High Clusterin Expression Correlates with a Poor Outcome in Stage II Colorectal Cancers

David Kevans,1 Jane Foley,1 Martin Tenniswood,2 Kieran Sheahan,1 John Hyland,1 Diarmuid O’Donoghue,1 Hugh Mulcahy,1 and Jacintha O’Sullivan1

1Centre for Colorectal Disease, St. Vincent’s University Hospital, Dublin, Ireland and 2Gen*NY*Sis Centre for Excellence in Cancer Genomics, University at Albany, Rensselaer, New York

Abstract

The role of clusterin in tumor growth and progression remains unclear. Overexpression of cytoplasmic clusterin has been studied in aggressive colon tumors; however, no correlation between clusterin expression and survival in colorectal cancer has been identified to date. We assessed levels of clusterin expression in a group of stage II colorectal cancer patients to assess its utility as a prognostic marker. The study included 251 patients with stage II colorectal cancer. Tissue microarrays were constructed and immunohistochemistry done and correlated with clinical features and long term outcome. Dual immunofluorescence and confocal microscopy were used with terminal deoxynucleotidyltransferase–mediated dUTP nick-end labeling probes and clusterin antibody to assess the degree of co-localization. Percentage epithelial cytoplasmic staining was higher in tumor compared with nonadjacent normal mucosa (P < 0.001). Within the stromal compartment, percentage cytoplasmic staining and intensity was lower in tumor tissue compared with normal nonadjacent mucosa (P ≤ 0.001). Survival was significantly associated with percentage epithelial cytoplasmic staining (P < 0.001), epithelial cytoplasmic staining intensity (P < 0.001), percentage stromal cytoplasmic staining (P = 0.002), and stromal cytoplasmic staining intensity (P < 0.001). Clusterin levels are associated with poor survival in stage II colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(2):393–9)

Introduction

Clusterin is a disulfide linked heterodimeric secreted glycoprotein that is ubiquitously expressed in most mammalian tissues and found in plasma, urine, and cerebrospinal fluid across a broad range of species (1, 2). Clusterin is a heavily glycosylated 76 to 80 kDa protein (3). Nonglycosylated clusterin isoforms have also been identified with nuclear (50 kDa) and cytoplasmic (60 kDa) localizations (3, 4).

Clusterin can bind to and form complexes with immunoglobulins, lipids, heparin, bacteria, complement components, paraoxonase, β amyloid, and leptin (1, 5-11) and is thought to be involved in lipid transportation, tissue remodeling, cellular debris clearance, complement inhibition, cell aggregation and adhesion, and matrix metalloproteinase inhibition (1, 3, 12). However, at this time, no distinct function of clusterin has been identified. It has been hypothesized that clusterin is an extracellular chaperone protecting cells from stress-induced insults caused by degraded and misfolded protein precipitates (13). Clusterin may be important in tumor growth and disease progression (2, 3); however, its precise role remains unclear with studies supporting both proapoptotic (14) and antiapoptotic functions (15, 16). Several studies have examined the prognostic significance of clusterin in human cancer with conflicting results. Cytoplasmic clusterin expression correlates with poor prognosis in prostate adenocarcinoma (17), renal cell carcinoma (18), ovarian carcinoma (19), cervical cancer (20), and hepatocellular carcinoma (21). In contrast, cytoplasmic clusterin expression correlates with a good prognosis in pancreatic adenocarcinoma (22) and non–small cell lung cancer (23). Clusterin expression has also been examined in breast carcinoma but not found to be of prognostic significance (24). Increased cytoplasmic clusterin expression has been reported in colorectal adenocarcinomas (4, 25) and in high grade colon tumors, and metastatic lymph nodes (2).

The aim of this study was to assess the levels of cytoplasmic clusterin expression in a well defined cohort of stage II colorectal cancer patients to assess its utility as a prognostic biomarker.

Materials and Methods

Patients. The study included 251 patients (median age, 72 y; range, 32-88; 136 male, 115 female) with stage II colorectal cancer diagnosed and treated in the Centre for Colorectal Disease, St Vincent’s University Hospital, Dublin, Ireland. Two hundred forty patients had T3N0M0 and 11 had T4N0M0 colorectal cancer. Seven patients received postoperative adjuvant chemotherapy. Ethical approval was granted by the St Vincent’s Hospital Ethics and Medical Research Committee. After surgery, tumors were fixed in 10% formalin and embedded in paraffin. Normal-appearing colorectal mucosal tissue remote from the tumor was obtained for 202 of the 251 cases. Patients were followed up at 6 monthly intervals and median follow-up of patients alive at the end of study was 5.9 y.
Clusterin and Survival in Stage II Colorectal Cancer

Tissue Microarray. H&E-stained slides from formalin-fixed, paraffin-embedded tumor and nonadjacent normal tissue were used to identify specific areas of the tissue. These areas were aligned with the paraffin block from which four 6-mm cores were removed and transferred to a recipient block using a Tissue Microarrayer (Beecher Instruments). Four-micrometer sections were cut for immunohistochemistry studies and mounted onto Super-Frost Plus adhesive slides (Menzel-Glaser).

Immunohistochemistry. Four-micrometer sections were baked for 2 h at 60°C, deparaffinized in xylene and rehydrated in alcohol and deionized water. Antigen retrieval was done using 1,500 mL antigen retrieval solution [15 mL of 1 mol/L sodium citrate and 15 mL of 1 mol/L citric acid in deionized water (pH 6.0)]. This solution was heated in a pressure cooker (Menarini Diagnostics) with the lid off for 10 min at full power in an 800 W microwave. Slides were placed in the pressure cooker, lid sealed, and heated at full power until the pressure valve popped up (5-7 min). Slides were then incubated at full power for a further 4 min. The pressure cooker was removed from the microwave and the lid was removed once the pressure equalized (~10 min). Slides were washed in PBS/0.05% Tween for 10 min. Nonspecific binding was blocked using 10% casein in PBS for 10 min at room temperature. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 7 min at room temperature. Slides were incubated with a mouse monoclonal clusterin 2D9 antibody (26) for 1 h at room temperature in a humidified chamber. An IgG mouse control and elimination of primary antibody were used as negative controls. Slides were washed in PBS/0.05% Tween for 5 min. Dako Envision reagent (DAKO) was then added to slides for 30 min at room temperature followed by a 5 min PBS/0.05% Tween wash. Slides were then incubated with diaminobenzidine chromogenic substrate system (DAKO) for 10 min, washed under running water for 5 min, and then counterstained with hematoxylin. Slides were then dehydrated by immersion in increasing concentrations of alcohols, placed in xylene, and coverslipped using pertex. Staining was assessed at ×40 magnification. Clusterin immunoreactivity was assessed separately in the epithelial and stromal compartments by an observer who was blinded to clinical outcome using the following scoring variables: percentage cytoplasmic staining (0-100%) and cytoplasmic intensity: 0 (absent), 1 (weak), 2 (moderate), 3 (strong), and 4 (very strong). Only two cases were negative (no core positive). Therefore these cases were not considered as a separate category and were included in strong staining intensity group for all analyses.

Reproducibility of Clusterin Immunohistochemistry. A subset of 100 cases was reviewed by a second blinded observer to assess interobserver agreement that was expressed for each scoring parameter as percentage agreement and a k value. To further validate the immunohistochemical scoring, a computerized analysis was done. Digitization of whole tissue microarray slides was accomplished using a Hamamatsu Nanozoomer (Hamamatsu Photonics Ltd.). Analysis of the digital slides was done in a two-step process. First, an intracellular staining detection algorithm developed by SlidePath (SlidePath Ltd.) was manually applied to a number of tumor regions using a software tool called Image Analysis Optimiser (SlidePath Ltd.) to determine preferential algorithm settings for clusterin staining. The clusterin optimized algorithm was then applied across the tissue microarray slides (n = 44 tumor cases). This analysis does not differentiate between epithelial and stromal staining, instead it quantifies overall clusterin immunoreactivity according to two variables: staining concentration (correlates with staining intensity) and % positive pixels (correlates with staining % positivity). Staining concentration measures the concentration of clusterin within a tissue sample by quantifying the number of positive pixels, the staining absorbance within the tissue and the tissue threshold intensity. The % positive pixels quantifies the percentage of tissue pixels that are positive for clusterin in a given tissue sample. Clusterin staining concentration and % positive pixel values were then compared with manually generated clusterin immunohistochemical scores and degree of correlation quantified.

Immunofluorescence. The DeadEnd Fluorometric terminal deoxynucleotidyl-transferase–mediated dUTP nick-end labeling (TUNEL) System (Promega Corporation) was used to assess DNA fragmentation in tissue, which is a marker of apoptosis. A combined dual labeling assay was done on 86 tumor cases to assess for TUNEL and clusterin fluorescence. Briefly, histologic sections from each tissue microarray block were deparaffinized in xylene and rehydrated through a series of graded alcohols. Tissue was treated with 20 μg/mL proteinase K for 10 min at room temperature. Slides were fixed in 4% paraformaldehyde for 15 min at room temperature and treated with equilibration buffer for 10 min. Terminal Deoxynucleotidyl Transferase Recombinant enzyme and fluorescently labeled nucleotide mix were added and incubated at 37°C for 1 h. The negative control slide was treated with nucleotide mix alone without terminal deoxynucleotidyl transferase recombinant enzyme and incubated at 37°C for 1 h. Stop buffer (2× SSC) was added to all slides to terminate the reaction. Three 5-min PBS washes were done after which slides were incubated with mouse monoclonal clusterin 2D9 (26) antibody for 1 h. Biotinylated anti–mouse IgG (Vector Laboratories) 1:200 was incubated on slides for 30 min, followed by three 5-min PBS washes. Slides were then incubated with diaminobenzidine chromogenic substrate system (DAKO) for 10 min, washed under running water for 5 min, and then counterstained with hematoxylin. Slides were then dehydrated by immersion in increasing concentrations of alcohols, placed in xylene, and coverslipped using pertex. Staining was assessed at ×40 magnification. Clusterin immunoreactivity was assessed separately in the epithelial and stromal compartments by an observer who was blinded to clinical outcome using the following scoring variables: percentage cytoplasmic staining (0-100%) and cytoplasmic intensity: 0 (absent), 1 (weak), 2 (moderate), 3 (strong), and 4 (very strong). Only two cases were categorized as having very strong staining intensity. Therefore these cases were not considered as a separate category and were included in strong staining intensity group for all analyses.

Reproducibility of Clusterin Immunohistochemistry. A subset of 100 cases was reviewed by a second blinded observer to assess interobserver agreement that was expressed for each scoring parameter as percentage agreement and a k value. To further validate the immunohistochemical scoring, a computerized analysis was done. Digitization of whole tissue microarray slides was accomplished using a Hamamatsu Nanozoomer (Hamamatsu Photonics Ltd.). Analysis of the digital slides was done in a two-step process. First, an intracellular staining detection algorithm developed by SlidePath (SlidePath Ltd.) was manually applied to a number of tumor regions using a software tool called Image Analysis Optimiser (SlidePath Ltd.) to determine preferential algorithm settings for clusterin staining. The clusterin optimized algorithm was then applied across the tissue microarray slides (n = 44 tumor cases). This analysis does not differentiate between epithelial and stromal staining, instead it quantifies overall clusterin immunoreactivity according to two variables: staining concentration (correlates with staining intensity) and % positive pixels (correlates with staining % positivity). Staining concentration measures the concentration of clusterin within a tissue sample by quantifying the number of positive pixels, the staining absorbance within the tissue and the tissue threshold intensity. The % positive pixels quantifies the percentage of tissue pixels that are positive for clusterin in a given tissue sample. Clusterin staining concentration and % positive pixel values were then compared with manually generated clusterin immunohistochemical scores and degree of correlation quantified.

Statistical Analysis

Continuous data are presented as medians and interquartile ranges. Data were assessed using Wilcoxon’s rank sum test, Wilcoxon’s signed-rank test, the Kruskal-Wallis test or Spearman’s rank correlation coefficient as
appropriate. Differences between proportions were assessed using the χ² test. Kaplan-Meier survival curves were constructed with cancer-related mortality as the end-point. Differences in survival between groups were assessed using the log-rank test. The distribution of clusterin staining positivity and intensity was dichotomized around median values to develop usable groups to determine the effect of staining on survival using the logrank test. The resulting groups contained different numbers of patients but were as close to equal as it was possible to achieve. Interobserver agreement was expressed using the κ statistic. Correlation between computer generated immunohistochemical variables and observer-generated variables was done using spearman’s rank correlation test with degree of correlation expressed as an r value. All P values are two-sided and P values of <0.05 were considered statistically significant in all analyses.

Results

Clusterin Staining in Paired Tumor and Normal Nonadjacent Mucosa. Figure 1A to D shows representative images of clusterin staining seen in tumor tissue ranging from weak to very strong staining intensity. Figure 1E and F show representative images of clusterin staining in paired tumor and normal nonadjacent mucosa. In general, there were higher levels of stromal staining in normal nonadjacent mucosa (E) compared with tumor tissue (F) with the opposite relationship observed for epithelial staining with higher clusterin staining levels seen in tumor (F) compared with nonadjacent normal mucosa (E).

Overall, percentage clusterin cytoplasmic staining was greater in the epithelium of tumor tissue compared with nonadjacent normal mucosa (P < 0.001; Fig. 2). In contrast, percentage stromal cytoplasmic staining was greater in normal nonadjacent mucosa compared with tumor (P = 0.001). Epithelial cytoplasmic staining intensity was similar in both tumor and normal adjacent mucosa (P = 0.33), whereas stromal cytoplasmic staining intensity was greater in nonadjacent normal cases (P < 0.001; Fig. 3).

Tumor Clusterin Staining Correlated with Clinical Variables. Table 1 shows the relationship between tumor clusterin percentage cytoplasmic staining and clinical variables. No significant association was seen between tumor epithelial or stromal staining and gender, age at diagnosis, tumor site, or tumor differentiation (Table 1). Similarly, no association was seen between clusterin staining intensity and clinical variables (data not shown).

Tumor Clusterin Staining and Survival. The overall 5-year survival of the patient population was 69% and the 7-year survival was 68%. Figure 4 shows outcome related to clusterin staining levels and staining intensity in tumor tissue. Survival was associated with percentage epithelial cytoplasmic staining (P < 0.001), epithelial cytoplasmic intensity (P < 0.001), percentage stromal cytoplasmic staining (P = 0.002), and stromal cytoplasmic intensity (P < 0.001). As an example, 7-year survival was 86% for the 115 patients with low levels of tumor epithelial cytoplasmic intensity compared with 55% for those 136 patients with high staining intensity (P < 0.001).

Reproducibility of Clusterin Staining. Interobserver agreement for clusterin immunostaining (n = 100 tumor cases) was as follows: percentage epithelial cytoplasmic staining, 75% agreement (κ = 0.5; 95% confidence interval, 0.33-0.67); epithelial cytoplasmic staining intensity, 80% agreement (κ = 0.59; 95% confidence interval, 0.42-0.74); percentage stromal cytoplasmic staining, 74% agreement (κ = 0.48; 95% confidence interval, 0.3-0.65); stromal cytoplasmic staining intensity, 89% agreement (κ = 0.62; 95% confidence interval, 0.42-0.82).

Comparing observer generated immunohistochemical variables with computer generated variables (n = 44 tumor cases) correlation was as follows: epithelial cytoplasmic percentage positivity versus % positive pixels, r = 0.5, P = 0.001; epithelial cytoplasmic staining intensity versus staining concentration, r = 0.813, P < 0.001; stromal cytoplasmic percentage positivity versus % positive pixels, r = 0.64, P = 0.001; Stromal cytoplasmic staining intensity versus staining concentration, r = 0.72, P < 0.001.

Evaluation of Association Between Clusterin and Apoptosis. Dual labeling with TUNEL (green fluorescence) and clusterin (red fluorescence) was done for 86 cases. Representative confocal microscopy images are shown in Fig. 5. No correlation was found between clusterin immunostaining using any scoring parameter and apoptosis assessed by TUNEL staining. For example, considering tumor epithelial % positivity, 42% (8 of 19) of the high clusterin expressing group were TUNEL negative, whereas 55% (37 of 67) were TUNEL positive, P = 0.44. Considering tumor stromal cytoplasmic % positivity, 63% (12 of 19) of the high clusterin expressing group were TUNEL negative, whereas 49% (33 of 67) were TUNEL positive, P = 0.31.

Discussion

This study is focused solely on patients with stage II colorectal cancer. The postoperative management of these patients is controversial. These anatomically homogenous cancers have spread beyond the bowel wall but do not seem to have metastasised to lymph nodes or distant organs at the time of surgery. Despite this, over one quarter develop recurrent cancer and ultimately die of their disease (27). Adjuvant chemotherapy has been used in this group, but the high cure rate with surgery alone makes it difficult to show a survival advantage for these treatments in randomized control trials, even if such an advantage exists (28). Identification of biomarkers predictive of disease progression would, therefore, be of immense clinical benefit. From a pathophysiologic viewpoint, the discovery of the factors driving tumor progression in these locally advanced cancers might also lead to a more focused and targeted therapy.

We report here, for the first time, that high cytoplasmic clusterin staining is associated with clinical progression in patients with stage II colorectal cancer. Percentage clusterin cytoplasmic staining and staining intensity were predictive of prognosis in tumor epithelial and stromal cells. We have also shown that the clusterin immunohistochemical scoring variables used in this study were reproducible when assessed by a second observer and also by a computerized increased scoring system (Slidepath), an important finding if clusterin is to be of utility a prognostic marker.
Consistent with previously published work (4), we found that clusterin epithelial cytoplasmic immunostaining was significantly higher in tumor compared with nonadjacent normal mucosa. This association supports the hypothesis that clusterin levels increase with disease progression (2). We showed the opposite association for stromal cytoplasmic immunostaining with significantly higher staining and intensity levels detected in nonadjacent normal mucosa compared with matched tumor tissue \((P \leq 0.001)\), an association that has also been previously described (4). Why differences in stromal clusterin expression between tumor and nonadjacent normal mucosa are observed is not clear. It is well-known that tumor cells recruit a wide variety of supporting cells to their microenvironment, resulting in a significant variation in the cellular composition of tumor versus nonadjacent normal stromal compartments. It is probable that the relationship we observed in stromal clusterin staining levels between tumor and nonadjacent normal mucosa reflect these differences.

Although this study showed an association between high cytoplasmic clusterin and adverse outcome for stage II colorectal cancer, there are conflicting data regarding the prognostic importance of this protein in different cancer types (17-23); some studies associate high clusterin levels with poor outcome (17-21) and others with...

**Figure 1.** Heterogenous epithelial cytoplasmic clusterin staining was observed in tumor cases \((n = 251)\). Representative examples of weak (A), moderate (B), strong (C), and very strong (D) staining intensity are shown above. Stronger stromal cytoplasmic staining was seen in nonadjacent normal (E) compared with tumor tissue (F), whereas the opposite pattern was observed for epithelial staining with stronger staining seen in tumor (F) compared with nonadjacent normal mucosa (E). Scale bar, 50 \(\mu m\).

**Figure 2.** Epithelial and stromal percentage clusterin staining in normal nonadjacent colonic tissue and matched tumor tissue in 202 patients with stage II colorectal cancer (*, Wilcoxon’s signed rank test).

**Figure 3.** Epithelial and stromal clusterin intensity in normal nonadjacent colonic tissue and matched tumor tissue in 202 patients with stage II colorectal cancer (*, Wilcoxon’s signed rank text).
a good prognosis (22, 23). This may be related to a number of clinical and technical factors. There is a great variability in the post translational modifications of clusterin in different species and in different tissue types (29, 30). This contributes to expression of distinct glyco-forms of the protein for which different antibodies are known to have varying affinities. It is also important to note that no single antibody to clusterin is capable of reacting with all glyco-forms. In this study, we used a mouse monoclonal antibody previously developed at our institutions (26) that is specific for a single α and β epitope and has a higher affinity for deglycosylated protein.

Elucidating the functional significance of clusterin has proven difficult and controversial. It remains unclear whether clusterin has a direct antiapoptotic effect or is merely a marker of cells with a resistant phenotype. In different models, clusterin has been identified in dying cells, surviving cells, invading phagocytic cells, and localized in areas of damaged tissue (31-35). It has been shown that intracellular clusterin can interfere with Bax activation in mitochondria blocking caspase activation through the intrinsic pathway and inhibiting apoptosis (36). It also can inhibit c-Myc induced apoptosis (36), whereas expression has also been shown to correlate with p53 activity. p53 suppresses basal as well as radiation-induced secreted clusterin in both breast and colon cancer cell lines by repressing clusterin gene promoter activity and transcription (37). Transcription factors implicated in cell survival, B-MYB, and nuclear factor-κB have also been shown to directly regulate clusterin expression (16, 38). It is currently proposed that tumor cell survival is related to overexpression of the secreted clusterin isoform and loss of the nuclear clusterin isoform (2). Our data did not show any association between clusterin expression and apoptosis and therefore could neither substantiate nor refute this hypothesis.

In vitro studies have shown that resistance to cancer treatment (chemotherapy and radiation) is mediated by enhanced expression of clusterin (1, 3, 15). Downregulation of clusterin using antisense oligonucleotides enhances the cytotoxicity of various chemotherapeutic agents (1, 39) as well as ionizing radiation (40). Because the suppression of clusterin expression renders human cancer cells sensitive to chemotherapeutic drug-mediated apoptosis, it is currently an antisense target in clinical trials. Preclinical and clinical phase I trials have also shown that inhibition of clusterin levels using antisense oligonucleotides increases apoptosis after conventional chemotherapeutic treatments (41, 42). Our study has shown that high clusterin levels are closely associated with outcome in stage II colorectal cancer and it is possible that the novel therapeutic strategy of clusterin

Table 1. Clinical details related to clusterin staining in the tumor tissues of 251 patients with stage II colorectal cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clusterin percentage staining [median (interquartile range)]</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epithelium</td>
<td>Stroma</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>136</td>
<td>88 (63-98)</td>
<td>0.48*</td>
</tr>
<tr>
<td>Female</td>
<td>115</td>
<td>83 (50-100)</td>
<td>3 (0-8)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>105</td>
<td>83 (48-100)</td>
<td>0.57*</td>
</tr>
<tr>
<td>≥70</td>
<td>146</td>
<td>88 (63-98)</td>
<td>0 (0-8)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>68</td>
<td>93 (79-100)</td>
<td>0.10</td>
</tr>
<tr>
<td>Left colon</td>
<td>72</td>
<td>87 (51-98)</td>
<td>1 (0-7)</td>
</tr>
<tr>
<td>Right colon</td>
<td>109</td>
<td>82 (57-97)</td>
<td>0 (0-5)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>27</td>
<td>76 (28-100)</td>
<td>0.28</td>
</tr>
<tr>
<td>Moderate</td>
<td>190</td>
<td>87 (63-98)</td>
<td>0 (0-7)</td>
</tr>
<tr>
<td>Poor</td>
<td>28</td>
<td>95 (44-100)</td>
<td>4 (0-8)</td>
</tr>
</tbody>
</table>

*Wilcoxon’s rank sum test.

Data on tumor site within the large bowel were not available for two cases.

Kruskal-Wallace test.

Data on tumor differentiation were not available for 6 cases.

Figure 4. Epithelial and stromal cytoplasmic clusterin percentage staining and intensity in tumor tissues related to survival in 251 patients with stage II colorectal cancer.
silencing to overcome chemoresistance may be of particular relevance for this subset of colorectal cancers. In conclusion, our data show that epithelial and stromal cytoplasmic clusterin immunostaining in tumor tissue are reproducible variables that are strongly associated with adverse outcome in stage II colorectal cancer. Clusterin may be a useful prognostic biomarker in stage II disease to segregate those patients with a good versus poor outcome.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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.

References
15. Miyake H, Nelson C, Rennie PS, Gleave ME. Acquisition of


34. Marinelli M, Quaglino D, Bettuzzi S, Strocchi P, Davalli P, Corti A. Increased levels of clusterin mRNA in the ventral prostate of the aging rat are associated to increases in cuboidal (atrophic) cell population and not to changes in apoptotic activity. Biochem Cell Biol 1994;72:515–21.


High Clusterin Expression Correlates with a Poor Outcome in Stage II Colorectal Cancers

David Kevans, Jane Foley, Martin Tenniswood, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/18/2/393

Cited articles
This article cites 42 articles, 15 of which you can access for free at:
http://cebp.aacrjournals.org/content/18/2/393.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/18/2/393.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.