Activation of the Mitochondrial Apoptotic Pathway Produces Reactive Oxygen Species and Oxidative Damage in Hepatocytes That Contribute to Liver Tumorigenesis

Hayato Hikita, Takahiro Kodama, Satoshi Tanaka, Yoshinobu Saito, Yasutoshi Nozaki, Tasuku Nakabori, Satoshi Shimizu, Yoshito Hayashi, Wei Li, Minoru Shigekawa, Ryotaro Sakamori, Takuya Miyagi, Naoki Hiramatsu, Tomohide Tatsumi, and Tetsuo Takehara

Abstract

Chronic hepatitis, including viral hepatitis and steatohepatitis, is a well-known high-risk condition for hepatocellular carcinoma. We previously reported that continuous hepatocyte apoptosis drives liver tumors in hepatocyte-specific Bcl-xl or Mcl-1 knockout mice. In this study, we further examine the underlying cellular mechanisms of generating tumors in apoptosis-prone liver. In cultured hepatocytes, the administration of ABT-737, a Bcl-xl/-2/-w inhibitor, led to production of reactive oxygen species (ROS) as well as activation of caspases. Mitochondria isolated from murine liver, upon administration of truncated-Bid, a proapoptotic Bcl-2 family protein, released cytochrome c and produced ROS, which was dependent on mitochondrial respiration. Hepatic apoptosis, regeneration, accumulation of oxidative damages, and tumorigenesis observed in hepatocyte-specific Mcl-1 knockout mice were substantially attenuated by further deficiency of Bax or Bid, suggesting that a balance of mitochondrial Bcl-2 family proteins governs generation of oxidative stress and other pathologies. Whole-exome sequencing clarified that C>A/G>T version, which is often caused by oxidative DNA damage in proliferating cells, was a frequently observed mutation pattern in liver tumors of Mcl-1 knockout mice. The administration of antioxidant L-N-acetylcysteine did not affect apoptosis, compensatory regeneration, or fibrotic responses but significantly reduced oxidative DNA damage and incidence and multiplicity of live tumors in Mcl-1 knockout mice. In conclusion, activation of the mitochondrial apoptotic pathway in hepatocytes accumulates intracellular oxidative damages, leading to liver tumorigenesis, independently of liver regeneration or fibrosis. This study supports a concept that antioxidant therapy may be useful for suppressing liver carcinogenesis in patients with chronic liver disease.

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide. Most HCC develops in patients with chronic hepatitis, including chronic hepatitis C, chronic hepatitis B, alcoholic liver disease, and nonalcoholic steatohepatitis (NASH; ref. 1). In the livers of these patients, hepatocyte apoptosis is frequently observed and regarded as one of the characteristic features. In clinical trials, the administration of oral caspase inhibitors that specifically block apoptosis significantly reduced serum alanine aminotransferase (ALT) levels in patients with chronic hepatitis (2, 3), supporting the idea that apoptosis may prevent cancer development. Collectively, whether the hepatocyte apoptosis that is observed in chronic hepatitis is mechanistically linked to HCC development was unclear.

Apoptosis is regulated by a fine balance of antiapoptotic bcl-2 family proteins and proapoptotic bcl-2 family proteins. Under conditions of cellular stress, proapoptotic BH3-only proteins, such as Bid, Bim, and Puma, activate the proapoptotic proteins, Bak and Bax. Bak and/or Bax when activated homo-oligomerize to form a channel on the mitochondrial outer membrane and release cytochrome c from its intermembrane space to the cytosolic region, leading to the activation of caspases to execute cell death (7). Antiapoptotic bcl-2 family proteins, including Bcl-2, Bcl-xl, Mcl-1, Bcl-w, and Bfl-1, protect the mitochondrial pathway of apoptosis by inhibiting Bak and Bax. We previously reported that among the antiapoptotic bcl-2 family proteins, Bcl-xl and Mcl-1, are critical molecules that protect hepatocytes from apoptosis because hepatocyte-specific Bcl-xl or Mcl-1 knockout (KO) mice displayed hepatocyte apoptosis over time after birth (8, 9). Recently, we and another group reported that these mice...
Mice

Hepatocyte-specific Mcl-1 KO mice (mcl-1\textsuperscript{flox/flox} Alb-Cre) were previously described (9). Bax KO mice (bax\textsuperscript{−/−}) were obtained from the Jackson Laboratory. Bid KO mice (bid\textsuperscript{−/−}; ref. 16) were kindly provided by Dr. Xiao-Ming Yin (Indiana University, IN). Bax/Mcl-1 double KO mice (bax\textsuperscript{−/−} mcl-1\textsuperscript{flox/flox} Alb-Cre) and Bid/Mcl-1 double KO mice (bid\textsuperscript{−/−} mcl-1\textsuperscript{flox/flox} Alb-Cre) were generated by mating the strains. Some mice were continuously treated with NAC, an antioxidant, in drinking water after weaning. NAC was dissolved in water (10 g/L) and freshly prepared three times a week. Mice were maintained in a specific pathogen-free facility and treated with humane care with approval from the Animal Care and Use Committee of Osaka University Medical School.

Hepatocyte apoptosis and liver fibrosis assays

Serum ALT levels and caspase-3/-7 activity were measured as previously described (9). For histological analysis, livers were formalin-fixed, embedded in paraffin, and thin sliced. The liver sections were stained with hematoxylin and eosin (H&E). To detect cells with oligonucleosomal DNA breaks, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) was performed according to a previously reported procedure (17). For calculating the fibrotic area, liver sections stained with Sirius red were analyzed using image analysis software (winROOF visual system; Mitani Co.).

Immunohistochemistry

Ki-67, PCNA, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were labeled in paraffin-embedded liver sections using an anti-k-i67 antibody (Dako), anti-PCNA antibody (Cell Signaling Technology), and anti-8-OHdG antibody (Nikkken Sei), respectively. The detection of immunolabeled proteins was performed using an avidin–biotin complex within the Vectastain ABC Kit (Vector Laboratories).

Real-time reverse-transcription PCR (RT-PCR)

Total RNA was prepared using the RNeasy Kit (Qiagen). For cDNA synthesis, 1 μg of total RNA was reverse-transcribed using the High Capacity RNA-to-DNA Master Mix (Applied Biosystems). Real-time reverse-transcription PCR (RT-PCR) was performed using an Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems). The following TaqMan Gene Expression Assays were used: mouse-coll1a1 (Mm00801666_g1), mouse-coll1a2 (Mm0483888_m1), and mouse-β-actin (Mm00607939_s1). All expression levels were corrected with the quantified expression level of β-actin mRNA.

Whole-exome sequencing and analysis

DNA was extracted from 30 mg of liver tumors and their surrounding nontumor lesion by Maxwell16 (Promega). Extracted DNA was fragmented by Acoustic Solubilizer (Covaris) and prepared concentrated sequencing library using SureSelectXT
Regent Kit (Agilent Technologies) and SureSelectXT mouse all exon (Agilent Technologies). Prepared library was sequenced by HiSeq2000 (Illumina). Sequenced data were analyzed by Virmid to detect somatic mutations (18).

Statistical analysis
The data are presented as the mean ± standard deviation. Differences between two groups were determined using an unpaired Student's t test. Multiple comparisons were performed by ANOVA, followed by Fisher post hoc correction. Carcinogenesis rates were analyzed by a $\chi^2$-test. $P < 0.05$ was considered statistically significant.

Results
Activation of the mitochondrial pathway of apoptosis increases ROS production
To examine the relationship between apoptosis and ROS production, we cultured CL2 cells, a nontransformed murine liver cell line, with ABT-737, a Bcl-xl/-2/-w inhibitor. The ABT-737 increased caspase-3/-7 activity and decreased cell viability in CL2 cells in a dose- and time-dependent manner (Fig. 1A). Administration of 4 μmol/L ABT-737 for 24 hours increased caspase-3/-7 activity in CL2 cells (Fig. 1B). This dose of ABT-737 did not affect cell viabilities or the mitochondrial inner membrane potential (Fig. 1B and C), suggesting that most cells with an activated mitochondrial pathway do not collapse into apoptosis in this culture condition. Importantly, hepatocytes treated with ABT-737 had clearly increased intracellular ROS levels (Fig. 1D). Similar results were obtained by experiments using primary hepatocytes isolated from murine liver (Fig. 1B and D). These results suggested that the activation of the mitochondrial pathway of apoptosis increases ROS in hepatocytes.

The mitochondrial pathway of apoptosis is mediated by disrupting the mitochondrial outer membrane. We next examined whether mitochondrial outer membrane disruption could generate ROS. To this end, mitochondria were isolated from murine liver and incubated with or without t-Bid, a proapoptotic activator...
of the BH3-only proteins. The administration of 200 nmol/L t-Bid significantly increased the levels of ROS as well as cytochrome c in the supernatant (Fig. 2A). In this condition, the mitochondrial inner membrane potential, a generator of mitochondrial energy production, was maintained after incubation with t-Bid (Fig. 2B). However, incubation of mitochondria isolated from hepatocyte-specific Bak/Bax KO mice with 200 nmol/L t-Bid increase neither the cytochrome c nor the ROS levels in the supernatant (Fig. 2C), suggesting that t-Bid-induced cytochrome c release and ROS increase are dependent on Bak and Bax. The mitochondrion is an organelle that produces cellular energy through respiration in the presence of oxygen. To examine the role of mitochondrial respiration in ROS generation, mitochondria were incubated with both rotenone and malonic acid, which inhibit complex I and II of the respiratory chain, respectively. The ROS levels in the supernatant of the treated mitochondria clearly decreased in both the presence and absence of t-Bid (Fig. 2D). These results indicated that Bak/Bax-mediated disruption of the mitochondrial outer membrane not only induces apoptosis but also affects and enhances the production of ROS, which is dependent on the mitochondrial respiratory chain.

Suppression of the mitochondrial pathway of apoptosis reduces oxidative damage in hepatocytes and liver tumor development in Mcl-1 KO mice

Next, we addressed the relationship between the activation of the mitochondrial pathway of apoptosis and oxidative stress in hepatocytes in vivo. The mitochondrial pathway of apoptosis is regulated by a fine balance between antiapoptotic proteins, such as Bcl-xL or Mcl-1, and proapoptotic proteins, such as Bak or Bax (19). As a mouse model with continuous activation of the mitochondrial apoptotic pathway in hepatocytes, we previously reported hepatocyte-specific Bcl-xL or Mcl-1 KO mice, which display spontaneous apoptosis in hepatocytes scattered throughout the liver and increased serum ALT levels over time after birth (8, 9). We also reported that oxidative stress as well as hepatocyte regeneration and liver fibrosis increased in hepatocyte-specific Bcl-xL or Mcl-1 KO mice (10). To further examine whether suppression of the mitochondrial pathway of apoptosis reduces oxidative damage, we generated Bax/Mcl-1 double KO mice by crossing Bax KO mice and hepatocyte-specific Mcl-1 KO mice. As expected, the increased serum ALT levels and caspase-3/7 activities in Mcl-1 KO mice were significantly attenuated by the Bax deficiency (Fig. 3A). H&E staining and TUNEL staining of liver sections showed that the bax deficiency attenuated the number of hepatocytes with typical apoptotic morphology and the number of TUNEL-positive cells in Mcl-1 KO mice (Supplementary Fig. S1 and Fig. 3B). Accompanied by the attenuation of hepatocyte apoptosis, hepatocyte regeneration also decreased in Bax/Mcl-1 double KO mice compared with Mcl-1 KO mice, as evidenced by Ki-67 immunohistochemistry (Supplementary Fig. S1 and Fig. 3B). The increased expression levels of col1a1 and col1a2, which encodes the major component of type I collagen, in Mcl-1 KO mice were decreased by the bax deficiency, indicating liver fibrosis observed in Mcl-1 KO mice was also attenuated by

<table>
<thead>
<tr>
<th>Figure 2.</th>
<th>Truncated-Bid (t-Bid) makes mitochondria release cytochrome c and increase ROS production. Liver mitochondria are isolated from C57BL6/J mice (A, B, and D) or hepatocyte-specific Bak/Bax double KO mice (C) and incubated with 200 μmol/L of t-Bid for 30 minutes in the presence or absence of indicated reagents. ROS levels were measured by DCF assay. Note that ROS oxidizes nonfluorescent DCFH to highly fluorescent DCF in this assay.</th>
<th>A, cytochrome c and ROS levels in the supernatants of Bak/Bax double KO mitochondria after incubation of t-Bid (N = 8 each). B, the mitochondrial membrane potential measured by JC-10 (N = 4 each). Triton X was used as a positive control for loss of mitochondrial membrane potential. C, cytochrome c and ROS levels in the supernatants of Bak/Bax KO mitochondria after incubation of t-Bid (N = 8 each). D, liver mitochondria isolated from C57BL6/J mice were incubated with t-Bid together with malonic acid and rotenone for 30 minutes. Malonic acid and rotenone were used for suppression of mitochondrial respiratory chain. ROS levels in the supernatants after incubation (N = 8 each). *, P &lt; 0.05.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mitochondria from wild-type liver</td>
<td>Mitochondria from wild-type liver</td>
</tr>
<tr>
<td>B</td>
<td>5% Triton X</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>Mitochondria from Bak/Bax KO liver</td>
<td>Mitochondria from Bak/Bax KO liver</td>
</tr>
<tr>
<td>D</td>
<td>Rotenon Malonic acid</td>
<td>+ + +</td>
</tr>
<tr>
<td>t-Bid (nmol/L)</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>t-Bid (nmol/L)</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>t-Bid (nmol/L)</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>t-Bid (nmol/L)</td>
<td>0</td>
<td>200</td>
</tr>
</tbody>
</table>
Importantly, the number of 8-OHdG-positive nuclei in the liver sections of Mcl-1 KO mice also decreased with Bax deficiency (Fig. 3D). Similar results were obtained with Bid deficiency in hepatocyte-specfic Mcl-1 KO mice; caspase-3/7 activity, as well as the number of 8-OHdG-positive nuclei, in liver sections significantly decreased in Bid/Mcl-1 double KO mice compared with Mcl-1 KO mice (Supplementary Fig. S2, Fig. 3D). These results indicated that the suppression of the mitochondrial pathway of apoptosis in hepatocytes reduces the accumulation of oxidative damage that is observed in the Mcl-1 KO liver.

We examined liver tumor incidence in Bax/Mcl-1 double KO mice and their littermates, Mcl-1 KO mice, in 1 year. Although approximately two thirds of the Mcl-1 KO mice developed liver tumors, most of which were multiple, only one of nine Bax/Mcl-1 double KO mice developed a solitary liver tumor (Fig. 3E). Similarly, the incidence rate of liver tumors in Mcl-1 KO mice was decreased by Bid deficiency (Fig. 3E). These findings indicated that the suppression of the mitochondrial pathway of apoptosis not only reduces the accumulation of oxidative damage but also the incidence of tumors in apoptosis-prone liver.

C>A/G>T transversion is frequently observed in tumors of Mcl-1 KO mice

To address the mechanisms of carcinogenesis in Mcl-1 KO mice, we sequenced whole exome of four liver tumors and their counterparts.
surrounding nontumor lesions in Mcl-1 KO mice using next-generation sequencing with target enrichment system (Supplementary Table S1). After analyzing by Virmid to detect somatic mutation (18), we detected 101, 63, 54, and 64 single nucleotide variants (SNV), which had been filtered by probabilities (Fig. 4A). Among them, 17.8% of SNVs were located in coding lesions (CDS; Fig. 4B) and 10 to 35 mutated genes for each tumor were found (Supplementary Table S2). However, there was not common one in them. However, in all examined liver tumors, C>T/G>A transition and C>A/G>T transversion were frequently observed among all somatic mutation patterns (Fig. 4C). Oxidative stress converts especially guanine in DNA into 8-OHdG by oxidation (20). Because the conversion of 8-OHdG frequently causes a transversion from guanine (G) to thymidine (T) in proliferating cells, this transversion may be one of the mechanisms by which oxidative stress causes malignant transformation of the hepatocytes. Together with the fact that transversion is more rarely occurred than transition (21), this result supported the idea that the liver is exposed to oxidative stress in Mcl-1 KO mice and that oxidative stress-induced SNVs may be involved in malignant transformation of the hepatocytes.

Administration of NAC, an antioxidant, does not affect apoptosis, regeneration or fibrosis but reduces incidence and multiplicity of tumor in Mcl-1 KO liver

To examine the role of hepatocyte oxidative damage in liver carcinogenesis, we provided Mcl-1 KO mice with NAC in their drinking water (10 g/L) for 1 year after weaning. The administration of NAC did not affect serum ALT levels and caspase-3/-7 activity in Mcl-1 KO mice (Fig. 5A). No significant difference in the number of TUNEL-positive hepatocytes was observed between Mcl-1 KO liver and NAC-treated Mcl-1 KO liver (Fig. 5B and C). Immunohistochemistry for Ki-67 and PCNA revealed that NAC treatment did not have any effect on liver compensatory regeneration followed by hepatocyte apoptosis (Fig. 5B and C). The liver fibrotic responses were not altered by NAC treatment as evidenced by the expression levels of col1a1 and col1a2 or Sirius red staining (Fig. 5D and E).

At the age of 1 year, the number of 8-OHdG positive nuclei in the liver of NAC-treated Mcl-1 KO mice significantly decreased (Fig. 6A), suggesting that NAC treatment reduced the accumulation of oxidative DNA damage. Importantly, NAC treatment significantly decreased the incidence rate of liver tumors in Mcl-1 KO mice (Fig. 6B and C). In addition, among mice that developed liver tumors, multiplicity of liver tumors significantly decreased with NAC treatment [NAC-untreated mice, 90% (18/20) vs. NAC-treated mice, 44% (4/9), P < 0.05]. In contrast, the maximum tumor size did not differ between NAC-treated mice and NAC-untreated mice (5.0 ± 6.3 mm vs. 5.0 ± 6.7 mm). Tumors in the NAC-treated Mcl-1 KO mice were histologically defined as well-differentiated HCCs, similar to those in the NAC-untreated Mcl-1 KO mice (Fig. 6B).

Discussion

Oxidative stress is produced by the relative overproduction of ROS in comparison with antioxidants. In this study, we demonstrated that ABT-737, which can inhibit Bcl-xl, induces ROS production in hepatocytes and that t-Bid, a proapoptotic Bcl-2 family protein activated by the death receptor proximal initiator caspase-8, enhances ROS production from liver mitochondria. Yin and colleagues previously reported that the administration of TNFα or Fas ligand generates ROS in wild-type hepatocytes but not in BID KO hepatocytes (22). This study further provides direct evidence that the activation of the mitochondrial pathway of apoptosis make mitochondria generate ROS production in hepatocytes. This study also provides in vivo evidence that oxidative damage in hepatocytes produced by the activation of the mitochondrial pathway of apoptosis is one of the mechanisms linked carcinogenesis in apoptosis-prone liver. Many studies have reported that chronic infections of hepatitis C virus and hepatitis B virus increase oxidative stress (23–26). In this study, we demonstrated that the hepatocyte apoptosis observed in viral hepatitis and nonviral hepatitis is sufficient for inducing enhanced ROS production and oxidative damage in hepatocytes leading to liver cancer development.

A hepatocyte contains approximately 1,000 mitochondria, where the electron transport chain produces ATP by oxidative phosphorylation. Although ATP production is essential for the maintenance of life, this process inevitably produces a small amount of ROS. However, mitochondria are also involved in the regulation of apoptosis in type II cells, such as hepatocytes. Namely, various apoptotic stimuli activate Bak/Bax and form pores on the mitochondrial outer membrane, leading to the release of cytochrome c from the mitochondrial intermembrane space (7, 27). The released cytochrome c activates downstream caspases, such as caspase-3 and -7, resulting in the execution of apoptosis. In this study, we showed that disruption of the mitochondrial outer membrane by the addition of t-Bid enhances...
production of ROS, which is mainly dependent on the mitochondrial respiratory chain.

In apoptosis-prone liver, ROS generated from mitochondria may produce oxidative DNA damages in neighboring hepatocytes as well as hepatocytes concerned if they can survive with lower levels of caspase activation than those sufficient for achieving cell death. ROS converts guanine in DNA into 8-OHdG by oxidation. Because 8-OHdG frequently causes a transversion from guanine (G) to thymidine (T) in proliferating cells, this transversion may be one of the mechanisms by which oxidative DNA damages drives malignant transformation of the hepatocytes in regenerating liver (28). This possibility is supported by our finding using next-generation sequencing that C>A/G>T transversion was frequently observed pattern in liver tumors of Mc-1 KO mice. High frequencies of C>T/G>A transition, T>C/A>G transition, and C>A/G>T transversion were characteristic features of mutation patterns in human HCCs (29–33) compared to other human tumors where C>T/G>A transition is the most abundant alternation (34). This characteristic features were conserved in our murine model (Fig. 4C). However, we could not find responsible mutated genes for tumorigenesis. Among many candidate genes, we further focused on mutations in p53 or beta-catenin genes, which were reported to be found in approximately half of human HCCs (31–33, 35), and examined almost all coding lesions of p53 (mean coverage 7985) and all coding lesions of beta-catenin (mean coverage 7609) by deep sequencing (Supplementary Materials and Methods, Supplementary Table S3). We analyzed five tumors from Mcl-1 KO mice and five normal livers from wild-type littermates and compared with the reference sequence (NCBI Reference Sequence: NC_000077.6 for p53, NC_000075.6 for beta-catenin). However, it revealed no difference with more confidence than P < 0.01 in any base position in tumors compared to wild-type livers (Supplementary Fig. S3). These data suggested that mutations in these two genes were not detected with these samples. They were not thought to be a cause of tumorigenesis in Mcl-1 KO liver. The discrepancy may be explained by the possible involvement of other carcinogenic factors in human HCCs. Further study will be needed in this point.

The antioxidant vitamin E was recently reported to reduce hepatocyte apoptosis and serum ALT levels in patients with NASH (36). However, ALT levels and hepatocyte apoptosis were not decreased by NAC treatment in this study. Oxidative stress may be
one of the causes for the hepatocyte apoptosis in patients with NASH. In contrast, in our model, the ablation of an antiapoptotic protein directly activated the mitochondrial pathway of apoptosis, explaining why the antioxidant did not reduce liver injury in our model. Importantly, in this study, the antioxidant significantly reduced the incidence rate of liver tumors, although it did not reduce serum ALT levels or the degree of hepatocyte apoptosis. This result raised an interesting idea that the long-term use of antioxidants is useful for preventing liver cancer development in patients with hepatitis even if their ALT levels cannot be completely controlled by these drugs. Another important point is the fact that the antioxidant suppressed liver tumor development without any effect on liver regeneration or fibrotic responses. Liver injury consequently induced compensatory regeneration and fibrosis. The acceleration of liver regeneration may contribute to tumor formation in the livers with continuous hepatocyte apoptosis. Indeed, NAC treatment did not affect the maximum tumor size, suggesting that compensatory regeneration might contribute to the progression of liver tumors. However, our finding clearly demonstrated that continuous regeneration is not a mechanism for tumor initiation in livers with continuous hepatocyte apoptosis.

In conclusion, the activation of the mitochondrial pathway of apoptosis in hepatocytes not only activated caspases to execute cell death but also enhanced ROS production, which is dependent on mitochondrial respiratory chain. Enhanced ROS production may lead to oxidative damage in the hepatocytes concerned or in neighboring hepatocytes. As the antioxidant NAC reduces the incidence of tumors in apoptosis-prone livers without affecting the levels of apoptosis or compensating regeneration, antioxidant therapy may be useful for preventing tumor development in patients with chronic hepatitis.
References


Activation of the Mitochondrial Apoptotic Pathway Produces Reactive Oxygen Species and Oxidative Damage in Hepatocytes That Contribute to Liver Tumorigenesis

Hayato Hikita, Takahiro Kodama, Satoshi Tanaka, et al.


Updated version Accessed the most recent version of this article at: doi:10.1158/1940-6207.CAPR-15-0022-T

Supplementary Material Accessed the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/06/04/1940-6207.CAPR-15-0022-T.DC1
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/06/11/1940-6207.CAPR-15-0022-T.DC2

Cited articles This article cites 36 articles, 3 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/8/8/693.full.html#ref-list-1

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.