Age-associated Increase of Codon 72 Arginine p53 Frequency in Gastric Cardia and Non-Cardia Adenocarcinoma

Zun-Wu Zhang,1 Paul Newcomb, Andrew Hollowood, Roger Feakins, Moganaden Moorghen, Alan Storey, Michael J. G. Farthing, Derek Alderson, and Jeff Holly

Division of Surgery [Z-W. Z., P. N., A. H., D. A., J. H.], and Department of Histopathology [M. M.], Bristol Royal Infirmary, University of Bristol, Bristol BS2 8HW; Department of Histopathology [R. F.] and Cancer Research UK; Skin Tumour Laboratory, Centre for Cutaneous Research [A. S.], St. Bartholomew’s and the Royal London School of Medicine and Dentistry, London E1 2AD; and Faculty of Medicine, University of Glasgow, Glasgow, [M. J. G. F.], United Kingdom

ABSTRACT

Purpose: A common polymorphism of the tumor suppressor gene TP53 at codon 72 has been associated with human cancer susceptibility and prognosis. To examine the role of the polymorphism in the gastric adenocarcinoma, we examined 397 patients with or without the cancer.

Experimental Design: DNA samples were extracted from archived gastric tumor tissues and/or normal tissues of gastric adenocarcinoma and noncancer patients. The TP53 codon 72 genotypes were determined by PCR-RFLP.

Results: The overall genotype frequencies for Pro/Pro, Arg/Pro, and Arg/Arg were 7.3, 45.1, and 47.6%, respectively. A significant stepwise increased frequency of codon 72 Arg p53 with age was observed in patients with gastric cancer, but not in noncancer patients (P = 0.01). Patients with gastric cardia cancer had a significantly higher frequency of homozygous Arg allele than those with non-cardia tumors (P = 0.03) or than noncancer patients. After adjustment for age and gender, a logistic regression analysis suggested that the risk for a p53 Arg homozygous patient to develop cardia cancer is 3.1 95% confidence interval, 1.4–7.3) times greater than for p53 Pro homozygous and p53 Arg/Pro heterozygous patients. No close relationship was observed among patient gender, tumor histological type, p53 protein expression, and codon 72 genotype distribution.

Conclusions: These findings indicate that codon 72 Arg p53 may be associated with a prolonged survival for patients who have had gastric adenocarcinoma, especially non-cardia adenocarcinoma. It may confer, however, a different role on patients who suffer cardia gastric adenocarcinoma.

INTRODUCTION

A common polymorphism of the tumor suppressor gene TP53 at codon 72 has been associated with human cancer susceptibility (1–6). The polymorphism results in either a variant protein with a Pro residue (CCC) or an Arg residue (CGC; Refs. 7 and 8). There are a number of differences between the p53 variants in their ability to bind components of the transcriptional machinery, to activate transcription, to induce apoptosis, and to repress the transformation of primary cells (9). Patients with the Pro/Pro genotype, especially patients who were smokers, were more likely to develop lung cancer than those with other genotypes (10–12). In contrast, nonsmokers with lung cancer have an increased frequency of the homozygous Arg genotype (1, 4). Increased frequency of the Pro allele (Pro/Pro or Arg/Pro genotypes) was also found in patients with breast cancer (5). Although controversial findings are reported on the relationship between the polymorphism and cervical cancer (13, 14), in vitro study suggested that the Arg form of p53 was significantly more susceptible to the E6 oncoprotein (derived from tumor-associated human papillomaviruses)-mediated degradation than was the Pro form (13). Thus, p53 variants may serve as risk factors for major human neoplasms and may play a role in modulating environmental risk factors for cancer.

Gastric cancer is one of the most common malignancies worldwide. Although the overall incidence of distal (non-cardia) gastric cancer has been decreasing over the past few decades, the incidence of adenocarcinomas of the proximal stomach (cardia) and esophagogastric junction is rising (15–18). Non-cardia gastric cancers have different risk factors and anatomical precursor lesions compared with tumors arising in the cardia. Chronic Helicobacter pylori infection and dietary factors, such as those high in salt or nitrate, and nutritional deficiencies have been associated with non-cardia gastric cancer (19). In contrast, cancer of the cardia, which is increasing in incidence, is most likely to be associated with gastroesophageal reflux (16). Gastric carcinogenesis is a complex, multistep, and multifactorial process, in which many factors are implicated. The majority of gastric cancers are thought to be caused by environmental factors that result in damage to the mucosa and that inhibit its ability to repair itself (20). This response is regulated, in part, by inhibitory and stimulatory factors that are products of proto-oncogenes and tumor suppressor genes (21). TP53, an important tumor suppressor gene, has been widely studied in gastric cancers (22). However, although more than 75% of gastric cancers showed p53 overexpression, less than 30% had mutations in this gene. The codon 72 polymorphism is located in exon 4 of the p53 gene, a region involving very few mutations (23). However, the different codon 72 genotypes may respond variably to gastric carcinogens. Therefore, one genotype may bear more risk of

Received 10/29/02; revised 2/20/03; accepted 2/21/03.
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 To whom requests for reprints should be addressed, at Division of Surgery, Level 7, Bristol Royal Infirmary, University of Bristol, Bristol BS2 8HW, United Kingdom. Phone: 0044-117-928-3282; Fax: 0044-117-925-2736; E-mail: z.w.zhang@bristol.ac.uk.
developing cancer compared with the others; there are very few studies, however, on TP53 polymorphisms and gastric cancer. Shepherd et al. (23) examined the relationship between codon 72 polymorphisms and their susceptibilities to gastric cancer in a group of American gastric cancer patients. They noticed a higher frequency of Arg homozygotes for patients with gastric cardia cancer compared with those with cancer in the antrum or corpus. In the present study, we examined the genotype frequency of codon 72 in gastric cancer patients and noncancer controls in a group of Caucasian subjects in England using PCR-based genotyping methods to further evaluate the possible relevance of this polymorphism to gastric cancer.

MATERIALS AND METHODS

Specimens. Formalin-fixed, paraffin-embedded gastric tissue samples were selected consecutively from the archival tissue banks in the Departments of Histopathology at Bristol Royal Infirmary, Bristol, and at St. Bartholomew’s Hospital, London, United Kingdom. A total of 397 specimens (120 gastric cancer cases and 277 noncancer controls, the latter consisting of patients with benign upper gastrointestinal disorders including chronic gastritis and peptic ulceration) were obtained. They were from Caucasian patients who had undergone endoscopy or gastrectomy between January 1997 and October 2000 at Bristol Royal Infirmary and between November 1992 and April 1995 at St. Bartholomew’s Hospital. Clinical data including diagnosis, race, age, and gender of each patient were obtained from patient records and endoscopy and histology reports. The classification of cardia and non-cardia gastric cancer was formed based on International Classification of Diseases for Oncology codes (24). Patients were excluded from the study if they had severe systemic diseases or cancers of other systems. All of the histological diagnoses were reassessed on original H&E slides by two pathologists (R. F. and M. M.) who were blinded to clinical details and research outcome. This study was approved by the research ethics committees of The United Bristol Healthcare National Health Service (NHS) Trust and of East London and The City NHS Health Authority.

DNA Extraction. Gastric tissue blocks from noncancer subjects, gastric tumor tissue, and some well-separated normal tissue blocks from cancer patients were used for genomic DNA preparation. Sections cut from each tissue block (10-μm thick) were kept in a sterilized 1.5-ml Eppendorf tube. The blade was cleaned with ethanol between samples to eliminate possible cross-contamination of sample DNA. The content of the Eppendorf tube was deparaffinized in two serial washes of Clearene (Surgipath Europe, Ltd., Peterborough, United Kingdom) and two washes in ethanol. After air-drying, the DNA was extracted using QIAamp DNA mini kit (Qiagen, West Sussex, Crawley, United Kingdom) according to the manufacturer’s instructions.

Analysis of TP53 Codon 72 Polymorphism. PCR reaction mixture (25 μl) consisted of 2.5 μl of template DNA solution, 0.2 μM each outer oligonucleotide primer (forward, 5’-GCT CTG TTT ACC CAT CTA CAG-3’; reverse, 5’-TGA AGT CTC ATG GAA GCC AGC-3’), 200 μM each dNTP (Life Technologies, Inc., Ltd., Paisley, United Kingdom), 1.5 μM MgCl2, 2.5 μl of PCR buffer, and 2.5 units of Taq DNA polymerase (Life Technologies, Inc. Ltd.). The PCR amplification consisted of an initial 2-min incubation at 94°C, followed by 35 cycles of denaturing at 94°C for 30 s and annealing at 58°C for 30 s, with an extension at 72°C for 1 min. The reaction was terminated after a final extension of 5 min at 72°C. An additional PCR amplification reaction using inner oligonucleotide primers (forward, 5’-TCC CCC TTG CCG TTC CAA-3’; reverse, CGT GCA AGT CAC AGA CT-3’) was carried out using 2.5 μl of the 1-in-250-diluted first-round PCR product under the same conditions as for the first-round PCR reaction. Twenty-five μl of second round PCR product (279 bp) were digested overnight in a final volume of 30 μl using 10 units of BstUI restriction enzyme under the conditions recommended by the supplier (New England Biolabs Inc., Hitchin, Hertfordshire, United Kingdom). The restriction enzyme digests within the sequence corresponding to the Arg codon (CGC) at position 72 to generate two visible fragments of 160 bp and 119 bp and leaves the Pro allele uncleaved. The DNA fragments were then resolved by electrophoresis in 3% agarose, and the gels were stained with ethidium bromide. The positive control to confirm digestion of the PCR products was heterozygous DNA. The negative control was a complete PCR reaction mixture-omitting template DNA, to exclude the possibility of cross-contamination during sample preparation. The genotypes of more than 10% samples were reassessed to confirm the results. The DNA sequencing was performed on selected PCR products to further confirm the authenticity of the genotype analysis. In addition, we performed a genotyping assay of 36 cancer patients using DNA from well-separated normal tissues and gastric tumor tissues from the same patients. We found an identical genotype for all of the patients analyzed.

Immunohistochemistry. The p53 protein expression was evaluated immunohistochemically (25). The p53 antibody, which recognizes both mutant and wild-type p53, was obtained from Nova-Castra (Newcastle upon Tyne, United Kingdom). There were samples from 77 tumor tissues and 70 adjacent noninvolved tissues available for the assessment. A combined qualitative and quantitative approach, H score, was used to assess both the intensity and the percentage of positive cells. Twenty % of the cases were re-evaluated in a blinded fashion to guarantee the reproducibility.

Statistical Analyses. Frequency tables were constructed using the SPSS (standard version, March 2001) statistical package with statistical significance using the χ² test. The odd ratios and 95% CIs were calculated as an approximation of relative risk and adjusted for confounding factors such as age and gender using a logistic regression model. We also calculated the frequencies of codon 72 genotypes for three age groups (<65, 65–74, and 75+ years) according to previous epidemiological data (16).

RESULTS

Gastric cancer patients, with mean age of 75 ± 13, were significantly older than noncancer patients (61 ± 17; P < 0.0001). Male patients were overrepresented in the gastric can-

---

2 The abbreviation used is: CI, confidence interval.
cancer group (60.8%) when compared with those in the noncancer group (49.8%; \(\chi^2 = 4; \) df, 1; \(P = 0.04\)). The antral mucosal histology was assessed in the noncancer group. Thirty-eight % (96 of 254) of them had atrophy and 15% (38 of 254) had intestinal metaplasia. No dysplasia or other precancerous lesions were observed. The overall genotype frequencies for \(\text{Pro/Pro}, \text{Arg/Pro}, \text{and Arg/Arg} \) were 7.3, 45.1, and 47.6%, respectively, and fitted the Hardy-Weinberg equilibrium with the allele frequencies of 0.7 (\(\text{Arg}\)) and 0.3 (\(\text{Pro}\)). The genotype distribution was similar in men and women (\(\chi^2 = 3.3; \) df, 2; \(P = 0.2\)). The presence of \(\text{Arg}\) homozygous allele was strongly associated with age in gastric cancer patients (\(P = 0.008\)). As shown in Fig. 1, whereas the frequency of \(\text{Arg}/\text{Arg}\) allele in noncancer patients had no significant correlation with age (\(P = 0.96\)), a stepwise increase of \(\text{Arg}/\text{Arg}\) in gastric cancer patients was observed (\(P = 0.01\)). The age-associated increase in \(\text{Arg}/\text{Arg}\) genotype distribution was evident in both cardia and non-cardia gastric cancer groups; but it did not reach statistical significance in cardia groups (\(P = 0.06\)), which may be attributable to the limited patient number in each subgroup (Table 1).

We compared the distribution pattern of codon 72 genotypes in gastric cancer patients with that in noncancer patients. There was no significant difference in genotype distribution between cancer and noncancer patients (\(\chi^2 = 2.9; \) df, 2; \(P = 0.2\)). However, although the mean ages and gender distribution for cardia and non-cardia gastric cancer were not significantly different, cardia and non-cardia gastric cancer showed different patterns of codon 72 genotype distribution, with the homozygous \(\text{Arg}/\text{Arg}\) genotype occurring in 46.6% of 88 non-cardia tumors and in 71.9% of 32 cardia tumors (\(P = 0.03\); Table 1). There was no difference of the codon 72 genotype distribution between noncancer and non-cardia gastric cancer patients, but cardia gastric cancer patients had a significantly higher percentage of \(\text{Arg}/\text{Arg}\) than did noncancer patients (\(P = 0.01\); Table 1). After adjustment for patient age and gender, a logistic regression analysis showed that the risk for an \(\text{Arg}/\text{Arg}\) homozygous patient to have cardia cancer is 3.1 (95% CI, 1.4–7.3) times greater than it is for \(\text{Pro}/\text{Pro}\) homozygous and \(\text{Arg}/\text{Pro}\) heterozygous patients. There was no relationship between tumor histological types, differentiation, and codon 72 genotypes (Table 2).

**DISCUSSION**

We have observed a strong correlation between the distribution of \(\text{TP53}\) codon 72 genotypes and patient age in the gastric cancer group, but not in noncancer patients. The frequency of the homozygous \(\text{Arg}\) allele in cardia cancer was significantly higher than that in non-cardia gastric cancer or noncancer patients.

There have been studies examining the relationship between codon 72 variants and age in healthy subjects (26). Bonafe et al. (26) studied 611 healthy younger controls and 394 centenarians to assess the role of \(\text{p53}\) variants on survival and

---

**Table 1** Tumor location and codon 72 genotype distribution

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Pro/Pro</th>
<th>Pro/Arg</th>
<th>Arg/Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncancer controls(^a)</td>
<td>277</td>
<td>8.3%</td>
<td>46.6%</td>
<td>45.1%</td>
</tr>
<tr>
<td>&lt;65</td>
<td>150</td>
<td>9.3%</td>
<td>46%</td>
<td>44.7%</td>
</tr>
<tr>
<td>65–74</td>
<td>66</td>
<td>7.6%</td>
<td>45.5%</td>
<td>47%</td>
</tr>
<tr>
<td>75+</td>
<td>61</td>
<td>6.6%</td>
<td>49.2%</td>
<td>44.3%</td>
</tr>
<tr>
<td>Non-cardia(^b)</td>
<td>88</td>
<td>6.8%</td>
<td>46.6%</td>
<td>46.6%</td>
</tr>
<tr>
<td>&lt;65</td>
<td>16</td>
<td>6.3%</td>
<td>68.8%</td>
<td>25%</td>
</tr>
<tr>
<td>65–74</td>
<td>25</td>
<td>12%</td>
<td>56%</td>
<td>32%</td>
</tr>
<tr>
<td>75+</td>
<td>47</td>
<td>4.3%</td>
<td>34%</td>
<td>61.7%</td>
</tr>
<tr>
<td>Cardia(^c)</td>
<td>32</td>
<td>28.1%</td>
<td>71.9%</td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>6</td>
<td>66.7%</td>
<td>33.3%</td>
<td></td>
</tr>
<tr>
<td>65–74</td>
<td>9</td>
<td>22.2%</td>
<td>77.8%</td>
<td></td>
</tr>
<tr>
<td>75+</td>
<td>17</td>
<td>17.6%</td>
<td>82.4%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Noncancer versus cardia: \(\chi^2 = 9.1; \) df, 2; \(P = 0.01\).
\(^b\) Non-cardia versus cardia: \(\chi^2 = 6.9; \) df, 2 (\(P = 0.03\); codon 72 genotype distribution against patient age for non-cardia, \(\chi^2 = 10.2\) (\(P = 0.04\)).
\(^c\) Codon 72 genotype distribution against patient age for cardia \(\chi^2 = 5.5\) (\(P = 0.06\)).
longevity, but they found that the distribution pattern of codon 72 genotypes had no significant difference between the controls and the centenarians. This is consistent with our findings and suggests that the p53 variants are not associated with aging in healthy populations. The relationship between age and codon 72 in cancer patients has not been reported. Kawaijiri et al. (12) studied 347 healthy controls and 140 gastric cancer patients in a Japanese population and found that the genotype distribution among gastric cancer patients was significantly different from healthy controls, with 48.6% Arg/Arg and 3.6% Pro/Pro and Pro/Arg in gastric cancer compared with 41.5% and 10.9% in healthy controls (patients alone showed that genotype distribution, it was evident from their data that Arg/Arg genotype occurred in 40% of 150 cancer cases who aged 60 or over, compared with 27.8% of 36 who were less than 60. In contrast, in noncancer controls, there were 29.5% of 112 and 35% of 40 for those aged 60 or over and those younger than 60, respectively.

The mechanisms which lead to the age-associated increase of codon 72 Arg p53 in gastric adenocarcinoma are not clear. The preferential retention of codon 72 Arg p53 has been reported in squamous cell carcinoma (28, 29) and this could lead to an increased frequency of Arg allele in cancer patients. Data from current study, however, do not support this mechanism. The increased frequency of codon 72 Arg p53 was only associated with patient age and there was no difference between gastric cancer and noncancer controls. We compared the genotypes of 36 cancer patients using DNA from well-separated normal tissues and gastric tumor tissues of same patients and found an identical genotype for all of the patients analyzed. Furthermore, previous studies also showed that loss of heterozygosity (LOH) play little role in determining the pattern of codon 72 genotype in patients with adenocarcinoma (11, 23).

The most interesting explanation for the results reported is that the codon 72 Arg p53 acts as a survival factor in gastric cancer patients or those who have homozygous Arg allele confer a late start of gastric cancer when compared with those with Pro allele (proline homozygotes or Pro/Arg heterozygotes). Several lines of evidence support this. Firstly, the biochemical properties of the codon 72 variants are different. It has been shown that the Arg/Arg and Pro/Pro variants differ in their ability to bind components of the transcriptional machinery, to activate transcription, to induce apoptosis, and to repress the transformation of primary cells (9). The p53 Arg/Arg variant induces apoptosis with faster kinetics and suppresses transformation more efficiently than the p53 Pro/Pro variant. The p53 Arg/Arg variant is a better inducer of transcription because of its stronger affinity for the TAFII32 and TAFII70 transcription factors (9). One might postulate that the presence of the Pro allele, which does not induce apoptosis as effectively as the Arg allele, will be associated with therapeutic resistance and poor prognosis. It is indeed, as reported in a previous study, that lung cancer patients with the Pro/Pro genotype had a worse prognosis than those with the Arg/Pro genotype (6, 11). Furthermore, the natural history of gastric cancer development also supports the role of Arg/Arg genotype in gastric cancer patient survival. Early gastric cancer is usually diagnosed at about 60 years of age (30), and it takes approximately 8 more years to develop advanced gastric cancer (31, 32). With no treatment, 90% of advanced gastric cancer patients will die within 1 year after diagnosis. Even with treatment, the prognosis is still poor, and the available data suggest only 8% will survive 5 years (33). Thus, the majority of gastric cancer patients will probably die by age 73. This calculation may not be entirely true in England; but similar data were reported from the United States, where most deaths attributable to gastric cancer occur between the ages of 65 and 84 for all races, with a median age at death of 72 years for white males and 77 for white females (34). In the current study, we observed a dramatically increased frequency of Arg/Arg genotype in cancer patients at age 75 or more, for which the percentage of Arg/Arg genotype was twice that in cancer patients in the younger age group and in all of the noncancer controls. This may further indicate that the prognosis in patients with Pro allele was worse than that in those with Arg/Arg genotype or else that the latter have a late onset of gastric cancer. However, a well-

---

**Table 2** Gastric cancer histology, p53 protein expression, and codon 72 genotype distribution

<table>
<thead>
<tr>
<th>Histology</th>
<th>n</th>
<th>Pro/Pro</th>
<th>Pro/Arg</th>
<th>Arg/Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal</td>
<td>60</td>
<td>5%</td>
<td>38.3%</td>
<td>56.7%</td>
</tr>
<tr>
<td>Diffuse</td>
<td>58</td>
<td>5.2%</td>
<td>46.6%</td>
<td>48.3%</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>12</td>
<td>16.7%</td>
<td>50%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Moderate</td>
<td>42</td>
<td>35.7%</td>
<td>64.3%</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>64</td>
<td>6.3%</td>
<td>45.3%</td>
<td>48.4%</td>
</tr>
<tr>
<td>p53 protein expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour tissue (n)</td>
<td>77</td>
<td>135 ± 72 (4)</td>
<td>117 ± 77 (29)</td>
<td>143 ± 99 (44)</td>
</tr>
<tr>
<td>Adjacent normal mucosa (n)</td>
<td>70</td>
<td>30 ± 20 (4)</td>
<td>21 ± 58 (27)</td>
<td>14 ± 35 (39)</td>
</tr>
</tbody>
</table>

*Expressed as H scores ± SD.
designed follow-up study is needed to confirm this. Finally, in situ cancer of the cervix often fails to progress (35), and as many as 70% of breast neoplasms never develop into a clinically relevant disease (36). Premalignant colonic polyps have a long gestation period before showing invasive tendencies. Therefore, it is not surprising that there is a subtype of early gastric cancer that does not progress or else progresses so slowly that, even over a prolonged period, it does not become invasive (37).

Another possible explanation for the current results is that the codon 72 Arg p53 may be associated with an increased susceptibility of gastric cancer, particularly gastric cardia adenocarcinoma. We noticed a marked difference in the distribution pattern of codon 72 allele types between gastric cardia and non-cardia adenocarcinoma, with a significantly increased frequency of codon 72 Arg p53 in patients with cardia adenocarcinoma. A similar trend was reported in a recent study (23). We also found that patients with cardia adenocarcinoma had a significantly higher frequency of the Arg/Arg allele type than did patients without cancer, but a difference was not seen between non-cardia adenocarcinoma and noncancer patients. These findings suggest that codon 72 Arg p53 may be associated with an increased risk of cardia adenocarcinoma. Adenocarcinoma arising from the gastric cardia carries risk factors that are similar to those of esophageal carcinoma (e.g., gastro-esophageal acid reflux) and that differentiate it from non-cardia gastric adenocarcinoma. The differences in the role of codon 72 polymorphism between cardia and non-cardia gastric adenocarcinoma suggests the possible importance of environmental factors.

In summary, we have observed a positive correlation between the frequency of codon 72 Arg p53 and the age of gastric adenocarcinoma patients but not the age of noncancer patients. The percentage of homozygous Arg allele was significantly higher in cardia gastric cancer patients than in non-cardia gastric cancer patients or in noncancer patients. These findings indicate that codon 72 Arg p53 may be associated with a later onset of gastric cancer or with a prolonged survival of patients who have had gastric adenocarcinoma, especially non-cardia adenocarcinoma. It may, however, have a different role in patients with cardia gastric adenocarcinoma. Clearly, a well-designed follow-up study is needed to confirm these findings.

REFERENCES


Age-associated Increase of Codon 72 Arginine p53 Frequency in Gastric Cardia and Non-Cardia Adenocarcinoma

Zun-Wu Zhang, Paul Newcomb, Andrew Hollowood, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/6/2151

Cited articles
This article cites 30 articles, 16 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/6/2151.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/6/2151.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, contact the AACR Publications Department at permissions@aacr.org.