

# Cellular adherence elicits ligand-independent activation of the Met cell-surface receptor

(*c-Met*/cell adhesion/signaling/tyrosine phosphorylation/B16 melanoma)

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**ABSTRACT** Cell adherence has a fundamental role in the proliferation and motility of normal cells and the metastasis of tumor cells. To identify signaling pathways activated by the adherence of tumor cells, we analyzed the tyrosine phosphorylation of proteins in mouse melanoma cells before and after attachment to substrata. We discovered that cellular adherence activated the protein-tyrosine kinase of the cell surface receptor Met, whose ligand is hepatocyte growth factor and scatter factor. The activation was exceedingly prompt, affected the great majority of Met in the cells, persisted so long as the cells remained adherent, and was rapidly reversed as soon as the cells were detached from substrata. Activation of Met required that cells be adherent but not that they spread on the substratum, and it occurred in the absence of any apparent ligand for the receptor. Ligand-independent activation of Met occurred in several varieties of tumor cells but not in normal endothelial cells that express the receptor. The activation of Met described here may represent a means by which cells respond to mechanical as opposed to biochemical stimuli.

The adherence of cells to a substratum elicits a multitude of intracellular events. These events occur in response to signals that originate at the surface of the cell and are then propagated by diverse biochemical pathways (1, 2). The details of this propagation and the means by which it elicits phenotypic responses remain largely obscure.

The ability of cells to adhere to specific surfaces is vital in the process of tumor metastasis (3, 4). We hypothesized that the signaling response to cellular adherence might vary as a function of metastatic ability. To explore this possibility, we turned to variants of the B16 line of mouse melanoma cells. The F10 variant of these cells is highly metastatic to the lung when injected into a tail vein of mice, whereas the F1 variant is poorly metastatic (5, 6).

As a first step in exploring signaling within the F1 and F10 variants, we analyzed the phosphorylation of proteins on tyrosine. We detected no substantive difference between the variants. Instead, we were led to the unanticipated finding that attachment of either F1 or F10 cells to substrata activated the protein-tyrosine kinase of a cell-surface receptor known as Met. The activation of Met required that the B16 cells be adherent but not that they spread on the substratum, and it occurred in the absence of any apparent ligand for the receptor.

Met is a heterodimer encoded by the protooncogene *MET* (7). The gene gives rise to a single 170-kDa polypeptide that is then cleaved into  $\alpha$  and  $\beta$  subunits (45 kDa and 150 kDa, respectively). The two subunits remain joined by disulfide bonds. The  $\alpha$  subunit is exclusively extracellular, whereas the  $\beta$  subunit crosses the plasma membrane and has an intracellular domain that carries protein-tyrosine kinase activity. The established ligand for Met is a polypeptide known as both

hepatocyte growth factor and scatter factor (HGF-SF) (8, 9). Binding of the ligand activates the Met kinase, apparently by eliciting dimerization of the  $\beta$  subunits and consequent autophosphorylation (10). The eventual cellular responses can include mitogenesis, motility, and invasiveness (11–14).

It has been reported previously that Met can also be active in the absence of HGF-SF (15–17). We now show that this ligand-independent activity is elicited by the mere attachment of cells to substratum. Thus, Met can be activated by a means other than binding to the conventional ligand. Ligand-independent activation of the  $\beta$  receptor for platelet-derived growth factor has also been reported recently (18), although the requirements for that activation differ appreciably from those reported here for Met. In aggregate, these findings raise the possibility that previously unappreciated mechanisms can activate signaling from at least some transmembrane receptors, perhaps to inform the interior of the cell that a mechanical event has affected the cell.

## MATERIALS AND METHODS

**Cells.** The B16F10 murine melanoma cell line was obtained from the Division of Cancer Treatment Tumor Bank, National Cancer Institute–Frederick Cancer Research Facility (Frederick, MD). HT144 human melanoma and HT29 and HCT116 human colon carcinoma cell lines were from the American Type Culture Collection. HUVEC, human umbilical vein endothelial cells, were obtained from Clonetics. BBE, bovine brain capillary endothelial cells, were provided by R. I. Weiner (University of California, San Francisco).

**Immunoprecipitation and Immunoblotting.** Anti-phosphotyrosine monoclonal antibody 4G10 was provided by D. Morrison, B. Drunker, and T. Roberts (National Cancer Institute, Frederick, MD). Anti-Met antibody, a rabbit polyclonal anti-peptide antibody raised against murine Met, was from Santa Cruz Biotechnology. Anti-Fak antibody, a rabbit polyclonal antibody raised against chicken Fak and crossreacting with murine Fak, was from Upstate Biotechnology (Lake Placid, NY).

Tumor cells were starved in serum-free media supplemented with 1% bovine serum albumin for 18–24 hr before experiments. Lysis of cells and immunoblotting were performed as described (19), except that 3% bovine serum albumin was used instead of dry milk. Where required, cells were first detached from dishes with phosphate-buffered saline containing 0.04% EDTA before lysis.

Cell lysates were cleared by centrifugation at  $14,000 \times g$  for 5 min and incubated with antibodies for 2 hr, and then with protein G-Sepharose beads for 1 hr at 4°C. The beads were washed three times with lysis buffer. Immune complexes were either boiled in Laemmli's sample buffer for electrophoresis or analyzed by immune complex kinase assay. Kinase reactions were performed in 20  $\mu$ l of reaction buffer containing 30 mM

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Abbreviation: HGF-SF, hepatocyte growth factor and scatter factor.  
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Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 10 mM MnCl<sub>2</sub>, and 16 μCi of [ $\gamma$ -<sup>32</sup>P]ATP (1 Ci = 37 GBq) for 20 min at room temperature. Samples were boiled in Laemmli's sample buffer and then subjected to PAGE, followed by autoradiography.

**Protein Purification and Peptide Sequencing.** All purification steps were done at 4°C and each step was monitored by immunoblotting for phosphotyrosine. A 1-liter lysate of about  $4 \times 10^9$  attached cells containing 2375 mg of protein was clarified by centrifugation at  $30,000 \times g$  and passed through a wheat germ agglutinin-Sepharose column (Vector Laboratories) at a flow rate of 1 ml/min. After extensive washing, proteins were eluted with lysis buffer containing 0.5 M *N*-acetylglucosamine (Sigma) at a flow rate of 0.5 ml/min. Fractions (50 ml) were pooled and subjected to immunoprecipitation with 10 mg of 4G10 IgG and 5 ml of protein G-Sepharose (Pharmacia). The immune complexes were eluted with 10 ml of lysis buffer containing 50 mM phosphotyrosine (Sigma). The elution was concentrated with Centricon-100 (Amicon) and 20 μg of the 150-kDa protein was recovered in a 250 μl solution. The purified protein was analyzed by electrophoresis on an SDS/polyacrylamide gel and visualized by silver staining with a kit (Pharmacia).

Purified protein (100 μl) was fractionated in a polyacrylamide gel and stained in a solution containing 0.05% Coomassie blue G (Sigma), 10% methanol, and 5% acetic acid for 15 min. After destaining, the band containing the 150-kDa protein was excised and washed twice with 50% methanol. The protein was digested in gel with *Achromobacter* protease I (Wako Chemical USA) in 0.05 M Tris-HCl (pH 9.0) buffer containing 0.1% Tween 20 for 20 hr at 30°C. Peptide fragments were extracted in a solution of 50% acetonitrile and 0.065% trifluoroacetic acid, and separated with a Hewlett-Packard HPLC model 1090 using Vydac C18 column (1.0 × 250 mm/10 μm/300 Å). Separated peptides were sequenced using an automated protein sequencer (Applied Biosystems model 494).

**Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Expression Analysis.** Total RNAs and polyadenylated RNAs were prepared with kits from Qiagen (Chatsworth, CA) and Invitrogen, respectively. RNAs were reverse transcribed with random hexamers and subjected to PCR. HGF-SF primers, 5'-GTGAAGCTCTGCAGATGAGTGTGCCAAC-3' and 5'-TGAACACTGAGGAATGTCACAGAC-3', were used to amplify a 433-nucleotide product. β<sub>2</sub>-microglobulin primers (20) were used to amplify a 302-nucleotide product as control. PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

## RESULTS

**Adherent B16 Cells Display a General Increase in Phosphotyrosine Over Their Detached Counterparts.** We first found that the general level of tyrosine phosphorylation in B16F10 melanoma cells was greater in adherent than in detached cells (Fig. 1A, lanes 1 and 2). Two specific differences were especially prominent. The first was a 125-kDa protein whose phosphorylation was greatly increased in attached cells. We have identified this as the Fak kinase (see Fig. 5B), which has been implicated in the cellular response to adherence (21–23). The second prominent difference was a 150-kDa protein that was heavily phosphorylated in attached but not in detached cells. The remaining differences were less prominent and not pursued.

**The Phosphorylated 150-kDa Protein Is the β-Subunit of the Cell Surface Receptor Met.** The identity of the 150-kDa protein was not immediately apparent. The protein failed to react with antisera to a variety of integrins and cell surface receptors. We were able to show, however, that the protein is *N*-glycosylated, exposed on the surface of cells and attached to membranes (data not shown). These findings allowed us to

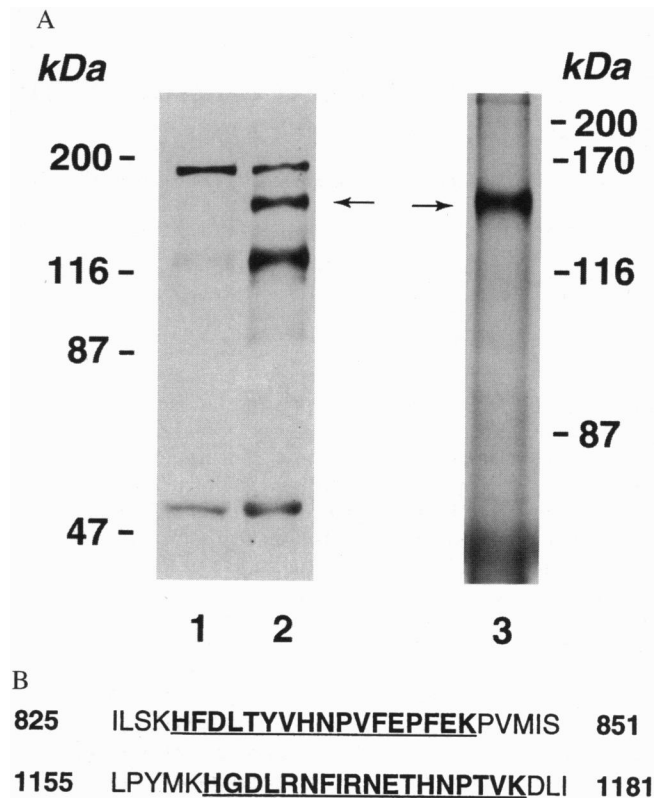


FIG. 1. Identification of tyrosine-phosphorylated Met in attached tumor cells. (A) Fractionation in polyacrylamide gels and immunoblotting. B16F10 cells were analyzed in either detached (lane 1) or attached (lane 2) forms. Cell lysates (normalized by content of protein) were fractionated in polyacrylamide gels and analyzed for phosphotyrosine by immunoblotting. The 150-kDa protein (arrows) was purified as described in the text and then analyzed in a polyacrylamide gel (lane 3). The protein was detected by silver-staining. (B) Sequences of peptides from the 150-kDa protein (underlined) are located within the sequence for Met β-subunit. The upper peptide resides within the extracellular domain of Met, the lower peptide within the intracellular domain.

design a protocol for purification that produced an apparently homogenous preparation of 150-kDa protein (Fig. 1A, lane 3).

Two peptides obtained from the purified protein were sequenced (Fig. 1B). A search of the protein-sequence data base revealed that both peptides were derived from the β-subunit of the cell surface receptor Met, one from the extracellular domain of the receptor, the other from the intracellular domain. We conclude that the 150-kDa protein is the β-subunit of Met, which is known to undergo autophosphorylation on tyrosine when the receptor is activated (10). The α-subunit of Met is not tyrosine phosphorylated and, thus, would not appear in our analyses of tyrosine phosphorylation in cellular lysates.

**Met-β Is Phosphorylated on Tyrosine in a Variety of Adherent Cells.** To pursue further the phosphorylation of Met-β in adherent cells, we used immunoprecipitation followed by immunoblotting. The immunoprecipitations were performed with an antiserum directed against Met-β. As a result, the precipitations recovered both the 150-kDa Met-β and the uncleaved 170-kDa precursor of the receptor (Fig. 2 Top). Met-β was much more abundant than the precursor, but each protein was equally abundant in adherent and detached cells (Fig. 2 Top). As anticipated, Met-β was phosphorylated on tyrosine in attached but not detached cells (Fig. 2 Middle). In contrast, the 170-kDa precursor was not substantially

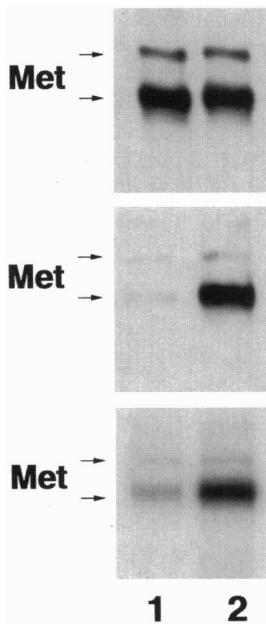


FIG. 2. Autophosphorylation of Met- $\beta$  in melanoma cells. Lysates of detached (lane 1) and attached (lane 2) B16F10 cells were normalized by content of protein and immunoprecipitated with antibody against Met- $\beta$ . The precipitates were fractionated in gels and analyzed by immunoblotting for either Met- $\beta$  or phosphotyrosine (*Top* and *Middle*, respectively). (*Bottom*) Separate immunoprecipitates were used in an assay for protein-tyrosine kinase, manifest as autophosphorylation of the receptor. Arrows designate the 170-kDa precursor of Met and the 150-kDa Met  $\beta$ -subunit.

phosphorylated in either setting. The specificity of the antisera for Met- $\beta$  precluded detection of the  $\alpha$ -subunit in these analyses.

We found further examples of tyrosine phosphorylation of Met in other lines of tumor cells that express the receptor (although there was one exception, the HT144 line of human melanoma cells) (Table 1). In every instance, the phosphorylation disappeared when the cells were detached from the substratum (data not shown). In contrast, no tyrosine phosphorylation of Met was observed in cultures of normal endothelial cells that express Met (Table 1).

When we began this study, we anticipated that variations in the capacity to metastasize might be accompanied by variations in the signaling response to cellular adhesion. In the one comparison that we have made, however, this expectation was not fulfilled; Met- $\beta$  was equally phosphorylated in both the F1 and F10 variants of B16 melanoma cells (Table 1 and data not shown), despite the large difference in their ability to metastasize (5, 6).

**The Protein-Tyrosine Kinase of Met- $\beta$  Is Active in Adherent but not Suspended Cells.** Met- $\beta$  carries the protein-tyrosine kinase activity of the Met receptor (7, 10). Activation of this kinase might well account for phosphorylation of the subunit in attached cells. We explored this possibility with an immune-

Table 1. Tyrosine phosphorylation of Met in a variety of adherent cells

Cell line	Origin	Activation of Met
B16F1	Melanoma	+
B16F10	Melanoma	+
HT144	Melanoma	-
HT29	Colon carcinoma	+
HCT116	Colon carcinoma	+
HUVEC	Vascular endothelium	-
BBE	Capillary endothelium	-

complex assay, which detected kinase activity as autophosphorylation of Met- $\beta$ . The results indicated that the kinase of Met was substantially more active in attached cells than in suspended cells (Fig. 2 *Bottom*). In contrast, the 170-kDa precursor displayed very little kinase activity and there was no difference between attached and suspended cells. We conclude that the protein-tyrosine kinase of Met is constitutively active in adherent cells, but is rapidly silenced when cells are detached from the substratum.

**Readherence of Melanoma Cells Elicits Immediate Tyrosine Phosphorylation of Met- $\beta$ .** The observations reported to this point were made with cells that had been adherent to substratum for extended periods of time. To determine how quickly tyrosine phosphorylation might occur following newly established adherence, we analyzed cells that had been removed from substratum and were then allowed to readhere for various periods of time.

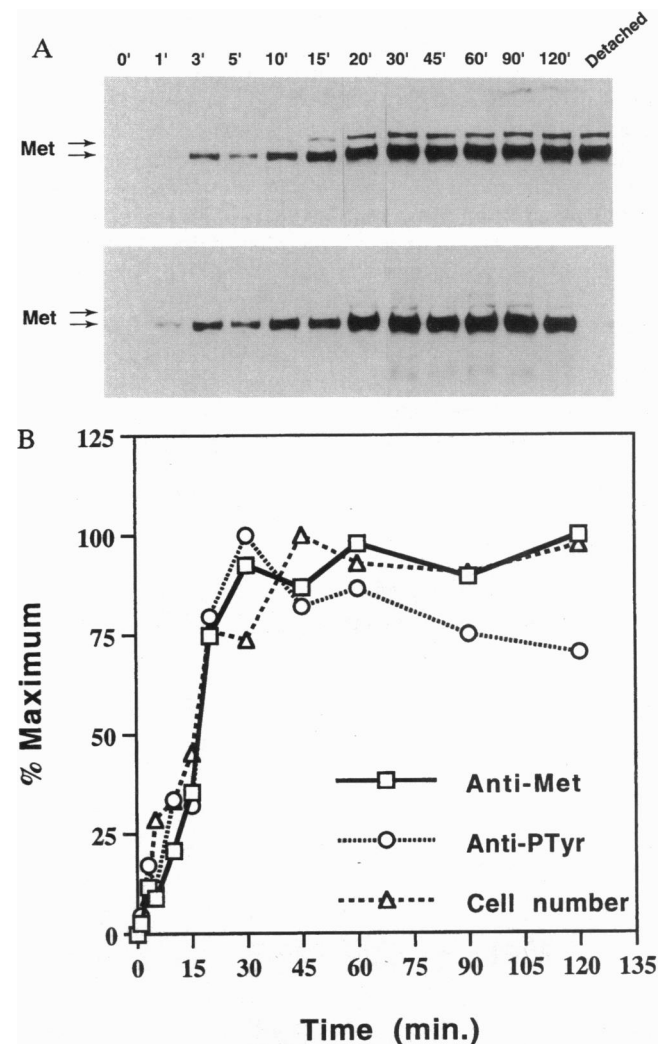


FIG. 3. Time course of Met- $\beta$  phosphorylation following adherence of melanoma cells. (*A*) Fractionation in gels and immunoblotting. Suspended B16F10 cells ( $n = 1,000,000$ ) were plated onto a 10-cm tissue culture dish in serum-free media at 37°C. At various time points, adherent cells were lysed after the floating cells were removed. Samples were immunoprecipitated with antibody to Met- $\beta$  and immunoblotted with antibodies to Met- $\beta$  (*Upper*) or phosphotyrosine (*Lower*). Arrows designate the 170-kDa precursor of Met and the 150-kDa Met  $\beta$ -subunit. (*B*) Quantitative analysis. Adherent cells were detached and counted. The immunoblots illustrated in *A* were stripped and reprobed with  $^{125}\text{I}$ -labeled secondary antibodies for phosphorim-

Met- $\beta$  was apparent in the fraction of newly adherent cells within one minute and reached a maximum by 30 min (Fig. 3A). Met accumulation reflects the number of cells that adhered. The appearance of phosphorylated Met followed a closely similar time course and then persisted without apparent diminution for as long as the cells remained adherent (Fig. 3A and data not shown). The persistence of the phosphorylation is in accord with our previous finding that the activation of Met- $\beta$  kinase and the consequent autophosphorylation are apparently constitutive events in adherent B16F10 cells (see above).

When analyzed quantitatively (Fig. 3B), the adherence of cells, the accumulation of Met, and the appearance of tyrosine-phosphorylated Met all followed the same time course. We conclude that activation of Met- $\beta$  kinase and autophosphorylation of the protein must occur without appreciable delay following adherence of the cells.

We also attempted to assess how rapidly Met- $\beta$  was dephosphorylated following detachment from substratum. The nature of the procedure for detachment limited the precision in the determination. But our results showed that in the shortest time required to detach cells and get them into lysis buffer ( $\approx 5$  min), dephosphorylation of Met- $\beta$  was complete (data not shown). The dephosphorylation of Met was partially blocked if the cells were detached in the presence of sodium orthovanadate, an inhibitor of tyrosine phosphatases (data not shown).

**Phosphorylation Affects the Majority of Met- $\beta$  in Adherent Cells.** We next sought to determine what fraction of the Met- $\beta$  in adherent B16F10 cells was phosphorylated. We first assessed the total amount of Met in adherent cells by performing a sequential immunoprecipitation and immunoblot for Met- $\beta$  (Fig. 4 Upper, lane 1). The initial precipitation was quantitative, because we could detect no residual Met- $\beta$  in the supernatant by immunoblotting (data not shown). The precipitated Met- $\beta$  was phosphorylated on tyrosine (Fig. 4 Lower, lane 1).

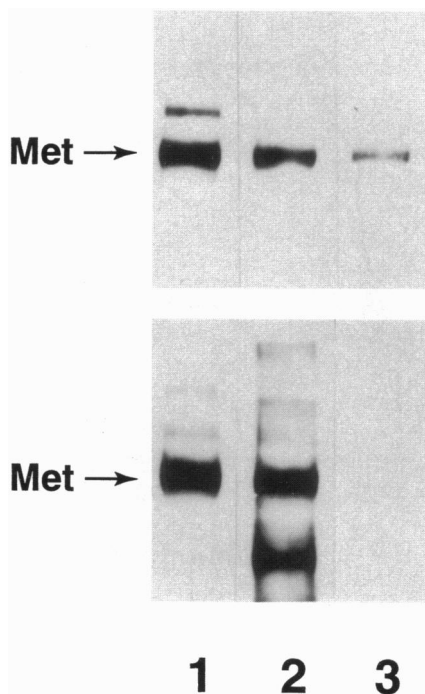


FIG. 4. Extent of tyrosine phosphorylation of Met. Equal volumes of lysates from attached B16F10 cells were immunoprecipitated for Met- $\beta$  (lane 1), phosphotyrosine (lane 2), or phosphotyrosine and Met- $\beta$  sequentially (lane 3). The immunoprecipitates were then fractionated in gels and analyzed by immunoblotting for either Met- $\beta$  (Upper) or phosphotyrosine (Lower). Arrows designate the 150-kDa Met  $\beta$ -subunit.

When the initial immunoprecipitation was performed with an antibody against phosphotyrosine, it appeared that the bulk of the detectable Met- $\beta$  was recovered (Fig. 4 Upper, compare lanes 1 and 2). In accord with this conclusion, the supernatant remaining after precipitation with antibody against phosphotyrosine contained only a small residue of Met, which was feebly phosphorylated on tyrosine (Fig. 4, lane 3). We conclude that tyrosine phosphorylation affects the great majority of Met- $\beta$  molecules in adherent B16F10 cells. As before, phosphorylation of the 170-kDa Met precursor was virtually undetectable (Fig. 4 Lower).

**Cell Spreading Is Not Required for Tyrosine Phosphorylation of Met- $\beta$ .** The initial attachment of cells to a substratum and their subsequent spreading on the surface are separable phenomena (1, 24, 25). We examined which of these two events might be required for activation of the Met kinase. First, we plated B16F10 cells in the presence of cytochalasin D, thereby disrupting the actin cables of cytoskeleton (26). Under these conditions, the melanoma cells attached firmly to the substratum but failed to spread (Fig. 5A). The failure to spread in the presence of cytochalasin D had no effect on the tyrosine phosphorylation of Met- $\beta$  (Fig. 5B Upper). Thus, it appeared that adherence alone was sufficient to activate the Met receptor.

In an effort to affirm this conclusion, we plated cells on a variety of substrata. These included poly-L-lysine, on which the B16F10 cells adhered but did not spread; and fibronectin, and type IV collagen, on which the cells both adhered and spread. Tyrosine phosphorylation of Met- $\beta$  was the same in all of these settings (data not shown). We again concluded that cellular adherence alone was sufficient for activation of the Met receptor.

The Fak kinase is found in focal adhesions, where it apparently participates in signaling after the adherence and spreading of cells (21–23). Analysis of Fak provided a striking contrast to our findings with Met. The Fak kinase in B16F10 cells was active only under circumstances where the cells were both adherent and spread (Fig. 5B Lower, and data not shown). These results suggest that the attachment of cells to substratum

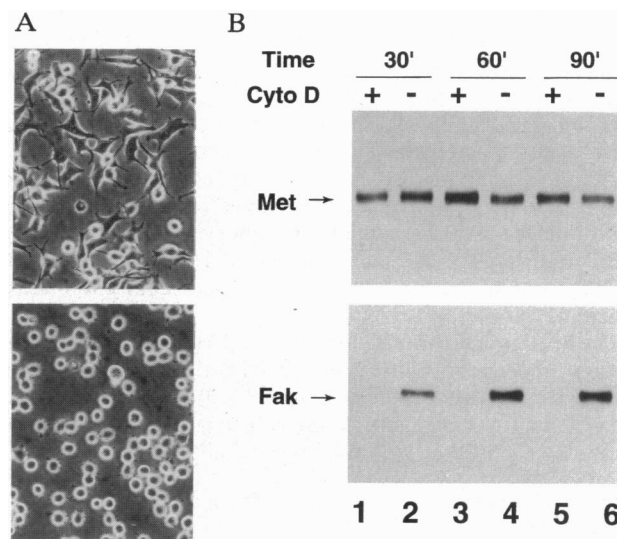


FIG. 5. Effect of cytochalasin D on phosphorylation of Met- $\beta$  and Fak. (A) B16F10 cells treated with cytochalasin D did not spread. Phase contrast photomicrographs of untreated (Upper) and treated (Lower) cells were taken after 60 min at a magnification of  $\times 100$ . (B) Phosphorylation of Met- $\beta$  but not Fak was unaffected by cytochalasin D treatment. Cells were reattached onto tissue culture dishes in serum-free medium containing 2.5  $\mu\text{g/ml}$  of cytochalasin D. Cell lysates were immunoprecipitated for either Met- $\beta$  (Upper) or Fak (Lower). The precipitates were then fractionated in gels and analyzed by immunoblotting for phosphotyrosine.

activates Met and Fak by different mechanisms, and that the activation of Met cannot be attributed to Fak.

**Activation of Met by Cellular Adherence Occurs in the Absence of Any Known Ligand for the Receptor.** By what means does cellular adherence activate autophosphorylation of Met- $\beta$ ? One possibility would be the prompt release of a ligand for the receptor, resulting in autocrine or paracrine activation. To address this possibility, we sought evidence that B16F10 cells might produce the established ligand for Met. Antisera against murine HGF-SF were not available to us, so we could not test directly for the ligand in B16 cells. Using the highly sensitive RT-PCR assay, however, we were unable to detect RNA for HGF-SF in either total or polyadenylated RNA from the B16F10 cells (Fig. 6). In addition, serum-free medium exposed to adherent B16F10 cells for 8 hr displayed no scatter activity when placed on Madin-Darby canine kidney cells, a standard test for SF (data not shown). We concluded that adherent B16F10 cells were not producing HGF-SF.

We also asked whether B16F10 cells were releasing a soluble ligand of any sort for Met. Cells were plated in serum-free medium, which was then harvested 30 min later. Incubation of suspended B16F10 cells in this conditioned medium for 20 min failed to elicit tyrosine phosphorylation of Met (data not shown). It is also unlikely that serum factors played any role in the activation of Met- $\beta$ . B16F10 cells could be plated in serum-free medium and then held in those conditions for 12 hr without effect on the tyrosine phosphorylation of Met- $\beta$  (data not shown).

We conclude that the adherence of B16F10 cells activates Met in the absence of its conventional ligand and without the detectable assistance of a soluble ligand of any sort. On the other hand, adherence is not essential for the activation of Met; application of HGF-SF to B16 cells in suspension elicited tyrosine phosphorylation of Met (data not shown).

## DISCUSSION

**Cellular Adherence Activates the Met Receptor.** The transmembrane receptor Met is characteristically activated by the polypeptide factor HGF-SF. It has been reported previously, however, that Met can also be constitutively active in tumor cells that are neither exposed to HGF-SF nor producing the factor themselves (15–17). Here we show that the simple attachment of cells to a substratum suffices to explain that constitutive activation.

Activation by attachment occurs in a variety of tumor cells that express Met, including carcinomas and melanomas, but not in normal endothelial cells, where Met is also expressed.

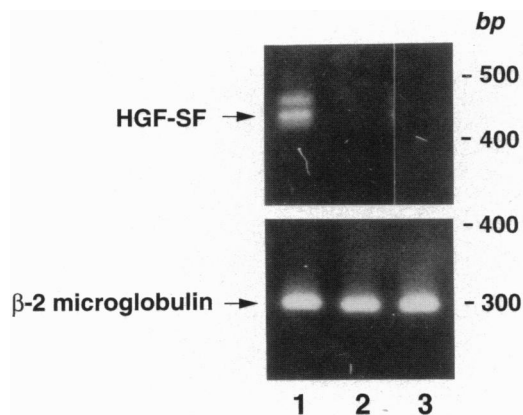


FIG. 6. Expression of HGF-SF in melanoma cells. RNAs from B16F10 and NIH 3T3 cells were analyzed by RT-PCR for HGF-SF and  $\beta_2$ -microglobulin as described. Lane 1, total RNA from NIH 3T3 cells; lane 2, total RNA from B16F10 cells; lane 3, polyadenylated RNA from B16F10 cells.

The activation is exceedingly prompt, persists so long as the cells remain adherent, and is rapidly reversed as soon as the cells are detached from substrata. It is possible that a similar activation would occur in response to intercellular adhesion, but we have yet to evaluate this possibility. The activation of Met reported here occurred even when cells were deposited on substrata at very low population densities, so intercellular contact played no part in the activation observed here.

The response of Met to adherence resembles that to HGF-SF: the protein-tyrosine kinase of the receptor is activated, leading to autophosphorylation of tyrosine in the  $\beta$ -subunit. We do not know, however, whether the same residues of tyrosine are affected in both settings, nor whether the same signaling pathways are activated downstream of Met. Indeed, we suspect that different residues of tyrosine on Met may be involved, because the combination of adherence and HGF-SF gives greater tyrosine phosphorylation than either stimulus alone, even though virtually all of the Met molecules are phosphorylated in response to adherence alone (unpublished results).

The initial attachment of cells to a substratum and subsequent spreading of the cells on the surface are separable phenomena (1, 24, 25). We found that only the former is required for activation of Met. Two very different circumstances were used to impede spreading and each allowed the activation of Met. The distinction between adherence and spreading was further dramatized by the finding that, in contrast to Met, the Fak kinase was activated only under conditions that allowed both attachment and spreading.

**Met Is Activated in the Absence of the Ligand HGF-SF.** We have been unable to implicate HGF-SF in the activation of Met that follows cellular attachment. The activation occurs in the absence of any external source of HGF-SF and the cells themselves do not produce the factor. Moreover, experiments with media conditioned by adherent cells indicate that no soluble inducer of any sort is involved in the activation. How then does the activation occur?

(i) The ability of Met to be activated by adherence may be unique to certain tumor cells; we have not observed the activation in any normal cells that express Met. Nevertheless, there is no reason to believe that Met itself is abnormal in the tumor cells, although it is sometimes overexpressed (15, 16). (ii) It is possible that some component of the extracellular matrix is serving as a surrogate ligand for Met. We cannot rigorously eliminate this explanation, although the activation occurs so rapidly that it is likely to precede the deposition of matrix on the substratum, and it does not occur in suspended cells, which carry extracellular matrix on their surface. (iii) The contact between an integrin and the substratum might trigger the activation. We have yet to evaluate this explanation rigorously, but in any event, it does not provide an immediate mechanism of activation for the Met kinase. (iv) Cross-talk from another receptor might activate Met, but this begs the question of how the postulated receptor is itself activated. (v) The Fak kinase is found in adhesion plaques and is activated by adhesion (21–23). Might Fak be involved in the activation of Met by cellular attachment? We doubt that this is the case because Met is activated under several circumstances when Fak is not. (vi) A sixth possibility invokes a direct mechanical effect on cells. Like many other transmembrane receptors, Met is activated by dimerization in the plane of the membrane (10). Perhaps cellular attachment elicits an architectural rearrangement of cytoskeletal and membranous elements that in turn drives dimerization of Met. Indeed, Faletto *et al.* (30), have reported previously that Met is constitutively aggregated in at least some cell lines. That aggregation may be a counterpart of the activation of Met that we reported here.

**The Activation of Met May Mediate the Cellular Response to Adherence.** It now appears possible that a number of cell surface receptors may transduce signals to the cellular interior as a result of mechanical stimuli. For example, a recent report

described the ligand-independent activation of the platelet-derived growth factor  $\beta$ -receptor in response to cell adhesion and spreading, distortion of cell shape and external strain (18). The activation superficially resembled that described here for Met, but there were notable differences as well. In particular, the ligand-independent activation of the platelet-derived growth factor  $\beta$ -receptor was not elicited by cell attachment alone, requiring both adherence and spreading, and it was accompanied by activation of Fak.

Several previous reports have implicated the activity of Met in tumorigenicity and metastasis, by virtue of its ability to elicit mitosis, motility, invasiveness and angiogenesis (14, 27–29). We failed to find any evidence that the ligand-independent activation of Met correlated with the metastatic capability of tumor cells. But we have examined only one pair of metastatic variants, and the assay by which their metastatic potential was determined may not be sensitive to the role of Met. Mitogenesis and motogenesis are also prominent cellular responses to the activation of Met by HGF-SF. We have no evidence that the ligand-independent activation of Met described here elicits either of these responses, but the matter requires further study.

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