# The Intestinal Stem Cell Marker SOX9 Predicts Relapse of Stage II Colon Cancer Patients

Espersen MLM[1,2] , Linnemann D[1], Alamili M[3], Christensen IJ[1], Troelsen JT[2], Høgdall E[1]

[1]Department of Pathology, Herlev University Hospital, Denmark
[2]Department of Science Systems and Models, Roskilde University, Denmark
[3]Department of Surgery, Køge University Hospital, Denmark

## OBJECTIVE

- To investigate if the intestinal stem cell marker SOX9 can predict stage II colon cancer patients with high risk of relapse.
- To investigate mismatch repair (MMR) deficiency in stage II colon cancer patients.

## BACKGROUND

- Due to the national colorectal screening program in Denmark, the number of patients with early stage colorectal cancer (stage I and II) is expected to increase.

  Approx. **20%** of the stage I and stage II colorectal cancer patients relapse

- To this date no optimal biomarker can identify colorectal cancer patients with high risk of relapse.
- Several studies report that SOX9 overexpression correlate to an unfavorable prognosis of colorectal cancer patients [1, 2].

## MATERIALS AND METHODS

**Figure 2**
Flowchart of the project.

- Primary FFPE tumor tissue from 144 stage II colon cancer patients
- Consecutively included (2005-2008):
  - Stage II colon cancer, ≥50 yrs. of age at diagnosis
- Excluding:
  - neoadjuvant chemotherapy, IBD, other primary cancers

**Patient materials**

**SOX9 analysis**

- EnVision™ FLEX (Dako), Autostainer Link (Dako)
- Anti-SOX9 (1+10.0000, Millipore)
- Full slides incl. the invasive front and the luminal surface
- Score:
  - % positive x intensity
  - Two independent individuals

**Digital analysis**

- Scanning of slides on Nanozoomer 2.0-HT (Hamamatzu)
- Scoring of staining using software from VisioPharm

**MMR assays**

- TMA (Automated, Beecher Instr.)
- MLH1, MSH2, MSH6 and PMS2 IHC (+/-) (RTU, Dako)
- Promoter methylation assay (SALSA® MS-MLPA®,MRC-Holland)

## RESULTS

**Figure 2**
A, B) SOX9 expression in healthy human colon tissue.
C, D) High SOX9 expression in stage II colon cancer tissue.
E, F) Low SOX9 expression in stage II colon cancer tissue.




- SOX9 is mainly expressed in the nuclei of epithelial cells located in the proliferative zone of the colon crypt.
- Both high, moderate, low and absent SOX9 expression was observed.

## RESULTS

- The inter-observer agreement on the SOX9 score was highly concordant with a weighted Cohen's κ coefficient of 0.84 (CI95%: 0.77-0.91).
- The majority MMR deficient tumors were hyper-methylated in the *MLH1* promoter.

**Figure 3**
Relapse free survival according to the SOX9 expression level at the invasive front of stage II colon tumors.
A) High level, moderate level, and low level of SOX9.
B) Adjusted model with high level and moderate level of SOX9 combined compared to low level of SOX9.

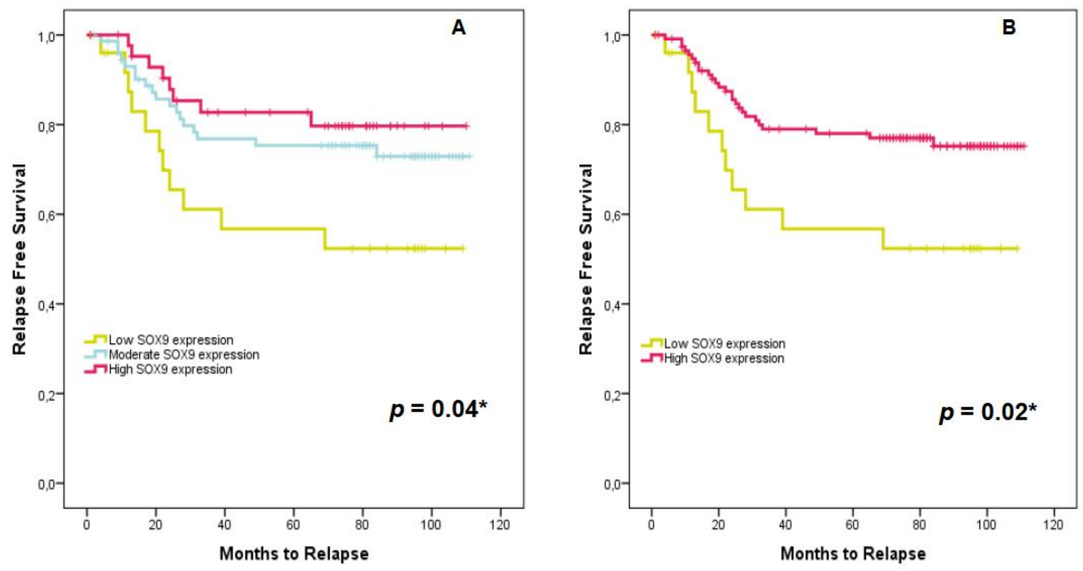


**Table 1**
Cox proportional hazards model for prediction of relapse with SOX9 expression as a continuous variable. The hazard ratio (HR) is presented with a difference of 3 in the scores.

| | | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | **No. of patients (%)** | **HR (95% CI)** | ***p* value** | **HR (95% CI)** | ***p* value** |
| **Overall** | 144 (100) | | | | |
| **Relapse** | | | | | |
| Yes | 37 (25.7) | | | | |
| No | 111 (74.3) | | | | |
| **SOX9 expression[a]** | | 0.73 (0.56-0.94) | 0.01* | 0.75 (0.58-0.96) | 0.02* |
| **MMR status** | | 0.19 (0.05-0.80) | 0.02* | 0.24 (0.06-0.99) | 0.05* |
| Deficient | 33 (22.9) | | | | |
| Proficient | 111 (77.1) | | | | |
| **Histological risk factors[b]** | | 3.01 (1.48-6.08) | >0.00* | 2.65 (1.31-5.39) | 0.01* |
| Yes | 72 (49) | | | | |
| No | 75 (51) | | | | |

a) SOX9 expression at the invasive front. b) Risk factors include <12 investigated lymph nodes, T4, low differentiation pattern, vein infiltration, nerve infiltration, direct invasion into other tissues, perforation of the tumor, and non-radical surgery.

## CONCLUSION

- Low level of SOX9 at the invasive front of the primary tumor is an independent predictor of relapse of stage II colon cancer patients in both univariate and multivariate analysis.
- MMR deficiency is negatively associated with relapse of stage II colon cancer patients.

## REFERENCES

[1] Lü *et al.*: Analysis of SOX9 expression in colorectal cancer. *Am J Clin Pathol* 2008;130:897-904.
[2] Candy *et al.*: Notch-induced transcription factors are predictive of survival and fluorouracil response in colorectal cancer patients. *Br J Cancer* 2013; 109:1023-30.

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