Alternagin-C, a Disintegrin-like Protein, Induces Vascular Endothelial Cell Growth Factor (VEGF) Expression and Endothelial Cell Proliferation in Vitro*

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Alternagin-C (ALT-C), a disintegrin-like protein purified from the venom of the Brazilian snake Bothrops alternatus, interacts with the major collagen I receptor, the $\alpha_2\beta_1$ integrin, inhibiting collagen binding. Here we show that ALT-C also inhibits the adhesion of a mouse fibroblast cell line (NIH-3T3) to collagen I ($IC_50$, 2.2 $\mu$m). In addition, when immobilized on plate wells, ALT-C supports the adhesion of this cell line as well as of human vein endothelial cell (HUVEC). ALT-C (3 $\mu$m) does not detach cells that were previously bound to collagen I. ALT-C (5 nM) induces HUVEC proliferation in vitro, and it inhibits the positive effect of vascular endothelial growth factor (VEGF) or FGF-2 on the proliferation of these cells, thus suggesting a common mechanism for these proteins. Gene expression analysis of human fibroblasts growing on ALT-C- or collagen-coated plates showed that ALT-C and collagen I induce a very similar pattern of gene expression. When compared with cells growing on plastic only, ALT-C up-regulates the expression of 45 genes including the VEGF gene and down-regulates the expression of 30 genes. Fibroblast VEGF expression was confirmed by RT-PCR and ELISA assay. Up-regulation of the VEGF gene and other growth factors could explain the positive effect on HUVEC proliferation. ALT-C also strongly activates Akt/PKB phosphorylation, a signaling event involved in endothelial survival and angiogenesis. In conclusion, ALT-C acts as a survival factor, promoting adhesion and endothelial cell proliferation.

Cell attachment to the extracellular matrix depends mostly on the integrins, a large family of glycoproteins expressed at the cell surface (1). Integrins are heterodimers formed of non-covalently associated $\alpha$- and $\beta$-subunits (2). In many cells in culture, integrin-mediated adhesion results in specialized adhesion sites, named focal contacts (3). In these sites, structural and signaling proteins such as integrins, cytoskeletal proteins, and kinases are concentrated and initiate signal transduction pathways (4). Aggregation of integrin receptors, ligand occupancy, and tyrosine kinase-mediated phosphorylation are the key events that result in diverse processes such as cell migration and differentiation, tissue remodeling, cell proliferation, angiogenesis, and tumor cell invasion and metastasis (1, 5).

Antagonists of integrins have been developed in order to provide powerful therapeutic approaches for the treatment of several types of cancer, such as antibodies to the $\alpha_v\beta_3$ integrin (6). Synthetic peptides with the sequence Arg-Gly-Asp (RGD) can competitively block the binding of several integrins to their ligands and efficiently reduce platelet aggregation and the number of experimental metastasis (7). RGD peptides induce the disassembly of focal contacts in melanoma cells and disrupt the actin cytoskeleton (8). Disintegrins are small peptides derived from viperidae snake venoms with an internal RGD or RGD motif (9). Disintegrins can bind to integrins and interfere with integrin function. In platelets, disintegrins inhibit the adhesion of fibrinogen to its receptor, the integrin $\alpha_{IIb}\beta_3$, resulting in inhibition of platelet aggregation (10, 11). Some RGD-disintegrins have been shown to inhibit tumor cell-extracellular matrix adhesion (12) and decrease the number of experimental metastasis (13, 14). RGD-disintegrins bind mostly to $\alpha_{IIb}\beta_3$, $\alpha_\text{v}\beta_3$, and $\alpha_\text{v}\beta_5$ integrins in distinct cell types therefore inhibiting also cell adhesion to fibronectin (13). Acutin and triflavin, two RGD-disintegrins from Agkistrodon acutus and Trimeresurus flavourvidis venoms, respectively, inhibit angiogenesis and induce apoptosis in endothelial cells (15, 16). The VAP1 protein (vascular apoptosis-inducing protein) isolated from Crotalus atrox venom is a metalloprotease/disintegrin that induces apoptosis (17). Since it was shown that the RGD-dependent $\alpha_v\beta_3$ integrin provides a survival signal to proliferative endothelial cells during new blood vessel growth (18, 19), it is thought that the anti-adhesive activity of RGD-disintegrins on endothelial cells may contribute to their anti-angiogenic activity.

A different class of disintegrin is also found in some snake venoms that do not have the RGD motif. These proteins are larger than the RGD-disintegrins (about 30 kDa) and they have an extra C-terminal, cysteine-rich domain (20–24). These disintegrin-like proteins do not bind to the integrin $\alpha_{IIb}\beta_3$, $\alpha_\text{v}\beta_3$, or $\alpha_\text{v}\beta_5$, but they interact with the collagen receptor, the $\alpha_\text{v}\beta_3$ integrin therefore inhibiting cell adhesion to collagen I (23).

The majority of the RGD and non-RGD disintegrins is synthesized in the venom gland as precursor forms having a pro- and metalloprotease domains, and proteolytic processing of
these proteins releases the disintegrin-like/cysteine-rich domain (20–21,23). It is possible to isolate the full-length protein or the processed domains from the venom. Related proteins (the ADAMs, for a disintegrin and metalloproteinase) are found in mammals as well as in several other organisms, in which they are involved in several physiological processes such as fertilization, cell differentiation, and shedding of receptors (25). The ADAMs have a similar domain organization with extra domains including transmembrane and intracellular domains (26). Both ADAMs and snake venom metalloproteinases (SVMPs) belong to the Reprlysins protein family of metalloproteinases (27).

We have previously described the isolation and characterization of ALT-C, a disintegrin-like protein from Bothrops alternatus snake venom (23). ALT-C is synthesized as a precursor form with a metalloprotease domain, from which is released after proteolytic processing, yielding a form with disintegrin and cysteine-rich domains (23). ALT-C binds to the αβ3 integrin-transfected-K562 cells therefore inhibiting collagen I adhesion (IC50, 100 nM). When immobilized on plate wells, ALT-C promotes the adhesion of αβ3 integrin-transfected-K562 cells but not the adhesion of control cells. ALT-C does not bind to the integrins α1β1, αβ1, αβ3, αβ1, and αβ3 (23). In human neutrophils, ALT-C also induces migration via integrin signaling, with activation of focal adhesion kinase (FAK) and its association with phosphatidylinositol 3-kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; TGFβ1, transforming growth factor-β; VEGF, vascular endothelial growth factor. These effects are opposite to those observed for most RGD-disintegrins, which inhibit angiogenesis and induce apoptosis (15, 30). To our knowledge, this is the first report of a disintegrin acting as a survival factor.

In the present work we show that ALT-C induces endothelial cell proliferation in vitro, and these effects could be mediated at least in part by an increased expression of vascular endothelial growth factor. These effects are opposite to those observed for most RGD-disintegrins, which inhibit angiogenesis and induce apoptosis (15, 30). To our knowledge, this is the first report of a disintegrin acting as a survival factor.

**EXPERIMENTAL PROCEDURES**

**Materials**—The venom of B. alternatus was kindly provided by the venom commission of Instituto Butantan, São Paulo, SP, Brazil. Alternacin-C (ALT-C) was purified from B. alternatus as previously described (23). Recombinant human FGF-2, VEGF165, and the mouse anti-VEGF monoclonal antibody (clone 26503) were purchased from R&D Systems, Minneapolis, MN. Genistein, LY294002, PMA, and anti-VEGF monoclonal antibody (clone 26503) were purchased from Calbiochem, San Diego, CA. Protein A/G-agarose, anti-Akt, and anti-phosphotyrosine antibodies were from Santa Cruz Biotechnology, streptavidin-conjugated horse-radish peroxidase was from Caltag Laboratories.

**Cell Lines**—Mouse embryo fibroblasts NIH-3T3 were from American Type Culture Collection. Human fibroblasts were obtained from Clonetics™. HUVECs (10^4 cells/well) were seeded in 199 medium plus 10% fetal bovine serum were seeded in 199 medium supplemented with 5% fetal calf serum and grown for 2 h. In order to investigate the possibility that the cell counts obtained after 72 h merely reflected a proportional difference in initial cell adhesion rates for each condition, an adhesion assay was run in parallel, and revealed after allowing cells to adhere for 2 h. The number of cells was then quantified by the MTT procedure as above.

**Gene Expression**—Plate wells were coated overnight with ALT-C (10 μg/ml) or collagen type I (40 μg/ml) and then blocked with 1% BSA. Human fibroblasts (passage 4) at 80% confluence in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum were seeded (2 × 10^5 cells/ml) in 10-cm plastic dishes on ALT-C, collagen type I or directly on plate wells. After 24 h at 37 °C in 5% CO2, cells were removed, and cells were lysed with lysis buffer from QIAgen RNAeasy kit for isolation of total RNA.

**Target Preparation, GeneChip™ Hybridization, and Data Analysis**—Labeled cRNA was synthesized from total RNA according to standard Affymetrix protocols. Brieﬂy, 20 μg per sample of total RNA and a 20 μg per sample of RNA containing a T7 RNA polymerase promoter were used to generate double-stranded cDNA. A T7 based in vitro transcription reaction (Enzo BioArray High Yield RNA Transcript labeling kit) was used to generate biotin-labeled and amplified cRNA from double-stranded cDNA. Total RNA integrity and cRNA size distribution were measured by the 2100 Bioanalyzer (Agilent Technologies), using the RNA 6000 Nano LabChip Kit (Agilent Technologies) protocol. Briefly, 2 μg of cRNA was denatured and incubated at 60 °C for 10 min prior to hybridization. The labeled cRNA was then hybridized to Affymetrix HG-U133A and HG-U133 Plus2 arrays. In the HG-U133A array, the global signal was normalized to a reference experiment by arranging the microarray signals. Feature extraction and robust multivariate analysis were performed using邑oGnome Software. The HG-U133 Plus2 array, which is an upgraded version of HG-U133A, was used to validate changes observed in the HG-U133A microarray experiment. Feature extraction and robust multivariate analysis were performed using邑oGnome Software.
Cell Proliferation Induced by a Disintegrin-like Protein

results

ALT-C Inhibits the Adhesion of Mouse Fibroblasts to Collagen I—It was previously reported by our group that ALT-C inhibits the adhesion of αβ1-transfected cells to collagen type I, with an IC50 of 100 nM (23). Here we show that the binding of mouse fibroblasts (NIH-3T3) to collagen I is also inhibited by ALT-C (Fig. 1). ALT-C inhibited collagen binding to fibroblasts with an IC50 of 2.2 μM. Inhibition was not 100% even in higher concentrations probably due to the presence of other collagen receptors in fibroblasts. These results suggest that the integrin αβ1 is involved in the adhesion of this cell type in the binding of collagen I.

ALT-C Supports the Adhesion of Fibroblasts and Endothelial Cells—It was previously demonstrated that ALT-C supports the adhesion of αβ1-transfected K562 cells but not the control cells (23). Here we show that ALT-C also significantly supported the adhesion of fibroblasts and endothelial cells in a mass-dependent fashion (Fig. 2, A and B). Adhesion to collagen I was considered to be 100%. These results confirm the effect of ALT-C as an adhesion molecule.

In agreement with these data, ALT-C was unable to detach cells that were previously adhered to collagen I (Fig. 3A). We have done a collagen concentration x adhesion curve for both fibroblasts and HUVECs, in which we could see that even collagen concentrations lower than 0.01 μg/well support cell adhesion (not shown). The collagen concentration that gives 50% of adhesion was 0.04 and 0.12 μg/well for HUVECs and fibroblasts, respectively. The adhesion of mouse fibroblasts to collagen I (0.12 μg/well) was not affected by incubation with ALT-C (1–4 μM) for 2 h (Fig. 3A). Comparable results were obtained for HUVECs (Fig. 3B); however, lower ALT-C concentrations must be used since HUVECs from primary culture are more sensitive than the fibroblast cell line used in this work. Only at the dose of 100 nM ALT-C induced a low but significant detachment (10%). Thus, ALT-C strongly favors cell adhesion and does not reproduce the anti-adhesive actions already described for some RGD-type disintegrins (33–34).

ALT-C Induces Endothelial Cell Proliferation—The ability to antagonize cell adhesion has been described as a main feature

FIG. 1. ALT-C inhibits mouse fibroblasts adhesion to collagen I. 96-well plates were coated with collagen type I (1 μg/well) in 0.1% acetic acid overnight at 4 °C. After blocking with 1% BSA, CMFDA-labeled cells (10^5 cells/well) were incubated with ALT-C and seeded in the wells. The plates were incubated at 37 °C for 30 min. After washing, remaining cells were lysed, and the plate was read for the release of fluorescence. The results were obtained from three independent experiments and in triplicate. The results for ALT-C were normalized by the collagen values without ALT-C in each experiment. The error bars show the S.E. of six samples with less deviation from the mean. The means for all ALT-C concentrations were significantly different from the collagen I using Dunnett’s test; **p < 0.01.

Analysis of VEGF Expression by RT-PCR and ELISA—Human fibroblasts were grown on 6 cm dishes coated with ALT-C (10 μg/ml), collagen type I (40 μg/ml) or collagen I (400 nM). Cells were lysed, 24, and 48 h after treatment, and total RNA was isolated using TRIzol (Invitrogen). Samples were treated with DNase before the experiments in order to discharge any contaminant DNA. RT-PCR was run using the Superscript one-step RT-PCR kit (Invitrogen), according to manufacturer’s instructions. The following VEGF primers were used: forward, GACCGGAGAAAGCATTGTGG; reverse, TGCAACCGAGTGCTGTTTG. β-Actin primers were used in the same conditions as endogen controls (forward, CGGGACGGCCGCTCTTGCACCAGGG, and reverse, CGGAGGAAGAGGATGGCGGCAGTGG).

PCR products were analyzed in a 2% agarose gel electrophoresis. For GCACCAGGG, and reverse, CGGAGGAAGAGGATGGCGGCAGTGG).
of anti-angiogenic disintegrins. Since the disintegrin-like
ALT-C promoted endothelial cell adhesion, we further investi-
gated its effects on endothelial proliferation. The stimulation of
endothelial cell proliferation achieved when ALT-C was immo-
bilized on plastic was proportional to the concentration of
ALT-C coated to plastic surfaces (Fig. 4A). In the presence of
5% fetal calf serum, no significant variations were seen in the
initial adhesion rates among the different conditions (adhesion
measured after 2 h from seeding cells), including cells adhering
on wells coated only with BSA. However, only in wells coated
with ALT-C the number of cells increased after 72 h, and the
increase was dependent on ALT-C concentration (Fig. 4A).

HUVECs were also responsive to treatment with different con-
centrations (1–100 nM) of soluble ALT-C (Fig. 4B). The induc-
tion of proliferation observed when treating cells with 5 nM
ALT-C was comparable to that observed with cultures treated
with 10 ng/ml FGF-2 or 10 ng/ml VEGF, two potent angiogenic
factors (Fig. 4B). Interestingly, concentrations of ALT-C
greater than 10 nM significantly reduced this effect. ALT-C (10
and 100 nM) significantly inhibited the VEGF and FGF-2 ef-
facts, respectively (Fig. 4C). ALT-C did not induce proliferation
of mouse fibroblasts in any of the doses and periods tested
(Fig. 4D).

Gene Expression Induced by ALT-C—In order to explain the
effects of ALT-C on cell proliferation, we performed a gene
expression assay using the GeneChip™ technology. When
compared with human fibroblast growing on plastic, ALT-C
induced a significant increase in several genes related to cell
cycle control, including VEGF (Fig. 5) and other growth factors
such as inducible early growth response (TGFβ), interleukin 11
(IL-11), early growth response 2 and 3 (EGR2 and 3), and
insulin-induced gene (IIG1). A total of 45 genes were up-regu-
lated and 30 genes were down-regulated with this experiment. The expression of VEGF may explain the positive effect of ALT-C on HUVEC proliferation.

The effect of immobilized ALT-C is probably similar to the collagen effect since the differences in gene expression of these two proteins were much smaller (Fig. 6). A total of 8 genes were up-regulated, and 4 genes were down-regulated when comparing the expression induced by ALT-C and collagen I. The significance of this difference is not understood yet. VEGF expression induced when human fibroblasts were grown on both ALT-C- or collagen-coated dishes for 24 h was also confirmed by RT-PCR (Fig. 7A). However, striking differences were seen when collagen-bound fibroblasts were treated with soluble ALT-C. VEGF expression was significantly increased after 48 h of incubation, while cells growing on immobilized ALT-C or collagen showed no difference (Fig. 7B).

**ALT-C Induces Akt/PKB Phosphorylation in HUVECs**—Phosphatidylinositol 3-kinase (PI3K)-Akt/PKB signaling axis is activated by many angiogenic growth factors (36). To determine whether the Akt/PKB activating pathways were essential for the proliferative effect induced by ALT-C on endothelial cells, we incubated HUVEC monolayers with ALT-C (5 nM) for 15 min, in the absence or presence of different kinase inhibitors. The interaction of ALT-C with endothelial cells strongly
activated Akt/PKB phosphorylation, as compared with control cultures (Fig. 8). Akt/PKB is a downstream target of activated PI3K (37) and PKC has been pointed as a key mediator of endothelial cell proliferation and differentiation (38, 39). Accordingly, ALT-C-activated Akt/PKB phosphorylation was abrogated by both LY294002, a selective inhibitor of PI3K, and BIM, a broad inhibitor of PKC family members. Tyrosine kinases may also contribute to the activation of Akt/PKB by ALT-C, since the treatment of HUVECs in the presence of genistein strongly inhibited Akt/PKB phosphorylation (Fig. 8).

**DISCUSSION**

We have previously demonstrated that ALT-C is a ligand for the major collagen I receptor, the integrin α1β1 and also supports K562 cell adhesion mediated by this integrin (23). In this report we show that ALT-C also supports the adhesion of other cell types such as mouse fibroblasts and HUVECs. Moreover, the adhesion of fibroblasts to collagen I is inhibited by ALT-C, supporting the evidence that the α1β1 integrin is a major collagen receptor in these cells. As would be expected for an adhesive protein, ALT-C failed to induce fibroblast and HUVEC detachment from collagen-coated surfaces, even in low collagen concentrations. It has been suggested that jararhagin, a metalloprotease with disintegrin-like and cysteine-rich domains, acts as a collagen agonist of the α1β1 integrin, causing the activation of this integrin and producing collagen-like cell signaling events such as the up-regulation of matrix metalloproteases (29). Since ALT-C does not have the metalloprotease domain, the results presented here provide strong evidence that the disintegrin and cysteine-rich domains are responsible for integrin activation. Recently, it has been demonstrated that two peptides derived from the Cys-rich domain of jararhagin and from a homologue, atrolysin a from Crotalus atrox snake venom, inhibit collagen I binding to platelets. This effect does not involve GPIV, another collagen receptor in platelets (40).

Our present data also show that ALT-C provides a suitable support for the adhesion of HUVECs. Moreover, in response to growing concentrations of ALT-C, either immobilized to plastic or incubated with HUVECs in the soluble form, it strongly induced endothelial cell proliferation. ALT-C up-regulates the expression of VEGF in human fibroblasts, which could explain the increase in HUVEC proliferation. We do not know if ALT-C could induce VEGF expression in HUVEC cells but it remains a possibility to be confirmed. Interestingly, ALT-C did not induce proliferation of mouse fibroblasts, thus suggesting a cell-specific effect.

The proliferative effect of ALT-C alone was similar to those exerted by VEGF and FGF-2, and the presence of ALT-C partially inhibited the endothelial cell proliferation induced by VEGF and FGF-2. Taken together, these data suggest that these proteins may partially act by a common cross-talk of signaling cascades (41). ALT-C also up-regulates the expression of other growth factors involved in cell proliferation such as IL-11, TGFβ3, and EGR2 and 3, which are probably also involved on its positive effect on HUVEC proliferation. IL-11 induces proliferation of human T-cells (42), stimulates hematopoiesis and inhibits apoptosis in a variety of cells (43). EGR2 and 3 stimulate the activities of several transcription factors that are associated with cell proliferation such as c-Fos, SRF, and c-Myc (44).
Interestingly, TGFβ/H9252 regulates several inhibitory cell-cycle proteins such as p27 and p15 (45). It seems that a delicate balance between the levels of different growth factors may exist, and the effect of factors that activate the cell cycle overwhelms the effect of factors with opposite actions.

Cell detachment usually results in anoikis, a form of apoptotic cell death that occurs upon loss of matrix attachment (46), except for transformed cells expressing activated Src and Ras oncogenes (47). As demonstrated for fibroblasts, ALT-C does not induce endothelial detachment from collagen, gelatin or fibronectin-coated surfaces (data not shown) and, apparently depending on cell type, it induces cell proliferation, therefore acting as a survival factor. It has been shown that integrins and growth factor receptors coordinately regulate the expression of pro-apoptotic proteins to prevent anoikis (48). The small differences in the gene expression pattern of cell adhesion induced by immobilized ALT-C or collagen I are in agreement with the idea that this disintegrin-like protein could act as a collagen-mimetic (29). However, the effect of soluble ALT-C was very different from collagen, since it induced an important and prolonged increase in VEGF expression. These results suggest a role for ALT-C on HUVEC signaling by providing cells with sustained survival signals.

The serine/threonine protein kinase Akt/PKB has been described as a key regulator of cell viability (49, 50). Activation of Akt/PKB occurs through the direct binding, in the plasma membrane, of the phosphoinositide products of PI3K reaction to its pleckstrin homology domain (51). It has been demonstrated that matrix adhesion and Ras transformation both activate the PI3K → Akt/PKB survival pathway (52) and that Akt/PKB is targeted for destruction by caspases during anoikis of endothelial cells (53). VEGF activation of Akt/PKB signaling in endothelial cells is also dependent on matrix attachment, and constitutively active Akt/PKB blocks the apoptosis induced by endothelial cell detachment (54).

On the other hand, many mitogens are activators of PKC family members, which have also been implicated as important mediators of endothelial cell viability (55). Phorbol esters, which mimic diacylglycerol and hence activate PKC isoenzymes, activate endothelial proliferation and induce the formation of tube-like structures by endothelial cells in vitro (38, 39).

Since ALT-C acted as a strong promoter of endothelial cell

**FIG. 7.** ALT-C induces VEGF expression in human fibroblasts. Fibroblasts were grown on ALT-C (10 μg/ml), collagen type I (Collagen, 40 μg/ml) or collagen I treated with ALT-C (400 nM) (Coll + ALT-C). Cells were lysed 4, 24, or 48 h after treatment for total RNA and protein isolation. A, detection of VEGF mRNA. RNA samples (24 h) were treated with DNase before the experiments in order to discharge any contaminant DNA. RT-PCRs were run using the VEGF primers as described under “Experimental Procedures.” β-Actin primers were used as endogenous control. B, detection of VEGF by immunoassay. ELISA assays for VEGF detection were performed using the kit Quantikine immunoassay for human VEGF as described under “Experimental Procedures.” Error bars show the S.E. of two independent experiments in triplicate. The mean significantly different using Bonferroni’s post-hoc is shown by ***, p < 0.001.

**FIG. 8.** ALT-C induces Akt/PKB phosphorylation in HUVECs. Cells were incubated with 5 nM ALT-C or PMA 15 min at 37 °C, with or without inhibitors genistein (80 μM), LY294002 (3 μM), or BIM (10 nM). Cell lysate was immunoprecipitated with anti-Akt/PKB antibodies and probed with anti-Akt/PKB and anti-phosphotyrosine antibodies by Western blotting. Statistical significance was assessed by ANOVA followed by Bonferroni’s t test, and p < 0.05 was taken as statistically significant.
adhesion and proliferation, we examined whether the interaction of ALT-C with HUVECs promotes the phosphorylation of Akt/PKB or involves the participation of PKC. Under the conditions of our assays, ALT-C promoted an important increase in Akt/PKB phosphorylation, which was blocked by the presence of PI3K and PKC inhibitors. Moreover, in the same assays, Akt/PKB phosphorylation was increased by PMA (a phorbol ester) to levels comparable to those resulting from ALT-C treatment of HUVECs. These data demonstrate that the binding of ALT-C to endothelial cells, or that the cell adhesion to this disintegrin-like molecule, initiates signaling cascades leading to the activation of PI3K. This result indicates that PKC family members also participate in this response. In fact, it was shown that PMA activation of HUVECs also leads to the activation of PI3K.

The fact that gemcitabine, a potent tyrosine-kinase inhibitor, also resulted in the blockade of ALT-C-induced Akt/PKB phosphorylation in endothelial cells is consistent with our previous observations. It has been demonstrated that disintegrins can induce intracellular signaling events such as an increase in the content of F-actin and cytoskeleton organization. These observations suggest that similar functions could be work common to integrins and tyrosine kinases receptors. 16. Sheu, J. R., Yen, M. H., Kan, Y. C., Hung, W. C., Chang, P. T., and Luk, H., N. Cancer Res. 15. Yeh, C. H., Peng, H. C., and Huang, T. F. (1998) Blood 238, 188–196
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