDK1 (in Fig. 4 a model is proposed to explain these interactions). Our results reveal a new connection between signaling cascades triggered by MET to elicit the G2/M transition.

In conclusion, our results reveal a new crosstalk between Src and Akt pathways. Src is required to recruit PDK1, an upstream Akt activator in the MET signaling complex. Src activity is involved in STAT3 and Erk2 phosphorylation while the Src-SH2 domain is needed for Akt1 phosphorylation on T308. Src has been described to regulate PDK1 activity for the insulin receptor, and to allow the formation of a PDK1-Src complex that protects PDK1 from degradation [46], but this regulation was never described for MET. Among the multiple functions played by Src in MET-signaling [13-20], Src usually contributes to anchorage independency and tumorigenicity. But Src is also involved in the regulation of the Akt anti-apoptotic effect [47], and could play a substantial role in the control of the cell cycle progression and the G2/M transition. The PDK1-Src interaction appears as a new possible target to counteract Akt-Met or Src-MET driven effects as anti-proliferative therapies.

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Conflicts of Interest: The authors declare no conflict of interest.

Data availability: The data that support the findings of this study are included in this manuscript.

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Figure legends

Fig. 1: SH2 domain of Src but not its tyrosine kinase activity allowed Akt1 activation by MET-activated PI3-Kinase. MET signaling was determined after microinjection and expression in *Xenopus* oocytes for 48 h. HGF (+), boiled (*) or not for 2 min, was applied for 1 h after microinjection of SH2 domains of Src, N- or C-SH2 of the p85 subunit of PI3-Kinase, or incubation with a Src inhibitor (PP1), or PI3-Kinase inhibitor wortmannin (W). Oocytes were harvested 15h after stimulation. (A) After anti-MET immunoprecipitations in the membranes, samples were submitted to anti-MET and anti-phospho-MET Y1234 and Y1235 Western blots. (B) Western blot and immunoblotting were realized on total lysates. (C) The percentage of G2/M transition (% G2/M) is expressed as a mean (+/-) standard deviation of twenty oocytes from three independent animals.

Fig. 2: MET recruits PDK1 via the Src-SH2 domain to activate the Akt1 pathway. Western blot analysis of the Src-PDK1 interaction in oocytes expressing MET for 48 h. Microinjection of SH2 domains of Src, N-SH2 of the p85 PI3-Kinase and Grb2, or incubation with PP1 were realized 1 h before stimulation. (A) Immunoprecipitations of MET or Src performed on membrane fractions, and followed by Western blots. (B) In the remaining cytosol from panel A, immunoprecipitations were performed using anti-GST antibodies to trap Src-SH2-GST, p85 PI3-Kinase-NSH2-GST, or Grb2-SH2-GST fusion proteins. (C) Immunoprecipitations and Western blots of Src were realized in the membrane and the cytosol fractions. (D) GST immunoprecipitations and Western blots performed on total (membrane and cytosol) fractions after microinjections of Src-SH2-GST, p85 PI3-Kinase-NSH2-GST, or Grb2-SH2-GST on unstimulated or stimulated expressing oocytes. (E) Percentage of G2/M transition obtained after microinjection of water, fusion protein's elution buffer (0 ng), Src-SH2-GST, p85 PI3-Kinase-NSH2-GST, or Grb2-SH2-GST at various concentrations.

Fig. 3: Docking of Src-SH3 by the activated MET receptor. Oocytes expressing MET for 48 h were analyzed after microinjection or not with the GST-Src-SH3 domain 1 h before stimulation or not by HGF. (A) Percentage of G2/M transition. (B) Immunoprecipitations of MET from membrane fractions. (C) Immunoprecipitations of PDK1 were performed in the cytosols from panel B. (D) Immunoprecipitation of Src were realized in the membrane and the cytosol fractions. (E) Immunoprecipitations of GST Src-SH3 domains from membrane fractions. Lane 2 from panel B was used as positive control (control) and loaded

on the gel for Src and PDK1. (F) Immunoprecipitations of PDK1 were performed in the cytosols from panel E. (G) Immunoprecipitations of Src were realized in the membrane and the cytosol fractions.

Fig. 4: Model proposed to illustrate the involvement of Src in MET-induced PI3K-PDK1–Akt pathway.

1: HGF/SF binding on MET triggers tyrosine kinase (TK) activity leading to its autophosphorylation on multiple tyrosine residues [1]. **2:** Upon Multi-substrate Docking Site (MDS) phosphorylation on tyrosines 1349 and 1356, MET recruits several signaling mediators including Gab1 and Grb2. Gab1 is recruited on the C-terminal lobe of the kinase *via* its Met-Binding Domain (MBD) [1,3]. **3:** Grb2 recruitment and binding to phosphorylated MET is promoted through its C-terminal SH3 domain interaction with Gab1 [1, 3, 44]. Furthermore, the Grb2 N-terminal SH3 domain allows SOS binding and Ras activation upstream of the Erk pathway [1,3]. **4:** MET tyrosine kinase phosphorylates several signaling mediators such as Gab1 on multiple tyrosines and, likely, PDK1 on tyrosine 9 [3]. **5:** Some Gab1 phosphorylated tyrosines allow PI3K interaction *via* the SH2 domains of the p85 regulatory subunit and the production of subsequent PIP3 at the plasma membrane [1,3]. **6:** Pleckstrin Homology domain (PH) stabilizes the MET-Gab1 complex and therefore sets a positive feedback loop between Gab1 and PDK1 [1, 3, 9]. **7:** PIP3 induces membrane co-recruitment of PDK1 and Akt through their PH domains [39, 40]. **8:** Simultaneously, Src through its SH3 and SH2 domains interacts respectively with both Gab1 and PDK1 phosphorylated on tyrosine 9, which are already recruited to the plasma membrane. Thus, due to these multiple interactions, Src and PDK1 stabilize each other in the MET membrane-signaling complex. **9:** Src can phosphorylate multiple MET signaling mediators such as Gab1 on multiple tyrosines different from these directly targeted by MET [41, 46], and PDK1 on tyrosine 373 and 376 which allow a full kinase activity [44]. **10:** PDK1 can phosphorylate Akt on threonine 308 and activate downstream signaling pathways [39,40].

A

IP anti-MET
(membrane)

B

C

A IP anti-MET (membrane)

IP anti-Src (membrane)

B IP anti-GST (cytosol)

C IP anti-Src

D IP anti-GST (total fraction)		<i>Src-SH2</i>	<i>p85PI3K-NSH2</i>	<i>Grb2-SH2</i>	<i>Src-SH2</i>	<i>p85PI3K-NSH2</i>	<i>Grb2-SH2</i>
	10	11	12	13	14	15	
HGF	-	-	-	+	+	+

E % G2/M

A

B IP anti-MET
(membrane)

E IP anti-GST Src-SH3
(membrane)

C IP anti-PDK1 (cytosol)

F IP anti-PDK1 (cytosol)

D IP anti-Src

G IP anti-Src