For Better or Worse: Clinical Correlation of SOX9 and Irradiated Rectal Cancer

Xiuzhen Duan, Brandon Trac, and Xianzhong Ding,
Department of Pathology
Loyola University Medical Center
Maywood, IL 60153
USA
SOX9 and Cancer Stem-like Cells

- It is well accepted that cancer stem-like cells are the main driving force behind tumor formation, metastasis, and chemoresistance.

- Cancer stem like cells exhibit diverse cell properties including self renewal, differentiation capacity, and resistance to apoptosis and chemotherapy.
SOX9

• Sex-determining region Y (SRY)-box 9 protein (SOX9) is a member of the SOX family of transcription factors

• SOX9 participates in a variety of functions, such as tumor cell differentiation, stem-like cell lineage formation and epithelial mesenchymal transition

• Role of SOX9 in colon cancer: controversial

• Effect of chemoradiation on SOX9 expression unclear
Title: SOX9, miR-495, miR-590-3p, and miR-320d were identified as chemoradiotherapy-sensitive genes and miRNAs in colorectal cancer patients based on a microarray dataset


Abstract: We aimed to identify chemoradiotherapy (CRT)-sensitive biomarkers in colorectal cancer (CRC) patients. The GSE15781 dataset used in this study contains 42 samples: 22 CRC tissues (non-CRT: n=13; CRT: n=9) and 20 normal colorectal tissues (non-CRT: n=10; CRT: n=10). Following pretreatment, differentially expressed genes were selected using the limma package. Potential CRT-sensitive genes were identified with Venn analysis and then enriched in function and pathway clusters using the DAVID online tool. Moreover, protein-protein interaction (PPI) network analysis was implemented using the STRING database. The TRRUST database was used to establish a transcription factor (TF)-target transcriptional network. A miRNA–mRNA network was constructed based on relevant databases. miRNA and mRNA expression levels were analyzed using real-time quantitative PCR. A group of 259 candidate CRT-sensitive genes were identified that were mainly enriched in cell cycle regulation, adhesion-associated processes, and the p53 signaling pathway. A PPI network was established that contained striking nodes, including ITGA2, MYC, ESR1, and dihydropyrimidine dehydrogenase (DPYD), among which ESR1 was linked to MYC, and the two nodes were also highlighted in the TF-target regulation network. SRY-box 9 (SOX9) was another key TF. Hsa-miR-590-3p, hsa-miR-495, hsa-miR-320c, and hsa-miR-320d were predominant in the miRNA–mRNA network. Expression levels of SOX9, DPYD mRNA, miR-495, and miR-590-3p were clearly reduced after X-ray treatment in irradiated HT-29 cells, whereas that of miR-320d was notably enhanced. SOX9 may be a CRT-sensitive gene in CRC patients, and hsa-miR-590-3p, hsa-miR-495, and hsa-miR-320d may be CRT-sensitive microRNAs in CRC patients. Therefore, SOX9, hsa-miR-590-3p, hsa-miR-495, and hsa-miR-320d may be used as sensitive biomarkers in CRC patients.
SOX9 expression predicts relapse of stage II colon cancer patients

Maiken Lise Marcker Espersen MSc\textsuperscript{a,b}, Dorte Linnemann MD, DMS\textsuperscript{c}, Ib Jarle Christensen MSc\textsuperscript{a}, Mahdi Alamili MD, PhD\textsuperscript{c}, Jesper T. Troelsen PhD, DMS\textsuperscript{b}, Estrid Høgdall PhD, DMS\textsuperscript{a,*}

\textsuperscript{a}Department of Pathology, Herlev University Hospital, DK-2730 Herlev, Denmark
\textsuperscript{b}Department of Science, Systems and Models, Roskilde University, DK-4000 Roskilde, Denmark
\textsuperscript{c}Department of Surgery, Køge University Hospital, DK-4600 Køge, Denmark

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Summary The aim of this study was to investigate if the protein expression of sex-determining region y-box 9 (SOX9) in primary tumors could predict relapse of stage II colon cancer patients. One hundred forty-four patients with stage II primary colon cancer were retrospectively enrolled in the study. SOX9 expression was evaluated by immunohistochemistry, and mismatch repair status was assessed by both immunohistochemistry and promoter hypermethylation assay. High SOX9 expression at the invasive front was significantly associated with lower risk of relapse when including the SOX9 expression as a continuous variable (from low to high expression) in univariate (hazard ratio [HR], 0.73; 95% confidence interval [CI], 0.56-0.94; \( P = .01 \)) and multivariate Cox proportional hazards analyses (HR, 0.75; 95% CI, 0.58-0.96; \( P = .02 \)), adjusting for mismatch repair deficiency and histopathologic risk factors. Conversely, low SOX9 expression at the invasive front was significantly associated with high risk of relapse, when including SOX9 expression as a dichotomous variable, in univariate (HR, 2.32; 95% CI, 1.14-4.69; \( P = .02 \)) and multivariate analyses (HR, 2.32; 95% CI, 1.14-4.69; \( P = .02 \)), adjusting for histopathologic risk factors and mismatch repair deficiency. In conclusion, high levels of SOX9 of primary stage II colon tumors predict low risk of relapse, whereas low levels of SOX9 predict high risk of relapse. SOX9 may have an important value as a biomarker when evaluating risk of relapse for personalized treatment.

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Robust biomarkers that can precisely stratify patients according to treatment needs are in great demand. The literature is inconclusive for most reported prognostic markers for colorectal cancer (CRC). Hence, adequately reported studies in large representative series are necessary to determine their clinical potential. We investigated the prognostic value of three Wnt signaling-associated proteins, β-catenin, E-cadherin, and SOX9, in a population-representative single-hospital series of 1290 Norwegian CRC patients by performing immunohistochemical analyses of each marker using the tissue microarray technology. Loss of membranous or cytosolic β-catenin and loss of cytosolic E-cadherin protein expression were significantly associated with reduced 5-year survival in 903 patients who underwent major resection (722 evaluable tissue cores) independently of standard clinicopathological high-risk parameters. Pre-specified subgroup analyses demonstrated particular effect for stage IV patients for β-catenin membrane staining (P = 0.018; formal interaction test P = 0.025). Among those who underwent complete resection (714 patients, 568 evaluable), 5-year time-to-recurrence analyses were performed, and stage II patients with loss of cytosolic E-cadherin were identified as an independent high-risk subgroup (P = 0.020, formal interaction test was not significant). Nuclear β-catenin and SOX9 protein, regardless of intracellular location, were not associated with prognosis. In conclusion, the protein expression level of membranous or cytosolic β-catenin and E-cadherin predicts CRC patient subgroups with inferior prognosis.

Keywords: beta-catenin, E-cadherin, SOX9 transcription factor, prognostic biomarkers, colorectal cancer, biomarker discovery, guideline adherence
Aims and Methods

• The objectives of this study is examining SOX9 expression in pre- and post-irradiated rectal cancer

• 25 patients with locally advanced rectal cancer
  – Moderately differentiated adenocarcinoma
  – Received neoadjuvant chemoradiation therapy followed by surgery
Aims and Methods

• SOX9 expression
  – Evaluated by IHC
  – 4-tier grading system
    • 0: no expression
    • 1: low expression
    • 2: moderate expression
    • 3: high expression
  – Percentage of SOX9 positive cells
Grade of SOX9 expression

Pre-CRT  post-CRT  Pre-CRT  post-CRT

Non-tumor mucosa  Adenocarcinoma
Pre-CRT           post-CRT           Pre-CRT            post-CRT
Non-tumor mucosa                  Adenocarcinoma

% positive cells

0  25  50  75  100

Pre-CRT  post-CRT  Pre-CRT  post-CRT
Non-tumor mucosa  Adenocarcinoma
SOX9 expression and tumor response to neoadjuvant therapy

• 2 cases with complete response to neoadjuvant therapy
  – High expression of SOX9

• No correlation of tumor volume reduction and SOX9 expression prior to chemotherapy

• No association with lymph node metastasis and late distant metastasis
Conclusions

- Role of SOX9 in colon cancer is still controversial

- Might be a potential marker to predict tumor response to chemoradiation and oncological outcomes

- Large cohort studies are needed to be conclusive