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ZKSCAN3 Facilitates Liver Metastasis of Colorectal Cancer Associated with CEA-expressing Tumor

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Abstract. *Aim: Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) is overexpressed in invasive colorectal cancer (CRC) cells and regulates the expression of several genes favoring tumor progression, including vascular endothelial growth factor (VEGF) and integrin β4. We evaluated the association of ZKSCAN3 and colorectal cancer liver metastasis (CLM) to determine whether it is related to invasive signaling pathways. Materials and Methods: The ratios of expression by primary tumor to normal tissue and metastatic tumor to normal tissue were compared between ZKSCAN3-overexpressing and underexpressing primary tumor groups. Results: In terms of CLM, the ZKSCAN3 overexpression was positively correlated with carcinoembryonic antigen (CEA), VEGF, and AKT expression. The protein-expression analysis showed that ZKSCAN-specific siRNA knockdown reduced CEA expression in LoVo and LS174T CRC cells. Matrigel invasion by ZKSCAN3-overexpressing HCT116 cells was increased when examined on CEA-coated filters compared with phosphate-buffered saline-treated controls. Additionally, matrix metalloproteinase 9 (MMP9) expression was greater in cells with reference allele (GG) than substitution allele (CC) for ZKSCAN3 rs733743 ($p=0.032$). ZKSCAN3 protein expression of the high serum CEA group was increased in hepatic metastatic tissue compared with the primary tumor tissue, while in the group with normal serum CEA it decreased or was similar. Reference ZKSCAN3 alleles were correlated with male dominance, a family history of malignancy, high serum CEA concentration and stage IV CRC in 450 patients with sporadic CRC. In conclusion, ZKSCAN3 appears to promote colorectal tumor progression and invasion. ZKSCAN3 may facilitate hepatic metastasis of CRC associated with CEA particularly in cases with CEA-producing tumor.*

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A rich history of investigations has uncovered several genes and pathways critical to the initiation and progression of colorectal cancer (CRC), including the Wingless-type MMTV integration site (WNT), RAS, mitogen-activated kinase-like protein (MAPK), phosphatidylinositol 3-kinase (PI3K), transforming growth factor-β (TGFβ), DNA mismatch-repair, and P53 pathways (1). Structural changes in extracellular matrix (ECM) proteins are a prerequisite for cell migration during tissue remodeling and are accomplished *via* complex control of expression and activities of matrix metalloproteinases (MMPs) (2). Overexpression of MMPs leads to degradation of the ECM, an essential step for tumor invasion and metastasis. Some MMP family members, such as MMP2 and MMP9, are of particular interest with respect to their roles in development and progression of CRC (3). In addition, angiogenesis controlled by angiogenic factors, such as the vascular endothelial growth factor (VEGF) family of cytokines, is a critical component of tumor growth and metastasis. VEGFA and VEGFB participate in early tumor development during adenoma formation, whereas VEGFC functions in the advanced stages of CRC (4).

Carcinoembryonic antigen (CEA) is a member of a family of highly related glycoproteins involved in cell adhesion. The

12 CEA-related cell adhesion molecules (CEACAMs) have surprisingly diverse functions in cell adhesion and intra- and extracellular signaling, affecting complex biological processes in tumor cells such as cancer progression, inflammation, angiogenesis, and metastasis. Both CEACAM5 (traditionally known as CEA) and CEACAM6 play roles in cell adhesion, differentiation, invasion, and metastasis (5).

Activation of the epidermal growth factor receptor (EGFR) pathway triggers a complex program of intracellular signals. The two major pathways activated by the EGFR pathway are the RAS/RAF/MEK/ERK pathway, which controls gene transcription, cell-cycle progression, and cell proliferation, and the PI3K/PTEN/AKT pathway, which activates a cascade of anti-apoptotic and pro-survival signals (6).

Wood *et al*. categorized the genomic landscape of CRC as being composed of a few commonly targeted gene “mountains” with a much larger number of gene “hills” altered at low frequency (7). These characteristics reflect the heterogeneity and complexity of the neoplasm. Recently, zinc finger with KRAB and SCAN domain 3 (ZKSCAN3), a novel zinc finger transcription factor, was found to be overexpressed in colonic tumors and multiple myeloma and to activate a gene-expression program driving colonic cancer and multiple myeloma progression (8, 9). More importantly, ZKSCAN3 expression was higher in deeply invasive tumor cells than in superficial non-invasive tumor cells, suggesting that it may play a crucial role in driving tumor-cell migration and invasion (8).

In this study, we evaluated the association of ZKSCAN3 with CRC liver metastasis (CLM) to determine whether ZKSCAN3 is related to invasive signaling pathways or acts as a ligand for other invasive molecules.

Materials and Methods

Genome-wide single nucleotide polymorphism (SNP) screening. In our previous study, we attempted to determine surrogate SNP markers that were chemosensitive to targeted regimens using a three-step approach that included genome-wide SNP screening (10). From the initial genome-wide screening, we selected 13 SNPs in 13 genes. ZKSCAN3 SNP *rs733743* was related to marginal chemosensitivity to cetuximab regimens in an *in vitro* transfection assay in that study, therefore, it was finally excluded for being an inefficient marker for cetuximab. Subsequent to that study, ZKSCAN3 was further investigated in this study regarding whether it may act as a transcription activator and promotes cancer cell progression and migration in various tumor types (8, 9, 11).

Western blot analysis. Equal amounts of protein (30 µg) were resolved in 10% SDS-PAGE. Gels containing proteins were transferred to nitrocellulose membranes and the membranes were blocked with 5% skimmed milk dissolved in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h. The membranes were washed three times with TBST, incubated in primary antibodies diluted in TBST at 4˚C overnight with gentle agitation, and washed again. They were then incubated in secondary antibodies conjugated to horseradish peroxidase. The target proteins were detected by enhanced chemiluminescence western blotting detection reagents (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The following antibodies were used: ZKSCAN3 (OriGene Technologies, Rockville, MD, USA), VEGF (Cell Signaling Technology, Danvers, MA, USA), cyclin D2 (Cell Signaling Technology), AKT (Cell Signaling Technology), phospho-AKT (S473; Cell Signaling Technology), integrin β4 (Cell Signaling Technology), KRAS (Abcam, Cambridge, MA, USA), CEA (T84.66; ATCC, Rockville, MD, USA), integrin α5β1 (Biorbyt, Cambridge, Cambridgeshire, UK), and actin (Bethyl, Montgomery, TX, USA).

We performed the western blot using the tissues of patients with liver metastasis from CRC. We divided the eight patients into two groups (a ZKSCAN3-overexpressing group and a ZKSCAN3-underexpressing group) according to ZKSCAN3 expressions of primary tumor tissue as compared with normal colonic epithelium.

CRC cell lines. The following five human CRC cell lines were used: RKO (colon carcinoma, poorly differentiated), LoVo (colorectal adenocarcinoma, derived from a metastatic site), LS174T (colorectal adernocarcinoma, Dukes' stage B), HCT116 (colorectal carcinoma), and SW480 (colorectal adenocarcinoma, Dukes' stage C). All cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were propagated every 3 days and cultured in RPMI-1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37˚C under an atmosphere of 5% CO_2 in air.

Stable cell line production. Stable HCT116 cell lines containing ZKSCAN3 cDNA (OriGene Technologies, Rockville, MD, USA) were generated by selecting transfected cells in 1 mg/ml G418 (Sigma, St. Louis, MO, USA) for 10 days. At least two clones overexpressing *ZKSCAN3* (G1 and G2) were established for the HCT116 cell lines and stable transfection was confirmed by sequencing and quantitative real-time polymerase chain reaction (PCR) and Western blotting of ZKSCAN3.

In vitro siRNA knockdown of ZKSCAN3. Transfections were performed in LoVo and LST174T cells using Lipofectamine 2000 according to the manufacturer’s guidelines (Invitrogen, Waltham, MA, USA). For transient *ZKSCAN3* knockdown, cells were incubated with *ZKSCAN3* siRNA (Sigma MISSION, siRNA, SASI_Hs02_00356760; 5’-CUU CUA GGC UUA CUC CAG A-3’; Sigma, St. Louis, MO, USA) for 48 h, then harvested and processed by western blotting to determine ZKSCAN3 and CEA expression.

Invasion assay. Culture plates (24-well) with a BD Biocoat™ Matrigel Invasion Chamber (BD 354480; Biosciences, Palo Alto, CA, USA) were used for invasion assays. Aliquots of CEA [5 µg in phosphate-buffered saline (PBS)/filter] and PBS control were coated onto the lower surface of the filters. The filters were then incubated at room temperature for 1 h. 3T3 fibroblast-conditioned medium was placed in the lower chamber as a chemoattractant (12). ZKSCAN3-overexpressing cells (2×10^5) suspended in the serum-free RPMI medium were seeded onto the upper chamber. Assays were performed after incubating the chambers for 16 h at 37˚C with 5% CO_2. Cells on the upper surface of the filters were completely

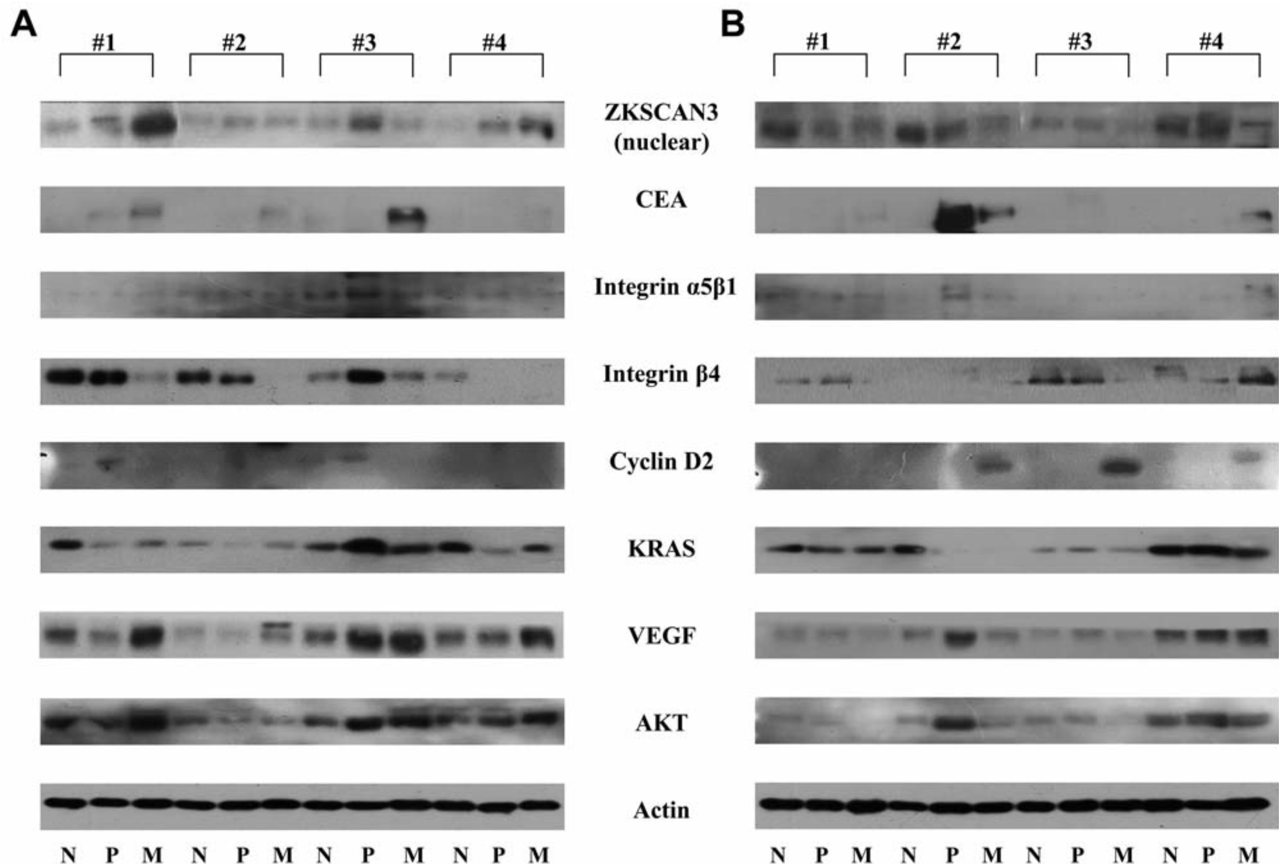

Figure 1. *Expression profiling identifies candidate Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3)-related targets in colorectal cancer tumor tissues. Expression in primary tumor to normal and metastatic tumor to normal were compared in samples from patients with ZKSCAN3 underexpression (A) and overexpression (B) (see Table I for quantitative data). Total proteins were analyzed by western blotting with antibodies against presumptive pathway proteins. For ZKSCAN3, nuclear/cytoplasmic fractionation with colorectal cancer (CRC) tumor proteins was performed using the NE-PER nuclear and cytoplasmic extraction reagents according to the manufacturer's instructions (Peirce Biotechnology, Rockford, IL, USA). N, Normal colonic epithelium; P, primary colorectal cancer; M, colorectal cancer liver metastasis.*

removed by wiping the filters with a cotton swab. The filters were fixed in ice-cold methanol for 2 minutes and stained with 0.2% crystal violet for 10 minutes. Cells attached to three different fields were counted under a light microscope (×100), and each assay was performed in triplicate.

Gelatin zymography assay. MMP2 and MMP9 activities in culture media were examined using gelatin zymography. Concentrated conditioned media (×10) mixed with sample buffer were electrophoresed on a 10% SDS-PAGE gel with 0.1% gelatine (Invitrogen, Grand Island, NY, USA) incorporated as a substrate for gelatinolytic proteases under non-reducing conditions at 125 V for 2 h. The gel was incubated at 37˚C for 16 h in fresh developing buffer and stained with 0.5% Coomassie brilliant blue R-250 (Bio-Rad, Hercules, CA, USA). Bands on the gels were measured using densitometric analyses (Bio-Rad).

Clinical associations with the reference allele of ZKSCAN3. A total of 450 patients with CRC whose tissues were stored at our center's tissue bank were chosen to identify correlations between genotyping and clinical prognostic parameters. This cohort included 331 patients with sporadic CRC with curative operations and 119 patients with metastatic CRC who underwent palliative or curative operations between January 2004 and December 2009. Genomic DNA was extracted from buffy coats of blood samples which were taken before surgery using a nucleic lysis buffer (Promega Korea, Seoul, Korea). The study protocol was approved by the Institutional Review Board of Human Genetic and Genomic Research (registration no.2009-0091; valid until 31 Dec 2018), in accordance with the Declaration of Helsinki.

Statistical analysis. Categorical variables were compared using chi-square tests, and continuous variables were compared using independent sample paired Student's t-test or analysis of variance (ANOVA). Comparisons between the ZKSCAN3-overexpressing and -underexpressing groups were performed using paired Student's *t*-test. All statistical tests used two-sided verification and a value of $p<0.05$ was considered as statistically significant. All statistical analyses were performed using IBM SPSS 21.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Table I. *Analysis of the expression of various molecules according to Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) expression in eight patients with liver metastasis from colorectal cancer (see Figure 1 for qualitative data).*

	ZKSCAN3 overexpression			ZKSCAN3 underexpression		
	P:N (mean±SEM)	M:N (mean±SEM)	*p*-Value	P:N (mean±SEM)	M:N (mean±SEM)	*p*-Value
CEA	1.279±0.078	1.354±0.062	0.001	1.508±0.128	2.527±0.652	0.140
Integrin β4	1.156±0.132	0.765±0.077	0.003	1.067±0.108	1.063±0.232	0.987
Integrin α5 β1	1.092±0.062	1.131±0.043	0.533	0.961±0.066	0.907±0.059	0.128
RAS	0.951±0.173	0.896±0.103	0.523	0.919±0.121	0.783±0.100	0.048
VEGF	1.029±0.080	1.265±0.047	<0.001	1.132±0.063	1.024±0.065	0.008
AKT	1.133±0.102	1.338±0.128	0.001	1.134±0.053	0.940±0.032	0.002
PAKT	0.974±0.029	0.979±0.044	0.882	1.053±0.104	0.910±0.104	0.027
Cyclin D2	1.018±0.033	1.061±0.037	0.442	0.920±0.070	0.934±0.054	0.833

N, Normal colonic epithelium; P, primary colorectal cancer tissue; M, liver metastasis tissue; P:N, ratio of expression in primary tumor to that in normal tissue; M:N, ratio of expression in metastatic tumor to that in normal tissue; CEA, carcinoembryonic antigen; VEGF, vascular endothelial growth factor.

Results

ZKSCAN3 overexpression in patients with CRC. Tumorigenesis-associated proteins possibly affected by ZKSCAN3 overexpression were concurrently examined using appropriate antibodies in the normal tissue and primary and metastatic tumors of each patient (Figure 1). The ratios of primary tumor to normal tissue and metastatic tumor to normal tissue were compared between the ZKSCAN3-overexpressing and -underexpressing groups. In terms of liver metastasis, ZKSCAN3 overexpression was positively correlated with CEA, VEGF, and AKT expression, whereas it was negatively correlated with integrin β4 expression. Otherwise, CEA, VEGF, and AKT were not correlated with liver metastasis in the ZKSCAN3-underexpressing group (Table I).

ZKSCAN3 knockdown reduces CEA expression. Based on the protein-expression assay results, we presumed that ZKSCAN3 and CEA may facilitate liver metastasis of CRC. To determine the relationship between CEA and ZKSCAN3, we hammed protein expression using five cell lines. ZKSCAN3 and CEA were strongly expressed in LoVo and LS174T cell lines (Figure 2A).

We determined the effect of silencing of ZKSCAN3 using LoVo and LS174T CRC cell lines. Western blotting indicated approximately 20% knockdown of endogenous ZKSCAN3 in LoVo and LS174T cells transfected with specific siRNA (Figure 2B). Western blotting also showed that ZKSCAN-specific siRNA transfection reduced the protein expression of CEA in LoVo and LS174T cells (Figure 2C).

ZKSCAN3 correlates with CEA increases tumor invasion. It has been suggested that both the ability of tumor cells to detach and an increase in motility are related to the development of metastatic potential and invasiveness in CRC. Invasiveness, measured by the number of cells invading a Matrigel invasion chamber, was greater in ZKSCAN3-overexpressing cell lines (Figure 3B). It has been suggested that ZKSCAN3 overexpression is related to tumor invasiveness. To investigate the relationship between ZKSCAN3 and CEA for invasiveness, we compared the invasiveness of ZKSCAN3-overexpressing HCT116 cell lines exposed to CEA-coated and PBS-coated filters. The invasiveness of ZKSCAN3-overexpressing HCT116 cell lines tended to be greater for CEA-coated filters than for PBS-coated filters (Figure 3C).

MMP2 and MMP9 expression in ZKSCAN3 overexpressed cell lines. The activities of MMP-2 and MMP-9 were assayed using quantitative zymography. Both active MMP2 and MMP9 (66 and 82 kDa, respectively) were highly expressed in ZKSCAN3-overexpressing cells. MMP9 expression was significantly greater in cells with wild-type ZKSCAN3 *rs733743* reference allele (GG) than in those with the substitution allele (CC) (p=0.032). However, there was no significant difference between the two allele types in MMP2 activity (Figure 4).

Expression of ZKSCAN3 in patients with a high serum level of CEA group and those with a normal level. To determine the relations of ZKSCAN3 with CEA in CLMs, we compared groups of patients who had CLMs by serum CEA in relation to ZKSCAN3 protein expression of primary tumor tissue and CLM tissue.

The preoperative serum CEA concentrations of the high level group were 440, 377, 359 and 184 ng/ml, and those of the normal level group were 0.78, 0.99, 1.1 and 1.3 ng/m, respectively. ZKSCAN3 protein expression by western blotting of the group with a high serum CEA level (with

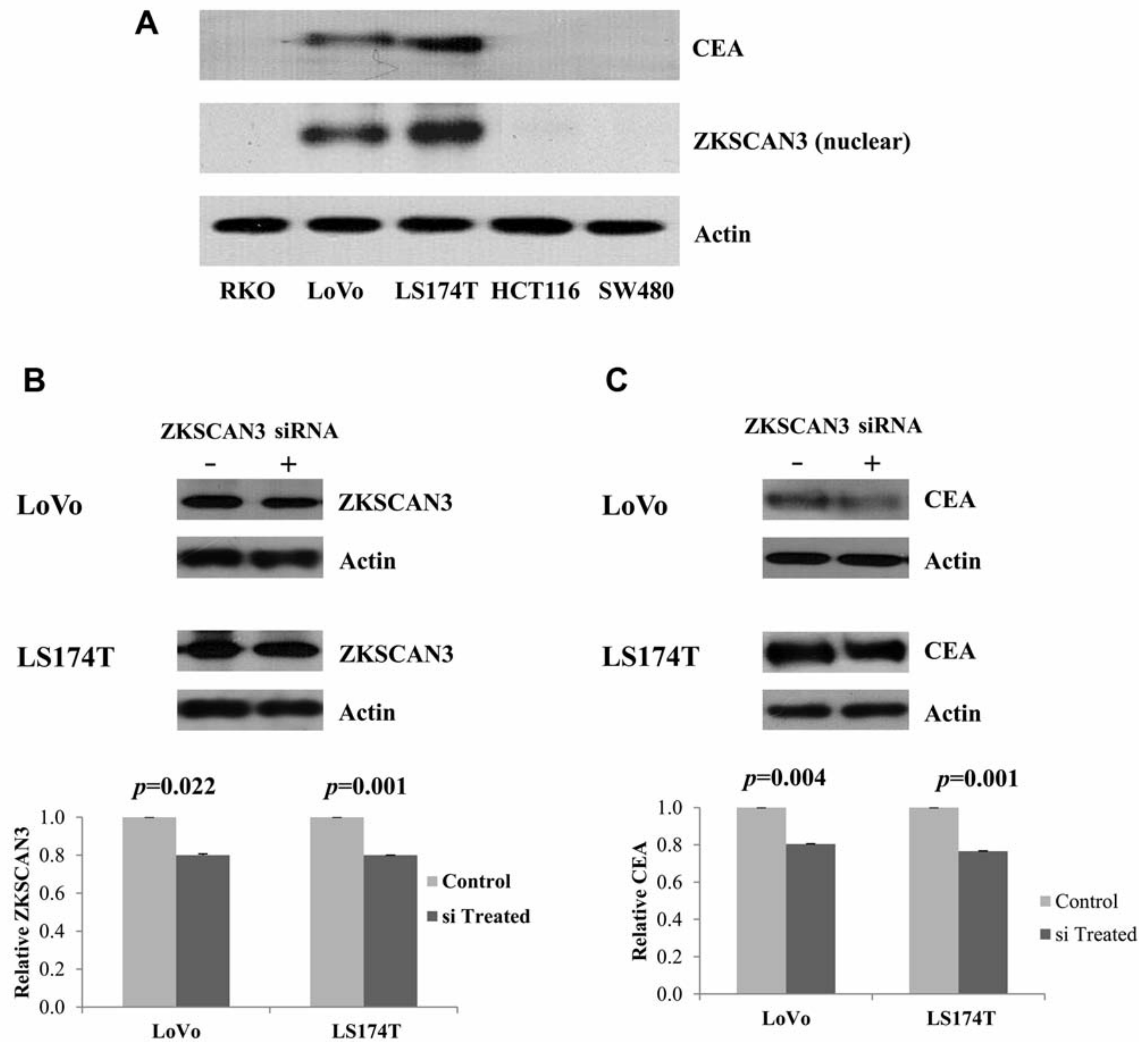

Figure 2. *Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) is related to carcinoembryonic antigen (CEA) expression. A: Expression profiling identified ZKSCAN3- and CEA-expressing cell lines from several colorectal cancer cell lines. Total cell extracts were subjected to western blotting using antibody to CEA, and nuclear extracts were isolated and subjected to western blotting with antibody to ZKSCAN3. Actin was used as a loading control. B: LoVo and LS174T cells were transfected with control and ZKSCAN3-specific siRNAs (48 h). Nuclear extracts were isolated and subjected to western blotting with antibody to ZKSCAN3. Actin was used as the loading control, and the relative protein density of blots was measured. C: Total cell extracts were subjected to western blotting using antibody to CEA. Actin was used as the loading control and the relative protein density of blots was measured. Values are means±SEM of triplicates. p-Values are from Student's t-test.*

CEA-producing tumor) showed increased protein expression in the CLM tissue in comparison to the primary tumor tissue. On the other hand, the group with a normal serum CEA level generally showed decreased or similar ZKSCAN3 protein expression in the CLM tissue in comparison to the primary tumor tissue (Figure 5).

Clinicopathological features are associated with ZKSCAN3 SNP rs733743. Clinicopathological characteristics were compared between patients who were homozygous for reference allele (GG) and those who were homozygous or heterozygous for the substitution allele (CC) (Table II). Reference ZKSCAN3 alleles were correlated with male

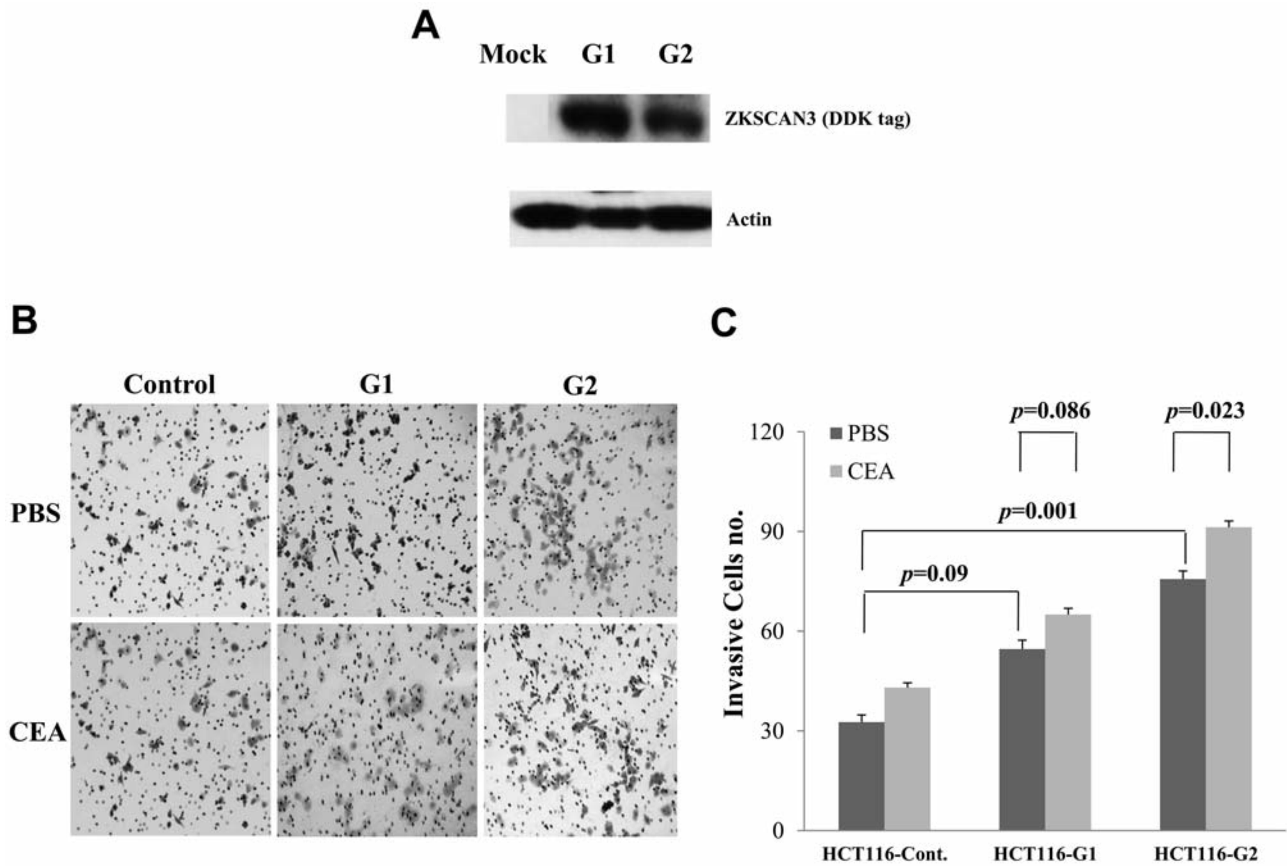

Figure 3. *The comparison of the effects of Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) overexpression on invasion by HCT116 cell lines G1 and G2 using phosphate-buffered saline (PBS)- and carcinoembryonic antigen (CEA)-coated filters. A: Stable expression of ZKSCAN3 by allele was confirmed by western blot, using antibody against ZKSCAN3-DDK. B and C: Invasiveness was higher in CEA-coated filters in ZKSCAN3-overexpressing HCT116 cell lines. Values are means±SEM of quadruplicates. p-Values are from Student's t-test.*

dominance, a family history of malignancy, high serum CEA concentration and stage IV CRC.

Discussion

Recent studies strongly suggest that CRC progression is the consequence of various combinations of a large number of gene products, each providing some advantage with respect to tumor growth/survival (7, 13-15). A previous study reported that ZKSCAN3, which is a zinc finger protein required for *Drosophila* hindgut development related to the bowel, is a new player in CRC, contributing to the progression of this malignancy (16). Yang *et al*. reported that patients with stage IV CRC had ZKSCAN3-positive tumor cell nuclei, whereas non-malignant adjacent tissue showed diminished ZKSCAN3 immunoreactivity (8). ZKSCAN3 is also overexpressed in a subset of CRC carrying the wild-type alleles which are common targets in this malignancy (*APC, KRAS, p53*). Experiments that interfered with ZKSCAN3 expression in RKO CRC cells carrying wild-type *ZKSCAN3* suggested that it expression may also contribute to tumor progression in a subset of CRCs, thereby stimulating tumor progression (8).

In this study, ZKSCAN3 was strongly expressed in metastatic CRC site-derived LoVo cell line. In addition, ZKSCAN3-overexpressing cell lines showed greater invasiveness than normal cell lines, similarly found in another study (8). An analysis of ZKSCAN3-overexpressing tumors derived from primary tumor sites in our study showed that CEA, VEGF, and AKT were more closely associated with metastatic tumors than primary tumors.

Previous studies determined that ZKSCAN3 coordinates a gene-expression program that includes VEGF, integrin β4, and cyclin D2, which is an important driver of colonic cancer and multiple myeloma progression (9, 17). VEGF can increase tumor metastatic potential and its receptor, VEGFR,

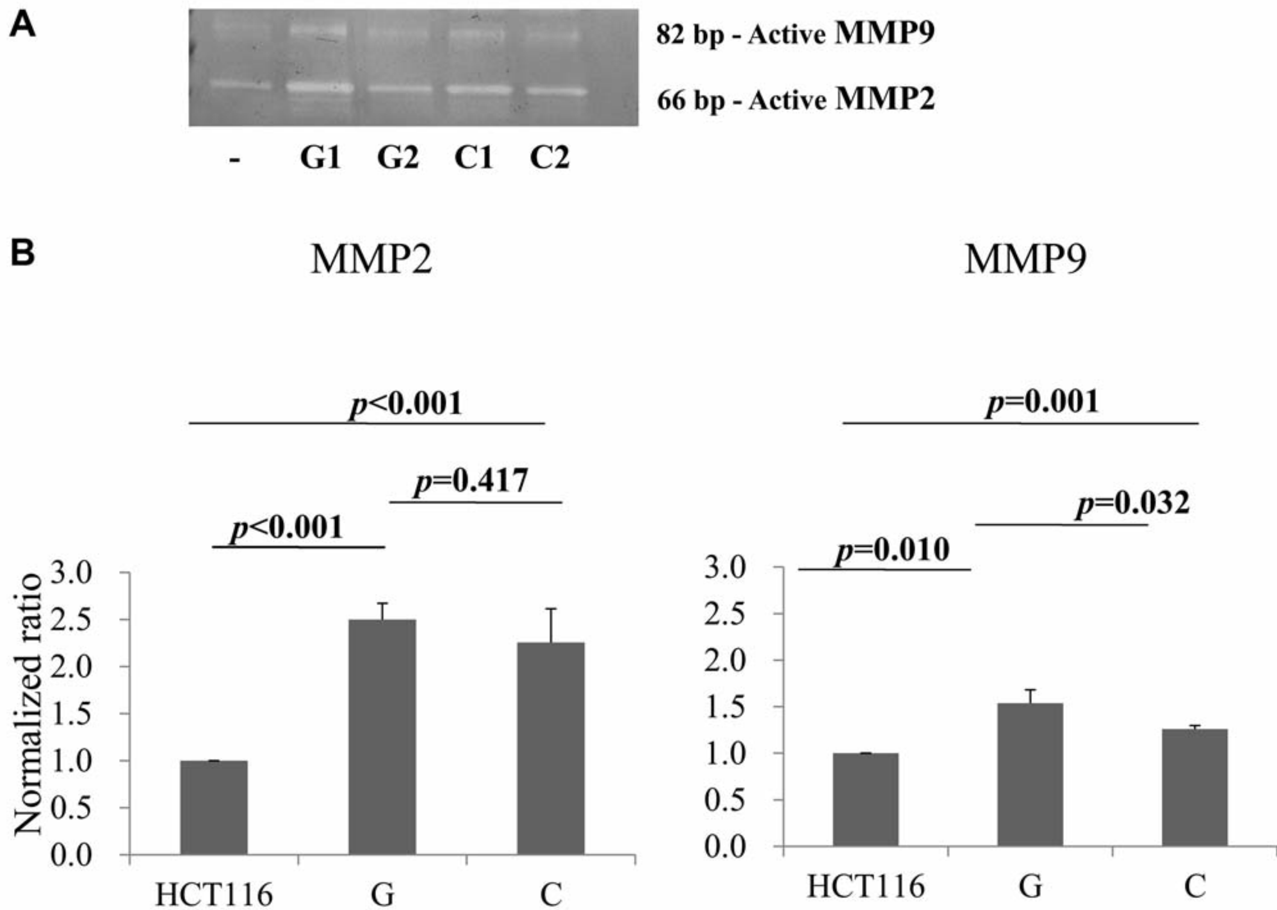

Figure 4. *Activity of metalloproteinase 2 (MMP2) and MMP9 according to Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) single nucleotide polymorphism (SNP) (rs733743) reference allele (G allele), substitution allele (C allele) and control HCT116 cells (–). A: MMP2 and MMP9 expressions of ZKSCAN3 respective allele were confirmed by MMP zymography. B: Values are means±SEM of triplicates. p-Values are from Student's t-test.*

is a therapeutic target. The expressional regulation of VEGF family members has been well investigated. For instance, hypoxia induces VEGFA expression in a PI3K/AKT-dependent pathway (18). The PI3K/AKT signaling pathway is also involved in insulin-like growth factor 1 induced VEGFC expression in lung cancer cells (19). PI3K can mediate the phosphorylation and activation of its downstream serine/threonine kinase, AKT, participating in some important biological activities such as survival, proliferation, migration, and differentiation in human cancer (20). AKT activation also contributes to tumorigenesis and metastasis in various types of human cancer (21). The PI3K/AKT signal transduction pathway probably plays a pivotal role in the extradomain A-mediated regulation of VEGFC expression in human colorectal carcinomas (22).

In our current study, ZKSCAN3 and CEA were simultaneously overexpressed in LoVo and LS174T CRC cells. The knockdown of ZKSCAN3 in LoVo and LS174T cells resulted in reduced CEA expression. These data indicate that CEA is a target and possibly one of multiple downstream effectors of ZKSCAN3. The invasiveness of ZKSCAN3-overexpressing HCT116 cells was greater with CEA-coated filters than without it. This appears to suggest that ZKSCAN3 expression is correlated with CEA, possibly affecting progression and invasion.

ZKSCAN3 protein expression in the group with a high serum level of CEA was increased in the CLM tissue in comparison to the primary tumor tissue, while that in the group with a normal serum level of CEA was decreased or similar. These findings suggest that ZKSCAN3 is associated with liver metastasis particularly in CEA-producing tumor. A direct relationship between ZKSCANN3 and CEA, DNA-binding site and downstream targets, including CEA, needs to be further identified.

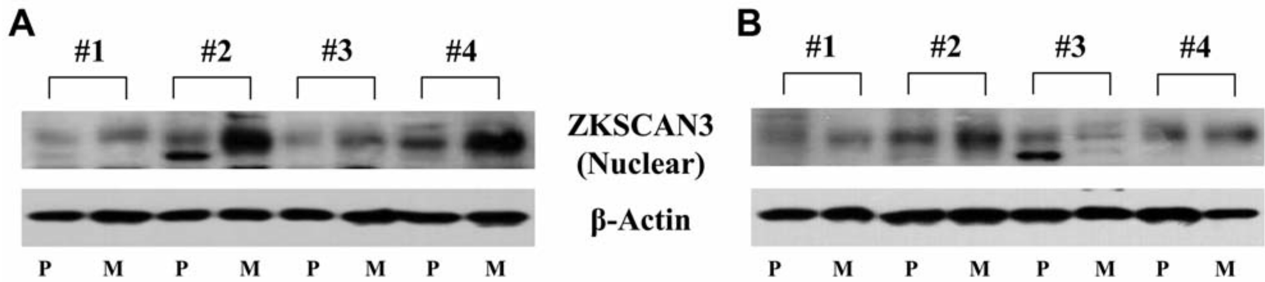

Figure 5. *Expression of Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3) in patients with high (A) and normal (B) serum carcinoembryonic antigen (CEA) levels in primary colorectal tumor tissue (P) and hepatic metastasis tumor tissue (M). Nuclear extracts were isolated and subjected to western blotting with antibody against ZKSCAN3. High serum CEA level: 440, 377, 359, and 184 ng/ml CEA, respectively; normal serum CEA level: 0.78, 0.99, 1.1, and 1.3 ng/ml CEA, respectively.*

We also verified the relationship between wild-type ZKSCAN3 and clinical parameters. In the SNP study, wild-type ZKSCAN3 was related to male sex, a family history of malignancy, CEA-producing tumors, and metastatic CRC. These findings appear to indicate ZKSCAN3 to be associated with metastatic CRC and CEA-producing tumors.

CEA plays an active role in the development of CLM (23). Jessup *et al*. demonstrated that irrespective of its function as a homophilic cell-adhesion molecule, pretreatment with soluble CEA increased the development of experimental liver metastatic nodules in nude mice, even when using weakly metastatic CRC cells negative for CEA expression (24). This occurs because CEA binds to a putative CEA receptor identified as heterogeneous nuclear ribonucleoprotein M at the surface of liver Kupffer cells through interactions with the PELPK motif present at the hinge region between the CEA N and A1 domains. Activation of the liver-specific Kupffer macrophages through CEA–CEA receptor binding leads to pro-inflammatory cytokine secretion [interleukin (IL)1α, IL1β, IL6, and tumor necrosis factor α] in the hepatic sinusoid (23, 25). These cytokines in turn up-regulate a number of cell-adhesion molecules such as intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule-1 (VCAM1), and E-selectin on the adjoining endothelium, thereby increasing the binding of circulating CRC cells (26). Arrested tumor cells in the microvasculature normally cause ischemic injury, with concomitant increases in nitric oxide and reactive oxygen species, but those expressing CEA stimulate the release of the anti-inflammatory cytokine IL10 responsible for the inhibition of hepatic injury cytotoxicity, thereby increasing the survival of weakly metastatic cells that exhibit increased metastasis (27). In addition, direct association of CEA with the death receptor 5 (DR5) (known as TRAIL receptor 2) expressed on CRC cells reduces anoikis and consequently increases metastasis (28).

The process of tumorigenesis and metastasis includes cancer cell migration and penetration through the ECM (29). Balanced activity between MMP and tissue inhibitors of metalloproteinases is responsible for the proper maintenance of tissue. Disruption of this balance affects tumorigenesis and metastasis of cancer (30, 31). High expression levels of

Table II. *Clinicopathological characteristics of 450 sporadic patients with colorectal cancer by reference-type and substitution alleles of Zinc finger with KRAB and SCAN domain 3 (ZKSCAN3).*

Variable	ZKSCAN3 GG of rs733743) (n=326)	ZKSCAN3 (GC or CC of rs733743) (n=124)	*p*-Value
Gender			0.023
Male	204 (62.6)	63 (50.8)	
Female	122 (37.4)	61 (49.2)	
Age, years			0.904
≤50	78 (23.9)	29 (23.4)	
>50	248 (76.1)	95 (76.6)	
Serum CEA, ng/ml			0.037
≤6	224 (69.6)	97 (79.5)	
>6	98 (30.4)	25 (20.5)	
Family history of malignancy	117 (35.9)	29 (23.4)	0.011
Primary tumor site			0.884
Colon	200 (61.3)	77 (62.1)	
Rectum	126 (38.7)	47 (37.9)	
T Stage			0.591
T1 or 2	29 (9.1)	13 (10.7)	
T3 or 4	291 (90.9)	108 (89.3)	
N Stage			0.901
Node-negative	156 (48.6)	58 (47.9)	
Node-positive	165 (51.4)	63 (52.1)	
Stage 4	95 (29.1)	24 (19.4)	0.035
Lymphovascular invasion	96 (29.9)	37 (30.1)	0.971
Perineural invasion	19 (29.2)	4 (21.1)	0.482
Differentiation			0.819
WD/MD	287 (88.6)	108 (87.8)	
PD/Muc	37 (11.4)	15 (12.2)	
Curative tumor resection	256 (78.5)	102 (82.3)	0.544

CEA, Carcinoembryonic antigen; WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated; MUC, mucinous.

MMP2 in the malignant epithelium, as well as in the surrounding stroma, are associated with reduced survival of patients with CRC (3). In addition, *MMP2* mRNA expression is higher in colorectal tumor tissue than in normal colorectal tissue (30). MMP9 expression is associated with the prognosis of patients with CRC, and patients with higher MMP9 expression have poorer survival (32). In this study, ZKSCAN3-overexpressing cells expressed higher levels of both MMP2 and MMP9 compared to HCT 116 control cells. MMP9 expression was higher in cells expressing reference alleles (GG) of ZKSCAN3 *rs733743* than in cells with the substitution allele (p=0.032). Therefore, ZKSCAN3 expression was related to MMP2 and MMP9 and reference alleles (GG) of ZKSCAN3 *rs733743*, particularly to MMP9.

In conclusion, ZKSCAN3 is related to CRC progression and invasion. ZKSCAN3 may facilitate hepatic metastasis of CRC associated with CEA, particularly in cases with serum CEA-producing tumor.

Disclosure Statement

None.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study.

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