**GNAS1 T393C Polymorphism Is Associated with Clinical Course in Patients with Intrahepatic Cholangiocarcinoma**

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**Abstract**

**BACKGROUND/AIMS:** The GNAS1 locus encodes the Gaαs protein, which stimulates the formation of cycloadenosinemonophosphate (cAMP). The cAMP pathway mediates pleiotropic effects, including the regulation of apoptosis and proliferation. We have recently shown that TT genotypes of the single-nucleotide polymorphism T393C in the gene GNAS1 predict the clinical outcome of patients with various carcinomas. 

**METHODS:** Eighty-seven patients with intrahepatic cholangiocarcinoma (ICC) were retrospectively genotyped to elucidate a potential association between T393C genotypes and clinical outcome. **RESULTS:** ICCs of patients with homozygous TT genotypes revealed a higher proliferation rate and a lower apoptotic rate. Homozygous TT patients were at highest risk for cancer-related deaths (hazard ratio = 2.74; 95% confidence interval = 1.03–7.28) compared with C-allele carriers. Kaplan-Meier curves for disease-specific over-occurred less frequently than extrahepatic bile duct carcinoma, but ranks second to hepatocellular carcinoma among primary malignant liver tumors. In western countries, ICC accounts for about 10% of primary liver malignancies, with increasing incidence in the last two decades [1,2]. Our group recently showed that R0 resection can provide prolonged survival, even in patients with advance ICC [3]. Poor results after R1 resection and high operative morbidity and mortality do not justify palliative resections but underline the need for an improved preoperative assessment of resectability to avoid unnecessary surgical therapy. The identification of patients with high-risk ICC may thus be helpful for optimizing decision making for surgical treatment. Therefore, it is desirable to identify molecular markers that individually predict tumor behavior and may facilitate individualized therapy even before initial surgery. The most common genetic alterations in ICC are mutations of RAS and TP53 genes. However, besides a broad range in the geographic incidences of k-RAS mutations in ICC (from 4% among Thai patients [4] to 100% among British patients [5]), the application of these markers requires tumor tissue, whereas an ideal prognostic factor is based on tissue material acquired before surgery. Such factors might be preoperatively determined tumor-related cell-free nucleic acids in plasma and serum. Recently, the preoperative plasma transcript AA454543 level has been identified as a prognostic parameter in hepatocellular carcinoma [6,7]. However, such novel and promising prognostic markers are still based on the presence of tumor mass. A genetic host factor may offer a better stratification of patients into more refined risk categories without the need for tumor tissue. Necessary genetic information might be derived from a blood sample even years before cancer develops.

**Keywords:** Single-nucleotide polymorphism, cholangiocarcinoma, disease-free survival, GNAS1, prognosis.

**Introduction**

Cholangiocarcinoma (CCC) is composed of cells resembling those of bile ducts. CCC arises either from the extrahepatic duct, including the hilar bifurcation, or from intrahepatic ducts. Intrahepatic cholangiocarcinoma (ICC) occurs less frequently than extrahepatic bile duct carcinoma, but ranks second to hepatocellular carcinoma among primary malignant liver tumors. In western countries, ICC accounts for about 10% of primary liver malignancies, with increasing incidence in the last two decades [1,2]. Our group recently showed that R0 resection can provide prolonged survival, even in patients with advance ICC [3]. Poor results after R1 resection and high operative morbidity and mortality do not justify palliative resections but underline the need for an improved preoperative assessment of resectability to avoid unnecessary surgical therapy. The identification of patients with high-risk ICC may thus be helpful for optimizing decision making for surgical treatment. Therefore, it is desirable to identify molecular markers that individually predict tumor behavior and may facilitate individualized therapy even before initial surgery. The most common genetic alterations in ICC are mutations of RAS and TP53 genes. However, besides a broad range in the geographic incidences of k-RAS mutations in ICC (from 4% among Thai patients [4] to 100% among British patients [5]), the application of these markers requires tumor tissue, whereas an ideal prognostic factor is based on tissue material acquired before surgery. Such factors might be preoperatively determined tumor-related cell-free nucleic acids in plasma and serum. Recently, the preoperative plasma transcript AA454543 level has been identified as a prognostic parameter in hepatocellular carcinoma [6,7]. However, such novel and promising prognostic markers are still based on the presence of tumor mass. A genetic host factor may offer a better stratification of patients into more refined risk categories without the need for tumor tissue. Necessary genetic information might be derived from a blood sample even years before cancer develops.
We have recently shown that T393C polymorphism in the gene GNAS1, which encodes the Gαs subunit of heterotrimeric G protein, is significantly associated with the clinical outcome of patients suffering from bladder cancer [8], colorectal cancer [9], chronic lymphocytic leukemia [10], and renal cell carcinoma [11]. We previously demonstrated that the T allele of GNAS1 T393C polymorphism is associated with increased Gαs mRNA expression in a variety of tissues, including tumor tissue [8], and might induce a change in the mRNA folding structures predicted by MFOLD [9,12]. MFOLD is a set of programs developed by Zuker that uses dynamic computer programming to predict RNA secondary structures. In vitro experiments suggest that an increased expression of Gαs is associated with enhanced apoptosis and that the second messenger, cyclic adenosine monophosphate (cAMP), which functions downstream of G proteins, plays a major role in this proapoptotic process [13–17].

The aim of the present study was, thus, to investigate a potential association between genotypes of T393C polymorphism and disease progression in patients with ICC. Moreover, we investigated a possible genotype-dependent correlation with tumor cell proliferation (using Ki67 immunohistochemistry) and apoptosis (applying the terminal deoxyribonucleotide transferase–mediated dUTP nick end labeling (TUNEL) method).

Materials and Methods

Patients

This study comprises a total of 87 consecutive ICC patients (with a mean age of 59 ± 11.2 years) seen at the Clinic of General Surgery and Transplantation of the University Hospital Essen (Essen, Germany) between August 1998 and June 2004 who underwent surgical exploration for intended liver resection. In 47 patients (54%), liver resection was performed. The diagnosis of ICC was based on histology obtained by preoperative or intraoperative biopsy or by examination of resected liver specimens. Patients with hilar CCC, gallbladder carcinoma, or mixed hepatocellular carcinoma/CCC and cirrhosis were excluded from this study. Among the resection group, one patient suffered from primary sclerosing cholangitis without cirrhosis; hepatolithiasis was not present in any case. Tumors were classified according to the pTNM system (sixth edition [18]). Detailed clinical data, including preoperative therapy, operative details, and pathological findings such as surgical radicability, tumor staging, and clinical follow-up, were available. Data were completed by January 2005, with a minimum follow-up period of 6 months or until death. The mean follow-up was 13.8 months, and the maximum follow-up was 73 months. For statistical analysis, two groups were created: (1) the exploration group consisting of ICC with no further surgical resection after explorative laparotomy, and (2) the resection group consisting of all cases with liver resection with detailed clinicopathological data. Twenty-three of 28 deaths of patients with resected ICC were strictly cancer-related (recurrence). One patient died of cerebral infarction, one died of respiratory insufficiency, and two died of multorgan failure. Regarding the exploration group, no data on the exact cause of death were available. All patients of this subgroup died within a short time (mean survival time, 8 months) due to advance-stage cancer.

Blood Donors

The Caucasian control sample consisted of 163 age-matched and sex-matched healthy white Caucasian individuals of either gender who were recruited at the local Department for Transfusion Medicine, University Hospital Essen. All samples were collected at random from subjects donating blood; the details of this sample have been published previously [19]. The control sample consisted of 69 males and 94 females, and the mean age was 58.5 ± 4.2 years. Healthy blood donors gave written consent for the inclusion of their blood in this study.

The present study was strictly performed according to the Declaration of Helsinki. The study was approved by the Ethics Committee of the University Hospital of Essen.

Genotyping

For the genotyping of T393C polymorphism, genomic DNA was isolated from several 10- to 20-μm-thick sections from routinely processed paraffin blocks (tumor tissue and normal tissue or partly exclusive non-neoplastic tissue in the exploration group) and placed in a 1.5-ml microfuge tube. The samples were dewaxed on a shaker incubator at 45°C for 5 minutes. After centrifugation at room temperature, the supernatant was removed. Pellets were washed in 1000 μl of ethanol and again centrifuged at full speed for 5 minutes. The supernatant was removed, and the open microfuge tube was incubated at 45°C for 2 to 5 minutes until the ethanol had evaporated. DNA was purified with the QIamp DNA Mini Kit (Qiagen, Hilden, Germany). Tissue pellets were resuspended in 180 μl of buffer ATL/20 μl of proteinase K and incubated overnight on a shaker incubator at 56°C. Further processing of the samples was performed according to the recommendations of the supplier. Genotypes for T393C polymorphism were determined as previously described [8].

Ki67 Immunostaining and TUNEL

Ki67 immunohistochemistry was performed on 5-μm-thick paraffin sections. Dewaxed and rehydrated sections were incubated with hydrogen peroxide to block endogenous peroxidase. Immunostaining of Ki67 was carried out with an automated staining device (DAKO Autostainer, Glostrup, Denmark). After antigen retrieval, prediluted monoclonal anti-Ki67 antibody (Biogenex, San Ramon, CA) was incubated for 30 minutes, and antibody demonstration was achieved using the commercially available anti-mouse IgG detection kit (EnVision; DakoCytomation, Carpenteria, CA). Primary antibodies were replaced with mouse immunoglobulin, which served as negative controls. Growth fraction (GF) was defined as the percentage of Ki67+ randomly chosen nuclei per 600 tumor cells. In situ DNA fragmentation, using the TUNEL technique, was performed on paraffin-embedded sections with the use of the ApoTag Plus Peroxidase In Situ
Apoptosis Detection Kit (Intergen Company, Oxford, UK), in accordance with the manufacturer’s recommendations. The number of apoptotic tumor cells was determined per 600 randomly chosen tumor cells. Corresponding hematoxylin–eosin sections were analyzed to avoid miscounting necrotic cells.

Statistical Analysis
All data were statistically analyzed with SPSS V.12 (SPSS, Inc., Chicago, IL) for Windows (Microsoft Corporation, Redmond, WA). Because the T393C single-nucleotide polymorphism (SNP) shows a gene–dose effect [8], linear analysis of variance (ANOVA) was used for the comparison of continuous parametric variables. Relationships between ordinal parameters were investigated using two-tailed chi-square analysis or Fisher’s exact test (where case numbers were low). Overall survival (OS) curves were estimated using the Kaplan-Meier method, and differences in survival curves were compared by the log-rank test. For multivariate analysis, the Cox regression model was used. All parameters that showed statistical significance on univariate survival analysis were included in the Cox regression analysis. Overall, 95% confidence intervals (95% CIs) were used throughout. Compatibility with the Hardy-Weinberg equilibrium was calculated with the public domain program HWE, which was developed by J. Ott (http://linkage.rockefeller.edu/ott/linkutil.htm).

Results
T393C genotype distribution and the demographic characteristics of the whole series (exploration and resection group) are displayed in Table 1.

The mean age was 59.5 years, and the mean follow-up was 13.8 months. The frequency of T allele (fT) in the patient group was 0.41, and genotype distribution was compatible with the Hardy-Weinberg equilibrium. To investigate whether T393C polymorphism is predictive of an increased risk for developing ICC, we compared genotypes and allele frequencies with those from 163 age-matched and sex-matched healthy white blood donors (CC, n = 43; TC, n = 87; TT, n = 33) [11]. Genotype distribution and T-allele frequency (fT = 0.47) were not significantly (P = .18) different from those of the patient group (fT = 0.41), which argues against the association of T393C genotypes with an increased susceptibility for ICC. In the resection group, gender, mean age, histologic grading, staging (T classification), lymph or blood vessel infiltration, lymph node status, distant metastasis, multifocal tumor growth, International Union Against Cancer (UICC) stage, tumor size, and resection status were not associated with genotype (Table 2).

GNAS1 T393C Genotypes and Proliferation/Apoptosis
ICC cases of the resection group were subject to analyses of proliferation and apoptosis. Three cases were not available for analysis due to lack of suitable materials. There was a significantly higher number of Ki67+ tumor cells per 600 tumor nuclei in TT genotypes compared to grouped CC/CT genotypes (ANOVA test, P = .008). Tumors with a TT genotype exhibited the lowest mean value of apoptotic tumor cells. The rate of apoptosis gradually increases from TC genotypes to CC genotypes (Table 3).

Clinical Outcome By GNAS1 T393C Genotypes
Recurrence-free survival and tumor-specific OS dependent on T393C genotypes were analyzed using Kaplan-Meier survival curves (Figure 1). R1/R2–resected patients exhibited a reduced tumor-specific OS, whereas patients who underwent explorative laparotomy without further surgical therapy exhibited the lowest tumor-specific OS (Figure 1A). Survival analysis of the complete series, including cases with explorative laparotomy and curative resection, showed that disease-specific OS was significantly dependent on T393C genotype (P = .02), with TT genotypes showing reduced survival compared to patients carrying at least one C allele (Figure 1B). The level of significance increased when CC and CT genotypes were grouped together (P < .008). Regarding the resection group, tumor-specific OS and time to local recurrence were again significantly dependent on T393C genotype (Figure 2). Patients with a TT genotype displayed a higher risk for death compared to patients with CC/CT genotype (P = .03; Figure 2A). The unfavorable prognostic effect of tumors with TT genotypes again increased when CC and CT genotypes were combined for Kaplan-Meier analysis (P < .007). The significance increased in the subgroup of patients with primarily R0-resected ICC (P < .001; Figure 2B). In addition, TT-homozygous patients with primarily an R0 resection status were at higher risk for local recurrence than were heterozygous or CC-homozygous patients (P = .03; Figure 2C). Parallel univariate survival analysis showed a significant association of resection status (P < .001), tumor size (P = .01), multifocal tumor growth (P = .02), higher UICC stage (P = .04), vascular invasion (P = .06), and the presence of lymph node metastasis (P = .05) with a reduced tumor-specific OS. To clarify the independent prognostic value of T393C polymorphism in patients with resected ICC, multivariate analysis of relevant parameters that revealed a prognostic significance on univariate survival analysis was performed. Cox regression analysis demonstrated R classification to be the best prognostic factor for disease-specific OS, followed by T393C polymorphism. T393C polymorphism was identified as a prognostic parameter, with T393

Table 1. GNAS1 T393C Genotype Distribution and Demographic Characteristics in 87 Patients (Resection and Exploration Group) with ICC.

<table>
<thead>
<tr>
<th></th>
<th>All (N = 87)</th>
<th>TT (n = 15; 17.2%)</th>
<th>TC (n = 41; 47.1%)</th>
<th>CC (n = 31; 35.6%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>40/46</td>
<td>7/8</td>
<td>19/21</td>
<td>14/17</td>
<td>.88</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>59.5 ± 11.2</td>
<td>58 ± 9.9</td>
<td>61.5 ± 11.1</td>
<td>57.6 ± 11.8</td>
<td>.30</td>
</tr>
</tbody>
</table>

P values were calculated using chi-square analysis and ANOVA for continuous variables.
homoygosity being an independent prognostic marker for reduced tumor-specific OS (hazard ratio = 2.47; 95% CI = 1.03–7.28) (Table 4).

**Discussion**

Although clinicopathological parameters such as UICC stages and resection status [3,20] may serve as prognostic markers in ICC, markers facilitating a more precise prediction of the clinical outcome of individual patients are still desirable. The majority of prognostic markers are based on features of the tumor tissue itself (e.g., expressions of proteins or genes; somatic mutations of tumor-specific oncogenes or tumor-suppressor genes). In this study, we examined the putative effect of a host factor, the GNAS1 T393C polymorphism. In contrast to conventional tumor-based prognostic markers, SNPs can be determined independently of the availability and/or the quality of tumor biopsy materials.

Our data demonstrate a significant association of both disease-specific OS and recurrence-free survival with the homozygous TT genotype of the GNAS1 gene. Patients with T393 homozygosity exhibited a decreased survival time regardless of their resection status and show earlier tumor recurrence. Even in the group of patients exclusively undergoing explorative laparotomy, the proportion of homozygous TT carriers increased compared to that of healthy blood donors.

To date, only a few studies dealing with the potential function of T393C polymorphism in the GNAS1 gene have been published. *In vitro* experiments suggested that an increased expression of Gαs is associated with enhanced apoptosis [21–23]. Activated Gαs generates the second messenger cAMP, which seems to play a major role in this proapoptotic process [13–15]. Recent investigations by our group showed a significant association of the C allele with an unfavorable clinical course in bladder cancer, sporadic colorectal cancer, and clear cell renal cell carcinoma [9,8,11]. Moreover, these studies yielded additional insights into the putative functional effect of GNAS1 polymorphism: (1) GNAS1 TT genotype was associated with increased Gαs

### Table 2. GNAS1 T393C Genotype Distribution, Demographic Characteristics, and Clinicopathological Characteristics in 47 Patients of the Resection Group.

<table>
<thead>
<tr>
<th>Gender (M/F)</th>
<th>TT (n = 7; 14.9%)</th>
<th>TC (n = 21; 44.7%)</th>
<th>CC (n = 19; 40.4%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (N = 47)</td>
<td>4/3</td>
<td>11/10</td>
<td>5/14</td>
<td>.17</td>
</tr>
<tr>
<td>Age (in years) at diagnosis (mean ± SD)</td>
<td>58.3 ± 11.8</td>
<td>58.9 ± 6.6</td>
<td>59 ± 12.1</td>
<td>.89</td>
</tr>
<tr>
<td>Grading [n (%)]</td>
<td>G1 2 (43)</td>
<td>G2 28 (59.6)</td>
<td>G3 17 (36.2)</td>
<td>.49</td>
</tr>
<tr>
<td>T393 (n = 7)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>.21</td>
</tr>
<tr>
<td>UICC stage grouping (sixth edition)</td>
<td>I 9</td>
<td>II 3</td>
<td>III (A–C) 30</td>
<td>.21</td>
</tr>
<tr>
<td>Staging [n (%)]</td>
<td>T1 11 (23.4)</td>
<td>T2 7 (14.9)</td>
<td>T3 23 (48.9)</td>
<td>.09</td>
</tr>
<tr>
<td>Lymph vessel infiltration</td>
<td>L0 34 (72.3)</td>
<td>L1 13 (27.7)</td>
<td>L2 26 (55.3)</td>
<td>.37</td>
</tr>
<tr>
<td>Blood vessel infiltration</td>
<td>V0 26 (55.3)</td>
<td>V1 21 (44.7)</td>
<td>V2 29 (61.7)</td>
<td>.50</td>
</tr>
<tr>
<td>Nodal status</td>
<td>0 18 (38.3)</td>
<td>1 29 (61.7)</td>
<td></td>
<td>.50</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>No 42 (89.4)</td>
<td>Yes 5 (10.6)</td>
<td></td>
<td>.49</td>
</tr>
<tr>
<td>Solitary tumor</td>
<td>20</td>
<td>3</td>
<td>11</td>
<td>.80</td>
</tr>
<tr>
<td>Multifocal tumor</td>
<td>26</td>
<td>15</td>
<td>10</td>
<td>.50</td>
</tr>
<tr>
<td>Tumor size</td>
<td>8.8</td>
<td>8.5 ± 3.1</td>
<td>8.6 ± 4.0</td>
<td>.50</td>
</tr>
<tr>
<td>Resection status</td>
<td>R0 26 (55.3)</td>
<td>R1/R2 21 (44.7)</td>
<td></td>
<td>.50</td>
</tr>
</tbody>
</table>

*P values were calculated using chi-square analysis and ANOVA for continuous variables.*

### Table 3. GNAS1 T393C Genotype Distribution, Apoptosis (TUNEL), and Proliferative Activity (Ki67) in the Resection Group.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis (n = 46) (% positively stained tumor cells)</td>
<td>1.9 ± 2.0</td>
<td>1.2 ± 0.7</td>
<td>1.8 ± 1.8</td>
<td>2.3 ± 2.6</td>
<td>.51</td>
</tr>
<tr>
<td>GF (n = 45) (% Ki67+ tumor cell nuclei)</td>
<td>8.5 ± 6.3</td>
<td>14.1 ± 6.5</td>
<td>6.6 ± 5.7</td>
<td>8.3 ± 6.0</td>
<td>.02</td>
</tr>
</tbody>
</table>

*P values were calculated using ANOVA for continuous variables. Data are presented as mean ± SD.*
mRNA expression in a variety of human tissues, including tumor tissues [8]; and (2) as predicted by MFOLD, T→C substitution of the GNAS1 gene changed the mRNA folding structures [9]. These findings tempt us to hypothesize that genotype-dependent differences in mRNA decay due to structural alteration may cause differences in Gαs mRNA expression. However, so far, no functional studies verifying this hypothesis exist.

Figure 1. Kaplan-Meier survival plot for disease-specific OS in the complete series on R status and exploration group (A) and on different T393C genotypes (B).

Figure 2. Kaplan-Meier survival curves in the subgroup of resected ICC (n = 47) (A) and in the subgroup of R0-resected tumors (n = 26) (B) in relation to different T393C genotypes. Survival plot for local recurrence-free survival in the subgroup of R0-resected ICC (n = 20) in relation to different T393C genotypes (C).
It is intriguing to note that, in contrast to our findings on many other carcinoma types, homozygous TT carriers with ICC were associated with the least favorable clinical course. Nevertheless, the biologic relevance of the finding of the present study is supported by a significantly higher proliferation rate (demonstrated immunohistochemically by the proliferation marker Ki67) in patients with homozygous TT genotype. Our finding of elevated GF suggests an impaired balance between cell loss and cell gain, resulting in a shift toward tumor net growth due to increased proliferation. However, an increased proliferation rate is the result of a complex biologic signaling cascade, which still remains to be elucidated.

In a very recent study on breast carcinoma, we observed the same finding [24]. Homozygous TT carriers with breast carcinoma exhibited the least favorable clinical course. This finding highly suggests that the putative biologic effect of the T393C SNP may act differently in different tumor types. In vitro studies have demonstrated that there exist fundamental differences in the mechanism of G\(\alpha_S\) stimulation of different adenylyl cyclases, which may be a possibility for the varied responses by different cells and tissues to receptor ligands elevating cAMP levels [25]. Besides the principal effectors induced by cAMP, protein kinase A and protein kinase C, novel receptors for cAMP have been recently identified. These proteins, designated as EPAC (exchange protein directly activated by cAMP) or RasGRP (Ras guanine nucleotide—releasing protein), are able to mediate the effects of cAMP in a protein kinase—independent fashion. Among these are the B-Raf and MEK/Erk pathways, which are able to induce cell immortalization, growth factor—independent growth, insensitivity to growth-inhibitory signals, and avoidance of apoptosis [26–28]. Future research has to elucidate whether the various genotypes of T393C polymorphism of the GNAS1 gene can be associated in a tumor type—independent manner with the activation of different pathways mediating tumor progression. It would be desirable to explain the underlying biologic processes of the different clinical impacts of GNAS1 genotypes; however, such functional studies were beyond the scope of this study.

We are aware that the findings of the present study are based on a rather small number of cases and, therefore, the biologic significance of the results presented may be limited. Nevertheless, we want to point out that the present cohort of ICC is, to the best of our knowledge, one of the largest series published. Moreover, the present study group is rather homogenous because all cases with liver cirrhosis, regardless of its origin, were excluded from our study to maintain the high homogeneity of our cohort and to rule out cirrhosis-related influence on patient survival.

In conclusion, our data identified T393 GNAS1 homozygosity as an independent prognostic parameter in ICC. However, T393 homozygosity, which was shown to be associated with a favorable clinical outcome in sporadic colorectal cancer, clear cell renal cell, carcinoma, and bladder cancer, seems to contribute to the tumor progression of ICC in a different manner we cannot explain yet. However, just like in ICC, T393 homozygosity in breast carcinoma [25] seems to be associated with an unfavorable clinical course, thus supporting our findings. Although we could observe elevated proliferative activity and a trend toward decreased apoptosis in TT-homozygous tumors, further studies are necessary to elucidate the complex and obviously differing functional effects induced by GNAS1 polymorphism in human carcinomas.

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References


Synergistic effects of retinoic acid and 8-Cl-cAMP on apoptosis require caspase-3 activation in human ovarian cancer cells. Oncogene 18, 1755 – 1763.


