



Expression of HIF-1 α , ANXA3, CD133 and their associations with clinicopathological parameters in human colon carcinoma

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Background: Our previous study found an association between the expression of hypoxia-inducible factor-1 α (HIF-1 α) and annexin A3 (ANXA3) in colon cancer. ANXA3 correlated with expansion of CD133⁺ tumor cells in hepatocellular carcinoma cancer stem-like cells (CSCs), for which CD133 has been recognized as a typical marker in many cancer cells, including: gastric cancer, lung cancer and colorectal cancer. But the expression and association of HIF-1 α , ANXA3 and CD133 in colon cancer has not been reported. The purpose of the present study was to evaluate the correlation among the expression of HIF-1 α , ANXA3 and CD133 in human colon cancer and to investigate its clinicopathological parameters.

Methods: The data for 35 patients diagnosed with colon adenocarcinoma in The First Affiliated Hospital of Chongqing Medical University and who had undergone colectomy, tumor and adjacent normal colon tissues were collected. The expressions of HIF-1 α , ANXA3, and CD133 were measured by immunohistochemistry in colon cancer and surrounding non-tumor tissues and measured by using a semiquantitative score system. Finally, relationships between HIF-1 α , ANXA3, and CD133 immunohistochemical staining and clinicopathologic variables were analyzed using the Fisher's exact probability test. Associations between the expression levels of HIF-1 α , ANXA3, and CD133 were analyzed by the Spearman's rank correlation.

Results: The positive rate of expression of HIF-1 α in colon cancer and normal colon tissue was 80% (28/35) and 14% (5/35), 77% (27/35) and 20% (7/35) for ANXA3, and 71% (25/35) and 23% (8/35) for CD133, respectively. The coefficient of correlation for the association among HIF-1 α , ANXA3 and CD133 showed that the expression of HIF-1 α was positively related with ANXA3 and CD133 in colon cancer tissues ($r_1=0.408$, $P_1=0.015$, $r_2=0.474$, $P_2=0.004$) and a positive correlation was observed between the expression of ANXA3 and CD133 ($r_3=0.409$, $P_3=0.015$). Expression of HIF-1 α , ANXA3 and CD133 were associated with tumor size, lymphatic metastasis and clinic stage of colon cancer (all $P<0.05$).

Conclusions: HIF-1 α , ANXA3 and CD133 were overexpressed in human colon cancer and showed positive correlations among themselves. The expression of HIF-1 α , ANXA3 and CD133 were closely related to the size of the tumor, lymphatic metastasis and clinical stage of colon cancer, which indicated that they could be promising biomarkers for the study of colon CSCs and treatment of colon carcinoma.

Keywords: Hypoxia-inducible factor-1 α (HIF-1 α); annexin A3 (ANXA3); immunohistochemistry; colon cancer

Submitted Mar 28, 2022. Accepted for publication Jun 20, 2022.

doi: 10.21037/tcr-22-1277

View this article at: <https://dx.doi.org/10.21037/tcr-22-1277>

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Introduction

Colon cancer is one of the most fatal solid tumors in the gastrointestinal tract. Despite many large, well-documented studies of the treatment of colon cancer, the death rate and life expectancy are still high. In 2020, there were 1.88 million known new cases and 910,000 deaths around the world (1), so it is imperative to explore new therapeutic strategies.

Hypoxia is one of the most typical characteristics of solid tumors and is related to dysregulated cell proliferation, anti-apoptosis, migration and invasion, and multidrug resistance in various tumor cells (2-4). As a transcription factor, hypoxia-inducible factor-1 (HIF-1) is the master regulator of the cellular hypoxic response and comprises the HIF-1 α and HIF-1 β subunits. The von Hippel-Lindau protein (pVHL) is part of the ubiquitin E3 ligase complex that targets proteins for proteolysis and can quickly degrade HIF-1 α under normoxia (5). Inactivation of the VHL tumor suppressor increases the expression of HIF-1 α in various tumor cells. Previous studies have found that overexpression of HIF-1 α can upregulate multiple hypoxia-inducible genes (6-8). HIF-1 α is one kind of nuclear protein with transcriptional activity under the hypoxic environment, which is overexpressed due to the rapid proliferation of tumor cells. Zhou and his colleagues (9) found that HIF-1 α was associated with tumor-node-metastasis (TNM) stage, histological grade, showing high diagnostic and prognostic value for patients with colon cancer. Annexin A3 (ANXA3) is a member of the annexin family that is a class of calcium-dependent phospholipid-binding proteins. Expression of the annexin family is found in both animals and plants. The annexins consist of five groups, A-E. Group A annexins are found in vertebrates and comprise 12 types of annexin A (annexin A1-A11 and annexin A13). Intensive studies have focused on ANXA3 in various cancers such as ovarian cancer, gastric cancer, breast cancer, prostate cancer and thyroid cancer, but its role in cancer is still in dispute and its function in colon carcinoma remains unknown. Our previous study found that HIF-1 α had a significant association with ANXA2 in colon cancer (10). Accumulating evidences suggested that there are some similarities between ANXA2 and ANXA3 in the development and progression of numerous tumors (11,12). In gastric cancer, ANXA3 was up-regulated in cancer tissues rather than adjacent normal tissues, further exploration of ANXA3 and patients' clinicopathological features found that high ANXA3 expression was related to bigger tumor size, poor

TNM stage, shorter overall survival and limited disease-free survival (13). Recently we found that the expression of HIF-1 α was associated with the expression of ANXA3 in colon cancer (14). Recent studies hint that ANXA3 play an important role in the maintenance of hepatocellular carcinoma cancer stem-like cells (CSCs) (15,16). CD133 has been recognized as a typical marker of CSCs in many cancers and is reportedly overexpressed in several tumors, including gastric cancer, lung cancer and colorectal cancer (17-20). A systematic review suggested that a systematic review TNM stage and lymphatic/vascular infiltration of gastric cancer (21). However, the relationship between HIF-1 α , ANXA3 and CD133 has not been reported.

In the present study, we evaluated that relationship by measuring the expressions of HIF-1 α , ANXA3, and CD133 by immunohistochemistry and analyzing the correlation of the 3 proteins with statistical software. We also evaluated the relationship between HIF-1 α , ANXA3, CD133 expression and the clinicopathological parameters in colon cancer. We present the following article in accordance with the REMARK reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1277/rc>).

Methods

Human tissue samples

Colon cancer tissue and adjacent normal tissue (10 cm from tumor margin) were obtained from the surgical specimens of 35 patients diagnosed with colon adenocarcinoma, who underwent radical colectomy without preoperative chemoradiotherapy at the Department of Gastrointestinal Surgery, The First Affiliated Hospital of Chongqing Medical University (Chongqing, China). The histologic types of the specimens were independently determined by 2 pathologists. All the patients' clinical data, including age, gender, TNM stage (UICC), histological stage and lymph node status, were collected. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (IRB No. 2020-861) and written informed consent was obtained from all patients.

Immunohistochemistry

Fresh specimens were fixed in 4% neutral formalin and embedded in paraffin. Immunohistochemical staining

was performed with an Immunohistochemical SP9000 kit (Zhongshan Golden Bridge, Beijing, China) according to the manufacturer's instructions. Briefly, 4- μ m sections were cut from formaldehyde-fixed, paraffin wax-embedded tumor tissue blocks. All the slides were dewaxed and rehydrated. Endogenous peroxidase activity was quenched by 3% H₂O₂ for 10 min, the slides were microwaved in citrate buffer for 15 min for antigen retrieval, and then blocked by 5% bovine serum albumin for 30 min at room temperature. Immunostaining was performed with anti-HIF-1 α and anti-ANXA3 rabbit polyclonal antibodies (GTX 127309, GTX103330 Gene Tex, USA 1:200, 1:150), and anti-CD133 rabbit polyclonal antibody (18470-1-AP, Proteintech, China). The slides were then incubated overnight at 4 °C with the primary antibodies in a humid chamber. The slides were incubated with biotinylated secondary goat anti-rabbit antibody and avidin-biotin-peroxidase complex for 20 min at room temperature, followed by incubation with 3,3-diaminobenzidine (DAB). Finally, the slides were counter-stained with hematoxylin.

Evaluation of immunohistochemical staining

The immunohistochemical results were independently evaluated by 2 investigators according to staining intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the percentage of cells stained was assessed using a semiquantitative 4-point scale (0, <10% of cancer cells stained; 1, 10–20% of cancer cells stained; 2, 21–50% of cancer cells stained; 3, >50% of cancer cells stained). If the product of staining intensity and percentage of positive cells was ≥ 3 , it was deemed to be immunoreaction positive (+).

Statistical analysis

All statistical analyses were processed using statistical software SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Associations between expression levels of HIF-1 α , ANXA3 and CD133 were analyzed by the Spearman's rank correlation coefficient. Relationships between HIF-1 α , ANXA3 and CD133 immunohistochemical staining and clinicopathological features were analyzed using Fisher's exact probability test. $P < 0.05$ was considered statistically significant.

Results

Expressions of HIF-1 α , ANXA3 and CD133 in colon cancer tissues

The HIF-1 α , ANXA3 and CD133 proteins were expressed predominantly in colon cancer tissues, and faintly in normal colon tissues. HIF-1 α was mainly expressed in the cytoplasm and nucleus of colon cancer cells, whereas ANXA3 and CD133 were mainly distributed in the cytomembrane (*Figure 1*). In colon cancer tissues the positive rates of HIF-1 α , ANXA3 and CD133 expression were 80% (28/35), 77% (27/35), and 71% (25/35), respectively. In normal colon tissues the positive rates of HIF-1 α , ANXA3, and CD133 were 14% (5/35), 20% (7/35), and 23% (8/35), respectively. The expression levels of HIF-1 α , ANXA3 and CD133 were significantly higher in colon cancer tissues compared with normal colon epithelium ($P < 0.01$). Correlational analyses showed that HIF-1 α expression positively correlated with that of ANXA3 and CD133 ($r_1 = 0.408$, $P_1 = 0.015$, $r_2 = 0.474$, $P_2 = 0.004$) and a positive correlation between the expression of ANXA3 and CD133 was demonstrated in the 35 colon cancer tissue samples (*Table 1*).

Correlation of HIF-1 α , ANXA3, CD133 expressions with clinicopathological features in human colon cancer

The association between HIF-1 α , ANXA3, and CD133 expressions, and the clinicopathological features of the 35 patients is shown in *Table 2*. We found that the size of the tumor, lymphatic metastasis and clinical stage were positively associated with high expressions of HIF-1 α , ANXA3 and CD133.

Discussion

In the present study, the expressions of HIF-1 α , ANXA3 and CD133 were significantly increased in colon cancer tissues when compared with normal colon tissues. The HIF-1 α expression was significantly associated with that of ANXA3 and CD133, and we detected a positive correlation between the expression of ANXA3 and CD133 in the 35 colon cancer cases. The expressions of HIF-1 α , ANXA3 and CD133 correlated with the tumor size, lymphatic metastasis and the TNM classification of colon cancer.

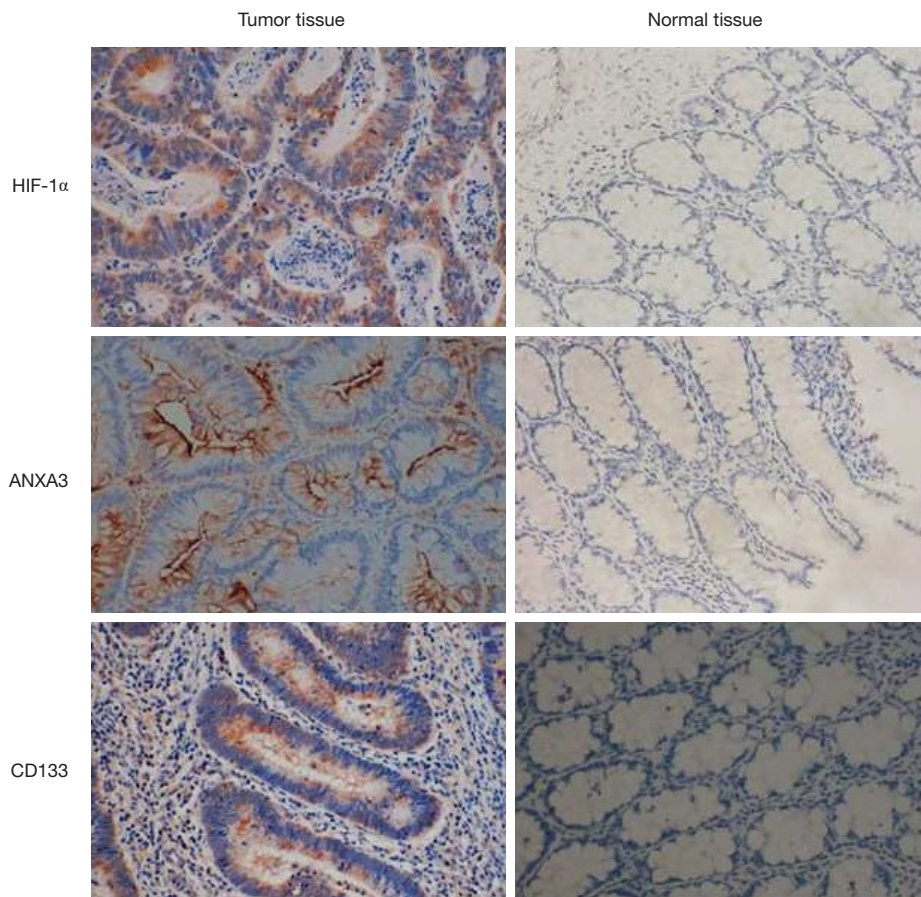


Figure 1 Expressions of HIF-1 α , ANXA3, and CD133 proteins in colon cancer tissues and normal colon tissues (IHC staining, $\times 200$). HIF-1 α , hypoxia-inducible factor-1alpha; ANXA3, annexin A3.

Table 1 Correlation analysis of HIF-1 α , ANXA3, and CD133 expression in colon cancer tissue

Gene expression	HIF-1 α				ANXA3			
	+	-	P	r	+	-	P	r
CD133			0.004*	0.474			0.015*	0.409
+	23	2			22	3		
-	5	5			5	5		
ANXA3			0.015*	0.408				
+	24	3			-	-		
-	4	4			-	-		

*, P<0.05. +, immunohistochemical result positive; -, immunohistochemical result negative. HIF-1 α , hypoxia-inducible factor-1alpha; ANXA3, annexin A3.

Table 2 Correlation between HIF-1 α , ANXA3, and CD133 expression in colon cancer and clinicopathological features

Clinicopathological feature	n	HIF-1 α			ANXA3			CD133		
		+	-	P	+	-	P	+	-	P
Age (years)				0.672			0.246			1.000
≤ 65	20	15	5		17	3		14	6	
> 65	15	13	2		10	5		11	4	
Sex				0.177			0.311			0.155
Male	18	16	2		15	3		11	7	
Female	17	12	5		12	5		14	3	
Tumor size (cm)				0.006*			0.032*			0.002*
≤ 3	13	7	6		7	6		5	8	
> 3	22	21	1		20	2		20	2	
Lymphatic metastasis				0.010*			0.003*			0.006*
Absent	14	8	6		7	7		6	8	
Present	21	20	1		20	1		19	2	
Stage				0.001*			0.011*			0.001*
I, II	15	8	7		8	7		6	9	
III, IV	20	20	0		19	1		19	1	

*, P<0.05. +, immunohistochemical result positive; -, immunohistochemical result negative. HIF-1 α , hypoxia-inducible factor-1alpha; ANXA3, annexin A3.

Hypoxia is a common trait of solid tumors, and HIF-1 α has important functions in the development of various tumors but especially solid tumors. A fast-growing tumor quickly outgrows its vascularization, which deprives the tumor cells of oxygen. This intra-tumor hypoxia inhibits the activity of prolyl hydroxylase domains, which can lead to stabilization and overexpression of HIF-1 α , resulting in the upregulation of numerous genes including vascular endothelial growth factor, phospholipase D2, survivin etc. (22-24). In recent years, the function of HIF-1 α has been deeply probed and overexpression of HIF-1 α promotes the development of glioblastoma, gastric cancer, hepatocarcinoma and colorectal cancer (25-28) and there is much evidence that HIF-1 α promotes a stem cell-like phenotype in many tumor cells (29-31).

It has been verified that ANXA3 plays a key role in cancer progression, metastasis and multidrug resistance (32,33). Liu *et al.* found that high expression of ANXA3 was associated with the clinical stage, lymphatic metastasis and recurrence of lung adenocarcinoma (34). Many studies have demonstrated that ANXA3 acts as a cancerogenic protein

in various tumors. Overexpression of ANXA3 has been found in breast cancer, colorectal cancer and ovarian cancer (14,35,36) and decreased expression of ANXA3 has been detected in follicular thyroid carcinoma, papillary thyroid cancer and prostate cancer (37-39). Hence, the function of ANXA3 in human tumors remains controversial. Studies suggested that ANXA3 plays an important role in the maintenance of hepatocellular carcinoma CSCs (15,16), but its role in colon CSCs has not been reported.

CD133, which is a 120-kDa glycoprotein with 5 transmembrane domains, is a commonly used CSC marker. Since CSCs in solid cancers were first reported in the early half of the 2000s (40,41), accumulating evidence has supported the existence of CSCs in colon cancer (42-44). We detected CD133 protein expression in $\approx 71\%$ of cases and it was closely related to the expressions of HIF-1 α and ANXA3. The correlation between HIF-1 α , ANXA3 and CD133 reminds us to further investigate the relationship in the signal transduction pathway of colon cancer cells. Therefore, large-scale further research is needed to elaborate the specific pathway.

Acknowledgments

The authors gratefully thank the Central Laboratory of The First Affiliated Hospital of Chongqing Medical University for providing equipment support.

Funding: This work was supported by the National Natural Science Foundation of China (Nos. 81201916 and 8207101617).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1277/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1277/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1277/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (IRB No. 2020-861), and written informed consent was obtained from all patients.

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(English Language Editor: K. Brown)

Cite this article as: Chai X, Wu X, Ren J, Du K, Wu X, Feng F, Zheng J. Expression of HIF-1 α , ANXA3, CD133 and their associations with clinicopathological parameters in human colon carcinoma. *Transl Cancer Res* 2022;11(6):1644-1651. doi: 10.21037/tcr-22-1277