The oncogenic roles of nuclear receptor coactivator 1 in human esophageal carcinoma

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Abstract

Nuclear receptor coactivator 1 (NCOA1) plays crucial roles in the regulation of gene expression mediated by a wide spectrum of steroid receptors such as androgen receptor (AR), estrogen receptor α (ER α), and estrogen receptor β (ER β). Therefore, dysregulations of NCOA1 have been found in a variety of cancer types. However, the clinical relevance and the functional roles of NCOA1 in human esophageal squamous cell carcinoma (ESCC) are less known. We found in this study that elevated levels of NCOA1 protein and/or mRNA as well as amplification of the NCOA1 gene occur in human ESCC. Elevated levels of NCOA1 due to these dysregulations were not only associated with more aggressive clinic-pathologic parameters but also poorer survival. Results from multiple cohorts of ESCC patients strongly suggest that the levels of NCOA1 could serve as an independent predictor of overall survival. In addition, silencing NCOA1 in ESCC cells remarkably decreased proliferation, migration, and invasion. These findings not only indicate that NCOA1 plays important roles in
1 | INTRODUCTION

Signaling pathways involved in the sex steroid receptors play important roles not only in cancers typically responsive to sex hormones (i.e., breast and prostate cancers) but also those classically considered as nonhormone responsive such as lung, liver, kidney, skin, and gastrointestinal cancers.1-3 Functionally, sex steroid receptors such as estrogen receptor (ER) and androgen receptor (AR) serve as transcription factors by recruiting multiple coactivators.4,5 By collaborating with transcription factors, coactivators play crucial roles in chromatin remodeling and regulation of gene expression.6-12 Nuclear receptor coactivator 1 (NCOA1, also known as SRC-1) is the founding member of the nuclear receptor coactivator family.2,13 In addition to serving as a coactivator of AR14,15 and ER,16,17 NCOA1 is also involved in transcriptional regulation of genes by interacting with other transcriptional factors. It has been reported that dysregulation of NCOA1 is involved in the initiation and progression of different cancers.18-21 NCOA1 overexpression is associated with lymph node metastasis and enhances cell proliferation in prostate cancer.14 The levels of NCOA1 also correlate with malignancy, recurrence, and therapeutic resistance.22-24 For example, elevated levels of NCOA1 can enhance breast cancer cell proliferation25,26 and metastasis.16,27,28 By enhancing Wnt/β-catenin signaling, NCOA1 is capable of promoting hepatocellular carcinoma progression.2

Esophageal squamous cell carcinoma (ESCC) is the third most common malignancy of the digestive tract and the fifth leading cause of cancer-related death worldwide.29-36 The prognosis of ESCC remains to be poor with a 5-year survival rate <20%.37,41 In this study, we found that NCOA1 is highly enriched in human ESCC, and the levels of NCOA1 are clinically important in ESCC progression and patient prognosis. Functional studies demonstrated that NCOA1 is capable of promoting ESCC cell growth and progression. Multiple lines of evidence derived from the current study underscore the potential for NCOA1 to be developed as a biomarker of ESCC and a molecular target of treatment.

2 | MATERIALS AND METHODS

2.1 | Population

Totally, 80 paraffin-embedded tissue samples from human primary ESCC and their paired adjacent normal tissues were collected in the Affiliated Tumor Hospital of Shantou University Medical College from 2010 to 2011. All ESCC patients were clinically diagnosed and confirmed histologically. No subjects underwent adjuvant or chemotherapy or radiation treatment prior to surgery. The study protocol was reviewed and approved by the Ethics Committee of Shantou University Medical College.

2.2 | Tissue microarray array (TMA) and immunohistochemistry (IHC)

Paraffin blocks with tumor tissues were identified by hematoxylin and eosin (H&E), and areas with respective histopathological features were marked on the block. The cylindrical tumor cores were punched and transferred to the recipient block using a 2.0-mm diameter precision punch. Blocks of the tissue microarray (TMA) were cut into 4-μm sections and processed for IHC as described previously.42-45 TMA slides were incubated with anti-NCOA1 antibody (sc-8995, Santa Cruz) at room temperature for 1 hour followed by incubation with the HRP-conjugated secondary antibody at room temperature for 30 minutes. Immunostaining was visualized by 3, 3′-diaminobenzidine (DAB), and the cell nuclei were counterstained by hematoxylin. Immunostaining intensities were semi-quantitatively graded by the percentage of positive cells. Each staining was evaluated by two independent investigators who were unaware of the clinical and pathological information. Receiver operating characteristic (ROC) curve analysis was performed to assess cutoff score for overexpression of NCOA1.

2.3 | Bioinformatics analysis

The ESCC dataset (GSE63941) from Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) was used to estimate the mRNA levels of NCOA1 in ESCC cell
lines. A dataset (titled “Hu Esophagus 2 statistics”) from the Oncomine database (https://www.oncomine.org/) was used to analyze the copy numbers of NCOA1 in ESCC tissues. A TCGA dataset named Esophageal Carcinoma (TCGA, Provisional), which includes esophageal adenocarcinoma (EA) and ESCC patients, was obtained from the cBioPortal (https://www.cbioportal.org/). The expression profiles of ESCC specimens (n = 95) were extracted and used to access the NCOA1 expression.

2.4 | Cells and cell culture

The ESCC cell lines including KYSE510 (obtained from the tumor cell bank of the Chinese Academy of Medical Science), HKESC-1, and HKESC-2 (kindly provided by Dr SW Tsao, the University of Hong Kong) were cultured in RPMI-1640 Medium (Invitrogen, Carlsbad, CA), TE-1 and TE-12 cells (kindly provided by Dr SW Tsao, the University of Hong Kong) were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT). The immortalized esophageal cell lines (NE2, kindly provided by Dr SW Tsao, the University of Hong Kong) were maintained in medium of 1:1 mixture of DK-SFM: Epi-Life serum-free medium (Invitrogen). All cells were maintained in monolayer at 37°C in humidified air with 5% CO₂.

2.5 | Generation of stable cell lines

KYSE510 cells were transfected with plasmid expressing shRNA targeting NCOA1 (shNCOA1 #1 and shNCOA1 #2) or shControl vector (shCtrl) using lipofectamine 3000 (Life Technologies, Carlsbad, MD, USA) according to the manufacturer’s instructions. Stable cells were selected by culturing the cells in the media with puromycin for 2 weeks. The sequences of shNCOA1 #1 and shNCOA1 #2 are CCTCAGGGCAGAGAACCATCT and CACGACGAAATAGCCATAC, respectively.

2.6 | Western blotting

Whole cell lysates were prepared by lysing the cells in lysis buffer, and cell lysates with equal amount of proteins were separated on 10% SDS-PAGE and transferred to PVDF membranes. The membranes were then incubated with primary antibodies at 4°C overnight. The primary antibodies used in this study were anti-NCOA1 (sc-8995; Santa Cruz, CA, USA) and anti-Tubulin (sc-9104; Santa Cruz). Membranes were then probed with secondary antibodies for 1 hour at room temperature. Blotted proteins were visualized by incubating in SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) followed by exposure to X-ray film (Eastman Kodak, Rochester, NY, USA).

2.7 | Cell proliferation assay

Real-time cell analysis (RTCA) was performed to estimate cell proliferation using the xCELLigence DP device (ACEA Biosciences) as described in the supplier’s instructions. In brief, two thousand cells were seeded in E-plates, and the plates were locked into the RTCA DP device supplied with humidified air with 5% CO₂ at 37°C. The proliferative ability was monitored by the xCELLigence RTCA Analyzer (Roche Applied Science, Mannheim, Germany).

2.8 | Quantitative real-time PCR

Total RNA was extracted from cells using TRIzol (Invitrogen) according to the manufacturer’s instruction and 2 μg RNA was reversely transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The cDNA was used as template for quantitative real-time PCR with the following primers for NCOA1: 5′-GAATCCTTGGGACCTCTT-3′ (forward) and 5′-TGGCTATTTCGTCGTGTT-3′ (reverse) and for β-actin 5′-GAACCCCAAGGCCAACCGAGA-3′ (forward) and 5′-TGACCCCGTCACCGGAGTCCATC-3′ (reverse).

2.9 | Cell migration and invasion assay

Cell migration and invasion assays were conducted using the xCELLigence RTCA Analyzer (Roche Applied Science, Mannheim, Germany). Briefly, 150 μL of RPMI-1640 supplemented with 10% FBS was added to the lower chamber in the CIM-16 plate (16-well, 8-μm pore filter) and 3 × 10⁴ cells in 100 μL of serum-free RPMI1640 were added to the upper chamber coated with or without Matrigel (BD Biosciences, Bedford, MA, USA). Cell index values that represented relative changes in electrical impedance on the underside of the 8-μm pore membrane were taken at a 3-hours interval.

2.10 | Gene set enrichment analysis

Microarray data (accession no. GSE23400) were obtained from the Gene Expression Omnibus of NCBI (https://www.ncbi.nlm.nih.gov/geo/) and subjected to Gene set enrichment analysis (GSEA) using GSEA software (version 2.0.13; https://www.broadinstitute.org/gsea/index.jsp).

2.11 | Statistical analysis

SPSS 13.0 (SPSS Inc, Chicago, IL) was used to analyze the data. The correlation between the expression of NCOA1 and clinic-pathological features of ESCC patients was examined.
by chi-square test. Student’s (or paired) t test was conducted for comparisons between two groups. All data were expressed as mean ± SD. A P-value <0.05 is considered statistically significant.

3 | RESULTS

3.1 | Overexpression of NCOA1 in human ESCC

Given the fact that dysregulation of NCOA1 was found in multiple types of cancers, we were interested in knowing if this coactivator plays any role in ESCC. To do so, we first examined both the mRNA levels and the copy numbers of NCOA1 in human ESCC. Analysis of the Oncomine database found that the copy number of NCOA1 in human ESCC specimens (n = 30) is higher than that in blood samples (n = 12; P < 0.001, Figure 1A) suggesting a potential gene amplification of NCOA1 in ESCC. In line with this finding, we found that 15 of 22 ESCC cell lines in a published dataset (GSE63941) with higher levels of NCOA1 mRNA than that of fibroblast from normal esophageal tissue (Figure 1B). Immunoblotting assays showed that compared to that in the NE2, an immortalized esophageal epithelial cell line, all 5 examined ESCC cell lines showed elevated protein levels of NCOA1 (Figure 1C). Next, we conducted a tissue microarray (TMA) to compare the NCOA1 protein levels in ESCC specimens with their adjacent non-tumor tissues (ANT) from 80 ESCC patients. The immunohistochemical staining results showed that the NCOA1 levels are significantly higher in the ESCC tissues than that of the paired ANT (P < 0.01, Figure 2). These results altogether suggest that higher NCOA1 is associated with human ESCC.

3.2 | The levels of NCOA1 in ESCC progression and patient overall survival

To determine the clinical importance of elevated levels of NCOA1 in human ESCC, we first compared NCOA1 expression with the other clinicopathological parameters in the aforementioned cohort of 80 ESCC patients and found that

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**FIGURE 1** NCOA1 is upregulated in human ESCC cells. A, Comparison of the gene copy numbers of NCOA1 in ESCC with that in the blood in Oncomine database. B, The mRNA levels of NCOA1 in ESCC cell lines (filled bars) and normal esophageal cells (open bars) were obtained from the ESCC dataset GEO, GSE63941. C, The protein levels of NCOA1 in a panel of ESCC cell lines estimated by a Western blot with tubulin as an internal control.
the NCOA1 level was not relevant to any clinical-pathological parameters except the tumor size ($P = 0.030$, Table 1). Analysis of the TCGA dataset did not reveal any correlation between the clinicopathological parameters and NCOA1 expression either (Table 2). Then, we validated a reasonable cutoff score for NCOA1 overexpression by performing a receiver operating characteristic (ROC) curve analysis (Figure 3A). Minimum threshold score for overexpression was set at 6 (immunochemistry score), which was closest to the point with both maximum sensitivity and specificity. The cases with scores $\leq 6$ were defined as a low expression of NCOA1, whereas those with scores $>6$ were defined as overexpression of NCOA1. Based on these criteria, Kaplan-Meier analysis found that ESCC patients with overexpression of NCOA1

| Variables          | No. of patients | NCOA1 level Low, no. (%) | High, no. (%) | $P$-value |
|--------------------|-----------------|--------------------------|---------------|-----------|
| Total samples      | 80              | 38 (47.5)                | 42 (52.5)     |           |
| Age (years)        |                 |                          |               |           |
| $\leq 60$          | 41              | 19 (46.3)                | 22 (53.7)     | 0.832     |
| $>60$              | 39              | 19 (48.7)                | 20 (51.3)     |           |
| Gender             |                 |                          |               |           |
| Female             | 19              | 10 (52.6)                | 9 (47.4)      | 0.796     |
| Male               | 61              | 28 (45.9)                | 33 (54.1)     |           |
| Tumor depth        |                 |                          |               |           |
| T1/T2              | 13              | 6 (46.2)                 | 7 (53.8)      | 0.519     |
| T3/T4              | 67              | 32 (47.8)                | 35 (52.2)     |           |
| Tumor size         |                 |                          |               |           |
| $<5$               | 49              | 21 (42.9)                | 28 (57.1)     | 0.030     |
| $\geq 5$           | 31              | 6 (19.4)                 | 25 (80.6)     |           |
| Stage              |                 |                          |               |           |
| I/II               | 22              | 11 (50.0)                | 11 (50.0)     | 0.203     |
| III/IV             | 58              | 27 (46.6)                | 31 (53.4)     |           |
| pN status          |                 |                          |               |           |
| N1-N3              | 45              | 20 (44.4)                | 25 (55.6)     | 0.535     |
| N0                 | 35              | 18 (51.4)                | 17 (48.6)     |           |

High in this analysis is based on a NCOA1 level $>6$; the remaining individuals were classified as low.
| Variables                  | No. of patients | NCOA1 level Low, no. (%) | High, no. (%) | P-value |
|---------------------------|----------------|--------------------------|--------------|---------|
| Total samples             | 95             | 29 (30.5)                | 66 (69.5)    |         |
| Age (years)               |                |                          |              |         |
| ≤60                       | 70             | 20 (28.6)                | 50 (71.4)    | 0.644   |
| >60                       | 25             | 9 (36.0)                 | 16 (64.0)    |         |
| Gender                    |                |                          |              |         |
| Female                    | 14             | 1 (7.1)                  | 13 (92.9)    | 0.057   |
| Male                      | 81             | 28 (34.6)                | 53 (65.4)    |         |
| Distant metastasis        |                |                          |              |         |
| M0                        | 51             | 28 (54.9)                | 23 (45.1)    | 1.000   |
| M1                        | 44             | 1 (2.3)                  | 43 (97.7)    |         |
| Tumor depth               |                |                          |              |         |
| T1/T2                     | 41             | 10 (24.4)                | 31 (75.6)    | 0.371   |
| T3/T4                     | 54             | 19 (35.2)                | 35 (64.8)    |         |
| Stage                     |                |                          |              |         |
| I/II                      | 65             | 17 (26.2)                | 48 (73.8)    | 0.236   |
| III/IV                    | 30             | 12 (40.0)                | 18 (60.0)    |         |
| Differentiation           |                |                          |              |         |
| Poor                      | 26             | 5 (19.2)                 | 21 (80.8)    | 0.211   |
| Moderate-Well             | 69             | 24 (34.8)                | 45 (65.2)    |         |
| Alcohol use               |                |                          |              |         |
| Yes                       | 69             | 20 (29.0)                | 49 (71.0)    | 1.000   |
| No                        | 26             | 9 (34.6)                 | 17 (65.4)    |         |
| pN status                 |                |                          |              |         |
| N1-N3                     | 40             | 14 (35)                  | 26 (65)      | 0.368   |
| N0                        | 55             | 15 (27.3)                | 40 (72.7)    |         |

High in this analysis is based on a NCOA1 level >1463.4; the remaining individuals were classified as low.

**FIGURE 3** NCOA1 overexpression and poor clinical outcomes of ESCC. A, ROC curve analysis was performed to identify the optimal cutoff value for the overexpression of NCOA1. The sensitivity and specificity of each cutoff point for NCOA1 were calculated, and the results were plotted as a ROC curve. IHC stained samples were grouped into high NCOA1 expression (n = 42) and low NCOA1 expression (n = 38) by ROC analysis. B, Kaplan-Meier curves showed the overall survival of 80 ESCC patients with high and low protein levels of NCOA1. C, The relationship between overall survival and mRNA levels of NCOA1 in a cohort of 95 ESCC patients.
have poor overall survival; the median survival time for ESCC patients with high NCOA1 was 16.0 months compared to 41.0 months for those with low NCOA1 expression ($P = 0.017$, log-rank test, Figure 3B). We further validated our findings in an ESCC-related dataset (n = 95) from TCGA. To validate a reasonable cutoff score for NCOA1 overexpression, a receiver operating characteristic (ROC) curve analysis was performed, and the cutoff score for NCOA1 overexpression was set at 1463.4 (mRNA expression value), which was closest to the point with both maximum sensitivity and specificity. The cases with scores $\leq 1463.4$ were defined as a low NCOA1, whereas those with scores $>1463.4$ were defined as overexpression of NCOA1 (Figure S1). Figure 3C showed that ESCC patients with high NCOA1 expression are closely associated with poorer overall survival ($P = 0.014$, log-rank test). In addition, Multivariate Cox regression analysis of the 80 patient

### Table 3

Univariate and multivariate Cox proportional hazards model showing variables that affect overall survival in ESCC patients (n = 80)

| Variables          | Univariate analysis | Multivariate analysis |
|--------------------|---------------------|-----------------------|
|                    | HR (95% CI)         | P-value   | HR (95% CI)         | P-value   |
| Age                |                     |           |                     |           |
| $\leq 60$ vs $>60$  | 1.793 (0.892-3.606) | 0.101     | 2.260 (0.982-5.202) | 0.055     |
| Gender             |                     |           |                     |           |
| Male vs Female     | 1.106 (0.496-2.463) | 0.806     | 1.715 (0.716-4.107) | 0.226     |
| pTNM stage         |                     |           |                     |           |
| III-IV vs I-II     | 2.391 (1.119-5.106) | 0.024     | 2.952 (0.788-11.052) | 0.108     |
| NCOA1 expression   |                     |           |                     |           |
| High vs Low        | 2.27 (1.128-4.570)  | 0.022     | 2.386 (1.049-5.426) | 0.038     |
| pN status          |                     |           |                     |           |
| N1-N3 vs N0        | 2.164 (1.000-4.682) | 0.050     | 1.300 (0.502-3.371) | 0.589     |
| Tumor depth        |                     |           |                     |           |
| T1/T2 vs T3/T4     | 2.572 (0.783-8.447) | 0.119     | 1.070 (0.277-4.127) | 0.922     |
| Tumor size         |                     |           |                     |           |
| $<5$ vs $\geq 5$   | 1.835 (0.911-3.695) | 0.089     | 2.017 (0.794-5.124) | 0.140     |

### Table 4

Univariate and multivariate Cox proportional hazards model showing variables that affect overall survival in ESCC patients from TCGA dataset (n = 95)

| Variables          | Univariate analysis | Multivariate analysis |
|--------------------|---------------------|-----------------------|
|                    | HR (95% CI)         | P-value   | HR (95% CI)         | P-value   |
| Age                |                     |           |                     |           |
| $\leq 60$ vs $>60$  | 1.710 (0.806-3.629) | 0.162     | 2.018 (0.883-4.611) | 0.096     |
| Gender             |                     |           |                     |           |
| Male vs Female     | 5.266 (1.221-22.713) | 0.026    | 7.807 (0.973-62.650) | 0.053     |
| Differentiation    |                     |           |                     |           |
| Poor-Moderate vs Well | 1.168 (0.534-2.555) | 0.698    | 0.896 (0.336-2.390) | 0.826     |
| pT status          |                     |           |                     |           |
| T3-T4 vs T1-T2     | 1.279 (0.615-2.662) | 0.510     | 1.274 (0.406-3.999) | 0.678     |
| pN status          |                     |           |                     |           |
| N1-N3 vs N0        | 2.015 (0.975-4.164) | 0.058     | 1.132 (0.343-3.730) | 0.839     |
| Distant metastasis |                     |           |                     |           |
| M1 vs M0           | 2.177 (0.649-7.298) | 0.208     | 2.399 (0.572-10.059) | 0.232     |
| pTNM stage         |                     |           |                     |           |
| III-IV vs I-II     | 2.391 (1.161-4.922) | 0.018     | 2.337 (0.591-9.239) | 0.226     |
| Alcohol use        |                     |           |                     |           |
| Yes vs No          | 2.008 (0.698-5.780) | 0.196     | 2.357 (0.769-7.223) | 0.134     |
| NCOA1 expression   |                     |           |                     |           |
| High vs Low        | 1.601 (0.692-3.705) | 0.272     | 2.936 (1.140-7.563) | 0.026     |
cohort (Table 3) and the TCGA dataset (Table 4) showed that NCOA1 expression is an independent predictor of prognosis for ESCC patients with a hazard ratio (HR) of 2.386 (95% confidence interval [CI] = 1.049‐5.426, \( P = 0.038 \)) and 2.936 (CI = 1.140‐7.536, \( P = 0.026 \)), respectively. These data indicate that the levels of NCOA1 in human ESCC are closely associated with patient outcome.

### 3.3 The effect of NCOA1 on growth, migration, and invasion of ESCC Cells

To dissect the underlying molecular mechanisms of elevated levels of NCOA1‐mediated cellular behaviors, we knocked down NCOA1 by shRNA technique and analyzed the effect on cell proliferation, migration, and invasion. Since KYSE510 has the highest NCOA1 expression among all the ESCC cells analyzed (Figure 1C), we decided to conduct these experiments in this specific cell line. First, we estimated the knockdown efficiencies of two shRNAs (shNCOA1 #1 and shNCOA1 #2) and found that both shRNAs knocked down NCOA1 efficiently although shRNA2 appears to be a bit better than shRNA1 (Figure S2). We then transfected KYSE510 cells with shRNA2 and conducted different assays using the xCELLigence system. Figure 4A showed that compared to the control knocking down NCOA1 significantly inhibited proliferation of KYSE510 cells (\( P < 0.05 \)). In addition, Figure 4B,C showed that knockdown NCOA1 in KYSE510 cell significantly decreased cell migration and invasion (\( P < 0.05 \)). These data altogether indicate that NCOA1 plays essential roles in ESCC cell growth, migration, and invasion. These observations are in line with the fact that elevated levels of NCOA1 in ESCC were accompanied by aggressive progression and poor patient outcomes.

### 3.4 The oncogenic role of NCOA1 in ESCC through multiple signaling pathways

Since NCOA1 has been reported to be involved in multiple signaling pathways in a wide spectrum of cancer types, we...
decided to conduct a gene set enrichment analysis (GSEA) of the published human ESCC expression profile (GSE23400). The results from this analysis suggest that in ESCC cells both VEGF and MEK pathways are robustly positively correlated with NCOA1 (Figure 5). Whether the effect of NCOA1 on ESCC cell growth, migration, and invasion are through either or both VEGF and MEK pathways need to be further investigated.

**4 | DISCUSSION**

Despite the fact that dysregulation of NCOA1 has been documented in multiple types of human cancers, the clinical importance of NCOA1 in tumor progression and clinical outcome of human ESCC were unappreciated. By analyzing multiple ESCC patient cohorts, we defined the heretofore undocumented associations between NCOA1, malignant properties, and poor survival of human ESCC and identified NCOA1 overexpression as an independent predictive factor for ESCC patient prognosis. In addition, we have shown that NCOA1 plays essential roles in growth, migration, and invasion of ESCC cells. These findings underscore the rationale for using NCOA1 as a biomarker and potential molecular target for potential treatment.

NCOA1 overexpression has been demonstrated in multiple cancers. The elevated levels of NCOA1 mRNA and increased gene copy number of NCOA1 found in this research suggest that both gene amplification and increased transcription could be part of the underlying mechanisms of NCOA1 overexpression in human ESCC. In addition, Cox analysis showed that the protein levels of NCOA1 are inversely proportional to ESCC patient survival. This suggests that NCOA1 could also serve as a potential biomarker for ESCC prognosis. All these findings are consistent with the report that patients with NCOA1 overexpression along with low expression of miR-105-1 were closely associated with both poor overall survival and progression-free survival in hepatocellular cancer.

Although NCOA1 is the founding member of the p160 family, its functional roles in oncogenesis and progression are not as well-established as that of SRC-3, another member of the family with well-defined oncogenic activity. It has been reported that NCOA1 can promote proliferation of both breast cancer and hepatocellular cancer cells. But results from mice with NCOA1 deletion suggest that NCOA1 is capable of promoting breast cancer metastasis without affecting primary tumor growth, and mechanistically proposed as through NCOA1 mediated crosstalk between tumor cells and their microenvironment. We demonstrated that NCOA1 plays essential roles in ESCC cell growth, migration, and metastasis suggesting that NCOA1 is important in each of these processes in ESCC cells. These ambiguous results suggest that NCOA1’s diverse and distinct functions are likely cancer-type specific.

**FIGURE 5** NCOA1 expression is positively correlated with both the VEGF and MEK pathways in ESCC. NCOA1 is positively associated with oncogenic pathways. Gene set enrichment analyses showed positive correlations between NCOA1 expression and a VEGF gene signature (VEGF_A_UP.V1_UP_137) and a MEK gene signature (MEK_UP.V1_UP_194) in a published cohort of ESCC patients (GSE23400)
Given the marked gender disparity in incidence and clinical outcomes of human ESCC, one intriguing question is how sex steroid signaling pathways are involved in the regulation of ESCC oncogenesis and progression. We and others have previously shown that AR and ERβ signaling pathways play important roles in tumorigenesis and progression of esophageal carcinoma. Coactivators such as NCOA1 are considered as master modulators in the coordination of nuclear receptor transcription and subsequent sex steroid-mediated signaling pathways. Based on the fact that NCOA1 is overexpressed in both patient samples and ESCC cancer cell lines, further study to better understand the interplay between NCOA1 and sex steroid receptors (ie, AR and ER) in ESCC is warranted.

In summary, we found that NCOA1 overexpression is a common phenomenon in both ESCC patients and ESCC cell lines. Overexpressed NCOA1 plays essential roles in each of the ESCC progression processes including proliferation, migration, and invasion. Therefore, the levels of NCOA1 could be used as a potential prognostic biomarker and targeting NCOA1 could be a strategy in the development of therapies for ESCC treatment.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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