Int Surg 2015;**100:**213–220
DOI: 10.9738/INTSURG-D-14-00022.1

High Expression of DARPP-32 in Colorectal Cancer Is Associated With Liver Metastases and Predicts Survival for Dukes A and B Patients: Results of a Pilot Study

Mario Kopljar[1], Leonardo Patrlj[1], Dragan Korolija-Marinic[1], Matija Horzic[1], Kristijan Cupurdija[1], Bore Bakota[2]

[1]*Department of Surgery, Clinical Hospital Dubrava, Zagreb, Croatia*

[2]*Department of Surgery, General Hospital Karlovac, Karlovac, Croatia*

The purpose of this study was to investigate prognostic significance of Dopamine and cAMP-Regulated neuronal Phosphoprotein 32 (DARPP-32) expression in primary colorectal cancer. The study material consisted of clinical and histopathological data of 100 patients operated for colorectal cancer between 1994 and 1997. For immunohistochemical analysis, specific rabbit antibodies for DARPP-32 were used and the percentage of stained tumor cells was calculated under gross magnification (400 times) on a sample of 500 tumor cells. DARPP-32 expression in the primary tumor was significantly greater in patients with distant metastases compared to patients with no distant metastases (p=0.002). In multivariate regression analysis, DARPP-32 expression in the primary tumor was a significant predictor of distant metastases. With a cut-off point of 76.5%, DARPP-32 expression in the primary tumor significantly influenced both overall and disease free survival, especially for Dukes A and B patients (p=0.037). The results of this study indicate that DARPP-32 may be a potential marker of worse prognosis and a valuable tool for managing further adjuvant treatment in patients with stages Dukes A and B colorectal cancer.

Key words: Colorectal neoplasms – Dopamine and cAMP-regulated phosphoprotein 32 – Humans – Nerve tissue proteins – Liver metastases

Corresponding author: Mario Kopljar, MD, PhD, Department of Surgery, Clinical Hospital Dubrava, Av. G. Suska 6, HR-10000 Zagreb, Croatia.
E-mail: kopljar@yahoo.com

Colorectal cancer is the second most common cause of cancer related death in Western Europe and the United States, with the incidence of 50/100,000 population.[1] In spite of significant developments in surgery and new chemotherapy drugs and protocols as well as radiotherapy regimens, this malignancy still has high mortality.[2]

The 5-year survival rate of colorectal cancer patients with Dukes A cancer ranges from 74 to 93%. Patients with Dukes B cancer have a 5-year survival of 40 to 82%, and those with positive lymph nodes (Dukes C) have a 5-year survival rate of 30 to 59%.[3,4] Recurrences are observed in as much as 34% of patients with Dukes A and B stage, compared with 59% in patients with lymph node metastases.[5]

Liver metastases are a well proven major determinant of survival in patients with colorectal cancer.[2,6] Therefore, better selection of patients with potential to develop liver metastases or those having occult metastases may increase the survival of those patients in whom adjuvant therapies would not otherwise be indicated.[2,5,7]

Recently, overexpression of dopamine and 3′5′-cyclic adenosine monophosphate regulated neuronal phosphoprotein 32 (DARPP-32) has been found in several gastrointestinal adenocarcinomas.[8] Although most of the research on this protein focused on its role in the central nervous system,[9–11] the finding of overexpression of this protein in cancer tissues brought up the hypothesis of its role in carcinogenesis.[8,12] Genetic studies led to the discovery of frequent 17q DNA amplifications in gastric cancer.[8] Subsequently, the gene located at this site, called PPP1R1B, has been sequenced and found to encode DARPP-32 molecule, that was brought into connection with several malignancies.[8,13–18] The DARPP-32 molecule is a protein with molecular mass of 32 kDa, consisting of 204 amino acids and 4 phosphorylation sites: Thr34, Thr75, Ser102, and Ser137. Depending on the phosphorylation of 1 of these 4 amino acids, the DARPP-32 molecule is acting as the signal integrator and as the regulator of the phosphorylase and kinase activities in eukaryotic cells.[19]

Basic research indicates that DARPP-32 may be associated with worse prognosis in some carcinomas.[20] However, it is remains unknown if evaluation of DARPP-32 expression in colorectal cancer patients may aid to evaluate prognosis.

The purpose of this study was to investigate possible associations of DARPP-32 expression in primary colorectal cancer with known prognostic determinants of colorectal cancer and therefore set the basis for further clinical research.

Materials and Methods

The study was conducted between 1994 and 1997 and included the analysis of 100 patients operated for colorectal cancer who had never received chemotherapy or radiotherapy before operation. Only patients with full records as described below were included.

For each included patient, age at presentation, sex, and location of the primary tumor were noted. The existence of liver metastases was determined by preoperative computed tomography (CT) scanning and intraoperative inspection and palpation. Tumor stage, according to TNM classification (AJCC, 6th revision from 2002),[21] as well as perineural and vascular invasion were determined by the pathologist using standard staining. To avoid subsequent differences in TNM classification, total number of lymph nodes and the number of positive lymph nodes was recorded, and metastases (M stage) was classified as absent (M0) or present (M1). Any intraperitoneal dissemination was also noted.

Calculated sample size was 86 patients, based on expected difference in DARPP-32 expression of 25% at $P < 0.01$ and power goal of 99%.[20] Taking into account a technical failure rate of 15%, the total number of 100 patients was estimated as sufficient to close the study.

A random sample of 100 patients who fulfilled the above-mentioned criteria were included into this study.

Patients were followed up for at least 5 years. Follow-up was performed every 3 months for 2 years, every 6 months for 3 years, and once every year thereafter. All patients underwent imaging examination (abdominal ultrasound, chest X-ray and pelvic CT/magnetic resonance imaging). Colonoscopy was performed once a year. Death of patients was recognized as the end of follow-up. Survival data were obtained from the Cancer registry of the Republic of Croatia and hospital records.

For every patient, three sites were examined by standard histology and immunohistochemistry: primary tumor, resection margin (at least 2 cm from the gross tumor), and lymph nodes. Both analyses were performed on 4-μm thick cuts. For immunohistochemistry, specific rabbit antibodies for DARPP-32 (Santa Cruz Biotechnology, Santa Cruz, CA, SAD, catalogue number sc-11365) were used, with stan-

Table 1 Descriptive data of included patients

Characteristics	Patients, n
Sex	
Female	44
Male	56
pT	
T1	2
T2	21
T3	66
T4	11
pN	
N0	51
N1	29
N2	20
M	
M0	83
M1	17
Dukes stage	
A	14
B	33
C	36
D	17
Peritoneal carcinosis	
Yes	9
No	91
Differentiation	
G1	42
G2	47
G3	11
Localization	
Cecum	8
Ascending colon	7
Hepatic flexure	5
Transverse colon	7
Splenic flexure	3
Descending colon	3
Sygmoid colon	19
Rectosygmoid	10
Rectum	38

dard immunohistochemistry kit (LSAB kit, DAKO, catalogue number K0690), 3,3′-diaminobenzidine chromogene (DAKO, catalogue number 3465), and nonspecific antibody of the same isotype as primary antibody (rabbit serum, DAKO, catalogue number X0902). For every sample, the percentage of stained tumor cells was calculated under gross magnification (400 times) on a sample of 500 tumor cells. The examining pathologist was blinded to the patient stage.

Table 2 Number of recurrences according to Dukes stage

Dukes stage	Patients, n	Hepatic recurrence/progression, n	Extrahepatic, n
A	14	1	0
B	33	6	0
C	36	12	2
D	17	11*	4

* Progression of hepatic metastases.

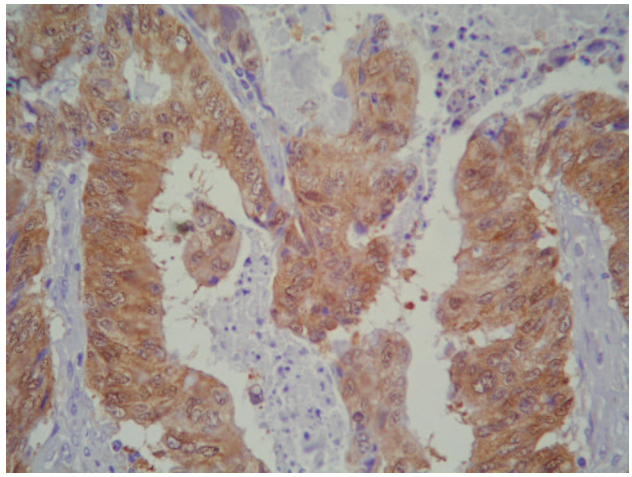

Fig. 1 Immunohistochemical staining of the colorectal cancer (T3N0M0). Predominantly cytoplasmatic staining is clearly observed. Magnification ×400.

The study was approved by the hospital ethics committee.

For statistical analysis, nonparametric Mann-Whitney and Wilcoxon's tests were used. For survival analysis, Log-rank test and Cox's regression analysis were performed. Values of $P < 0.05$ were considered statistically significant. For sensitivity analysis, cutoff values of DARPP-32 expression were calculated using the method of Budczies *et al*.[22] Univariate logistic regression was performed and variables with values of $P < 0.1$ were included in multivariate logistic regression.

Results

The mean age of included patients was 63.9 years (SD: 9.5). Descriptive data of included patients is presented in Table 1. The mode of recurrence is presented in Table 2. The overall 5-year survival for patients in stages Dukes A and Dukes B was 73.5%.

Staining of DARPP-32 was predominantly observed in the cytoplasm (Fig. 1). Percentage of cells expressing DARPP-32 in the primary tumor (median: 85%, range: 21–100%) was significantly greater that in tumor-free resection margin (median: 3%, range: 0–15%; Wilcoxon's test, $Z = 8.682$; $P = 0.00009$). Similarly, the percentage of cells expressing DARPP-32 in lymph node metastases (median: 90%, range: 25–100%) was significantly greater than in the tumor-free resection margin (median: 5%, range: 0–

Table 3 Univariate logistic regression analysis of different predictors for distant metastases

	Coef.	SE	OR	95% CI	*P*
DARPP-32 expression in the primary tumor	0.093	0.037	1.10	1.03–1.19	0.013
pT	0.908	0.488	2.48	1.00–6.83	0.062
pN	0.800	0.337	2.23	1.16–4.42	0.017
Peritoneal dissemination	16.095	1318.727	0.00	0.00–0.00	0.995
Vascular invasion	1.011	0.765	2.75	0.53–11.79	0.186
Perineural invasion	0.027	0.825	0.97	0.14–4.19	0.974
Differentiation	–0.292	0.419	0.75	0.31–1.66	0.486

Coef., coefficient of regression; pN, pathological N stage; pT, pathological T stage.

15%; Wilcoxon's test, $Z = 6.093$; $P = 0.00009$). There was a statistically significant correlation in DARPP-32 expression between primary tumor and lymph node metastases ($P = 0.0001$).

The percentage of DARPP-32 positive tumor cells in the primary tumor was significantly greater in patients with distant metastases (median: 92%, range: 71–100%) compared with patients with no distant metastases (median 84%; range: 21–100%; Mann-Whitney *U* test: $U = 369.5$; $P = 0.002$). There was no statistically significant difference in the expression of DARPP-32 in the primary tumor according to pT, pN, sex, peritoneal dissemination, vascular and perineural invasion, and tumor differentiation.

In univariate logistic regression analysis, only DARPP-32 expression and pathological N stage were found to be statistically significant predictors of distant metastases, while pathological T stage, vascular invasion, perineural invasion, peritoneal dissemination, and tumor differentiation were not found to be significant predictors of distant metastases (Table 3). When statistically significant predictors of distant metastases (*P* values less than 0.1) were fitted into multivariate regression analysis, significant influence of DARPP-32 expression was observed (Table 4).

The cutoff value of DARPP-32 expression in the primary tumor that best separates patients according to overall survival was calculated using the method described by Budczies.[22] This method determines the cutoff value that has the highest accuracy, as calculated from sensitivity and specificity. According to the results of this analysis, cutoff value was determined to be 76.5% (hazard ratio: 3.33, 95% confidence interval [CI]: 1.48–7.49).

Table 4 Multivariate logistic regression analysis of different predictors for distant metastases

	Coef.	SE	OR	95% CI	*P*
DARPP-32 expression in the primary tumor	0.09156	0.04032	1.10	1.03–1.20	0.023
pT	0.87378	0.63950	2.40	0.74–9.37	0.172
pN	0.48798	0.41011	1.63	0.73–3.71	0.234

Overall survival (OS) was significantly greater for patients with DARPP-32 expression in the primary tumor lower or equal to 76.5% (log-rank test, *P*=0.001; Fig. 2A). Similarly, disease-free survival (DFS) was significantly greater for patients with DARPP-32 expression in the primary tumor lower or equal to 76.5% (log-rank test, $P = 0.002$; Fig. 2B).

When only patients with Dukes A and B stages were analyzed, both OS and DFS were significantly greater for patients with DARPP-32 expression in the primary tumor lower or equal to 76.5% (log-rank test, $P = 0.037$ and $P = 0.036$, respectively; Fig. 3).

Discussion

Nearly half of the patients who undergo a potentially curative resection of colorectal cancer will relapse because of microscopic residual disease not detected at the time of the original operation.[23] One of the main problems in the treatment of patients with colorectal carcinoma is the identification of patients with such occult metastases that may benefit from adjuvant chemotherapy.[24] The criteria that select patients at high risk of recurrence, and hence, for consideration of adjuvant therapy, are enormously important since it is when cancer is microscopic that chemotherapy may be curative.[25] To date, the most useful criterion that predicts residual microscopic disease is the presence of lymph-node metastases, although other criteria such as vascular invasion, lymphatic invasion, size of the tumor, and differentiation of the tumor may also be important.[26]

Adjuvant chemotherapy can reduce relapse rate by 40% and decrease mortality by 33% in patients with Dukes C stage colon carcinoma.[27] In spite of that, one-third to one-half of the patients with Dukes C colon carcinoma live 5 years without adjuvant chemotherapy. In addition, approximately one-third of the patients with Dukes B stage colon carcinoma will develop a relapse or metastases.[28] Results from an analysis of pooled data from 7 randomized trials did not demonstrate overall survival benefit of

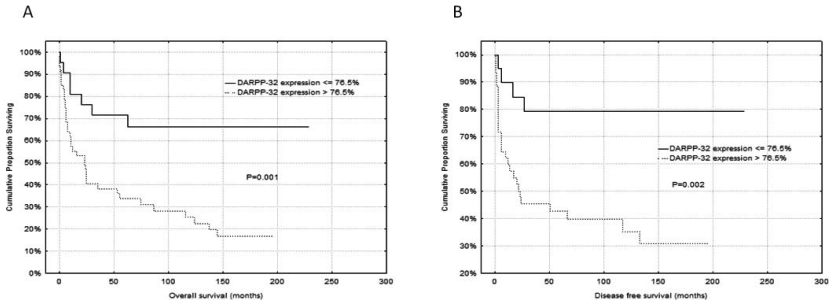

Fig. 2 OS and DFS according to DARPP-32 expression.

adding 5-FU–based adjuvant therapy to patients with resected stage II colon cancer.[29] Similarly, an analysis of outcomes of patients with stage II disease based on whether or not they had received adjuvant chemotherapy showed no statistically significant difference in 5-year OS between the groups (78% versus 75%, respectively), with an HR for survival of 0.91 (95% CI: 0.77–1.09) when patients receiving adjuvant treatment were compared with untreated patients.[30] This primarily indicates that patient selection for adjuvant therapy is not optimal.

The current definition of high-risk stage II colon cancer is clearly inadequate, in that many high-risk patients do not have a recurrence while some average-risk patients do.[31] Thus, it is important to search for a more accurate identification of patients with occult metastases in whom the adjuvant chemotherapy will have an effect. This clearly represents the rationale for continuous search for better survival predictors, especially for patients with stage II colorectal cancer.

Multiple *in vitro* and *in vivo* studies have found DARPP-32 (as well as its isoform t-DARPP) to be overexpressed in breast, prostate, colon, and gastric cancers[32] associated with reduced apoptosis[33,34] and resistance to chemotherapy.[14,35–37] However, it is important to note that DARPP-32 effects on cancer cells are under debate. On one side, data from breast cancer suggest that its expression is associated with reduced invasion and thus with reduced metastatic potential.[38] On the other side, data from gastric cancer involve this protein in the chemoresistance process.[14,39] So, it seems that the type of tumor histology is critical in addressing DARPP-32 effects on cancer cells because we can observe opposite effects.

In the study of Wang *et al.*,[20] statistical analysis showed no significant correlation between the expression of DARPP-32 and the differentiation, metastasis, and Dukes' stage of the colorectal carcinoma, which may be due to the small number of samples since only 33 carcinoma specimens were evaluated. The results from this study also failed to demonstrate significant correlation of DARPP-32 expression and Dukes' stage. However, the results of this study clearly demonstrate that DARPP-32 expression in the primary tumor was greater in patients with lymph node metastases compared with patients without lymph node metastases. The expression of DARPP-32 was also statistically significantly greater in patients with distant metastases compared with patients without distant metastases. Formation of distant, hematogenous metastases is a complex process—which favors the selection of tumor clones with the ability to separate from the main tumor mass, survive in the bloodstream and grow in distant organs—that requires tumor cells to acquire certain phenotypic features.[40,41] Therefore, these results support the theory that increased DARPP-32 expression could present a feature of the tumor with increased biological capability of dissemination, since all patients with

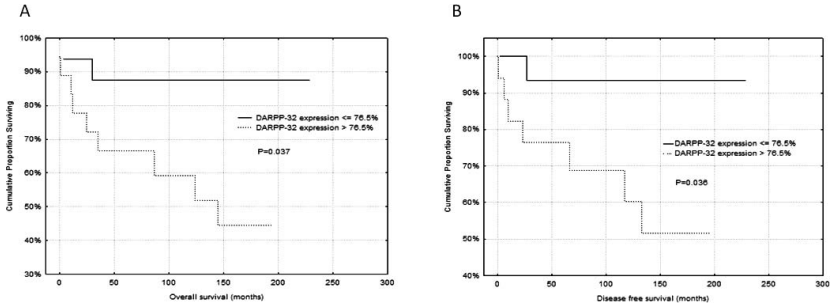

Fig. 3 OS and DFS according to DARPP-32 expression for Dukes A and B patients. The overall survival for Dukes A and B patients was 73.5%.

distant metastases in this study had more than 70% of tumor cells expressing DARPP-32.

To determine the significance of DARPP-32 expression as a factor that identifies biologically aggressive growth of the colorectal carcinoma, the regression analysis was performed to assess the influence of several clinical and pathohistological features on the presence of distant metastases. The results in this study confirm that only the expression of DARPP-32 in the primary tumor and the presence of regional lymph nodes metastases (pN grade) are statistically significant predictors of distant metastases. Additional analysis demonstrated statistically significant and independent effects of both DARPP-32 expression and regional lymph nodes metastases on the presence of distant metastases. Also, DARPP-32 expression in the primary tumor was predictive for disease-free survival in all patients, as well as in a subgroup of patients with Dukes A and B stages. The findings of this study, therefore, may have important clinical implications.

Expression of DARRP-32 in the primary tumor may have a significant impact on adjuvant treatment of node negative patients. According to current NCCN guidelines,[3] patients with T1-2 N0 M0 tumors are not candidates for adjuvant chemotherapy, and neither are some patients with T3 N0 M0 colon cancer unless poor prognostic features are present (e.g. poorly differentiated histology, lymphatic/vascular invasion, bowel obstruction, perineural invasion, localized perforation and close, indeterminate, or positive margins). Currently, the benefit of adjuvant chemotherapy for stage II colon cancer is only about 5%,[42,43] and this may well be improved by better patient selection, possibly by using DARPP-32 as one of the indicators for adjuvant treatment in node negative patients.

It is not likely that DARPP-32, as a possible prognostic factor, would influence the choice of neoadjuvant chemotherapy, since the role of neoadjuvant treatment is to downsize and possibly downstage advanced tumors prior to surgery. Possible significance of neoadjuvant treatment for colorectal cancer patients, based on high expression of DARPP-32, will have to be evaluated in further clinical trials.

If future research finds DARPP-32 to be associated with chemoresistance in colorectal cancer, that would most likely preclude certain lines of chemotherapy. However, the resistance to chemotherapy may not be general, but only related to some chemotherapeutics or lines of chemotherapy. Since node negative colorectal cancer patients are currently not considered for systemic treatment as it is, chemoresistance could probably play a significant role only in the case of disease progression (local recurrence or distant metastases).

The results of this study indicate that DARPP-32 may be the potential marker of worse prognosis and perhaps a valuable tool for managing further treatment in patients with colorectal cancer. We believe that reporting these preliminary data is of great importance in reaching broad scientific community. Bases on the results of this study, further prospective randomized trials are needed to properly evaluate the impact of DARPP-32 expression in prediction of distant metastases in patients with colorectal cancer.

Acknowledgments

Authors would like to thank Mladen Petrovečki, chair of medical informatics at the University of Rijeka Medical School, for advice on regression analysis.

Potential and real conflicts of interest: none declared.

Statistical analysis was checked by a statistician.

References

1. Williams NS, Northover JMA, Arnott SJ, Jass JR. Colorectal tumors. In: Peckham M, Pinedo H, Veronesi U, eds. *Oxford Textbook of Oncology*. Oxford, UK: Oxford University Press; 1995
2. Kopljar M, Brkljacic B, Doko M, Horzic M. Nature of Doppler perfusion index changes in patients with colorectal cancer liver metastases. *J Ultrasound Med* 2004;**23**(10):1295–1300
3. American Joint Committee on Cancer. Colon and rectum. *AJCC Cancer Staging Manual*. New York, NY: Springer; 2010: 143–164
4. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst* 2004;**96**(19):1420–1425
5. Leen E, Goldberg JA, Angerson WJ, McArdle CS. Potential role of Doppler perfusion index in selection of patients with colorectal cancer for adjuvant chemotherapy. *Lancet* 2000; **355**(9197):34–37

6. Garcia-Foncillas J, Diaz-Rubio E. Progress in metastatic colorectal cancer: growing role of cetuximab to optimize clinical outcome. *Clin Transl Oncol* 2010;**12**(8):533–542
7. Fong Y. Doppler perfusion index in colorectal cancer. *Lancet* 2000;**355**(9197):5–6
8. El-Rifai W, Smith MF Jr, Li G, Beckler A, Carl VS, Montgomery E *et al*. Gastric cancers overexpress DARPP-32 and a novel isoform, t-DARPP. *Cancer Res* 2002;62(14):4061–4064
9. Greengard P. The neurobiology of slow synaptic transmission. *Science* 2001;**294**(5544):1024–1030
10. Bibb JA, Snyder GL, Nishi A, Yan Z, Meijer L, Fienberg AA *et al*. Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* 1999;**402**(6762):669–671
11. Kurihara T, Lewis RM, Eisler J, Greengard P. Cloning of cDNA for DARPP-32, a dopamine- and cyclic AMP-regulated neuronal phosphoprotein. *J Neurosci* 1988;**8**(2):508–517
12. Varis A, Wolf M, Monni O, Vakkari ML, Kokkola A, Moskaluk C *et al*. Targets of gene amplification and overexpression at 17q in gastric cancer. *Cancer Res* 2002;**62**(9):2625–2629
13. Garcia-Jimenez C, Zaballos MA, Santisteban P. DARPP-32 (dopamine and 3′,5′-cyclic adenosine monophosphate-regulated neuronal. *Mol Endocrinol* 2005;**19**(12):3060–3072
14. Hamel S, Bouchard A, Ferrario C, Hassan S, Aguilar-Mahecha A, Buchanan M *et al*. Both t-Darpp and DARPP-32 can cause resistance to trastuzumab in breast cancer cells and are frequently expressed in primary breast cancers. *Breast Cancer Res Treat* 2010;**120**(1):47–57
15. Hong L, Wang J, Zhao Y, Han Z, Zhou X, Guo W *et al*. DARPP-32 mediates multidrug resistance of gastric cancer through regulation of. *Cancer Invest* 2007;**25**(8):699–705
16. Pimenta FJ, Horta MC, Vidigal PV, De Souza BR, De Marco L, Romano-Silva MA *et al*. Decreased expression of DARPP-32 in oral premalignant and malignant lesions. Anticancer Res. 2007;**27**(4B):2339–2343
17. Wang J, Pan YL, Liu N, Guo CC, Hong L, Fan DM. Expression and significance of DARPP-32 in gastric carcinoma. *Zhonghua Bing Li Xue Za Zhi* 2004;**33**(4):350–353
18. Zhu S, Belkhiri A, El-Rifai W. DARPP-32 increases interactions between epidermal growth factor receptor and ERBB3 to promote tumor resistance to gefitinib. *Gastroenterology* 2011;7:7
19. Le Novere N, Li L, Girault JA. DARPP-32: molecular integration of phosphorylation potential. *Cell Mol Life Sci* 2008;65(14):2125–2127
20. Wang MS, Pan Y, Liu N, Guo C, Hong L, Fan D. Overexpression of DARPP-32 in colorectal adenocarcinoma. *Int J Clin Pract* 2005;**59**(1):58–61
21. Horton JK, Tepper JE. Staging of colorectal cancer: past, present, and future. Clin *Colorectal Cancer* 2005;**4**(5):302–312
22. Budczies J, Klauschen F, Sinn BV, Gyorffy B, Schmitt WD, Darb-Esfahani S *et al*. Cutoff Finder: a comprehensive and straightforward Web application enabling rapid biomarker cutoff optimization. *PloS One* 2012;**7**(12):e51862
23. Des Guetz G, Uzzan B, Morere JF, Perret G, Nicolas P. Duration of adjuvant chemotherapy for patients with non-metastatic colorectal cancer. *Cochrane Database Syst Rev* 2010(1):CD007046
24. Onate-Ocana LF, Montesdeoca R, Lopez-Graniel CM, Aiello-Crocifoglio V, Mondragon-Sanchez R, Cortina-Borja M *et al*. Identification of patients with high-risk lymph node-negative colorectal cancer and potential benefit from adjuvant chemotherapy. *Jpn J Clin Oncol* 2004;**34**(6):323–328
25. Roxburgh C, McDonald A, Salmond J, Oien K, Anderson J, McKee R *et al*. Adjuvant chemotherapy for resected colon cancer: comparison of the prognostic value of tumour and patient related factors. *Int J Colorectal Dis* 2011;**26**(4):483–492
26. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, Flynn PJ *et al*. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol* 2004;**22**(16):3408–3419
27. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen CM, et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Ann Intern Med* 1995;**122**(5):321–326
28. Hobday TJ, Erlichman C. Adjuvant therapy of colon cancer: a review. *Clin Colorectal Cancer* 2002;**1**(4):230–236
29. Gill S, Loprinzi CL, Sargent DJ, Thome SD, Alberts SR, Haller DG *et al*. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004;**22**(10):1797–1806
30. Schrag D, Rifas-Shiman S, Saltz L, Bach PB, Begg CB. Adjuvant chemotherapy use for Medicare beneficiaries with stage II colon cancer. *J Clin Oncol* 2002;20(19):3999–4005
31. Benson AB 3rd, Hamilton SR. Path toward prognostication and prediction: an evolving matrix. *J Clin Oncol* 2011;**29**(35): 4599–601
32. Beckler A, Moskaluk CA, Zaika A, Hampton GM, Powell SM, Frierson HF Jr *et al*. Overexpression of the 32-kilodalton dopamine and cyclic adenosine 3′,5′-monophosphate-regulated phosphoprotein in common adenocarcinomas. *Cancer* 2003; **98**(7):1547–1551
33. Belkhiri A, Zaika A, Pidkovka N, Knuutila S, Moskaluk C, El-Rifai W. Darpp-32: a novel antiapoptotic gene in upper gastrointestinal carcinomas. *Cancer Res* 2005;**65**(15):6583–6592
34. Belkhiri A, Dar AA, Zaika A, Kelley M, El-Rifai W. T-DARPP promotes cancer cell survival by up-regulation of Bcl2 through Akt-dependent mechanism. *Cancer Res* 2008;**68**(2):395–403
35. Belkhiri A, Dar AA, Peng DF, Razvi MH, Rinehart C, Arteaga CL *et al*. Expression of T-DARPP mediates trastuzumab resistance in breast cancer cells. *Clin Cancer Res* 2008;**14**(14): 4564–4571
36. Hong L, Wang J, Han Y, Zhao Y, Gao J, Zhang X *et al*. Reversal of multidrug resistance of vincristine-resistant gastric adenocarcinoma cells through up-regulation of DARPP-32. *Cell Biol Int* 2007;**31**(9):1010–1015
37. Hong L, Zhao Y, Wang J, Han Y, Guo W, Jin H *et al*. Reversal of multidrug resistance of adriamycin-resistant gastric adeno-

carcinoma cells through the up-regulation of DARPP-32. *Dig Dis Sci* 2008;**53**(1):101–107

38. Hansen C, Greengard P, Nairn AC, Andersson T, Vogel WF. Phosphorylation of DARPP-32 regulates breast cancer cell migration downstream of the receptor tyrosine kinase DDR1. *Exp Cell Res* 2006;**312**(20):4011–4018
39. Hong L, Wang J, Zhao Y, Han Z, Zhou X, Guo W, et al. DARPP-32 mediates multidrug resistance of gastric cancer through regulation of P-gp and ZNRD1. *Cancer Invest* 2007;**25**(8):699–705
40. Fidler IJ, Radinsky R. Genetic control of cancer metastasis. *J Natl Cancer Inst* 1990;**82**(3):166–168
41. Kerbel RS. Growth dominance of the metastatic cancer cell: cellular and molecular aspects. *Adv Cancer Res* 1990;**55**:87–132
42. Figueredo A, Charette ML, Maroun J, Brouwers MC, Zuraw L. Adjuvant therapy for stage II colon cancer: a systematic review from the Cancer Care Ontario Program in evidence-based care's gastrointestinal cancer disease site group. *J Clin Oncol*. 2004;**22**(16):3395–3407
43. Portal vein chemotherapy for colorectal cancer: a meta-analysis of 4000 patients in 10 studies. Liver Infusion Meta-analysis Group. *J Natl Cancer Inst* 1997;**89**(7):497–505