The receptor tyrosine kinase Tie1 is expressed and activated in epithelial tumour cell lines

KATHRYN A. REES, HARPRIT SINGH and NICHOLAS P.J. BRINDLE

Department of Cardiovascular Sciences, University of Leicester, Leicester, LE2 7LX, UK

Abstract. The receptor tyrosine kinase Tie1 is expressed primarily in vascular endothelial cells. The receptor has also been detected in epithelial tumours in breast, thyroid and gastric cancers and in tumour cell lines where it appears as a 45 kDa truncated receptor fragment. In this study, we show that in addition to truncated Tie1, breast and colon tumour cell lines express a full-length Tie1 holoreceptor. In contrast to the situation in endothelial cells, Tie1 truncation is not activated by phorbol esters and generation of truncated Tie1 does not occur via a metalloprotease-inhibitor sensitive mechanism. Examination of the phosphorylation status of Tie1 revealed both the holoreceptor and truncated receptor to be constitutively activated in MCF-7 cells. These data indicate that Tie1 expressed in epithelial tumour cell lines is present in holoreceptor and truncated forms, and in MCF-7 cells both forms are constitutively phosphorylated and competent to signal. Our findings suggest therefore that anti-angiogenic strategies targeting the angiopoietin/Tie system in tumour microvasculature could also have additional direct effects on the tumour epithelial cells within those tumours in which there is also extravascular expression of the Tie1 receptor tyrosine kinase.

Introduction

The angiopoietins are a family of secreted glycoprotein ligands whose expression is markedly altered in a wide range of cancers, including breast, colorectal and gastric tumours (1). There are four members of the angiopoietin family, angiopoietin-1 (Ang1) through angiopoietin-4, with Ang3 and Ang4 being mouse and human orthologues respectively (2-4). The angiopoietins are all ligands for Tie2, a receptor tyrosine kinase predominantly expressed by vascular endothelial cells (5). As with angiopoietins, expression of Tie2

has been shown to be increased in cancer, indeed Tie2 expression correlates with poor survival and high metastatic risk in breast cancer, including node-negative breast cancers (6,7). Of the angiopoietins, the best characterized are Ang1 and Ang2. Ang1 is an activator of Tie2 whereas Ang2 can antagonize Ang1 activation (3). In quiescent vasculature Ang1 is produced by perivascular cells and acts to promote blood vessel stability (2,8). Expression of Ang2 is more dynamically regulated than that of Ang1 and up-regulation of Ang2 occurs under conditions of vessel remodelling where it can suppress the effects of Ang1 and thus permit vessel destabilization prior to angiogenesis or regression (3). Consistent with the notion that Ang2 promotes angiogenesis by destabilization of microvessels, the ratio of Ang2/Ang1 is elevated in most of the cancers in which expression has been examined (1).

Tie2 together with Tie1 constitute the Tie family of receptor tyrosine kinases and as with Tie2, Tie1 is expressed principally by vascular endothelial cells (9). Tie1 and Tie2 have a similar overall structure, with an extracellular domain composed of three immunoglobulin-like repeats, three epidermal growth factor homology domains followed by a further immunoglobulin motif and three fibronectin III domains (10-12). The intracellular portions of each receptor include a tyrosine kinase domain with kinase insert. Overall amino acid identity between the receptors is approximately 30% in the extracellular domain and 75% in the intracellular domain (10,11). In contrast to Tie2, relatively little is known about the Tie1. This is partly because a ligand for this receptor has not been available. However, studies with transgenic mice indicate that Tie1 is important for promoting endothelial survival (13,14). This is supported by experiments in which a chimaeric form of Tie1 expressed in mouse fibroblasts was shown to inhibit cell death induced by UV irradiation (15).

Tie1 undergoes regulated proteolytic cleavage in which the extracellular domain of the receptor is released from the cell as a result of metalloprotease action (16-18). Cleavage is activated by a number of factors including phorbol esters and vascular endothelial growth factor (VEGF) and generates a cell-associated receptor fragment of approximately 45 kDa consisting of Tie1 transmembrane and intracellular domains (16-18).

It has been found that Ang1 can activate Tie1 in endothelial cells and when the receptor is transfected into non-endothelial cells (19). As with Tie2, Ang2 antagonizes Ang1 activation of Tie1 in endothelial cells (19). Tie1 and

Tie2 physically interact in endothelial cells (20) and stimulation of Tie1 by Ang1 is enhanced in the presence of Tie2, although the ligand is still able to stimulate Tie1 even in the absence of Tie2 (19).

Tie1 expression is increased in a number of tumours, and the receptor has been suggested as a prognostic marker for gastric cancer where its expression correlates inversely with patient survival (21). Importantly, immunocytochemical analysis has shown that in addition to the vascular compartment, Tie1 is expressed in epithelial tumour cells in breast (22-24), gastric (21) and thyroid (25) cancers. Consistent with these findings, several breast and colon tumour cell lines have also been shown to express Tie1, where it appears to exist in the truncated 45 kDa form (24). In this study, we determined whether this tyrosine kinase is competent to signal in tumour cells in which it is expressed.

Materials and methods

Materials. Antibodies recognizing the carboxy-terminus of Tie1, and immunogen peptide were obtained from Santa Cruz Biotechnology, antibodies recognizing Tie1 extracellular domain were from R&D Systems. Recombinant Ang1 and Ang2 were from R&D Systems (Abingdon, Oxon, UK). All other materials were as previously described (20,26).

Cell culture. Human umbilical endothelial cells (HUVEC) were obtained from Promocell and cultured as described by the supplier. Cancer cell lines were originally obtained from European Cell Culture Collection. CACO-2 cells were maintained in MEM with 10% FCS, MCF-7 and MDA-MB-231 cells were maintained in DMEM with 10% FCS, MDA-MB-436 and HCT-15 cells were maintained in RPMI-1640 containing 10 and 20% FCS respectively. SW480 cells were maintained in Optimem I containing 10% FCS.

Immunoprecipitation and immunoblotting. Cells were maintained as described above. In experiments involving treatment with phorbol esters, metalloprotease inhibitor or angiopoietin cells were incubated in serum-free medium for approximately 1 h before treatment. Whole cell lysates were prepared by washing cells in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4; PBS) followed by addition of 2X Laemmli sample buffer containing 100 mM dithiothreitol. For immunoprecipitation cells were lysed with ice cold lysis buffer (50 mM Tris, pH 7.4; 50 mM NaCl; 1 mM sodium fluoride, 1 mM EGTA, 1 mM sodium orthovanadate, 1% Triton X-100, complete protease inhibitor cocktail), cleared of particulate matter by centrifugation at 13,000 x g for 10 min, pre-adsorbed with protein-G before immunoprecipitation with 2 μg primary antibody and protein-G for 3 h. Recovered immunoprecipitates were washed 3 times with lysis buffer and proteins eluted by heating at 105°C in Laemmli sample buffer containing 100 mM dithiothreitol. Proteins were resolved by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes and probed with appropriate antibodies as indicated in results. Immunoreactive proteins were visualized with peroxidase-conjugated secondary antibodies and chemi-
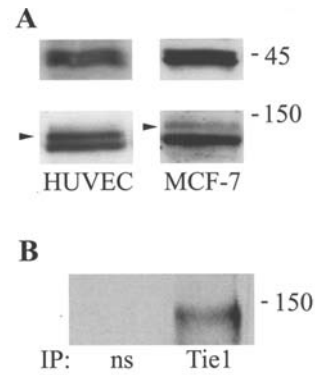

Figure 1. MCF-7 cells express full-length and truncated Tie1. Human umbilical vein endothelial cells or MCF-7 cells were harvested and lysed. (A) Proteins in whole cell lysates were resolved by SDS-PAGE and Tie1 detected by immunoblotting. The fully glycosylated holoreceptor is indicated with an arrowhead. (B) Cell lysates were immunoprecipitated with an irrelevant antibody (ns) or antibody recognizing Tie1 extracellular domain. Immunoprecipitated proteins were resolved by SDS-PAGE and Tie1 detected by immunoblotting. Molecular masses are indicated in kDa.

luminescent detection (27). Where required, membranes were stripped using Reblot, as described by the manufacturer (Chemicon International Inc., Temecula, CA, USA) before blocking and re-probing.

Results

Previous studies have demonstrated expression of the endothelial receptor tyrosine kinase Tie1 in a number of tumours in both vascular and tumour cell compartments (21-25). Consistent with this the receptor has been found to be expressed in several epithelial tumour cell lines including the breast cancer line MCF-7 (24). Significantly, Tie1 was detected as the 45 kDa receptor fragment in these cells (24). Although the receptor has been found in cancer cells its activation state is not known. We were interested therefore to determine the activation state of Tie. In initial experiments we sought to confirm expression of the truncated receptor in MCF-7 cells. As shown in Fig. 1A, immunoblotting MCF-7 cell lysates with an antibody recognizing the carboxy-terminus of Tie1 revealed a doublet of 45 kDa Tie1, as described previously by others (24). In addition to truncated Tie1, we also observed a protein doublet of approximately 145 kDa in MCF-7 cells, similar to that seen in endothelial cells (Fig. 1A). This protein corresponds to an upper band of fully glycosylated surface expressed receptor (Fig. 1A, arrowhead) and partially glycosylated Tie1 as described previously (16). To further test whether full-length Tie1 is present in MCF-7 cells we used antibodies recognizing the fully glycosylated extracellular domain of Tie1 and a non-specific antibody to immunoprecipitate from MCF-7 cell lysates (Fig. 1B). Antibodies recognizing the extracellular domain of Tie1, but not the control antibody, immunoprecipitated a single 145 kDa protein corresponding to fully-glycosylated Tie1 holoreceptor.

Together these data show that in addition to the previously reported 45 kDa Tie1 truncation product, full-length Tie1 is also expressed in MCF-7 cells. We therefore, tested whether full-length receptor is present in other
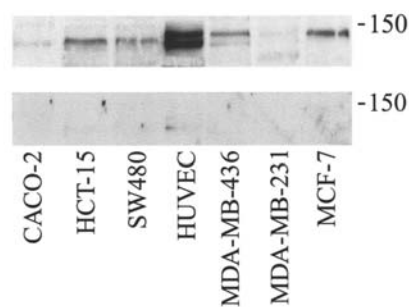
Figure 2. Full-length Tie1 is expressed by colon and breast epithelial tumour cell lines. Whole cell lysates were prepared from the cell lines indicated and proteins were resolved by SDS-PAGE and Tie1 detected by immunoblotting (upper panel). The lower panel shows the result of re-probing the blot with anti-Tie1 in the presence of 1 μg/ml immunogen peptide. Molecular masses are indicated in kDa.
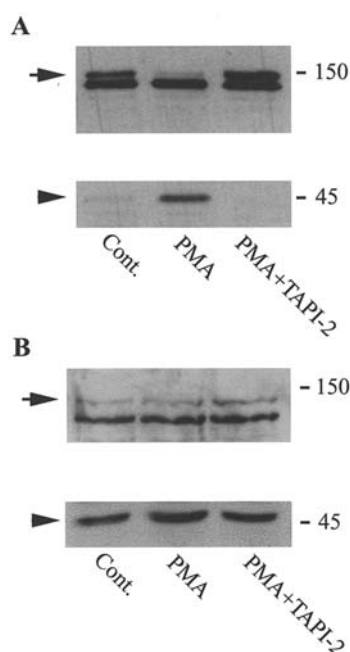

Figure 3. Effects of PMA and metalloprotease inhibitor on Tie1 cleavage. Human umbilical vein endothelial cells (A) or MCF-7 cells (B) were treated with control vehicle (Cont.), 10 ng/ml phorbol myristate acetate (PMA) for 30 min or PMA in the presence of 100 μM TAPI-2 (PMA + TAPI-2) for 30 min before harvesting and preparation of whole cell lysates. Proteins in whole cell lysates were resolved by SDS-PAGE and Tie1 detected by immunoblotting. Molecular masses are indicated in kDa.
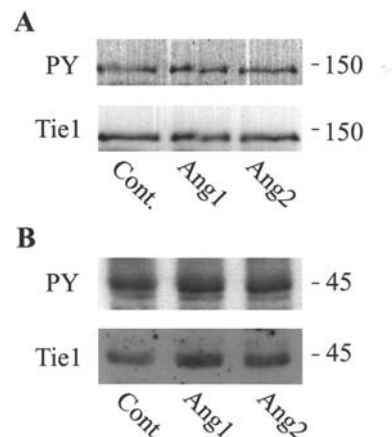

Figure 4. Tie1 is tyrosine phosphorylated in MCF-7 cells. Serum-deprived MCF-7 cells were treated with control vehicle (Cont.), 200 ng/ml Ang1 or 200 ng/ml Ang2 for 30 min as indicated. Cell lysates were prepared and Tie1 immunoprecipitated with an antibody recognizing the receptor extra-cellular domain (A) or intracellular carboxy-terminus (B). Immuno-precipitated proteins were resolved by SDS-PAGE and phosphorylation status (PY) detected by immunoblotting with anti-phosphotyrosine antibodies. Blots were re-probed with anti-Tie1 as indicated. Molecular masses are indicated in kDa.

epithelial tumour lines. A number of tumour cell lines were probed for the presence of Tie1 by immunoblotting. As shown in Fig. 2, a number of these cell lines, including the colon tumour lines HCT-15 and SW480, as well as the breast cancer lines MCF-7 and MDA-MB-436 expressed clearly detectable 145 kDa Tie1, whereas levels in CACO-2 and MDA-MB-231 were barely detectable. Re-probing blots with anti-Tie1 but in the presence of a peptide corresponding to the Tie1 immunogen prevented the antibody reacting with the 145 kDa protein confirming it as Tie1.

In endothelial cells Tie1 holoreceptor undergoes costitutive proteolytic cleavage to generate the 45 kDa truncation product comprising of transmembrane and intracellular domains (16,17). This process is stimulated by phorbol esters as well as the physiological activators VEGF, TNFα and changes in shear stress (16,18,28,29). Constitutive and activated cleavage of Tie1 is mediated by metalloprotease activity (17). It was of interest, therefore, to determine whether similar mechanisms operate on Tie1 in cancer cells. To do this, we challenged MCF-7 cells with PMA in the absence and presence of the metalloprotease inhibitor TAPI-2 and analysed Tie1 by immunoblotting with an anti-body recognizing the receptor intracellular domain (Fig. 3). Consistent with previous findings, PMA activated cleavage of Tie1 in endothelial cells causing loss of the surface expressed full-length 145 kDa protein and formation of a 45 kDa doublet fragment (Fig. 3A). In the presence of TAPI-2 PMA was without effect on full-length receptor and generation of 45 kDa Tie1 was largely ablated (Fig. 3A). In contrast, treatment of MCF-7 with the same concentration of PMA did not affect levels of full-length or 45 kDa Tie1. Similarly, TAPI-2 was without detectable effect on truncated or full-length Tie1 in MCF-7 cells (Fig. 3B). Together, these data show that Tie1 expressed in MCF-7 is regulated differently than Tie1 expressed in the normal endothelial cell background.

To examine the signalling potential of Tie1 in cancer cells the receptor was immunoprecipitated from MCF-7 cells and receptor activation assessed by examining tyrosine phosphorylation status. As we had found some full-length receptor in MCF-7 cells it was possible that Tie1 holore-ceptor could respond to angiopoietin ligands. Therefore, to investigate this we analyzed tyrosine phosphorylation state of full-length and truncated Tie1 immunoprecipitated from MCF-7 cells cultured under basal conditions and following treatment with Ang1 or Ang2. Tie1 holoreceptor was immuno-precipitated from MCF-7 cells with an antibody recognizing the receptor ectodomain and phosphorylation status assessed by anti-phosphotyrosine immunoblotting (Fig. 4A). Tie1 (145 kDa) holoreceptor was found to be phosphorylated in MCF-7 cells (Fig. 4A). Surprisingly, Ang2 did not inhibit the

constitutive phosphorylation of Tie1 in these cells; instead the ligand caused a small increase in phosphorylation of the receptor. Ang1 also caused a small additional activation of phosphorylation of full-length Tie1 (Fig. 4A). Phosphorylation status of the truncated 45 kDa Tie1 was also assessed by anti-phosphotyrosine immunoblotting of the receptor immunoprecipitated with an antibody recognizing the Tie1 intracellular domain (Fig. 4B). Under basal conditions truncated Tie1 was tyrosine phosphorylated and there was little detectable effect of Ang1 or Ang2 on phosphorylation status of the endodomain fragment.

Discussion

In this study, we show that a number of epithelial cancer cells express the endothelial receptor tyrosine kinase Tie1 as a holoreceptor. We further demonstrate that in contrast to the situation in endothelial cells, generation of truncated Tie1 does not occur via a metalloprotease-inhibitor sensitive mechanism and that the constitutive production of truncated Tie1 cannot be activated by PMA in the MCF-7 breast cancer cell line. Examination of the phosphorylation status of Tie1 revealed both holoreceptor and truncated receptor to be constitutively activated in MCF-7 cells.

Tie1 has been shown to be expressed in several tumour types in both vascular and tumour cell compartments (21-25). Furthermore, the receptor has been shown to be expressed in several tumour cell lines, including MCF-7, where it was found primarily in truncated 45 kDa forms. The present data demonstrate also expression of the full-length receptor. Certainly it would be expected that cells expressing truncated Tie1 would have some full-length receptor as this is the source of the truncated receptor. It is clear from studies in endothelial cells that Tie1 is sensitive to proteolytic cleavage (16). Tumour cell lines as well as tumours *in vivo* are known to express a number of proteases, including matrix metalloproteases, stromolysin and plasminogen activator, and the tumour microenvironment is known to be pro-proteolytic (30-32). It is likely, therefore that undetectable or low levels of full-length Tie1 but clearly evident truncated Tie1 indicates that normally in these cells most of the receptor is proteolytically cleaved and that holoreceptor represents a small fraction of the total receptor under these conditions.

The nature of the protease responsible for cleaving Tie1 in MCF-7 cells is not known. In contrast to endothelial cells, the metalloprotease inhibitor TAPI-2 failed to inhibit truncation of Tie1. It is also interesting that, again in contrast to the receptor in its normal endothelial cell background, PMA was without effect on Tie1 truncation in MCF-7 cells. This may indicate that cleavage of the receptor is already maximal in the cancer cells so additional activators would be ineffective. Also, in endothelial cells activation of Tie1 cleavage by PMA is mediated by metalloprotease activity (17). As we find metalloprotease inhibitor to be without effect on MCF-7 Tie1 cleavage it suggests that the normally regulated metalloprotease pathway of Tie1 cleavage is dominated by an alternative non-regulated, non-metalloprotease pathway in these cancer cells.

An important finding of the present study is that in MCF-7 cells the full-length receptor and truncation product

is phosphorylated under basal conditions. Constitutive activation of other receptor tyrosine kinases is commonly observed in tumour cells in culture as well as *in situ*, and can occur by mutation and overexpression mechanisms (33). Even though Tie1 holoreceptor was constitutively phosphorylated we found that Ang2, which usually acts to inhibit Ang1 signalling through the Tie receptors in endothelial cells (3,19), did not to suppress Tie1 phosphorylation in MCF-7 cells but, if anything, caused a small activation. The receptor was also still able to respond to Ang1 with a small increase in activation. There are a number of studies of angiopoietin expression during tumourigenesis and in most cases angiopoietin expression is substantially increased, particularly the relative expression of Ang2 compared to Ang1. Our data would suggest these ligands could act on Tie1 expressed in the tumour cells to maintain or even activate the receptor.

In summary, our data show that Tie1 is expressed in a number of breast and colon cancer cell lines as both full-length and truncated receptor forms. In MCF-7 cells the receptor and its truncation product is phosphorylated under basal conditions. These findings indicate that Tie1 expressed within tumour epithelium is constitutively active and competent to signal. The angiopoietins and their better characterized receptor Tie2 have recently emerged as potentially attractive targets for anti-angiogenic therapy of tumours (34,35). The finding that Tie1 is present and constitutively active, as judged by receptor phosphorylation status, would suggest that therapeutics aimed at sequestering angiopoietins may also have effects directly on the tumour cell to limit the ability of the angiopoietins to act on Tie1 in tumour cells. Furthermore, the tyrosine kinase domains of Tie1 and Tie2 are highly homologous. Thus, anti-angiogenic tyrosine kinase inhibitors aimed at suppressing endothelial Tie2 would be likely also to affect Tie1 and thus may have direct, probably desirable, additional direct inhibitory effects on any full-length as well as truncated Tie1 expressed in the tumour cells.

Acknowledgements

References

1. Tait CR and Jones PF: Angiopoietins in tumours: the angiogenic switch. J Pathol 204: 1-10, 2004.
2. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC and Yancopoulos GD: Isolation of angiopoietin-1, a ligand for the Tie2 receptor, by secretion-trap expression cloning. Cell 87: 1161-1169, 1996.
3. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN and Yancopoulos GD: Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science 277: 55-60, 1997.
4. Valenzuela DM, Griffiths JA, Rojas J, Aldrich TH, Jones PF, Zhou H, McClain J, Copeland NG, Gilbert DJ, Jenkins NA, Huang T, Papadopoulos N, Maisonpierre PC, Davis S and Yancopoulos GD: Angiopoietins 3 and 4: diverging gene counterparts in mice and humans. Proc Natl Acad Sci USA 96: 1904-1909, 1999.

5. Peters KG, Kontos CD, Lin PC, Wong AL, Rao P, Huang L, Dewhirst MW and Sankar S: Functional significance of Tie2 signaling in the adult vasculature. Recent Prog Horm Res 59: 51-71, 2004.
6. Dales JP, Garcia S, Bonnier P, Duffaud F, Meunier-Carpentier S, Andrac-Meyer L, Lavaut MN, Allasia C and Charpin C: Tie2/Tek expression in breast carcinoma: Correlations of immunohistochemical assays and long-term follow-up in a series of 909 patients. Int J Oncol 22: 391-397, 2003.
7. Meunier-Carpentier S, Dales JP, Djemli A, Garcia S, Bonnier P, Andrac-Meyer L, Lavaut MN, Allasia C and Charpin C: Comparison of the prognosis indication of VEGFR-1 and VEGFR-2 and Tie2 receptor expression in breast carcinoma. Int J Oncol 26: 977-984, 2005.
8. Kim I, Kim HG, So JS, Kim JH, Kwak HJ and Koh GY: Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Circ Res 86: 24-29, 2000.
9. Jones N, Iljin K, Dumont DJ and Alitalo K: Tie receptors: new modulators of angiogenic and lymphangiogenic responses. Nat Rev Mol Cell Biol 2: 257-267, 2001.
10. Partanen J, Armstrong E, Makela TP, Korhonen J, Sandberg M, Renkonen R, Knuutila S, Huebner K and Alitalo K: A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. Mol Cell Biol 12: 1698-1707, 1992.
11. Ziegler S, Bird T, Schneringer J, Schooley K and Baum P: Molecular cloning and characterization of a novel receptor protein tyrosine kinase from human placenta. Oncogene 8: 663-670, 1993.
12. Barton WA, Tzvetkova-Robev D, Miranda EP, Kolev MV, Rajashankar KR, Himanen JP and Nikolov DB: Crystal structures of the Tie2 receptor ectodomain and the angiopoietin-2-Tie2 complex. Nat Struct Mol Biol 13: 524-532, 2006.
13. Puri M, Rossant J, Alitalo K, Bernstein A and Partanen J: The receptor tyrosine kinase tie is required for integrity and survival of vascular endothelial cells. EMBO J 14: 5884-5891, 1995.
14. Sato T, Tozawa Y, Deutsch U, Wolburg-Bucholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W and Qin Y: Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature 376: 70-74, 1995.
15. Kontos CD, Cha EH, York JD and Peters KG: The endothelial receptor tyrosine kinase Tie1 activates phosphatidylinositol 3-kinase and Akt to inhibit apoptosis. Mol Cell Biol 22: 1704-1713, 2002.
16. Yabkowitz R, Myer S, Yanagihara D, Brankow D, Staley T, Elliot G, Hu S and Ratzkin B: Regulation of tie receptor expression on human endothelial cells by protein kinase c-mediated release of soluble tie. Blood 90: 706-715, 1997.
17. McCarthy MJ, Burrows R, Bell SC, Christie G, Bell PRF and Brindle NPJ: Potential roles of metalloprotease mediated ectodomain cleavage in signaling by the endothelial receptor tyrosine kinase Tie-1. Lab Invest 79: 889-895, 1999.
18. Yabkowitz R, Meyer S, Black T, Elliott G, Merewether LA and Yamane HK: Inflammatory cytokines and vascular endothelial growth factor stimulate the release of soluble tie receptor from human endothelial cells via metalloprotease activation. Blood 93: 1969-1979, 1999.
19. Saharinen P, Kerkela K, Ekman N, Marron M, Brindle N, Lee GM, Augustin H, Koh GY and Alitalo K: Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2. J Cell Biol 169: 239-243, 2005.
20. Marron MB, Hughes DP, Edge MD, Forder CL and Brindle NPJ: Evidence for heterotypic interaction between the receptor tyrosine kinases Tie-1 and Tie-2. J Biol Chem 275: 39741-39746, 2000.
21. Lin W-C, Li AF-Y, Chi C-W, Chung W-W, Huang CL, Lui W-Y, Kung H-J and Wu C-W: Tie-1 protein tyrosine kinase: a novel independent prognostic marker for gastric cancer. Clin Cancer Res 5: 1745-1751, 1999.
22. Cance WG, Craven RJ, Weiner TM and Liu ET: Novel protein kinases expressed in human breast cancer. Int J Cancer 54: 571-577, 1993.
23. Tseng L-M, Hsu C-Y, Wang H-C, Liu J, Chang H-M, Lo S-S, Wu C-W, Lui W-Y and Chi C-W: Tie-1 tyrosine kinase is an independent prognostic indicator for invasive breast cancer. Anticancer Res 21: 2163-2170, 2001.
24. Yang XH, Hand RA, Livasy CA, Cance WG and Craven RJ: Overexpression of the receptor tyrosine kinase Tie-1 intracellular domain in breast cancer. Tumour Biol 24: 61-69, 2003.
25. Ito Y, Yoshida H, Uruno T, Nakano K, Takamura Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, Kuma K and Miyauchi A: Tie-1 tyrosine kinase expression in human thyroid neoplasms. Histopathology 44: 318-322, 2004.
26. Hughes DP, Marron MB and Brindle NPJ: The anti-inflammatory endothelial tyrosine kinase Tie2 interacts with a novel nuclear factor-(kappa)b inhibitor ABIN-2. Circ Res 92: 630-636, 2003.
27. Matthews JA, Batki A, Hynds C and Kricka LJ: Enhanced chemiluminescent method for the detection of DNA dot-hybridization assays. Anal Biochem 151: 205-209, 1985.
28. Tsiamis AC, Morris PN, Marron MB and Brindle NPJ: Vascular endothelial growth factor modulates the Tie-2:Tie-1 receptor complex. Microvasc Res 63: 149-158, 2002.
29. Chen-Konak L, Guetta-Shubin Y, Yahav H, Shay-Salit A, Zilberman M, Binah O and Resnick N: Transcriptional and post-translation regulation of the Tie1 receptor by fluid shear stress changes in vascular endothelial cells. FASEB J 17: 2121-2123, 2003.
30. Duffy MJ and McCarthy K: Matrix metalloproteinases in cancer: prognostic markers and targets for therapy (Review). Int J Oncol 12: 1343-1348, 1998.
31. Stack MS, Ellerbroek SM and Fishman DA: The role of proteolytic enzymes in the pathology of epithelial ovarian carcinoma. Int J Oncol 12: 569-576, 1998.
32. Ludwig T: Local proteolytic activity in tumor cell invasion and metastasis. Bioessays 27: 1181-1191, 2005.
33. Zwick E, Bange J and Ullrich A: Receptor tyrosine kinases as targets for anticancer drugs. Trends Mol Med 8: 17-23, 2002.
34. Lin P, Buxton JA, Acheson A, Radziejewski C, Maisonpierre PC, Yancopoulos GD, Channon KM, Hale LP, Dewhirst MW, George SE and Peters KG: Antiangiogenic gene therapy targeting the endothelium-specific receptor tyrosine kinase Tie2. Proc Natl Acad Sci USA 95: 8829-8834, 1998.
35. White RR, Shan S, Rusconi CP, Shetty G, Dewhirst MW, Kontos CD and Sullenger BA: Inhibition of rat corneal angiogenesis by a nuclease-resistant RNA aptamer specific for angiopoietin-2. Proc Natl Acad Sci USA 100: 5028-5033, 2003.