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Abberant Immunohistochemical Expression of OCT3/4 and EMT Related Markers, Vimentin and E-cadherin, is Correlated with Adverse Histopathological Features in Colorectal Adenocarcinoma

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Rezumat

Expresia imunohistochimică aberantă a markerilor EMT, Vimentina și E-caderina și OCT3/4 este corelată cu parametri histopatologici adverși în adenocarcinomul colorectal

Scop: Deşi managementul clinic al cancerului colorectal a fost îmbunătățit semnificativ, acesta se confruntă cu o incidență în creştere în rândul tinerilor şi în rândul populațiilor din țările în curs de dezvoltare. Mai mult decât atât, diagnosticul este pus mai ales în stadii avansate, când resursele terapeutice sunt limitate. Prin urmare, avem nevoie de noi biomarkeri pentru diagnosticare şi noi ținte terapeutice. Evenimentul cheie care duce la invazie şi metastază este tranziția epitelial-mezenchimală (EMT), care poate fi studiată cu markeri IHC. Am urmărit corelarea expresiei markerilor legați de EMT (Vimentină şi E-caderină) şi a unui marker de celule stem (OCT 3/4) cu parametrii clinicopatologici ai tumorilor.

Material şi metode: Au fost evaluate probe de rezecție chirurgicală de la 30 de pacienți cu cancer de colon fără tratament neo-adjuvant, internați în perioada 2018-2021. Au fost efectuate teste imunohistochimice pentru a investiga expresia markerilor legați de EMT şi OCT 3/4 în celulele tumorale.

Rezultate: Pozitivitatea Vimentinei, OCT3/4 şi pierderea E-cadherinei au fost asociate semnificativ cu gradul tumorii, mugurii tumorali, frontul tumoral invaziv şi metastaze limfoganglionare.
Concluzii: Vimentina, E-caderina şi OCT 3/4 ar putea servi ca un panel de biomarkeri care pot ajuta la prognosticul pacienților, cu potențial de a fi ținte terapeutice.

Cuvinte cheie: adenocarcinom de colon, EMT, Vimentina, E-caderina, OCT3/4, muguri tumorali, front tumoral invaziv

Abstract
Background: Although clinical management for colorectal cancer has been markedly improved, it is faced with a growing incidence among the young and among those in developing nations. Furthermore, diagnosis occurs mostly in advanced stages, when the therapeutic resources are limited. Therefore we need new biomarkers for diagnostics and therapeutic targets. The key event that leads to invasion and metastasis is the epithelial to mesenchymal transition (EMT), which can be studied with IHC markers. We aimed to corelate the expression of EMT related markers (Vimentin and E-cadherin) and a stem cell marker (OCT 3/4) with the clinicopathological parameters of the tumors.
Material and Methods: Surgical resection specimens from 30 treatment-naive colon cancer patients, hospitalized from 2018 to 2021 were assessed. Immunohistochemical tests were performed to investigate the expression of EMT related markers and OCT 3/4 in tumor cells.
Results: Vimentin, OCT3/4 positivity and loss of E-cadherin were significantly associated with tumor grade, tumor budding, invasive tumor front, and lymph node metastasis.
Conclusions: Vimentin, E-cadherin and OCT 3/4 might serve as a panel of biomarkers that can aid in the prognostication of patients, with the added potential of being oncotargets.

Key words: colon adenocarcinoma, EMT, Vimentin, E-cadherin, OCT3/4, tumor budding, invasive tumor front

Introduction

Colorectal adenocarcinoma (COAD) is a global public health issue, as it remains the third most common cancer and the second highest leading cause of cancer-related deaths world-wide (1), with an increasing incidence among the young and among those living in developing nations (2). Despite considerable new advancements in screening, diagnosis and treatment protocols, including surgical interventions, chemotherapy and radiation therapy, the clinical management for patients with COAD remains to be improved. It is essential to grasp a better understanding of the development and evolution of this disease, in order to create novel strategies for the treatment and prognostic stratification of COAD.

The most nefarious hallmarks of cancer consist of the properties related to invasion and metastasis (3), which are responsible for the majority of cancer related deaths. In order to attain their metastatic potential, the tumor cells must undergo specific step-by-step trans-formations culminating with the epithelial to mesenchymal transition (EMT)(4). As its name implies, EMT is a well-coordinated biological cellular program characterized by cytoskeleton remodeling and changes in cell-matrix adhesion, in which the epithelial cells lose their exclusive features such as cell-to-cell junctions and the vertical polarity, while gaining properties of mesenchymal cells such

as spindle-like morphology that lacks vertical polarity, causing increased motility and invasiveness of the cancer cells (4-5). The epitome of the EMT process is the up-regulation of mesenchymal markers and down-regulation of structural adhesion proteins. During the EMT the tumor cells start to lose the expression of E-cadherin, an event that leads to the reduction of the cell-to-cell adhesion. This is paralleled by the elevated expression levels of the mesenchymal marker Vimentin (6-8). Besides serving as a functional and morphological framework for tissue invasion and metastasis, the EMT is also an obstacle in the success of cancer therapy by being a pivotal process in tumor heterogeneity and therapy resistance (9-10).

In COAD there seems to exist a morphological indicator of undergoing EMT, in the form of tumor buds (12), which are defined as the presence of single tumor cells or small tumor clusters composed of up to four cells at the invasive front of the tumor and it has been incorporated as an additional prognostic factor for COAD in the 8th Edition of the TNM Classification of Malignant Tumors (13) and the WHO classification of 2019 (15). It has been showed that tumor buds are at least partially synonymous with EMT, as they lose E-cadherin expression and co-express cytokeratin and Vimentin (14).

Vimentin is a 57 KD, type III intermediate filament protein that is ubiquitously expressed in normal mesenchymal tissues, in various stages of development, contributing to cellular and tissular structure and integrity (11) and it is strongly upregulated following injury to various tissues (16). VIM influences the cell shape and mobility during the EMT, being abnormally expressed in epithelial cells. Furthermore, gene knockdown of VIM resulted in decreases in cell mobility and invasiveness in colon carcinoma cell lines (17). Thus, it can be used to identify cancer cells undertaking EMT as there is a positive association of Vimentin expression with augmented invasiveness and metastasis (6,19).

E-cadherin (E-CAD) is a calcium-dependent transmembrane glycoprotein, encoded by the CDH1 gene, and a member of the "classical" or type I Cadherins subfamily (18). Although it does not possess enzymatic activity, it acts as a tumor suppressor in numerous human epithelial tumors, inhibiting migration and metastasis (20-21). E-CAD facilitates the assembly of intercellular junctions and it serves as an adhesion molecule. It is localized at the basolateral surface of epithelial cells, and it is involved in cell-to-cell interactions, both in normal and cancer cells (22). Unlike Vimentin, studies have shown that inhibition of CDH1 gene expression in colon cancer cell lines promotes cell migration and invasion (23-24), and it can be used to identify cells that undergo EMT by showing a negative correlation (i.e., loss of expression). Besides structural proteins like VIM and E-CAD, several other EMT associated transcription factors like Slug, Twist, Zeb1 and Zeb2 have been reported as markers for aggressive tumor markers in various cancers including colorectal carcinoma (25-26).

Recently, the octamer-binding transcription factor 4 (also known as OCT3/4, OTF3, or POU5F1), a transcription factor that ensures survival and maintains pluripoteniality of embryonic stem cells (27), has been showed to be involved in the progression of various types of cancer, including breast, non-small cell lung carcinoma, gliomas, gastric cancers, and COAD (28-34). Being a stem cell marker, it is currently widely used for the diagnosis of germ cell tumors (36). One previous study demonstrated that OCT4 gene knockdown induced EMT-like cell morphological changes and EMT marker expression alteration, indicating that OCT4 is involved in the EMT process in colorectal cancer cells (35).

Therefore, this study was performed in part to evaluate the expression of EMT markers, Vimentin and E-cadherin and the correlation with the clinicopathological parameters in COAD matched with normal tissue. Our secondary objective was to evaluate the expression of OCT3/4 in comparison with the EMT markers and the clinicopathological parameters in hoping to better understand the relationship between stem cells and EMT.

Material and Methods

Our tumor series comprised 30 colorectal carcinoma cancer patients, who underwent surgical resection, between 2018 and 2021. Inclusion criteria: patients with colorectal cancer confirmed on resection specimens, operated between 2018-2021, aged >18 years, with complete clinical and paraclinical data, no neoadjuvant treatments prior to surgical resection, with complete immunohistochemical results for Vimentin, E-cadherin, and OCT3/4. Patients with unresected colorectal cancers, those with chemotherapeutic treatments prior to colonic resection, those with incomplete clinical-paraclinical data, or those in whom immunohistochemical testing was not performed were excluded.

All the relevant clinicopathologic characteristics obtained from staging (sex, age, tumor localization, tumor stage, tumor grade, histological type, tumor budding, number of involved lymph nodes, presence of satellite nodules, lymph node stage, type of tumor front, venous-lymphatic invasion and perineural invasion) were assessed for all patients.

All tumors were chemo-/radiation-naïve, none of the patients received chemo-radiotherapy prior to surgery. Surgical specimens were histologically evaluated based on the 8th edition of the American Joint Committee on Cancer guidelines. Hematoxylin-Eosin (HE) stained sections were reviewed by two pathologists, who confirmed the diagnosis of COAD and staged the tumors.

Tumor Budding Evaluation

The evaluation of tumor buds was done with regards to the criteria established by the International Tumor Budding Consensus Conference (ITBCC) (36). Hotspot areas were identified at the invasive front by light microscopy on histology slides stained with the usual, HE stain, using a scanning magnification. Tumor buds were defined as single tumor cells or small clusters composed of maximum 4 tumor cells. The buds were counted on a single field of a 20 x objective lens, corresponding to a 0.785 mm^2 area. The cases were classified based upon the number of buds in order of severity into Bd1 –low (0-4 buds/0.785 mm^2), Bd2 - intermediate (5-9 buds), and Bd3 - high (≥10 buds).

Lymph Node Status Evaluation

Based on the total number of metastatic lymph nodes, the cases were further divided into N groups as follows: a) pN0 (no metastatic lymph nodes); b) pN1 (metastases in one or two lymph nodes); and c) pN2 (more than two metastatic lymph nodes). We also calculated the metastatic lymph node index by dividing the number of metastatic lymph nodes to the total number of lymph nodes identified in the surgical specimens.

Type of Tumor Front Evaluation

Tumor growth pattern at the tumor invasion front was assessed at scanning magnification and categorized as expansive or infiltrative as described by a previous study (37). The growth pattern was considered expansive when the tumor margin was non-infiltrative, pushing and well-circumscribed. It was considered infiltrative when tumor glands or irregular trabeculae or nests of tumor cells invaded in a haphazard manner with widespread infiltration of adjacent tissue without a clear, pushing border.

Venous Invasion Type

Venous invasion, when present, was divided into two groups, namely extramural type (EMVI - beyond muscularis propria) or intramural type (IMVI - restricted to submucosa or muscularis propria). This distinction was performed because it has been showed by multivariate analysis that EMVI is an independent adverse prognostic factor for COAD and is correlated with liver metastasis (38).

Immunohistochemistry (IHC)

IHC tests were performed on 2 μm sections from formalin-fixed paraffin-embedded tissues (fixed in 10% neutral buffered formalin), from each tumor, by utilizing the CONFIRM anti-Vimentin (V9) primary antibody (Ventana Medical Systems, USA). An automated immunostainer (Ventana Bench Mark XT) was used to perform the IHC procedure. For E-cadherin and OCT3/4 we used the 36B5 and N1NK antibody clones respectively (Leica Microsystems GmbH, Germany). An automated immunostainer (BOND-III Fully Automated IHC and ISH Staining System - Leica Microsystems GmbH, Germany) was also used for these antibodies. We anticipated the risk of false negative reactions due to heterogenous staining by assessing tissue microarrays, so we only used full slides. The Vimentin IHC slides were evaluated by examining cytoplasmic staining. Vimentin cytoplasmic expression in more than 5% of tumor cells was considered positive and less than 5% of tumor cells was considered negative (39). For E-cadherin, membranous staining was considered positive and a total loss of expression in more than 5% of the tumor cells, or a partial loss in more than 20% was considered significant. For OCT3/4 any convincing cytoplasmic or nuclear staining in more than 1% of the tumor cells was considered positive.

Statistical Analysis

The data were processed using SPSS version 23.0, running on a Windows 10 operating system. For descriptive statistics, the means and standard deviations were calculated, the medians and quartiles for the quantitative variables, and the frequency and percentage for qualitative variables, respectively.

Compared to the quantitative data, depending on the normality of the data, the Student t-test (Independent Sample T-test) (for two groups with normally distributed data) and Mann-Whitney (for data that do not have a normal distribution) were used, respectively.

Quantitative data were tested for normality (Shapiro-Wilk test) and variant homogeneity using the Levene test. Fisher's Exact Test was used for the data. The probability of error less than 5% ($p < 0.05$) was considered the threshold of statistical significance.

Results

Clinicopathological parameters

A total of 30 cases of colorectal cancers were analyzed. Of the 30 cases, 11 (37%) were male, and 19 female (63%). The age range was between 47 and 87 years, with a median of 67.5 years. The right side was involved in 18 cases (60%), the left being involved in 12 cases (40%). Histologically, 27 cases were conventional adenocarcinomas, and 3 cases were mucinous adenocarcinomas, 90%, and 10% respectively. Three tumors were well differentiated, 23 were moderately differentiated, and 4 were poorly differentiated (10%, 76% and 14% respectively). Regarding stage, 2 tumors were T2, 18 were T3 tumors and the remaining 10 being stage 4 (7%, 60%and 33% respectively). Of the 30 cases, lymphovascular invasion was found in 28 cases (93%), with 17 of the EMVI type (57%), the remaining 11 being of the IMVI type (36%). Tumor budding was mostly evaluated as Bd1, consisting of 18 cases (60%), while Bd2 was encountered in 7 cases (23%) and Bd3 in 5 (17%) cases. Perineural invasion was found in 17 cases (57%) the rest of 13 cases no showing PNI (43%). The tumor front was infiltrative in 11 cases and pushing in 19 cases (37% vs 63%). The mean number of lymph nodes harvested was 28.46, and the average number of lymph nodes with metastases was 2.5; 16 patients had a lymph node ratio of zero and 14 patients more than zero (*Table 1*).

Of the 30 cases, 11 showed abnormal cytoplasmic Vimentin positivity (37%), (*Fig. 1*). E-Cadherin loss of expression found in 17 cases (*Fig. 2*). Only 7 cases showed OCT3/4 positivity (*Fig. 3, Table 1*).

Table 1. Demographic and clinicopathological characteristics of patients

	Patient numbers
Sex	
M	11
F	19
Age	
>70	14
≤ 70	16
Side	
Left	12
Right	18
Histological type	
Conventional	27
Mucinous	3
Grading	
G1	3
G2	23
G3	4
T stage	
2	2
3	18
4	10
N Stage	
N0	15
N1	6
N2	9
Tumor front	
Infiltrative	11
Pushing	19
Tumor budding	
Bd1	18
Bd2	7
Bd3	5
Venous invasion	
Not identified	2
EMVI	17
IMVI	11
Perineural Invasion	
Yes	17
No	13
Lymph node ratio	
>0	14
=0	16
Vimentin	
Positive	11
Negative	19
E-cadherin	
No loss of expression	13
Loss of expression	17
OCT3/4	
Positive	7
Negative	23

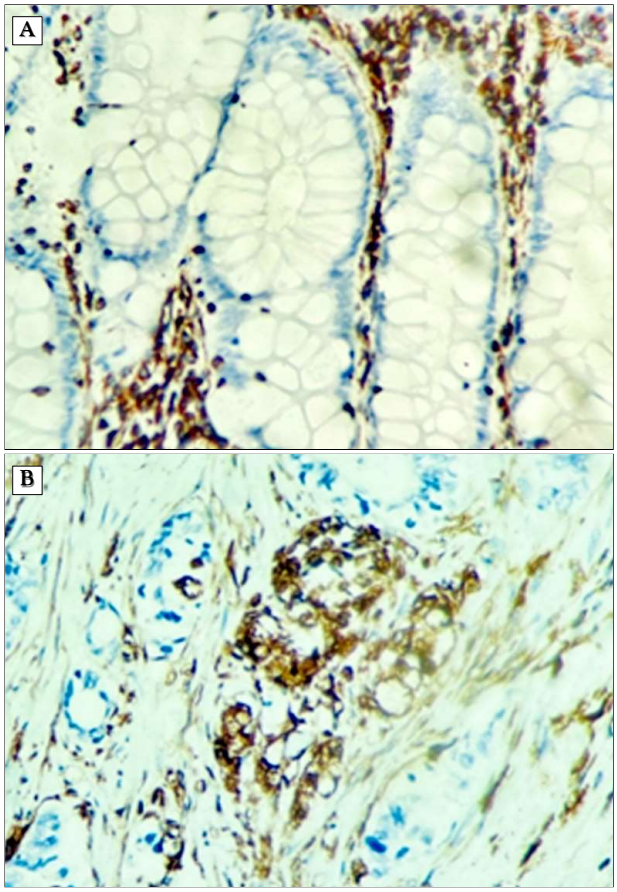


Figure 1. Vimentin is negative in normal colonic epithelial tissue **(A)** and positive in tumor glands **(B)**

Statistical analysis of Vimentin, E-cadherin and OCT 3/4 expression in relation to clinico-pathological parameters

For evaluating the correlation of Vimentin, E-cadherin and OCT3/4 expression with sex, age, sideness, pT category, pN category, number of metastatic lymph nodes, histological grade, type of tumor front, tumor buds, lympho-vascular and perineural invasion were used for statistical tests. We found no significant statistical correlation between the expression of Vimentin, E-cadherin or OCT3/4 and the age, sex, location of the tumors or histological type. Vimentin expression was corelated with tumor grade, being significantly more expressed in G3 tumors (p_value=0.003768). There was a significant correlation between Vimentin positivity and the degree of tumor budding, with an increased positivity in cases with Bd-2 and Bd-3 (p_value= 0.000009). Although there was no correlation with the presence of lymphovascular invasion (p_value=0.519540), there was a significant correlation between the positivity of Vimentin and the type of vascular invasion, being more

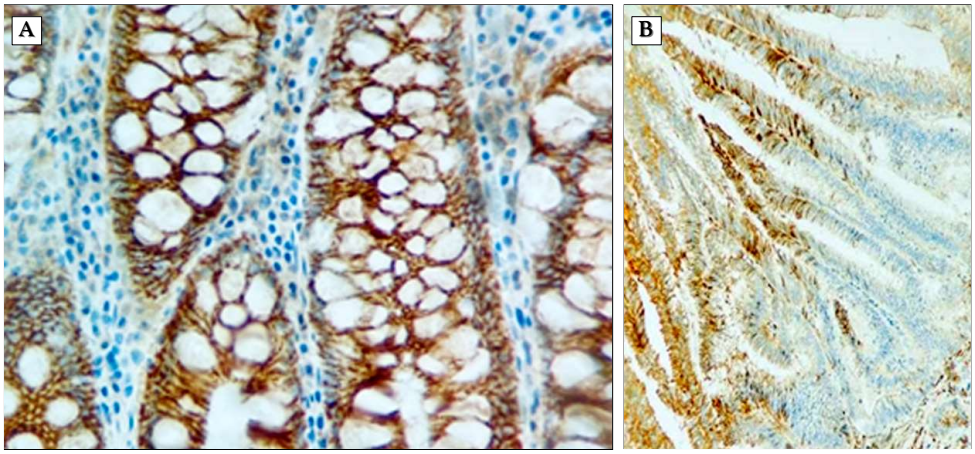


Figure 2. Normal colon epithelium showing no loss of expression of E-CAD **(A)**. COAD showing partial loss of E-CAD **(B)**

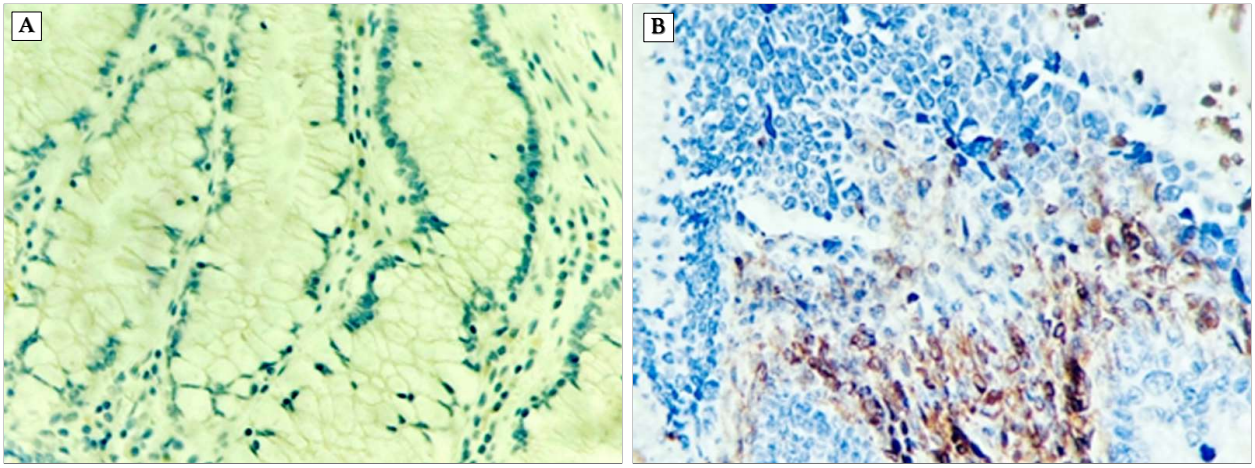


Figure 3. OCT3/4 is negative in normal epithelium **(A)**. COAD case showing OCT 3/4 positivity **(B)**

frequent in the EMVI type (p_value= 0.007857). There were also statistical correlations between Vimentin positivity and pTand pN categories, as it tended to be associated with higher categories (p_value=0.001360, and 0.000114, respectively). Cases with perineural invasion also tended to present Vimentin positivity (p_value=0.000317). There was a strong association of Vimentin expression with the invasive tumor front (p_value=0.000004) (*Table 2*).

Loss of expression of E-cadherin was increased in G3 tumors compared to G1 and G2 (P value=0.005902). Cases with low tumor budding (Bd-1) showed significantly lower loss of expression compared to cases with high tumor budding (Bd-3) (p_value=0.000267). There was no correlation with pT categories, but we found correlations between loss of E-CAD and high pN categories (p_value= 0.039094) and increased number of metastatic lymph nodes (p_value=0.015858). Unlike Vimentin, there was no correlation of E-CAD expression and perineural invasion or lymphovascular invasion (p_value=0.071120 and 0.491954, respectively), however, there was a strong correlation between loss of E-CAD expression and an invasive tumor front (p_ value=0.022746) (*Table 3*).

Besides the previously mentioned, OCT3/4 positivity did not show correlations with perineural invasion, lymphovascular invasion, pT and pN categories. We did, however, found correlations with high tumor grade (p_value= 0.032647), high tumor budding (p_value= 0.003195), number of

Table 2. Vimentin expression and correlation with clinicopathological parameters

	VIM=neg (N=19)	VIM=pos (N=11)	p_value (test)
Age	67.63±11.3930	67.27±10.8911	0.933303 (Independent Samples T Test)
Sex			0.466146(Fisher's Exact Test)
F	11/19 (57.9%)	8/11 (72.7%)	
M	8/19 (42.1%)	3/11 (27.3%)	
Histology			0.279310 (Fisher's Exact Test)
Conventional	16/19 (84.2%)	11/11 (100%)	
Mucinous	3/19 (15.8%)	0/11 (0%)	
Tumor grade			0.003768 (Likelihood Ratio)
G1	3/19 (15.8%)	0/11 (0%)	
G2	16/19 (84.2%)	7/11 (63.6%)	
G3	0/19 (0%)	4/11 (36.4%)	
Tumour Budding			0.000009 (Likelihood Ratio)
Bd-1	17/19 (89.5%)	1/11 (9.1%)	
Bd-2	2/19 (10.5%)	5/11 (45.5%)	
Bd-3	0/19 (0%)	5/11 (45.5%)	
Lymphovascular invasion	2/19 (10.5%)	0/11 (0%)	0.519540 (Fisher's Exact Test)
Lymphovascular invasion type			0.007857(Likelihood Ratio)
None	2/19 (10.5%)	0/11 (0%)	
EMVI	7/19 (36.8%)	10/11 (90.9%)	
IMVI	10/19 (52.6%)	1/11 (9.1%)	
N category			0.000114 (Likelihood Ratio)
0	14/19 (73.7%)	1/11 (9.1%)	
1	4/19 (21.1%)	2/11 (18.2%)	
2	1/19 (5.3%)	8/11 (72.7%)	
T category			0.001360 (Likelihood Ratio)
2	2/19 (10.5%)	0/11 (0%)	
3	15/19 (78.9%)	3/11 (27.3%)	
4	2/19 (10.5%)	8/11 (72.7%)	
Number of positive lymphnodes	0.00 (0.00, 0.00)	4.00 (2.00, 9.00)	0.000113 (Mann-Whitney U Test)
Location			1.000000 (Fisher's Exact Test)
Right colon	11/19 (57.9%)	7/11 (63.6%)	
Left colon	8/19 (42.1%)	4/11 (36.4%)	
PNI +	6/19 (31.6%)	11/11 (100%)	0.000317(Fisher's Exact Test)
ECAD	14/19 (73.7%)	3/11 (27.3%)	0.022746(Fisher's Exact Test)
OCT3/4 +	2/19 (10.5%)	5/11 (45.5%)	0.068031(Fisher's Exact Test)
Tumor front			0.000004 (Fisher's Exact Test)
Expansive	18/19 (94.7%)	1/11 (9.1%)	
Infiltrative	1/19 (5.3%)	10/11 (90.9%)	

metastatic lymph nodes (p_value=0.011315), and invasive tumor front (p_value= 0.004474). Lastly, OCT3/4 positivity was not correlated with Vimentin expression, but it did show correlation with loss of E-CAD (p_value=0.024725)(*Table 4*).

Discussions

Invasion and metastasis are the most dangerous characteristics of malignant tumors. The abnormal expression of cell adhesion molecules plays an important role in improving the mobility of tumor cells which leads to enhanced migration properties and to the formation of metastasis. During EMT, the epithelial cancer cells undergo transformation and they develop mesenchymal properties, losing E-cadherin (40) and gaining Vimentin expression. In this study, we investigated a heterogeneous (with regard to site, tumor budding, stage, tumor grade, invasion front, lymphovascular invasion, perineural invasion) cohort of colon cancer patients for expression of Vimentin, E-cadherin and the stem cell marker OCT3/4. Our study shows that expression of Vimentin in tumors correlates with tumor

Table 3. E-Cadherin expression and correlation with clinicopathological parameters

	ECAD=loss (N=13)	ECAD= no loss (N=17)	p_value (test)
Age	67.15±12.8182	67.76±9.8395	0.883516 (Independent Samples T Test)
Sex			0.708426 (Fisher's Exact Test)
F	9/13 (69.2%)	10/17 (58.8%)	
M	4/13 (30.8%)	7/17 (41.2%)	
Histology			0.237931 (Fisher's Exact Test)
Conventional	13/13 (100%)	14/17 (82.4%)	
Mucinous	0/13 (0%)	3/17 (17.6%)	
Tumor grade			0.005902 (Likelihood Ratio)
G1	0/13 (0%)	3/17 (17.6%)	
G2	9/13 (69.2%)	14/17 (82.4%)	
G3	4/13 (30.8%)	0/17 (0%)	
Tumour Budding			0.000267 (Likelihood Ratio)
Bd-1	3/13 (23.1%)	15/17 (88.2%)	
Bd-2	5/13 (38.5%)	2/17 (11.8%)	
Bd-3	5/13 (38.5%)	0/17 (0%)	
Lymphovascular invasion +	13/13 (100%)	15/17 (88.2%)	0.491954 (Fisher's Exact Test)
Lymphovascular invasion type			0.076991 (Likelihood Ratio)
None	0/13 (0%)	2/17 (11.8%)	
EMVI	10/13 (76.9%)	7/17 (41.2%)	
IMVI	3/13 (23.1%)	8/17 (47.1%)	
N category			0.039094 (Likelihood Ratio)
0	4/13 (30.8%)	11/17 (64.7%)	
1	2/13 (15.4%)	4/17 (23.5%)	
2	7/13 (53.8%)	2/17 (11.8%)	
T category			0.170612 (Likelihood Ratio)
2	0/13 (0%)	2/17 (11.8%)	
3	7/13 (53.8%)	11/17 (64.7%)	
4	6/13 (46.2%)	4/17 (23.5%)	
Number of positive lymphnodes	4.00 (0.00, 8.50)	0.00 (0.00, 1.00)	0.015858 (Mann-Whitney U Test)
Location			1.000000 (Fisher's Exact Test)
Right colon	8/13 (61.5%)	10/17 (58.8%)	
Left colon	5/13 (38.5%)	7/17 (41.2%)	
PNI +	10/13 (76.9%)	7/17 (41.2%)	0.071120 (Fisher's Exact Test)
OCT3/4 +	6/13 (46.2%)	1/17 (5.9%)	0.024725 (Fisher's Exact Test)
Tumor front			0.022746 (Fisher's Exact Test)
Expansive	5/13 (38.5%)	14/17 (82.4%)	
Infiltrative	8/13 (61.5%)	3/17 (17.6%)	

grade, T category, N category, tumor budding, EMVI and peri-neural invasion. The study also shows that loss of E-cadherin significantly correlated with increased tumor grade, increased tumor budding, higher N categories and invasive tumor front. OCT3/4, a stem cell marker that has been involved in EMT, indeed showed significant correlation with morphological clues of EMT such as tumor buds and invasive type front, but also with tumor grade and number of positive lymph nodes. The findings of the present study have also demonstrated that the alteration of expression of Vimentin, E-cadherin and OCT3/4 is absent in normal tissues, being only observed in tumors, which strongly suggests they can be used as biomarkers for aggressive disease.

Vimentin expression has been shown to be consistently associated with the EMT and thus with prognosis in various types of cancer, including breast carcinoma, head and neck squamous cell carcinoma, gastric adenocarcinoma, non small cell lung carcinoma, prostate adenocarcinoma and COAD(41-45). It has been shown that high Vimentin expression, together with another EMT transcription factor (Slug) is associated with lymph node

Table 4. OCT3/4 expression and correlation with clinicopathological parameters

	OCT3/4=neg (N=23)	**OCT3/4=pos (N=7)**	**p_value (test)**
Age	68.30 ±10.8058	64.76 ±12.1850	0.478452 (Independent Samples T Test)
Sex			1.000000 (Fisher's Exact Test)
F	14/23 (60.9%)	5/7 (71.4%)	
M	9/23 (39.1%)	2/7 (28.6%)	
Histology			1.000000 (Fisher's Exact Test)
Conventional	20/23 (87%)	7/7 (100%)	
Mucinous	3/23 (13%)	0/7 (0%)	
Tumor grade			0.032647(Likelihood Ratio)
G1	3/23 (13%)	0/7 (0%)	
G2	19/23 (82.6%)	4/7 (57.1%)	
G3	1/23 (4.3%)	3/7 (42.9%)	
Tumour Budding			0.003195 (Likelihood Ratio)
Bd-1	17/23 (73.9%)	1/7 (14.3%)	
Bd-2	5/23 (21.7%)	2/7 (28.6%)	
Bd-3	1/23 (4.3%)	4/7 (57.1%)	
Lymphovascular invasion +	21/23 (91.3%)	7/7 (100%)	1.000000 (Fisher's Exact Test)
Lymphovascular invasion type			0.148086 (Likelihood Ratio)
None	2/23 (8.7%)	0/7 (0%)	
EMVI	11/23 (47.8%)	6/7 (85.7%)	
MVI	10/23 (43.5%)	1/7 (14.3%)	
N category			0.072622 (Likelihood Ratio)
0	14/23 (60.9%)	1/7 (14.3%)	
1	4/23 (17.4%)	2/7 (28.6%)	
2	5/23 (21.7%)	4/7 (57.1%)	
T category			0.517369 (Likelihood Ratio)
2	2/23 (8.7%)	0/7 (0%)	
3	13/23 (56.5%)	5/7 (71.4%)	
4	8/23 (34.8%)	2/7 (28.6%)	
Number of positive lymphnodes	0.00 (0.00, 1.00)	4.00 (1.00, 12.00)	0.011315 (Mann-Whitney U Test)
Location			0.669319 (Fisher's Exact Test)
Right colon	13/23 (56.5%)	5/7 (71.4%)	
Left colon	10/23 (43.5%)	2/7 (28.6%)	
PNI +	11/23 (47.8%)	6/7 (85.7%)	0.103755 (Fisher's Exact Test)
Tumor front			0.004474 (Fisher's Exact Test)
Expansive	18/23 (78.3%)	1/7 (14.3%)	
Infiltrative	5/23 (21.7%)	6/7 (85.7%)	

metastasis and poor prognosis in CRC (46). These findings recommend Vimentin as a biomarker for aggressive tumors. Also, we should not underestimate the role that Vimentin can have in therapy. One study showed that miRNA-17-5p directly binds to the 3′UTR of VIM mRNA and it inhibited the metastasis of CRC into liver in vivo (47). Another study showed that the FiVe1 compound (FOXC2-inhibiting Vimentin effector-1) can selectively and irreversibly inhibit the growth of breast cancer cells during EMT and soft tissue sarcomas, by directly interacting with Vimentin, leading to a more epithelial state in mesenchymally transformed cells (48).

E-cadherin abnormal expression has been also extensively studied and its loss of expression has become synonymous with the EMT. The loss of E-cadherin is correlated with infiltrative tumor growth and lymph node metastasis in colon cancer (49). In hepatocellular carcinoma an association of loss of E-cadherin with adverse clinicopathological factors and prognosis has been demonstrated (50). In cervical cancer loss of E-cadherin is an independent prognostic factor (51). Besides, it can be targeted by small molecules that can restore expression and enhance the activity of cell-to-cell adhesions, with the potential of inhibiting

metastasis, being a candidate for targeted therapy (52).

OCT3/4 is a major regulator of differentiation, pluripotency, and self-renewal in embryonic stem cells (53). Its role in cancer is to confer "stemness" to cancer cells, contributing to carcinogenesis and metastasis, leading to poor outcomes (54). It has been found to be overexpressed in a variety of cancers such as colon adenocarcinoma, lung adenocarcinoma, prostate adenocarcinoma, cervical and breast carcinoma (55-59). It has been found to promote the EMT in hepatocellular carcinoma through activation of Snail signaling (60). It has been shown that OCT3/4 is associated with clinicopathological characteristics in esophageal carcinoma, where it is positively regulated by LEF1, this interaction being a candidate for targeted therapy (61). OCT3/4 is also ubiquitinated by several E3 ligases which can be candidates for OCT3/4-related therapeutics (62).

Conclusions

In summary, the expression of Vimentin and loss of E-cadherin are independently correlated with clinicopathological characteristics that are well-established adverse prognosis factors. OCT-4 is also highly expressed COAD tissue, and is a potential biomarker of epithelial to mesenchymal transition. Specific therapy against Vimentin, E-cadherin and tumor stem cells may block the EMT, which is a promising target of colon cancer treatment, and can assist in the clinical treatment of COAD. In daily anatomical pathology practice, the immunoprofile of COAD might have a prognosis impact. Further, larger, studies are necessary to check the prognosis role of EMT associated markers.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical Statement

Patient consent was waived due to the retrospective nature of this study. The study protocol was approved by the Ethics Committee of "Prof. Dr. Alexandru Treistorianu" Oncological Institute, Bucharest, Romania.

References

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Przeglad gastroenterologiczny.2019;14(2):89–103.
2. Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. Cancer Epidemiol Biomarkers Prev. 2009;18(6):1688-94.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
4. Nieto MA, Huang RYJ, Jackson RA, Thiery JP. EMT: 2016. Cell 2016; 166(1): 21–45
5. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009; 9(4):265–273.
6. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest 2009;119:1429-37.
7. Elzamly S, Badri N, Padilla O, Dwivedi AK, Alvarado LA, Hamilton M, et al. Epithelial-Mesenchymal Transition Markers in Breast Cancer and Pathological Responseafter Neoadjuvant Chemotherapy. Breast Cancer (Auckl). 2018;12:1178223418788074.
8. Ye Z, Zhou M, Tian B, Wu B, Li J. Expression of lncRNA-CCAT1, E-cadherin and N-cadherin in colorectal cancer and its clinical significance. Int J Clin Exp Med. 2015;8:3707.
9. Koren S, Reavie L, Couto JP, De Silva D, Stadler MB, Roloff T, et al. PIK3CA(H1047R) induces multipotency and multi-lineage mammary tumours. Nature. 2015;525(7567):114118.
10. Smith BN, Bhowmick NA. Role of EMT in Metastasis and Therapy Resistance. J Clin Med. 2016;5(2):17.
11. Coulombe PA, Wong P. Cytoplasmic intermediate filaments revealed as dynamic and multipurpose scaffolds. Nat Cell Biol. 2004;6:699-706.
12. Grigore AD, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor Budding: The Name is EMT. Partial EMT. J Clin Med. 2016;5(5):51.
13. Amin M, Byrd D, Edge S,Greene F. AJCC cancer staging manual. Cham: Springer International Publishing AG. 2016.
14. Meyer SN, Galvan JA, Zahnd S, Sokol L, Dawson H, Lugli A et al. Co-expression of cytokeratin and vimentin in colorectal cancer highlights a subset of tumor buds and an atypical cancer-associated stroma. Hum. Pathol. 2019;87:18–27.
15. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO Classification of Tumours of the Digestive System. Histopathology. 2020;76(2):182–8.
16. Gan Z, Ding L, Burckhardt CJ, Lowery J, Zaritsky A, Sitterley K, et al. Vimentin Intermediate Filaments Template Microtubule Networks to Enhance Persistence in Cell Polarity and Directed Migration. Cel Syst. 2016; 3:252-263.e8.
17. Mclnroy L, Maatta A. Down-regulation of vimentin expression inhibits carcinoma cell migration and adhesion. Biochem Biophys Res Commun. 2007;360:109-14.
18. Shiozaki H, Oka H, Inoue M, Tamura S, Monden M. E-cadherin mediated adhesion system in cancer cells. Cancer. 1996;77:1605–1613.
19. Niknami Z, Muhammadnejad A, Ebrahimi A, Harsani Z, Shirkoohi R. Significance of E-cadherin and Vimentin as epithelial-mesenchymal

transition markers in colorectal carcinoma prognosis. EXCLI journal. 2020; 19:917-926.
20. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2:442–54.
21. Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene 2005;24:7443–54.
22. Tsanou E, Peschos D, Batistatou A, Charalabopoulos A, Charalabopoulos K. The E-cadherin adhesion molecule and colorectal cancer. A global literature approach. Anticancer Res. 2008;28(6A):3815–3826.
23. Chen X, Wang Y, Xia H, Wang Q, Jiang X, Lin Z, et al. Loss of E-cadherin promotes the growth, invasion and drug resistance of colorectal cancer cells and is associated with liver metastasis. Mol Biol Rep. 2012;39(6):6707-6714.
24. Lu MH, Huang CC, Pan MR, Chen HH, Hung WC. Prospero homeobox 1 promotes epithelial-mesenchymal transition in colon cancer cells by inhibiting E-cadherin via miR-9. Clin Cancer Res. 2012;18(23): 6416-6425.
25. Kahlert C, Lahes S, Radhakrishnan P, Dutta S, Mogler C, Herpel E, et al. Overexpression of ZEB2 at the invasion front of colorectal cancer is an independent prognostic marker and regulates tumor invasion in vitro. Clin. Cancer Res. 2011;17(24):7654-7663.
26. Kurahara H, Takao S, Maemura K, Mataki Y, Kuwahata T, Maeda K, et al. Epithelial-mesenchymal transition and mesenchymal-epithelial transition via regulation of ZEB-1 and ZEB-2 expression in pancreatic cancer. Journal of surgical oncology. 2012;105(7):655–661.
27. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–872.
28. Asadi MH, Mowla SJ, Fathi F, Aleyasin A, Asadzadeh J, Atlasi Y. OCT4B1, a novel spliced variant of OCT4, is highly expressed in gastric cancer and acts as an antiapoptotic factor. Int J Cancer. 2011;128:2645–2652.
29. Chen Z, Xu WR, Qian H, Zhu W, Bu XF, Wang S, et al. Oct4, a novel marker for human gastric cancer. J Surg Oncol. 2009;99:414–419.
30. Kim RJ, Nam JS. OCT4 expression enhances features of cancer stem cells in a mouse model of breast cancer. Lab Anim Res. 2011;27:147–152.
31. Iida H, Suzuki M, Goitsuka R, Ueno H. Hypoxia induces CD133 expression in human lung cancer cells by up-regulation of OCT3/4 and SOX2. Int J Oncol. 2012;40:71–79.
32. Guo Y, Liu S, Wang P, Zhao S, Wang F, Bing L, et al. Expression profile of embryonic stem cell-associated genes Oct4, Sox2 and Nanog in human gliomas. Histopathology. 2011;59:763–775.
33. He W, Li K, Wang F, Qin YR, Fan QX. Expression of OCT4 in human esophageal squamous cell carcinoma is significantly associated with poorer prognosis. World J Gastroenterol. 2012;18:712–719.
34. Fujino S, Norikatsu M. Oct4 Gene Expression in Primary Colorectal Cancer Promotes Liver Metastasis. Stem cells international 2019: 7896524.
35. Dai X, Ge J, Wang X, Qian X, Zhang C, Li X. OCT4 regulates epithelial-mesenchymal transition and its knockdown inhibits colorectal cancer cell migration and invasion. Oncology reports, 2013;29(1):155–160.
36. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017;30(9):1299-1311.
37. Morikawa T, Kuchiba A, Qian ZR, Mino-Kenudson M, Hornick JL, Yamauchi M, et al. Prognostic significance and molecular associations of tumor growth pattern in colorectal cancer. Ann Surg Oncol. 2012;19(6):1944-1953.
38. Betge J, Pollheimer MJ, Lindtner RA, Kornprat P, Schlemmer A, Rehak P, et al. Intramural and extramural vascular invasion in colorectal cancer: prognostic significance and quality of pathology reporting. Cancer. 2012; 118(3):628-38.
39. Al-Maghrabi J. Vimentin immunoexpression is associated with higher tumor grade, metastasis, and shorter survival in colorectal cancer. International journal of clinical and experimental pathology 2020;13(3):493-500.
40. Berx G, van Roy F. Involvement of members of the cadherin superfamily in cancer. Cold Spring Harb Perspect Biol. 2009;1:a003129.
41. Li LI, Lv Y, Zhang Y, He L, Zhang H. Expression and clinical significance of Oct-4 and E-cad in non-small-cell lung cancer. Oncology letters. 2016; 11(1):234–236.
42. Kokkinos MI, Wafai R, Wong MK, Newgreen DF, Thompson EW, Waltham M. Vimentin and epithelial-mesenchymal transition in human breast cancer-observations in vitro and in vivo. Cells Tissues Organs. 2007;185(1-3): 191-203.
43. Satelli A, Batth I, Brownlee Z, Mitra A, Zhou S, Noh H, et al. EMT circulating tumor cells detected by cell-surface vimentin are associated with prostate cancer progression. Oncotarget. 2017;8(30):49329-49337.
44. Yin S, Chen FF, Yang GF. Vimentin immunohistochemical expression as a prognostic factor in gastric cancer: A meta-analysis. Pathol Res Pract. 2018 Sep;214(9):1376-1380.
45. Nijkamp MM, Span PN, Hoogsteen IJ, van der Kogel AJ, Kaanders JH, Bussink J. Expression of E-cadherin and vimentin correlates with metastasis formation in head and neck squamous cell carcinoma patients. Radiother Oncol. 2011;99(3):344-8.
46. Toiyama Y, Yasuda H, Saigusa S, Tanaka K, Inoue Y, Goel A, et al. Increased expression of Slug and Vimentin as novel predictive biomarkers for lymph node metastasis and poor prognosis in colorectal cancer. Carcinogenesis. 2013;34(11):2548-57.
47. Kim TW, Lee Y, Yun NH, Shin CH, Hong H, Kim H, et al. MicroRNA-17-5p regulates EMT by targeting vimentin in colorectal cancer. Br J Cancer. 2020;123(7):1123-1130.
48. Bollong MJ, Pietila M, Pearson AD, Sarkar TR, Ahmad I, Soundararajan R, et al. A vimentin binding small molecule leads to mitotic disruption in mesenchymal cancers. Proc. Natl. Acad. Sci. USA. 2017;114:E9903–E9912.
49. Kim SA, Inamura K, Yamauchi M, Nishihara R, Mima K, Sukawa Y, et al. Loss of CDH1 (E-cadherin) expression is associated with infiltrative tumour growth and lymph node metastasis. Br J Cancer. 2016;114(2):199-206.
50. Zhai X, Zhu H, Wang W, Zhang S, Zhang Y, Mao G. Abnormal expression of EMT-related proteins, S100A4, vimentin and E-cadherin, is correlated with clinicopathological features and prognosis in HCC. Med Oncol. 2014; 31(6):970.
51. Cheng Y, Zhou Y, Jiang W, Yang X, Zhu J, Feng D, et al. Significance of E-cadherin, β-catenin, and vimentin expression as postoperative prognosis indicators in cervical squamous cell carcinoma. Hum Pathol. 2012;43(8): 1213-20.
52. Song Y, Ye M, Zhou J, Wang Z, Zhu X. Targeting E-cadherin expression with small molecules for digestive cancer treatment. Am J Transl Res. 2019; 11(7):3932-3944.
53. Lengerke C, Fehm T, Kurth R, Neubauer H, Scheble V, Müller F, et al. Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma. BMC Cancer. 2011;11:42.
54. Lathia JD, Liu H. Overview of cancer stem cells and stemness for community oncologists. Target Oncol. 2017; 12:387-99.
55. Li SW, Wu XL, Dong CL, Xie XY, Wu JF, Zhang X. The differential expression of oct4 isoforms in cervical carcinoma. PLoS ONE. 2015;10:e0118033.
56. Linn DE, Yang X, Sun F, Xie Y, Chen H, Jiang R, et al. A role for oct4 in tumor initiation of drug-resistant prostate cancer cells. Genes Cancer. 2010; 1:908-916.
57. Wang QH, Zhang M, Shi CT, Xie JJ, Chen F, Shi QF, et al. High oct4 predicted worse prognosis of right-sided colon cancer patients. Future Oncol. 2018;14:2279-2291.
58. Zhang W, Ren Z, Jia L, Li X, Jia X, Han Y. Fbxw7 and skp2 regulate stem cell switch between quiescence and mitotic division in lung adenocarcinoma. BioMed Res. Int. 2019;2019:9648269.
59. Cho Y, Kang HG, Kim SJ, Lee S, Jee S, Ahn SG, et al. Post-translational modification of oct4 in breast cancer tumorigenesis. Cell Death Differ. 2018; 25:1781-1795.
60. Yin X, Bo Z, Zheng S, Gao D, Qiu S, Wu W, et al. Coexpression of gene Oct4 and Nanog initiates stem cell characteristics in hepatocellular carcinoma and promotes epithelial-mesenchymal transition through activation of Stat3/Snail signaling. J Hematol Oncol. 2015;8:23.
61. Zhao Y, Li C, Huang L, Niu S, Lu Q, Gong D, et al. Prognostic value of association of OCT4 with LEF1 expression in esophageal squamous cell carcinoma and their impact on epithelial-mesenchymal transition, invasion, and migration. Cancer Med. 2018;7(8):3977-3987.
62. Baek KH, Choi J, Pei CZ. Cellular Functions of OCT-3/4 Regulated by Ubiquitination in Proliferating Cells. Cancers (Basel). 2020;12(3):663.