Expression of Angiopoietins and Its Clinical Significance in Non-Small Cell Lung Cancer

Fumihiro Tanaka,2 Shinya Ishikawa, Kazuhiro Yanagihara, Ryo Miyahara, Yozo Kawano, Mio Li, Yosuke Otake, and Hiromi Wada

Department of Thoracic Surgery, Faculty of Medicine, Kyoto University, Kyoto, 606-8507 Japan [F. T., K. Y., R. M., Y. K., M. L., H. W.], and Department of Thoracic Surgery, Seishin-Iryo Center Hospital, Kobe 651-2273, Japan [Y. O.]

ABSTRACT

Angiopoietin (Ang)-1 and -2 have been recently identified as potent angiogenic factors which function in concert with vascular endothelial growth factor (VEGF), but no detailed clinical study on Ang expression has been reported. To assess the clinical significance of Ang expression in non-small cell lung cancer (NSCLC), a total of 236 patients with pathologically stage-I-IIIA disease were retrospectively reviewed. Expression of Ang-1, Ang-2, or VEGF was examined immunohistochemically; intratumoral microvessel density (IMVD) was examined with immunohistochemical staining against CD34, a marker of pan-endothelial cells (CD34-IMVD), and that against CD105, a marker of proliferative endothelial cells (CD105-IMVD). Positive expression of Ang-1 and that of Ang-2 were seen in 101 (42.8%) and 40 patients (16.9%), respectively. There was no significant correlation between Ang-1 expression and CD34-IMVD or CD105-IMVD. In contrast, the average CD105-IMVD for Ang-2-positive tumor was significantly higher than that for Ang-2-negative tumor (56.7 versus 38.5; P = 0.032). More interestingly, such an angiogenic effect of Ang-2 was seen only when VEGF expression was high; when VEGF expression was low, the average CD105-IMVD for Ang-2-positive tumor was significantly higher than that for Ang-2-negative tumor (89.1 versus 63.6; P = 0.045); when VEGF expression was low, the average CD105-IMVD for Ang-2-positive tumor and that for Ang-2-negative tumor were almost the same (27.4 and 27.1, respectively). Moreover, positive expression of Ang-2, not Ang-1, was a significant predictor to predict a poor postoperative survival (5-year survival rates for Ang-2-positive patients and -negative patients were 53.5 and 70.3%, respectively; P = 0.027), which was confirmed by a multivariate analysis. The influence of Ang-2 status on postoperative survival was enhanced when VEGF expression was high. That said, the 5-year survival of Ang-2-positive and VEGF-high patients was extremely low (41.4%) as compared with that for Ang-2-negative and VEGF-low patients (66.6%), as compared with that for Ang-2-positive and VEGF-low patients (63.6%), and as compared with that for Ang-2-negative and VEGF-low patients (71.8%). In conclusion, positive Ang-2 expression was significantly correlated with a poor prognosis, as well as with aggressive angiogenesis in resected NSCLC that was enhanced in the presence of high VEGF expression.

INTRODUCTION

Angiogenesis is an essential process in the development and progression of malignant tumors including NSCLC1 (1, 2). VEGF is the most potent angiogenic factor, and many clinical studies have demonstrated that increased expression of VEGF is associated with increased IMVD (a measurement of angiogenesis) and with a poor survival in NSCLC (3).

Received 6/17/02; accepted 10/04/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Grant-in-Aid 14370410 (to F. T.) for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
2 To whom requests for reprints should be addressed, at Department of Thoracic Surgery, Faculty of Medicine, Kyoto University, Shogoin-kawahara-cho 54, Sakyo-ku, Kyoto, 606-8507, JAPAN. Phone: 81-75-751-4975; Fax: 81-75-751-4974; E-mail: ftanaka@kuhp.kyoto-u.ac.jp.

PATIENTS AND METHODS

Patients and Tissue Preparation. A total of 237 patients with pathological stage-I-IIIA NSCLC, who underwent complete resection without any preoperative therapy at Kyoto University Hospital from January 1, 1985, through December 31, 1990, were retrospectively reviewed. One patient was excluded from the study because of operation-related death, and a final total of 236 patients were evaluated (Table 1; Refs. 19–21). Pathological stage was re-evaluated and determined with the present tumor-node-metastasis classification as revised in 1997 (22). Histological type and cell differentiation were determined using the current classification by WHO as revised in 2000 (23).

For analyses according to the differentiation of cancer cells, well-differentiated Sq and Ad were classified as well-differentiated tumors and moderately differentiated Sqa and Ad as moderately differentiated tumors. Large cell carcinoma and poorly differentiated Sq and Ad were classified as poorly differentiated tumors, and the other histological types were excluded in the analyses (20).

For all of these patients, the inpatient medical records, chest X-ray films, whole-body computed tomography films, bone scanning data, and records of surgery were reviewed. Intraoperative therapy was not performed in any patient. As postoperative adjuvant therapy, cisplatin-based chemotherapy, radiation, and oral administration of tegafur (a fluorouracil-derivative drug) were prescribed for 55, 35, and 58 patients, respectively (21). Follow-up of the postoperative clinical course was conducted by outpatient medical records and by inquiries by telephone or letter.

Downloaded from cancerres.aacrjournals.org on November 13, 2017. © 2002 American Association for Cancer Research.
All of the primary tumor specimens were immediately fixed in 10% (v/v) formalin, and then embedded in paraffin. Serial 4-μm sections were prepared from each sample and served for H&E staining and IHS. Results of IHS were evaluated by two authors (F. T. and S. I.) independently without knowledge of any clinical data.

Expression of Ang-1, Ang-2, and VEGF. Expression of Ang-1, Ang-2, or VEGF was evaluated immunohistochemically using a standard streptavidin-biotinylated horseradish-peroxidase complex method (LSAB-2 kit; DAKO, Kyoto, Japan). After retrieval of the antigen by heating the sections in a microwave oven three times for 5 min each, the sections were incubated with an anti-Ang-1 polyclonal antibody (goat IgG, 200 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA) diluted at 1/50, an anti-Ang-2 polyclonal antibody (goat IgG, 200 μg/ml; Santa Cruz Biotechnology) diluted at 1/50, or an anti-VEGF polyclonal antibody A-20 (rabbit IgG, 200 μg/ml, Santa Cruz Biotechnology) diluted at 1/50. The anti-VEGF antibody recognizes the 165-, 189-, and 121-amino splicing variants of VEGF. As a chromogen, 3,3′-diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO) was used, and the sections were counterstained with hematoxylin. For the negative controls, normal goat IgG was used as a substitute for the primary antibody against Ang-1 or Ang-2, and normal rabbit IgG was used for that against VEGF.

Ang-1 or Ang-2 expression was judged to be positive, when the percentage of cancer cells with positive staining exceeded 5%. VEGF expression was classified according to the following grading system as described previously (19). Briefly, a percentage score was defined as follows: score 0 if no VEGF-positive staining cell was documented; score 1 if the percentage of VEGF-positive staining cells was ≤25%; score 2 if the percentage was >25% and ≤50%; and score 3 if the percentage was >50%; an intensity score was defined as follows: score 0 if no staining was documented; score 1 if the staining intensity was weak; score 2 if the intensity was moderate; and score 3 if the intensity was high. The staining intensity of tumor cells was judged as high (score 3) when the staining intensity was comparable with that of smooth muscle cells of either bronchial wall or blood vessels. Grade of VEGF expression was represented as the sum of the percentage score and the intensity score (VEGF score), and VEGF expression was finally defined as follows: weak expression when the VEGF score is ≤4; and strong expression when the VEGF score is 5 or 6.

Quantification of Angiogenesis (IMVD). IHS for CD34 and CD 105 to highlight ECs was performed using a sensitive streptavidin-biotinylated horseradish-peroxidase complex system (TSA-Indirect kit; NEN Life Science Products, Boston, MA) as described previously (19). Briefly, dewaxed sections were incubated with an anti-CD34 mAb QBEnd10 (mouse IgG1, 50 μg/ml; DAKO) diluted at 1/50 or an anti-CD105 mAb SN6 h (mouse IgG1; 366 μg/ml; DAKO Japan) diluted at 1/100. The 10 most vascular areas within a section were selected for evaluation of angiogenesis, and vessels, labeled with the anti-CD34 mAb or the anti-CD105 mAb, were counted under light microscopy at ×200. The average counts were recorded as the CD34-IMVD or CD105-IMVD for each case.

Statistical Methods. The χ² was used to compare counts. Continuous data were compared using the Student t test if the distribution of samples was normal, or the Mann-Whitney t test if the sample distribution was asymmetrical. The postoperative survival rate was analyzed by the Kaplan-Meier method, and the differences in survival rates were assessed by the log-rank test. Multivariate analysis of prognostic factors was performed using Cox’s regression model. Differences were considered significant when P < 0.05. All of the statistical manipulation was performed using the SPSS for Windows software system (SPSS Inc., Chicago, IL).

RESULTS

Expression of Ang-1 and Expression of Ang-2 in NSCLC. Positive expression of Ang-1 and that of Ang-2 were mainly seen in the...
edge of tumor tissues and were seen not only in the cytoplasm of tumor cells but also in ECs (Fig. 1). However, the degree and the extent of Angs expression in ECs were extremely heterogeneous, and the staining intensity in ECs was generally not so strong as to be clearly distinguished from negative expression. For these reasons, in the present study, we have focused on Ang expression in tumor cells, and Ang-1 or Ang-2 expression was judged based on the percentage of positive-staining tumor cells as described in the previous section. The staining intensity of IHS for Ang-1 or Ang-2 was heterogeneous even in the same tissue, and we could not evaluate semiquantitatively the degree of Ang-1 or Ang-2 expression.

Expression of Ang-1 was judged to be positive in 101 (42.8%) of all of the patients. No significant correlation between Ang-1 expression and any patient characteristic was documented (Table 1). Expression of Ang-2 was positive only in 40 patients (16.9%), and positive Ang-2 expression was significantly less in well-differentiated tumor (Table 1).

Both Ang-1 expression and Ang-2 expression were positive [Ang-1(+)/Ang-2(+)] in 27 patients (11.4%), and both were negative [Ang-1(−)/Ang-2(−)] in 122 patients (51.7%); the numbers of Ang-1(+) and Ang-2(+) patients were 13 (5.5%) and 74 (31.4%), respectively.

### Angs Expression and IMVD

The average CD34-IMVD for Ang-1-negative tumor and that for Ang-1-positive tumor were 179.3 and 180.3, respectively, showing no correlation between Ang-1 status and CD34-IMVD. There was no significant correlation between Ang-1 status and CD105-IMVD, and the average CD105-IMVD for Ang-1-positive tumor was somewhat higher than that for Ang-1-negative tumor (48.0 versus 36.9; \( P = 0.093 \)). The average CD105-IMVD for Ang-2-positive tumor was significantly higher than that for Ang-2-negative tumor (56.7 versus 38.5; \( P = 0.032 \)), suggesting that Ag-2 plays an important role in angiogenesis. The average CD34-IMVD for Ang-2-positive tumor was somewhat higher than that for Ang-2-negative tumor (199.3 versus 177.0), but the difference was not statistically significant (\( P = 0.347 \)). When angiogenesis was analyzed in combination with Ang-1 status and Ang-2 status, Ang-1-negative and Ang-2-positive tumor or Ang-1-positive and Ang-2-negative tumor showed higher IMVD, suggesting that Ang-2, not Ang-1, played major roles in tumor angiogenesis (Table 2).

There proved to be a marked difference in CD34-IMVD or CD105-IMVD between VEGF-negative tumor and VEGF-positive tumor, demonstrating VEGF was a most potent angiogenic factor; the average CD34-IMVD for VEGF-low tumor and that for VEGF-high tumor were 166.9 and 204.8, respectively (\( P = 0.006 \)), and the average

| Pathological stage | Ad | Sq | Poorly differentiated | Moderately differentiated | Well differentiated | Total | \( n \) | \( n \) | \( n \) | \( n \) |
|-------------------|----|----|----------------------|--------------------------|-------------------|-------|-----|-----|-----|-----|-----|
| I                 | 138(58.5)| 44 (18.6) | 58 (25.2) | 91 (39.6) | 81 (35.2) | 236 (100%) | 101 (42.8%) | 40 (16.9%) | \( P \) | \( P \) |
| II                | 54 (22.9) | 30 (41.7%) | 2 (0.8) | 120 (50.8) | 206 (87.3) | \( P \) | \( P \) |
| IIIa              | 7126 | 122 (51.7%) | 27 (12.1%) | 49 (22.6%) | 28 (11.9%) | \( P \) | \( P \) |

\( ^a \) \( n \), number of patients.
\( ^b \) \( \text{Age (mean ± SD), } 62.4 ± 9.7 \text{ years.} \)
\( ^c \) Other histological types were excluded in the analysis.

### Table 2. IMVD and postoperative survival according to the status of combination of Ang-1 and Ang-2 expression

<table>
<thead>
<tr>
<th>Ang expression</th>
<th>IMVD (mean ± SE)</th>
<th>5-yr survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-1(−)/Ang-2(−)</td>
<td>CD34-IMVD ( ^a )</td>
<td>176.7 ± 9.1</td>
</tr>
<tr>
<td>Ang-1(−)/Ang-2(+)</td>
<td>CD34-IMVD ( ^a )</td>
<td>203.7 ± 25.2</td>
</tr>
<tr>
<td>Ang-1(+)/Ang-2(−)</td>
<td>CD34-IMVD ( ^a )</td>
<td>177.5 ± 11.3</td>
</tr>
<tr>
<td>Ang-1(+)/Ang-2(+)</td>
<td>CD34-IMVD ( ^a )</td>
<td>188.0 ± 19.2</td>
</tr>
</tbody>
</table>

\( ^a \) IMVD determined with an anti-CD34 mAb.
\( ^b \) IMVD determined with an anti-CD105 mAb.
\( ^c \) \( P < 0.05 \) for Ang-1(−)/Ang-2(−) versus Ang-1(−)/Ang-2(+), versus Ang-1(+)/Ang-2(+).
\( ^d \) \( P < 0.05 \) for Ang-1(−)/Ang-2(−) versus Ang-1(+)/Ang-2(−).
\( ^e \) \( P < 0.05 \) for Ang-1(−)/Ang-2(+) versus any other group.
CD105-IMVD for VEGF-low tumor and that for VEGF-high tumor were 27.2 and 69.7, respectively (P < 0.001).

Next, correlation between IMVD and Angs status in combination with VEGF status was examined, because many experimental studies showed that angiogenesis was promoted by Angs in concert with VEGF. The average CD34-IMVD for Ang-1-negative and VEGF-low tumor and that for Ang-1-positive and VEGF-low tumor were 166.7 and 168.8, respectively (P = 0.843); the average CD34-IMVD for Ang-1-negative and VEGF-high tumor and that for Ang-1-positive and VEGF-high tumor were 214.1 and 196.4, respectively (P = 0.446). In addition, the average CD105-IMVD for Ang-1-negative and VEGF-low tumor and that for Ang-1-positive and VEGF-low tumor were 25.1 and 29.1, respectively (P = 0.543); the average CD105-IMVD for Ang-1-negative and VEGF-high tumor and that for Ang-1-positive and VEGF-high tumor were 65.3 and 73.6, respectively (P = 0.513). These results showed that Ang-1 did not affect angiogenesis induced by VEGF. In contrast, Ang-2 status affected CD105-IMVD when VEGF expression was high as follows: when VEGF expression was high, the average CD105-IMVD for Ang-2-positive tumor was significantly higher than that for Ang-2-negative tumor (89.1 versus 63.6; P = 0.045). In contrast, when VEGF expression was low, the average CD105-IMVD for Ang-2-positive was almost the same as that for Ang-2-negative tumor (27.4 versus 27.1; P = 0.982).

Regardless of Ang-2 status, VEGF status was a significant factor to increase CD105-IMVD (P < 0.001).

Angs Expression and Postoperative Survival. The 5-year survival rate for Ang-2-positive patients was significantly lower than that for Ang-2-negative patients (61.8% versus 81.1%; P = 0.022; Table 3). A multivariate analysis confirmed that positive Ang-2 expression was a significant factor to predict a poor prognosis (Table 4).

A univariate analysis failed to demonstrate that VEGF expression was a significant prognostic factor (Fig. 3C), whereas VEGF expression was the most potent angiogenic factor. Because angiogenesis was highly promoted by Ang-2 in the presence of high VEGF expression stage were performed (Table 3). For pathological stage-I disease, the 5-year survival rate for Ang-2-positive patients was significant lower than that for Ang-2-negative patients (61.8% versus 81.1%; P = 0.022; Table 3). A multivariate analysis confirmed that positive Ang-2 expression was a significant factor to predict a poor prognosis (Table 4).

A univariate analysis failed to demonstrate that VEGF expression was a significant prognostic factor (Fig. 3C), whereas VEGF expression was the most potent angiogenic factor. Because angiogenesis was highly promoted by Ang-2 in the presence of high VEGF expression...
Ang-2 gene expression was assessed using reverse transcription-PCR and/or in situ hybridization, as assessed with reverse transcription-PCR, was inversely correlated with vessel maturation but failed to demonstrate a significant correlation between Ang-2 gene expression and IMVD. In the present study, increased Ang-2 expression was significantly correlated with higher CD105-IMVD in NSCLC, suggesting that Ang-2 plays important roles in tumor angiogenesis. Tumor with positive Ang-2 expression also showed somewhat higher CD34-IMVD as compared with tumor with negative Ang-2 expression, but the difference did not reach a statistical significance, which may be attributable to the validity in the reactivity of the anti-CD34 antibody used in evaluation of angiogenesis. Thus, antibodies against pan-endothelial antigen such as CD34 may react with not only newly forming vessels but also stable vessels just trapped in tumors, whereas antibodies against CD105, a proliferation-related endothelial antigen, can preferentially react with active ECs of angiogenic tissue (24). In fact, Kumar et al. (25) and we (19) have demonstrated that CD105-IMVD is a significant prognostic factor, and CD34-IMVD is not, in breast carcinoma and NSCLC, respectively. These results along with the results documented in the present study may suggest that CD105 is superior to pan-endothelial markers such as CD34 in evaluation of angiogenesis in clinical studies. The present study also demonstrated that CD105-IMVD for Ang-2-positive tumor remained low in the absence of high VEGF expression whereas CD105-IMVD for Ang-2-positive tumor was elevated to the highest value in the presence of high VEGF expression. These results are in accordance with experimental results that angiogenesis is promoted by Ang-2 in concert with VEGF (2, 7).

In the present study, we used only IMVD as a parameter of angiogenesis and showed that the status of Ang-1 did not affect angiogenesis. In future studies, other angiogenesis parameters, such as vessel maturity, length, and area, should be examined, because Ang-1 might affect other angiogenesis parameters.

With respect to a correlation between Angs expression and tumor progression or prognosis, only two clinical studies have been reported (12, 14). Eggert et al. (12) reported that increased Ang-2 gene expression, not Ang-1 gene expression, was significantly correlated with advanced tumor stages in neuroblastoma, but they failed to demonstrate a significant correlation between Ang-2 gene expression and postoperative survival. Etoh et al. (14) documented that increased Ang-2 gene expression was significantly correlated not only with an advanced tumor-stage but also with a poor prognosis in gastric carcinoma; this was the only clinical study that showed a significant correlation between Angs expression and a prognosis. Thus, the

**DISCUSSION**

In the present study, we clearly demonstrated that increased expression of Ang-2, not Ang-1, in tumor cells was significantly correlated with aggressive angiogenesis and a poor prognosis in NSCLC. Only two clinical studies have been reported on Ang-2 status in resected NSCLC (17, 18). In both studies, Ang-2 gene expression was assessed using reverse transcription-PCR and/or in situ hybridization, and no significant correlation between Ang-2 gene expression and angiogenesis or clinical outcome was documented in either study (17, 18). With respect to the correlation between Angs expression and angiogenesis, only one clinical study in glioma has been reported (10). In this study, Koga et al. (10) demonstrated that Ang-2 gene expression, as assessed with reverse transcription-PCR, was inversely correlated with vessel maturation but failed to demonstrate a significant correlation between Ang-2 gene expression and IMVD. In the present study, increased Ang-2 expression was significantly correlated with higher CD105-IMVD in NSCLC, suggesting that Ang-2 plays important roles in tumor angiogenesis. Tumor with positive Ang-2 expression also showed somewhat higher CD34-IMVD as compared with tumor with negative Ang-2 expression, but the difference did not reach a statistical significance, which may be attributable to the validity in the reactivity of the anti-CD34 antibody used in evaluation of angiogenesis. Thus, antibodies against pan-endothelial antigen such as CD34 may react with not only newly forming vessels but also stable vessels just trapped in tumors, whereas antibodies against CD105, a proliferation-related endothelial antigen, can preferentially react with active ECs of angiogenic tissue (24). In fact, Kumar et al. (25) and we (19) have demonstrated that CD105-IMVD is a significant prognostic factor, and CD34-IMVD is not, in breast carcinoma and NSCLC, respectively. These results along with the results documented in the present study may suggest that CD105 is superior to pan-endothelial markers such as CD34 in evaluation of angiogenesis in clinical studies. The present study also demonstrated that CD105-IMVD for Ang-2-positive tumor remained low in the absence of high VEGF expression whereas CD105-IMVD for Ang-2-positive tumor was elevated to the highest value in the presence of high VEGF expression. These results are in accordance with experimental results that angiogenesis is promoted by Ang-2 in concert with VEGF (2, 7). In the present study, we used only IMVD as a parameter of angiogenesis and showed that the status of Ang-1 did not affect angiogenesis. In future studies, other angiogenesis parameters, such as vessel maturity, length, and area, should be examined, because Ang-1 might affect other angiogenesis parameters.

With respect to a correlation between Angs expression and tumor progression or prognosis, only two clinical studies have been reported (12, 14). Eggert et al. (12) reported that increased Ang-2 gene expression, not Ang-1 gene expression, was significantly correlated with advanced tumor stages in neuroblastoma, but they failed to demonstrate a significant correlation between Ang-2 gene expression and postoperative survival. Etoh et al. (14) documented that increased Ang-2 gene expression was significantly correlated not only with an advanced tumor-stage but also with a poor prognosis in gastric carcinoma; this was the only clinical study that showed a significant correlation between Angs expression and a prognosis. Thus, the
present study added clinical evidence that Ang-2 played important roles in tumor progression through tumor angiogenesis and that Ang-2 could be a new prognostic marker. In addition, we demonstrated that the postoperative survival of patients with Ang-2-positive and VEGF-high tumor was extremely poor, which might be attributable to active angiogenesis in tumor tissues. Although Ang-2 significantly affected angiogenesis only when VEGF expression was high, a multivariate analysis of prognostic factors showed that Ang-2 proved to be an independent factor to predict a poor postoperative survival. The reason why Ang-2 status was an independent prognostic factor regardless of the VEGF status should be examined in a future study for a larger number of patients with more homogeneous characteristics.

In the present study, Angs expression was judged based on Angs expression in tumor cells, not in ECs, whereas Angs were expressed not only in ECs, but also in tumor cells (9–14, 18, 26). In the present study, we did not assess Angs expression in ECs, not only because the degree and the extent of Angs expression in ECs were very heterogeneous, but also because the intensity of Angs expression was so strong as to be clearly distinguished from negative expression. In fact, Ang-2 expression levels in whole tumor tissues were evaluated in most clinical studies, and detailed analyses of Ang-2 expression in tumor cells and that in ECs have not been reported. Only one clinical study has been reported on the quantitative evaluation of Ang-2 expression in ECs; that study used in situ hybridization to evaluate Ang-2 gene expression and demonstrated that only a correlation existed between the number of Ang-2-expressing vessels and VEGF expression (17). These results suggest that it may be difficult to assess Ang-2 expression quantitatively in ECs in clinical materials.

In conclusion, Ang-2 expression in NSCLC was significantly correlated with active angiogenesis, and can be a significant prognostic factor. In future studies, expression of Tie2 along with expression of the Ang ligands should be studied.

ACKNOWLEDGMENTS

We thank Tomoko Yamada for excellent preparation of the sections. We also thank Seiko Sakai for her assistance in the preparation of the manuscript.

REFERENCES

Expression of Angiopoietins and Its Clinical Significance in Non-Small Cell Lung Cancer

Fumihiro Tanaka, Shinya Ishikawa, Kazuhiro Yanagihara, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/23/7124

Cited articles
This article cites 24 articles, 10 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/23/7124.full#ref-list-1

Citing articles
This article has been cited by 20 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/23/7124.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link:
http://cancerres.aacrjournals.org/content/62/23/7124.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.