Correlation of Cancer Stem-Cell Markers OCT4, SOX2, and NANOG with Clinicopathological Features and Prognosis in Operative Patients with Rectal Cancer

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Purpose: To investigate the association of cancer stem-cell markers [octamer-binding transcription factor 4 (OCT4), sex determining region Y-box 2 (SOX2), and Nanog homebox (NANOG)] expression with clinicopathological properties and overall survival (OS) in operative rectal cancer (RC) patients receiving adjuvant therapy.

Materials and Methods: 153 patients with primary RC receiving surgery were enrolled. Tumor tissue and paired adjacent normal tissue sample were collected, and OCT4, SOX2, and NANOG expressions were assessed by immunofluorescent staining. The median follow-up duration was 5.2 years, and the last follow-up date was August 2016.

Results: Tumor tissue OCT4 (p<0.001), SOX2 (p=0.003), and NANOG (p<0.001) expressions were higher than those in adjacent tissue. OCT4 expression was positively correlated with pathological grade (R=0.185, p=0.022), tumor size (R=0.224, p=0.005), and N stage (R=0.170, p=0.036). NANOG expression was positively associated with tumor size (R=0.169, p=0.036). Kaplan-Meier suggested that OCT4+ was associated with worse OS compared with OCT4− (p<0.001), while no association of SOX2 (p=0.121) and NANOG expressions (p=0.195) with OS was uncovered. Compared with one or no positive marker, at least two positive markers were associated with shorter OS (p=0.001), while all three positive markers were correlated with worse OS compared with two or less positive markers (p<0.001). Multivariate Cox’s analysis revealed that OCT4+ (p<0.001) and N stage (p=0.046) were independent factors for shorter OS.

Conclusion: Tumor tissue OCT4 expression was correlated with poor differentiation, tumor size, and N stage, and it can serve as an independent prognostic biomarker in operative patients with RC receiving adjuvant therapy.

Key Words: OCT4, SOX2, NANOG, prognosis, rectal cancer

INTRODUCTION

Rectal cancer (RC), one of the most commonly diagnosed malignant tumors, is a crucial threat imperiling human health worldwide.¹ 2016 cancer statistics report indicated approximately 39220 new RC cases in the United States and estimated incidence of 11 per 100000 people in the developing countries.²³ Despite of great improvement in early diagnosis, operative treatment, adjuvant therapies (such as chemotherapy, radiotherapy and hormonotherapy) and integrated patients’ care, the prognosis of RC patients remains far from satisfaction.⁴

Cancer stem cells (CSCs), a small subset of tumor cells, are characterized by self-renewing capacity, heterogeneity and resistance to several therapies (such as chemotherapy and radiotherapy), and contribute to the process of tumor development, infiltration, metastasis and recurrence.⁵ As common stem related transcription factors, octamer-binding transcription factor 4 (OCT4), sex determining region Y-box 2 (SOX2), and Nanog homebox (NANOG) are considered as critical regulators of self-
renewal and pluripotency of embryonic stem, mediating tumor proliferation and tumor differentiation. And they have been identified as predictive biomarkers for poor prognosis in several carcinomas including hepatocellular cancer, esophageal squamous cell carcinoma (ESCC) and ovarian cancer. Several earlier studies indicate that OCT4, SOX2, and NANOG are frequently involved in digestive diseases, even though few studies have explored the effects of OCT4, SOX2, and NANOG expression on the prognosis in RC patients. Therefore, the purpose of this study was to investigate the association of cancer stem-cell marker (OCT4, SOX2, and NANOG) expressions with clinicopathological properties and overall survival (OS) in operative RC patients receiving adjuvant therapy.

MATERIALS AND METHODS

Participants
153 patients with primary RC receiving surgery at Department of General Surgery in the Second Affiliated Hospital of Harbin Medical University between December 2009 and December 2010 were consecutively enrolled in this cohort study. All patients were diagnosed by the validation of clinical properties, imageological examination and pathologic analysis. Patients who had received pre-surgery neo-adjuvant therapies, or were complicated with or history of other tumors, hematological malignancies or severe infection were excluded from this study. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University with approval No. 2009PHB012-01. All the patients signed the informed consents.

Treatment after surgery
In these 153 RC patients, 20 RC patients who were in TNM Stage I didn’t take adjuvant treatment after surgery, while 131 patients who in TNM Stage II and Stage III received 5-fluorouracil based chemotherapy, among which 31% patients received radiotherapy at the same time, but others did not. Two Stage IV patients gave up chemotherapy or radiotherapy due to poor outcomes of surgery.

Data collection
Baseline demographic, pathological and clinical characteristics of all patients, including age, gender, pathological grade, tumor size, positive lymph nodes, distant metastasis, and TNM stage, were collected. TNM stage was assessed according to the 7th edition of the American Joint Committee on Cancer (AJCC) cancer staging manual and the pathological grade was divided into well differentiation, moderate differentiation, and poor differentiation.

Follow-ups
Patients were intensively followed up during the first half year post-operation; once a month, followed by once every 3–9 months. The median follow-up duration was 5.2 years, and the last follow-up date was August 2016. OS was calculated from the time of the surgery to date of death from any cause or last follow-up.

Sample collection
Tumor tissue samples and paired adjacent normal tissue were obtained from all patients during the surgery, and they were subsequently fixed in formalin and embedded in paraffin for further determination.

Immunofluorescent staining
The formalin-fixed, paraffin-embedded sections (5 μm thick) were deparaffinated and dehydrated at 65°C for 3 h. After permeabilization in polybutylene terephthalate (PBST) [phosphate-buffered saline (PBS), 1% bovine serum albumin (BSA), 0.1% Triton] overnight, the sections were then treated with methanol containing 0.3% hydrogen peroxide and autoclaved at 121°C for 10 min for antigen retrieval. After blocking with 10% goat serum, one section was incubated at 4°C overnight with rabbit antibody against OCT4 with dilution 1:1600 (C52G3, CST, Boston, MA, USA) and mouse antibody against SOX2 with dilution 1:400 (L1D6A2, CST), while another section was incubated with mouse antibody against NANOG with dilution 1:2000 (1E6C4, CST). After washing with PBS three times, Alexa Fluor 488 Conjugate labelled (green) antibody against rabbit IgG with dilution 1:500 (4412, CST) and Alexa Fluor 594 Conjugate labelled (red) antibody against mouse IgG with dilution 1:500 (8890, CST) were added as secondary antibodies. After staining, the sections were counterstained with Hoechst 33342. Histological score (HSCORE) ranged from 0 (no staining) to 4 (maximal staining), which was calculated to assess immunofluorescent staining, and the computational formula as follows: HSCORE=ΣPi(i+1). HSCORE 0.7 was considered as a threshold to distinguish positivity and negativity of immunofluorescent staining.

Statistics
Statistics was carried out using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and 2010 office software (Microsoft, Boston, MA, USA). Data were presented as mean±standard deviation, count (percentage), or p value. OCT4, SOX2, and NANOG expressions in tumor tissues and paired adjacent tissues were compared by chi-square test. Correlation of expressions of these three factors with each other in patients was determined by Spearman test. Kaplan-Meier was drawn and log-rank test was used to investigate the correlation of OCT4, SOX2, and NANOG expressions with OS. Univariate Cox proportional hazard regression was performed to analyze the factors affecting OS, while factors with a p value <0.1 was further analyzed by multivariate Cox proportional hazard regression. p value <0.05 was considered significant.
RESULTS

Baseline characteristics
In the present study, the mean age of RC patients was 65.97±11.59 years, and male and female were 83 and 70, respectively (Table 1). The number of RC patients with well, moderate, and poor differentiation was 23 (15%), 109 (71%), and 21 (14%), respectively. The mean of tumor size was 4.71±1.22 cm, and 79 (52%) patients were with tumor size <5 cm, while 74 (48%) patients were with tumor size ≥5 cm. There were 3 (2%) patients in T1 stage, 17 (11%) patients in T2 stage, 131 (86%) patients in T3 stage and 2 (1%) patients in T4a stage. As for N stage, 90 (59%) patients were in N0, 24 (16%) patients in N1a, 15 (10%) patients in N1b, 3 (2%) patients in N1c, 12 (8%) patients in N2a, and 9 (6%) patients in N2b. One hundred and fifty one (99%) patients were in M0 stage, and 2 (1%) patients were in M1a stage. According to the T, N, and M stage, 20 (13%), 68 (44%), 52 (34%), 11 (7%), and 2 (1%) patients were in TNM stage I, stage IIA, stage IIIB, stage IIIC, and stage IVA, respectively.

Table 1. Baseline Characteristics of RC Patients

| Parameters               | RC patients (n=153) |
|--------------------------|---------------------|
| Age (yrs)                | 65.97±11.59         |
| Gender (male/female)     | 83/70               |
| Pathological grade, n (%)|                     |
| Well differentiation     | 23 (15)             |
| Moderate differentiation  | 108 (71)            |
| Poor differentiation     | 21 (14)             |
| Tumor size (cm)          | 4.71±1.22           |
| <5                       | 79 (52)             |
| ≥5                       | 74 (48)             |
| T stage, n (%)           |                     |
| T1                       | 3 (2)               |
| T2                       | 17 (11)             |
| T3                       | 131 (86)            |
| T4a                      | 2 (1)               |
| N stage, n (%)           |                     |
| N0                       | 90 (59)             |
| N1a                      | 24 (16)             |
| N1b                      | 15 (10)             |
| N1c                      | 3 (2)               |
| N2a                      | 12 (8)              |
| N2b                      | 9 (6)               |
| M stage, n (%)           |                     |
| M0                       | 151 (99)            |
| M1a                      | 2 (1)               |
| TNM stage, n (%)         |                     |
| I                        | 20 (13)             |
| IIa                      | 68 (44)             |
| IIIB                     | 52 (34)             |
| IIIC                     | 11 (7)              |
| IVA                      | 2 (1)               |

RC, rectal cancer.
Data was presented as mean value±standard deviation or counts (with or without percentage).

OCT4, SOX2, and NANOG expression in tumor tissues and paired adjacent normal tissues
OCT4, SOX2 and NANOG expressions were mainly expressed on the cytoplasm of cell (Fig. 1). As shown in Table 2, OCT4 (p<0.001), SOX2 (p=0.003), and NANOG (p<0.001) expressions in tumor tissue were higher than those in adjacent tissue. Co-expressions status of OCT4, SOX2, and NANOG in tumor tissues was evaluated, and Table 3 shows that there were 31 (20%) patients with no marker positive, 50 (33%) patients with only one marker positive, 48 (31%) patients with two markers positive [including: 1) OCT4+ and SOX2+ group (N=28, 18%); 2) OCT4+ and NANOG+ group (N=16, 10%); and 3) SOX2+ and NANOG+ group (N=4, 3%)], and 24 (16%) patients with all three markers positive.

Correlations of OCT4, SOX2, and NANOG expressions
Correlations of expressions of these three factors with each other in patients were assessed, and Table 4 shows that OCT4 expression was positively correlated with expression of SOX2 (R=0.316, p<0.001) and NANOG (R=0.164, p=0.043), while no correlation between SOX2 and NANOG expressions was found (R=-0.019, p=0.817).

Correlations of OCT4, SOX2, and NANOG expressions with clinical and pathological features of RC patients
Correlations of OCT4, SOX2, and NANOG expressions with clinical and pathological features of RC patients were assessed, and Table 5 shows that OCT4, SOX2, and NANOG expressions were correlated with clinical and pathological features of RC patients, respectively.

Fig. 1. Immunohistochemical staining for OCT4, SOX2, and NANOG expressions in tumor tissues and paired adjacent normal tissues. Immunohistochemical staining for OCT4 (A), OCT4+ (B), SOX2 (C), SOX2+ (D), NANOG+ (E), and NANOG+ (F). OCT4, SOX2, and NANOG expressions were mainly found on cell membrane. OCT4, octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; NANOG, Nanog homebox.
Co-expression of OCT4, SOX2, and NANOG in Tumor Tissues and Paired Adjacent Tissues

| Parameters | OCT4 | SOX2 | NANOG |
|------------|------|------|-------|
| Tumor tissue (%) | Positive 85 (56) | Negative 68 (44) | Positive 72 (47) | Negative 81 (53) | Positive 61 (40) | Negative 92 (60) |
| Adjacent tissue (%) | Positive 34 (22) | Negative 119 (78) | Positive 47 (31) | Negative 106 (69) | Positive 28 (18) | Negative 125 (82) |
| p value | <0.001* | 0.003* | <0.001* |

OCT4, octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; NANOG, Nanog homeobox.

Data was presented by counts (percentage). Comparison was determined by chi-square test.

*<p value <0.05 was considered significant.

Correlations of OCT4, SOX2, and NANOG expression with OS

The mean OS was 56 months, and the 5-year survival was 53.6% in these RC patients. Correlations of OCT4, SOX2, and NANOG expressions with OS were assessed. Fig. 2 shows that OCT4 expression was positively correlated with pathological grade (R=0.185, p=0.022), tumor size (R=0.224, p=0.005), and N stage (R=0.170, p=0.036), whereas NANOG expression was positively associated with tumor size (R=0.169, p=0.036). No correlation of OCT4, SOX2, and NANOG expressions with other clinical and pathological features was found in RC patients (Table 5).

Correlation of number of positive markers with OS

Compared with one or no positive marker, at least two positive markers were associated with worse OS (p<0.001) (Fig. 3B), while all three positive markers were correlated with even worse OS compared with two or less positive markers (p<0.001) (Fig. 3C). No correlation of no positive marker and at least one positive marker with OS was found (p=0.063) (Fig. 3A).

Analysis of factors affecting OS by univariate and multivariate model

Univariate Cox’s proportional hazards regression was used to evaluate the factors affecting OS in RC patients. Table 6 indicates that OCT4+ (p<0.001), poor differentiation (p<0.001), T stage (p=0.033), N stage (p<0.001), and TNM stage (p<0.001) were correlated with worse OS in RC patients. All factors with a p value <0.1 were further analysed by the multivariate Cox’s proportional hazards regression model. The result indicated that the OCT4+ (p<0.001) and N stage (p=0.046) were independent factors for shorter OS.

DISCUSSION

The present study found that: 1) Stronger expressions of OCT4, SOX2, and NANOG were found in tumor tissue than those in paired adjacent tissue; 2) OCT4 expression was positively correlated with poor differentiation, tumor size and N stage, while NANOG expression was positively associated with tumor size; 3) OCT4+ was correlated with worse OS than OCT4+ in RC patients, and OCT4+ and N stage could independently predict worse OS; and 4) All three positive markers seem to have greater value in predicting worse OS compared with only one or two positive markers in RC patients.

OCT4, located in 6 chromosomes (6p21.31), is one of the Pit-Oct-Unc (POU) transcription factor family members, which plays a vital role in maintaining stem cell self-renewal, pluripotency, and differentiation. Upregulating OCT4 expression induces the proliferation and metastasis of epithelial ovarian...
Table 5. Correlation of OCT4, SOX2, and NANOG Expression with Clinicopathological Features

| Parameters | Age | Gender (male) | Pathological grade | Tumor size | T stage | N stage | M stage | TNM stage |
|------------|-----|---------------|--------------------|------------|---------|---------|---------|-----------|
| OCT4 expression | R   | -0.122        | 0.155              | 0.185*     | 0.224*  | 0.143   | 0.170*  | -0.013    | 0.091     |
|             | p   | 0.133         | 0.055              | 0.022*     | 0.005*  | 0.077   | 0.036*  | 0.875     | 0.264     |
| SOX2 expression | R   | -0.023        | -0.054             | 0.079      | 0.047   | -0.126  | 0.045   | -0.109    | -0.113    |
|             | p   | 0.774         | 0.506              | 0.329      | 0.561   | 0.121   | 0.583   | 0.182     | 0.163     |
| NANOG expression | R   | -0.012        | -0.056             | -0.124     | 0.169*  | -0.051  | 0.139   | -0.094    | 0.062     |
|             | p   | 0.887         | 0.491              | 0.128      | 0.036*  | 0.534   | 0.087   | 0.249     | 0.450     |

OCT4, octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; NANOG, Nanog homebox.
Data was presented as correlation index R and p value. Correlation was determined by Pearson test. *p<0.05 was considered significant.

Fig. 2. The correlations of OCT4, SOX2, and NANOG expressions with OS. (A) Correlation of OCT4+ and OCT4− with OS. (B) Correlation of SOX2+ and SOX2− with OS. (C) Correlation of NANOG+ and NANOG− with OS. Kaplan-Meier curves were used to analyse the correlation of OCT4, SOX2, and NANOG expressions with OS. Comparison of two groups was made by log-rank test. p<0.05 was considered significant. OS, overall survival; OCT4, octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; NANOG, Nanog homebox.

Fig. 3. The correlation of number of positive markers with OS. (A) Association of no positive marker and at least one positive marker with OS. (B) Association of one or no positive marker and at least two positive markers with OS. (C) Association of two or less positive markers and all three positive markers with OS. Kaplan-Meier curves were used to evaluate the correlation of number of positive markers with OS. Comparison of two groups was made by log-rank test. p<0.05 was considered significant. OS, overall survival.
Table 6. Univariate and Multivariate Cox Analysis of the Factors Affecting OS in Rectal Cancer Patients

| Factor                | Univariate Cox |           |           | Multivariate Cox |           |           |
|-----------------------|----------------|-----------|-----------|------------------|-----------|-----------|
|                       | p value        | HR        | 95% CI    | p value          | HR        | 95% CI    |
| OCT4 (vs. OCT4−)      | <0.001*        | 4.724     | 2.626     | 8.496            | <0.001*   | 4.071     | 2.210     | 7.498     |
| SOX2 (vs. SOX2−)     | 0.126          | 1.440     | 0.903     | 2.295            | -         | -         | -         |
| NANOG (vs. NANOG−)    | 0.199          | 1.360     | 0.850     | 2.174            | -         | -         | -         |
| Age                   | 0.269          | 0.988     | 0.968     | 1.009            | -         | -         | -         |
| Gender (male)         | 0.261          | 1.310     | 0.818     | 2.098            | -         | -         | -         |
| Tumor size ≥5 cm (vs. <5 cm) | 0.125       | 1.158     | 0.960     | 1.397            | -         | -         | -         |
| Pathological grade    | <0.001*        | 2.273     | 1.450     | 3.563            | 0.264     | 1.470     | 0.748     | 2.890     |
| T stage               | 0.033*         | 1.961     | 1.057     | 3.641            | 0.728     | 1.187     | 0.452     | 3.114     |
| N stage               | <0.001*        | 2.011     | 1.479     | 2.736            | 0.046*    | 1.685     | 1.010     | 2.813     |
| M stage               | 0.140          | 2.910     | 0.705     | 12.007           | -         | -         | -         |
| TNM stage             | <0.001*        | 1.861     | 1.314     | 2.636            | 0.264     | 1.470     | 0.748     | 2.890     |

OS, overall survival; HR, hazard ratio; CI, confidence interval; OCT4, octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; NANOG, Nanog homebox.

Data was presented as p value, HR, and 95% CI. Univariate Cox proportional hazard regression was performed to analyze the factors affecting OS, while factors with p value <0.1 was further analyzed by multivariate Cox proportional hazard regression. Pathological grade was scored as 1-well differentiation, 2-moderate differentiation, 3-poor differentiation; T stage was scored as 1-T1, 2-T2, 3-T3, and 4-T4; N stage was scored as 0-N0, 1-N1, and 2-N2; M stage was scored as 0-M0 and 1-M1; TNM stage was scored as 1-Stage I, 2-Stage II, 3-Stage III, and 4-Stage IV. Based on these definitions, Cox proportional hazard regressions analysis was performed.

*p value <0.05 was considered significant.
to be positively associated with lymph node metastasis, and SOX2 expression predicts poor progression-free survival and OS in small-cell lung cancer patients after first-line chemotherapy. 36,37 On the other hand, however, overexpression of tissue SOX2 has also been shown to predict better outcome in NSCLC patients. 38 As for the NANOG expression, high expression of tissue NANOG was found in renal cancer patients, even though no association was found between NANOG expression and any clinicopathological features. 39 The results, therefore, are not always consistent, and some controversies about the effects of SOX2 and NANOG expressions on the prognosis of tumors still exist. 16,36,38,39 In the current study, we showed that NANOG expression was associated only with tumor size, however, no correlation of SOX2 and NANOG expressions with other clinicopathological properties as well as OS in operative RC patients receiving adjuvant therapy was observed.

We also found that the combination of all three positive markers seems to have even greater value in predicting worse OS compared with only one or two positive markers in RC patients. It is quite possible that CSCs markers (OCT4, SOX2, and NANOG expressions) are strongly associated with CSCs that are characterized by stem-cell properties. Therefore, all three positive markers could reflect high concentration of CSCs, which is related to the resistance of radio-, chemo-, and hormone-therapy, thereby increasing recurrences in RC patients.

Some limitations existed in this study: 1) Sample size was relatively small, possibly resulting in lower statistical efficiency compared with larger sample size studies. 2) There were two RC patients in stage IV enrolled in our study, which might affect the predictive value of tumor tissue OCT4, SOX2, and NANOG expressions on RC patients. In the present study, therefore, we excluded these two stage IV patients and analysed the correlations of OCT4, SOX2, and NANOG expressions with clinicopathological properties and OS in RC patients in stage I, II, and III. We found no difference in the prospective value between RC patients with or without stage IV patients. The effects of tumor tissue OCT4, SOX2, and NANOG expression in the stage IV patients should to be investigated in further study. 3) In the current study, OCT4, SOX2, and NANOG expressions in blood sample were not assessed, even though blood sample is more easily obtained because of its non-invasiveness, particularly the RC patients in stage IV who cannot undergo surgery or biopsy.

In conclusion, tumor tissue OCT4 expression was correlated with poor differentiation, tumor size, and N stage, and it could serve as an independent prognostic biomarker in operative patients with RC receiving adjuvant therapy.

ACKNOWLEDGEMENTS

This study was supported by Research and Development Project of Scientific Achievement Transformation of Harbin Medical University, the Mechanism of CircRNA in the Pathogenesis of Colorectal Cancer.

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REFERENCES

1. Li X, Lu H, Xu K, Wang H, Liang X, Hu Z. Negative lymph node count is an independent prognostic factor for patients with rectal cancer who received preoperative radiotherapy. BMC Cancer 2017;17:227.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:78-107.
4. Hoecht S, Hinkelbein W. Treatment of rectal cancer. N Engl J Med 2006;355:2486; author reply 2487-8.
5. Beck B, Blanpain C. Unravelling cancer stem cell potential. Nat Rev Cancer 2013;13:727-38.
6. Cao L, Li C, Shen S, Yan Y, Ji W, Wang J, et al. OCT4 increases BIRC5 and CCND1 expression and promotes cancer progression in hepatocellular carcinoma. BMC Cancer 2013;13:82.
7. Zhou X, Huang GR, Hu F. Over-expression of Oct4 in human esophageal squamous cell carcinoma. Mol Cells 2011;32:29-35.
8. Lin J, Zhang L, Huang H, Huang Y, Huang L, Wang J, et al. miR-26b/KLF4 axis inhibits epithelial ovarian carcinoma proliferation and metastasis through downregulating OCT4. Oncotarget 2015;6:23793-806.
9. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 2008;132:567-82.
10. Neumann J, Babr F, Horst D, Kriegl L, Engel J, Luque RM, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer 2011;11:518.
11. Lessey BA, Castellaum AI, Savin SW, Buck CA, Schimann R, Bilker W, et al. Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab 1994;79:643-9.
12. Kashyap V, Rezende NC, Scotland KB, Shaffer SM, Persson JL, Gudas LJ, et al. Regulation of stem cell pluripotency and differentiation involves a mutually regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. Stem Cells Dev 2009;18:1093-108.
13. Wang YD, Cai N, Wu XL, Cao HZ, Xie LL, Zheng PS. OCT4 promotes tumorigenesis and inhibits apoptosis of cervical cancer cells by miR-125b/BAK1 pathway. Cell Death Disc 2013;4:e760.
14. Tang YA, Chen CH, Sun HS, Cheng CP, Tseng VS, Hsu HS, et al. Global Oct4 target gene analysis reveals novel downstream PTEN and TNC genes required for drug-resistance and metastasis in lung cancer. Nucleic Acids Res 2015;43:1593-608.
15. Wu Y, Liu S, Xin H, Jiang J, Younghsi E, Sun S, et al. Up-regulation of microRNA-145 promotes differentiation by repressing OCT4 in human endometriosis adenocarcinoma cells. Cancer 2011;117:3989-98.
16. Yu B, Cai H, Xu Z, Xu T, Zou Q, Gu M. Expressions of stem cell transcription factors Nanog and Oct4 in renal cell carcinoma tissues and clinical significance. Artif Cells Nanomed Biotechnol 2016;44:1818-23.
17. Li X, Wang J, Xu Z, Ahmad A, Li E, Wang Y, et al. Expression of Sox2 and Oct4 and their clinical significance in human non-small-cell lung cancer. Int J Mol Sci 2012;13:7663-75.

https://doi.org/10.3349/ymj.2018.59.1.35
18. Li C, Yan Y, Ji W, Bao L, Qian H, Chen L, et al. OCT4 positively regulates Survivin expression to promote cancer cell proliferation and leads to poor prognosis in esophageal squamous cell carcinoma. PLoS One 2012;7:e49693.

19. Matsuoka J, Yashiro M, Sakurai K, Kubo N, Tanaka H, Muguruma K, et al. Role of the stemness factors sox2, oct3/4, and nanog in gastric carcinoma. J Surg Res 2012;174:130-5.

20. Atlasi Y, Mowlia SJ, Ziae AA, Bahrami AR. OCT-4, an embryonic stem cell marker, is highly expressed in bladder cancer. Int J Cancer 2007;120:596-602.

21. Chiou SH, Yu CC, Huang CY, Lin SC, Liu CJ, Tsai TH, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res 2008;14:4085-95.

22. Shen L, Huang X, Xie X, Su J, Yuan J, Chen X. High expression of SOX2 and OCT4 indicates radiation resistance and an independent negative prognosis in cervical squamous cell carcinoma. J Histochem Cytochem 2014;62:499-509.

23. 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;1:908-16.

24. Li B, Yao Z, Wan Y, Lin D. Overexpression of OCT4 is associated with gefitinib resistance in non-small cell lung cancer. Oncotarget 2016;7:77342-7.

25. Sullivan JP, Minna JD, Shau JW. Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy. Cancer Metastasis Rev 2010;29:61-72.

26. Li W, Zhou Y, Zhang X, Yang Y, Dan S, Su T, et al. Dual inhibiting OCT4 and AKT potently suppresses the propagation of human cancer cells. Sci Rep 2017;7:46246.

27. Emhemmed F, Ali Azouaou S, Thauad F, Schini-Kerth V, Désauuby L, Muller CD, et al. Selective anticancer effects of a synthetic flavagline on human Oct4-expressing cancer stem-like cells via a p38 MAPK-dependent caspase-3-dependent pathway. Biochem Pharmacol 2014;89:185-96.

28. Yong X, Tang B, Xiao YF, Xie R, Qin Y, Luo G, et al. Helicobacter pylori upregulates Nanog and Oct4 via Wnt/β-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. Cancer Lett 2016;374:292-303.

29. Teng HR, Li PN, Hou DR, Liu SW, Lin CT, Loo MR, et al. Valproic acid enhances Oct4 promoter activity through PI3K/Akt/mTOR pathway activated nuclear receptors. Mol Cell Endocrinol 2014;383:147-58.

30. Rectal Cancer Treatment (PDQ®): Health Professional Version. In: PDQ Cancer Information Summaries. Bethesda (MD): National Cancer Institute; 2002.

31. Al-Marzoqee FY, Khoder G, Al-Awadhi H, John R, Beg A, Vincze A, et al. Upregulation and inhibition of the nuclear translocation of Oct4 during multistep gastric carcinogenesis. Int J Oncol 2012;41:1733-43.

32. Wen Y, Hou Y, Huang Z, Cai J, Wang Z. SOX2 is required to maintain cancer stem cells in ovarian cancer. Cancer Sci 2017;108:719-31.

33. Xiao L, Song Y, Huang W, Yang S, Fu J, Feng X, et al. Expression of SOX2, NANOG and OCT4 in a mouse model of lipopolysaccharide-induced acute uterine injury and intrauterine adhesions. Reprod Biol Endocrinol 2017;15:14.

34. Piva M, Domenici G, Iriondo O, Rábano M, Simões BM, Corrales V, et al. Sox2 promotes tamoxifen resistance in breast cancer cells. EMBO Mol Med 2014;6:66-79.

35. Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238-42.

36. Sodja E, Rijavec M, Koren A, Sadikov A, Korošec P, Cufer T. The prognostic value of whole blood SOX2, NANOG and OCT4 mRNA expression in advanced small-cell lung cancer. Radiol Oncol 2016;50:188-96.

37. Yamawaki K, Ishiguro T, Mori Y, Yoshihara K, Suda K, Tamura R, et al. Sox2-dependent inhibition of p21 is associated with poor prognosis of endometrial cancer. Cancer Sci 2017;108:632-40.

38. Velcheti V, Schalper K, Yao X, Cheng H, Kocoglu M, Dhodapkar K, et al. High SOX2 levels predict better outcome in non-small cell lung carcinomas. PLoS One 2013;8:e61427.

39. Sholl LM, Barletta JA, Yeap BY, Chirieac LR, Hornick JL. Sox2 protein expression is an independent poor prognostic indicator in stage I lung adenocarcinoma. Am J Surg Pathol 2010;34:1193-8.