Combined Phenotype of 4 Markers Improves Prognostic Value of Patients With Colon Cancer

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Abstract: *Introduction:* Combination of multiple biomarkers representing distinct aspects of tumor biology will have a better prognostic value. This study was to identify prognostic subgroups of colon adenocarcinoma by combined analysis of synuclein-gamma (SNCG), a human homologue of piwi (Hiwi), phosphatase of regenerating liver-3 (PRL-3), arrest-defective protein 1, homolog A (ARD1) and clinicopathologic features in 225 colon adenocarcinoma specimens. *Methods:* Immunohistochemistry for 4 tumor markers was performed in whole tissue sections from 225 colon adenocarcinoma patients with complete clinicopathologic data and up to 10-year follow-up. The immunohistochemical expression patterns were examined individually and in multimarker combinations. Univariate and multivariate analyses were performed to identify independent predictive markers of poor outcome. *Results:* With the tumor marker positive rate [32.0% (62/225) for SNCG; 76.9% (173/225) for combined SNCG/Hiwi/PRL-3/ARD1] and the detecting accuracy [61.9% (252/407) for SNCG; 82.6% (336/407) for combined SNCG/Hiwi/PRL-3/ARD1] increasing, incremental value of combined SNCG/Hiwi/PRL-3/ARD1 ($P < 0.001$; hazard ratios (HR), 3.2) to poor outcome was found. Stratified by lymph node, Hiwi alone ($P = 0.004$; HR, 3.2) led to poor outcome in patients without lymph node metastasis (LN−), and SNCG ($P < 0.001$; HR, 2.5) had independently poor prognostic value for patients with lymph node metastasis (LN+). *Conclusions:* Multimarker phenotypes improved tumor positive rate, detecting accuracy and prognostic value. In addition, a subgroup of more aggressive tumors can be identified by evaluating Hiwi level in LN− cancer, and SNCG level in LN+ cancer.

Key Indexing Terms: Colon cancer; Lymph node metastasis; Immunochemistry; Combined analysis. **[Am J Med Sci 2012;343(4):295–302.]**

Colorectal cancer (CRC) is one of the major causes of cancer death worldwide; as many as 35% of patients with pN0 stage cancer develop extranodal metastasis within 5 years after surgery.[1] A significant proportion of patients develop recurrence, although they receive adjuvant therapies; moreover, patients with the same tumor stages may show different outcomes, suggesting that the conventional staging procedures may be unable to precisely predict cancer prognosis.[2,3] Therefore, new prognostic factors capable of identifying patients at high risk who need preventive chemotherapy or other adjuvant therapies should be found,[4,5] and combination of different markers representing different aspects of tumor biology will have a better prognostic value.[6,7]

Synuclein-gamma (SNCG), or breast cancer–specific gene, is overexpressed in a variety of human cancers,[8] and its overexpression stimulates cancer metastasis,[9] impairs cell cycle checkpoint[10] and promotes chemoresistance[11] in human breast cancer cells. Aberrantly expressed SNCG interacts with BubR1 and heat shock protein 70 (Hsp70) in late stages of breast cancer and ovarian cancer. Increased SNCG was reported to correlate with adverse outcome in breast cancer[12,13] and in colon cancer.[14] A recent study has shown that SNCG is closely involved in perineural invasion/distant metastasis and is a significant prognostic factor for pancreatic cancer.[15]

Hiwi, a human homologue of piwi, encodes a highly basic 861 amino acid protein responsible for stem cell self-renewal[16] and may play an impotent role in the maintenance of hematopoietic stem cells.[17] Hiwi was overexpressed in tumor vessels of lymphoma, uterine cervical cancer,[18] breast carcinoma,[18] ovarian cancer,[18] endometrial cancer,[18] esophageal cancer cells,[19] soft-tissue sarcoma[20] and seminomas.[16] Increased Hiwi[20] or coexpression with other 2 stem cell–associated genes, human telomerase reverse transcriptase and survivin, resulted in a significantly increased risk of soft-tissue sarcoma–related death.[21]

Phosphatase of regenerating liver-3 (PRL-3), a metastasis-associated gene, possesses a unique prenylation motif [CAAX box (C = cysteine, A = aliphatic amino acid, X = any amino acid)], which is posttranslationally modified by the addition of a farnesyl isoprenoid group,[22] and this feature is critical for its membrane association and intracellular localization.[22,23] PRL-3 is an important cell-cycle regulator,[24] and its overexpression promoted the metastatic ability of cells.[25] Patients with increased PRL-3 level had either a trend or a significant association with reduced survival in colorectal,[26,27] breast[28,29] or gastric[30,31] cancer.

Arrest-defective protein 1, homolog A (ARD1), an acetyltransferase, is involved in proliferation,[32,33] apoptosis,[32] metabolism,[32] cell-cell interaction[32] and neuronal dendritic development.[34] Dysregulation of ARD1 is associated with tumorigenesis and neurodegenerative disorder.[35] ARD1 protein was ubiquitously expressed in cancer tissues,[36] tumor cell lines and endothelial cells,[37] but controversial roles of ARD1 in cancer development have been reported.[35] The potential prognostic value of ARD1 expression for patients with cancer remains unclear.

The simultaneous measurement of multiple biomarkers provides an opportunity to investigate and compare their predictive ability in the same cohort. A few investigations have evaluated the incremental usefulness of multiple biomarkers from distinct biologic pathways for predicting the risk of death for colon cancer. SNCG, Hiwi, PRL-3 and ARD1 reflect different mechanisms in the development of tumor. We investigated the possible correlations of these 4

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biomarkers with clinical outcome, in an effort to identify high-risk patients with colon cancer to whom to administer tailored cancer treatment options.

MATERIALS AND METHODS

Patients

The study was approved and supervised by the Medical Ethics Committee of Peking University Cancer Hospital & Institute. Participants attending this study were informative for the well-characterized clinicopathologic variables, including patient outcome. None of the patients had received chemotherapy or radiation therapy before surgery, and none of them had a history of hereditary disease. Age at first diagnosis ranged from 23 to 85 years (mean ± standard deviation, 60.5 ± 12.1). The mean follow-up length for these patients was 45.0 ± 28.4 months, ranged from 1 month to 120 months. Among 225 patients with colon cancer, 45.8% (103/225) patients died of colon adenocarcinoma and 27.1% (61/225) patients developed recurrence after surgery during the follow-up period. Among the patients with recurrence, liver metastasis was present in 28 of 61 (45.9%), lung metastasis was present in 9 of 61 (14.8%), extensive intra-abdominal metastasis was present in 12 of 61 (19.7%), and the rest recurrent lesions were in colon, brain, bone and ovary. Overall survival (OS) time was calculated from the date of surgery to the date of death due to any cause. Disease-free survival (DFS) time was calculated for patients from the date of surgery to the date of disease progression (local recurrence or distant metastasis). Data on patients, who

TABLE 1. Influences of clinicopathologic factors, SNCG, Hiwi, PRL-3 and ARD1 and their combination on postoperative recurrence

Characteristics	No. cases	No. recurrence (%)	HR (95% CI)	*P*
Gender				
Male	120	28 (23.3)	1	0.173
Female	105	33 (31.4)	1.5 (0.8–2.7)	
Age (yr)				
≤60	87	22 (25.3)	1	0.625
>60	138	39 (28.3)	1.2 (0.6–2.1)	
Size (cm)				
≤4	89	19 (21.3)	1	0.108
>4	135	42 (31.1)	1.7 (0.9–3.1)	
Intravascular embolus				
Negative	168	40 (23.8)	1	0.056
Positive	57	21 (36.8)	1.9 (1.0–3.6)	
TNM stage				
I/II	113	14 (12.4)	1	<0.001
III/IV	112	47 (42.0)	5.1 (2.6–10.0)	
Depth of invasion				
pT1 and pT2	39	4 (10.3)	1	0.009
pT3 and pT4	186	57 (30.6)	3.9 (1.3–11.4)	
LN metastasis				
Negative	123	20 (16.3)	1	<0.001
Positive	102	41 (40.2)	3.5 (1.9–6.4)	
SNCG				
Negative	153	34 (22.2)	1	0.016
Positive	72	27 (37.5)	2.1 (1.1–3.9)	
Hiwi				
Negative	163	43 (26.4)	1	0.689
Positive	62	18 (29.0)	1.1 (0.6–2.2)	
PRl-3				
Negative	169	47 (27.8)	1	0.682
Positive	56	14 (25.0)	0.9 (0.4–1.7)	
ARD1				
Negative	106	25 (23.6)	1	0.261
Positive	119	36 (30.3)	1.4 (0.8–2.5)	
Combined SNCG/ARD1				
Negative	76	13 (17.1)	1	0.016
Positive	149	48 (32.2)	2.3 (1.2–4.6)	
Combined SNCG/Hiwi/ARD1				
Negative	52	7 (13.5)	1	0.012
Positive	173	54 (31.2)	2.9 (1.2–6.9)	

LN, lymph node; HR, hazard ratio; CI, confidence interval.