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Clinical Implications of Intestinal Stem Cell Markers in Colorectal Cancer

Maiken Lise Marcker Espersen,1,2 Jesper Olsen,2,3 Dorte Linnemann,1 Estrid Høgdall,1 Jesper T. Troelsen2

Abstract
Colorectal cancer (CRC) still has one of the highest incidence and mortality rate among cancers. Therefore, improved differential diagnostics and personalized treatment are still needed. Several intestinal stem cell markers have been found to be associated with CRC and might have a prognostic and predictive significance in CRC patients. This review provides an overview of the intestinal stem cell markers leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), B cell–specific Moloney murine leukemia virus insertion site 1 (BMI1), Musashi1 (MSI1), and sex-determining region y-box 9 (SOX9) and their implications in human CRC. The exact roles of the intestinal stem cell markers in CRC development and progression remain unclear; however, high expression of these stem cell markers have a potential prognostic significance and might be implicated in chemotherapy resistance.

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Keywords: Biomarkers, BMI1, LGR5, MSI1, SOX9

Introduction
Colorectal cancer (CRC) is one of the most common cancers in the developed world and carries the second highest mortality rate.1 Thus, there is a great need for improved differentiated diagnosis and personalized treatment of CRC patients.

Sporadic CRC arises as a consequence of lacking homeostatic control of proliferation and apoptosis within colon epithelial cells, driving the cells toward immortality and enhanced proliferation. This deregulation is caused by genetic and epigenetic alterations impairing essential pathways involving p53, PI3K, epidermal growth factor receptor (EGFR), and the canonical Wnt-signaling pathway. The Wnt signaling pathway is a major driver of CRC initiation and progression. Upon activation of the Wnt signaling pathway, β-catenin is translocated from the cytoplasm into the nucleus, where it associates with TCF/LEF transcription factors, thus regulating downstream Wnt target genes, such as CMYC.2,3 The essential Wnt-associated gene adenomatous polyposis coli (APC) is one of the most frequently mutated genes in early neoplastic transformation. Other Wnt signaling–associated genes have additionally been described as altered in CRC, including the ring finger protein 43 (RNF43) gene, which recently was described to be one of the most commonly mutated genes in CRC.2-7 Moreover, TP53 and the KRAS oncogene are also commonly affected in CRC, with the mutational status of KRAS oncogene being predictive for anti-EGFR monoclonal antibody therapy.8

Another hallmark of CRC is DNA mismatch repair (MMR) deficiency, which is reported in approximately 15% of all cases of CRC. The most commonly affected MMR genes are MLH1, MSH2, and MSH6. MMR deficiency causes accumulation of mutations and microsatellite instability (MSI), where microsatellite sequences in the genome are altered. MSI tumors are further subdivided according to the frequency of MSI into high frequency of MSI or low frequency of MSI. Colorectal tumors with impaired MMR are predominantly associated with right-sided colon tumors and correlate to a favorable prognosis.9

The traditional stochastic model of cancer development argues that in principle, all tumor cells are biologic equivalents and have the potential to proliferate and drive tumor growth.10 Within recent years, the traditional cancer model has been challenged by another model, the cancer stem cell model. The cancer stem cells model proposes that tumors are composed of a hierarchy of cells that are biologically distinct.10,11 Cells with stem cell properties reside within the tumor and are responsible for tumor initiation, progression, metastasis, recurrence, and resistance to chemotherapy.12
Lgr5 expressing cells are proposed to mark actively cycling stem cell markers and their potential role in human CRC.

**LGR5**

LGR5 was initially identified in 1998. The receptor did not receive much attention until 2007, where it was reported to be a potential stem cell marker of the small intestine and colon in mice. Lgr5 expressing cells are long-lived and have the ability to generate all cell types of the small intestine and colon epithelia. Lgr5 is expressed in cells at the bottom of the colonic crypts and in crypt base columnar cells interspersed between the Paneth cells at position 4 referring to the location of stem cells approximately 4 cells from the bottom of the crypts. Several stem cell markers have been identified, including B cell—specific Moloney murine leukemia virus insertion site 1 (Bmi1), telomerase reverse transcriptase (Tert), and homeodomain only protein X (Hopx). Leucine-rich repeat and immunoglobulin-like domains 1 (Lrig1), Musashi1 (MSI1), and Sox9 have been suggested as more general markers marking both stem cell populations. Most of the studies have focused on the adult stem cells of the small intestine. However, within recent years, several of these stem cell markers have been linked to CRC, and an increase in their expression level in the primary tumors of CRC patients has been correlated to a poor prognosis and chemotherapy resistance. Some of these markers have been more extensively investigated than others. The most investigated intestinal stem cell markers in a clinical setting are LGR5, MMR deficient tumors compared to MMR intact tumors. Furthermore, LGR5 expression might also correlate to lymph node and distant metastasis. A positive association between high expression of LGR5 at the invasive front of the tumor and advanced disease stage has been reported. In addition, LGR5 expression at the luminal surface was inversely correlated to the progression of disease. However, others have found no significant impact of the distribution of LGR5 expressing cells within CRC.

High LGR5 expression in CRC might correlate to lower disease-free survival, overall survival, and cancer-specific survival, indicating that LGR5 is a potential prognostic marker. However, this is inconsistent, with other studies finding that LGR5 does not have a...
**Table 1** Implications of LGR5 Expression in Human CRC

<table>
<thead>
<tr>
<th>Main Findings</th>
<th>Study Cohort (No. of Specimens)</th>
<th>AJCC Stage (No. of Patients Analyzed)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGR5 expression is not associated with prognosis.</td>
<td>Tumor (891)</td>
<td>NS</td>
<td>Ziskin et al., 2013</td>
</tr>
<tr>
<td>Increased LGR5 expression in CRC and correlates with female sex.</td>
<td>Tumor (102) Unpaired healthy (12)</td>
<td>I + II (35)</td>
<td>Fan et al., 2010</td>
</tr>
<tr>
<td>Increased expression of LGR5 in distant metastasis derived from tumors with LGR5 positive cells in tumor buds and vascular compartments of the primary tumor.</td>
<td>Tumor (89)</td>
<td>III (45)</td>
<td>Kleist et al., 2011</td>
</tr>
<tr>
<td>LGR5 expression at the invasive front is positively correlated to advanced disease.</td>
<td>Tumor (30)</td>
<td>I + II (17)</td>
<td>Takeda et al., 2011</td>
</tr>
<tr>
<td>LGR5 expression at the luminal surface is inversely correlated to disease stage.</td>
<td>Tumor (53) Paired healthy (53)</td>
<td>I (9)</td>
<td>He et al., 2014</td>
</tr>
<tr>
<td>LGR5 expression correlates with TNM stage, lymph node metastasis, and vascular invasion.</td>
<td>Tumor (296) Paired healthy (216)</td>
<td>I (60)</td>
<td>Hsu et al., 2013</td>
</tr>
<tr>
<td>High levels of LGR5 correlate to poor prognosis.</td>
<td>Tumor (89) Unpaired healthy (72)</td>
<td>III (45)</td>
<td>Takahashi et al., 2011</td>
</tr>
<tr>
<td>LGR5 expression at the luminal surface is inversely correlated to disease stage.</td>
<td>Tumor (89) Unpaired healthy (72)</td>
<td>III (45)</td>
<td>Takahashi et al., 2011</td>
</tr>
<tr>
<td>LGR5 expression correlates with AJCC stage and TNM stages. High LGR5 expression correlates with poor prognosis.</td>
<td>Tumor (89) Unpaired healthy (72)</td>
<td>III (45)</td>
<td>Takahashi et al., 2011</td>
</tr>
<tr>
<td>LGR5 mRNA is significantly up-regulated in CRC.</td>
<td>Tumor (39) Paired healthy (39)</td>
<td>I to IV (39)</td>
<td>McClanahan et al., 2006</td>
</tr>
<tr>
<td>Patients with high LGR5 expression in their primary tumors have a poorer prognosis.</td>
<td>Tumor (180) Paired healthy (180)</td>
<td>O (0)</td>
<td>Takahashi et al., 2011</td>
</tr>
<tr>
<td>LGR5 is an independent prognostic marker.</td>
<td>Tumor (192) Paired healthy (80)</td>
<td>I (47)</td>
<td>Wu et al., 2012</td>
</tr>
<tr>
<td>LGR5 correlates with lymph node metastasis, vascular invasion, lymphatic invasion, tumor depth, and tumor grade.</td>
<td>Tumor (50) Paired healthy (50)</td>
<td>I (25)</td>
<td>Uchida et al., 2010</td>
</tr>
<tr>
<td>LGR5 expression is increased in stage IV CRC patients compared to normal matched mucosa.</td>
<td>Tumor (42) Paired healthy (42)</td>
<td>IV (42)</td>
<td>Gao et al., 2014</td>
</tr>
<tr>
<td>LGR5 homozygous wt genotype in blood associated with a lower time to tumor reoccurrence in CRC patients than LGR5 heterozygote patients.</td>
<td>Tumor (234)</td>
<td>High risk II (105)</td>
<td>Gerger et al., 2011</td>
</tr>
<tr>
<td>LGR5 gene variation correlates negatively with LGR5 protein expression in CRC.</td>
<td>Tumor (89) Unpaired buccal swaps (72)</td>
<td>III (45)</td>
<td>Kleist et al., 2012</td>
</tr>
<tr>
<td>LGR5 expression is associated with a favorable prognosis.</td>
<td>Tumor (90)</td>
<td>II (90)</td>
<td>de Sousa E Melo et al., 2011</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC = American Joint Committee on Cancer; CRC = colorectal cancer; LGR5 = leucine-rich repeat-containing G-protein-coupled receptor-5; NS = Not specified; Paired healthy = healthy colon mucosa from same individual as investigated tumor; TNM = tumor, node, metastasis classification system; wt = wild type.

prognostic significance and high expression of Wnt-driven intestinal stem cell markers, including LGR5, within CRC tissue is associated with a favorable prognosis. Interestingly, increased LGR5 mRNA expression in the peripheral blood of CRC patients has also been associated with a poor outcome. This might reflect circulating cancer cells with stem cell–like properties playing a role in the metastatic event.

CRC cell line studies describe LGR5+ cells to be associated with chemotherapeutic resistance and resistance mechanisms. Accordingly, patients with low levels of LGR5 within their primary tumors have a significant better response rate to 5-fluouracil (5-FU)-based therapy than patients with high levels of LGR5. Studies on the implications of LGR5 in CRC patients are summarized in Table 1.

These studies suggest that LGR5 might be of relevance as a prognostic and predictive marker.

**BMI1**

BMI1 is a component of the polycomb repressive complex 1, which plays an important role in gene silencing by chromatin modification in cells, including, among others, embryonic and adult stem cells. Bmi1 was initially identified as an oncogene that, together with c-myc, plays a role in initiation of mouse B cell lymphomas. It was later found to be important in hematopoiesis and neural development. BMI1 targets the Ink4A/Arf locus, which encodes the critical cell cycle regulators p16 and p19ARF (p14ARF in humans). These are involved in the retinoblastoma protein (Rb) and p53 signaling pathways, regulating cell cycle and apoptosis.

In vivo lineage tracing suggests that Bmi1 expression marks small intestinal stem cells located at the +4 position from the crypt bottom in mice. These stem cells are functionally distinct from the Lgr5 expressing stem cell population. The +4 putative stem cells are characterized by being quiescent, being resistant to...
irradiation, and having regenerative potential after injury or ablation of Lgr5 expressing cells.\textsuperscript{19,83,84} Whether two functionally distinct intestinal adult stem cell populations exist and whether expression of Bmi1 actually marks +4 stem cells are still controversial.\textsuperscript{35,36}

Bmi1 has been described to be low expressed or absent in the nucleus of human colon epithelial cells at the very bottom of the crypt.\textsuperscript{42-44} The exact role of Bmi1 in the normal colon and in CRC is unclear. Table 2 lists studies that have investigated the implications of Bmi1 in human CRC. Several studies report overexpression of the Bmi1 at the protein and mRNA levels in CRC relative to healthy colon tissue.\textsuperscript{42-47} Human CRC cells have been proposed to require Bmi1 expression for maintenance of tumor growth.\textsuperscript{87} Furthermore, knockdown of Bmi1 severely affects the self-renewal capacity in vitro and impairs the cancer-initiating potential of human colon cancer cells in mice.\textsuperscript{87} Bmi1 expression might be inversely correlated to various cell cycle proteins, eg, p14 and p16, and positively correlated to c-MYC expression, although findings are contradictory.\textsuperscript{43-45} Inhibition of Bmi1 results in growth arrest of the preestablished tumors \textit{in vivo}.\textsuperscript{87} These results suggest Bmi1 as a relevant therapeutic target of CRC.

Bmi1 expression has also been correlated to several clinicopathologic factors, such as tumor size, serum carcinoembryonic antigen levels, and histologic differentiation grade.\textsuperscript{43,46} A gradient of Bmi1 expression can be observed in human colon precancerous and cancerous tissue. Here, low-grade intraepithelial dysplastic tissue has the lowest expression and high-grade dysplastic and cancerous tissue has the highest.\textsuperscript{45} Bmi1 expression is correlated to cancer stage, suggesting that Bmi1 might be associated with colon cancer progression.\textsuperscript{45-47}

The prognostic significance of Bmi1 expression in colorectal tumors is conflicting. More patients with Bmi1 positive tumors have tumor recurrence or metastases compared to patients with Bmi1 negative tumors.\textsuperscript{47} Furthermore, high Bmi1 expression in primary tumors from CRC patients is an independent prognostic factor for disease-free survival and for overall survival.\textsuperscript{46,47} However, high Bmi1 expression is also correlated with a better prognosis compared to patients with low expression.\textsuperscript{48} By combining several biomarkers with the Bmi1 expression, prognostic stratification is improved.\textsuperscript{48} In addition, another recent study reported that patients with decreased postoperative plasma mRNA levels of Bmi1 compared to patients with increased postoperative mRNA levels correlated with a favorable prognosis in CRC.\textsuperscript{49} These studies suggest that Bmi1 may be of relevance as a prognostic indicator in CRC. However, the exact directionality of its prognostic utility remains to be elucidated.

### Table 2 Implications of Bmi1 in CRC Patients

<table>
<thead>
<tr>
<th>Main Findings</th>
<th>Study Cohort (No. of Specimens)</th>
<th>AJCC Stage (No. of Patients Analyzed)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmi1 is overexpressed in CRC.</td>
<td></td>
<td></td>
<td>Reinish et al., 2006\textsuperscript{42}</td>
</tr>
<tr>
<td>Bmi1 expression correlates with gender, histologic tumor differentiation, tumor size, and serum CEA levels.</td>
<td>Tumor (11) Paired healthy (11)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Bmi1 expression has an inverse correlation to the expression of p16 and p14.</td>
<td>Tumor (87) Paired healthy (87)</td>
<td>NS; N and M stage provided</td>
<td>Kim et al., 2004\textsuperscript{43}</td>
</tr>
<tr>
<td>Bmi1 is overexpressed in human low-grade intraepithelial dysplasia, high-grade intraepithelial dysplasia, and cancer.</td>
<td>NS</td>
<td>NS</td>
<td>Tateishi et al., 2006\textsuperscript{44}</td>
</tr>
<tr>
<td>High expression of Bmi1 correlates with metastasis and advanced stage of cancer.</td>
<td>Tumor (43) Paired healthy (43)</td>
<td>I (9) II (18) III (10) IV(6)</td>
<td>Liu et al., 2010\textsuperscript{45}</td>
</tr>
<tr>
<td>High expression of Bmi1 is associated with a lower overall survival.</td>
<td>Tumor (98) Paired healthy (98)</td>
<td>II (29) III (69)</td>
<td>Du et al., 2010\textsuperscript{46}</td>
</tr>
<tr>
<td>Patients with Bmi1 positive tumors have a lower disease-free survival and a lower overall survival.</td>
<td>Tumor (203) Paired healthy (203)</td>
<td>I (24) II (81) III (80) IV (18)</td>
<td>Li et al., 2010\textsuperscript{47}</td>
</tr>
<tr>
<td>High expression of Bmi1 is associated with a better prognosis. Combination of Bmi1 with other biomarkers improves the prognostic stratification when compared to applying the biomarkers individually.</td>
<td>Tumor (247) Paired healthy (47)</td>
<td>I (52) II (110) III (65)</td>
<td>Benard et al., 2014\textsuperscript{48}</td>
</tr>
<tr>
<td>Patients with decreased postoperative plasma Bmi1 mRNA levels have a better prognosis than patients with increased postoperative Bmi1 mRNA levels</td>
<td>Tumor (45)</td>
<td>I + II (15) III + IV (12) NS (18)</td>
<td>Pun et al., 2014\textsuperscript{49}</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC = American Joint Committee on Cancer; Bmi1 = B cell—specific Moloney murine leukemia virus insertion site 1; CEA = carcinoembryonic antigen; CRC = colorectal cancer; NS = not specified; Paired healthy = healthy colon mucosa from same individual as investigated tumor.
MSI1 functions as suppressor by binding to its target mRNA, thus repressing translation of its downstream targets.92 Additionally, MSI1 compete with eukaryotic initiation factor 4G (eIF4G) for binding to the poly(A)-binding protein (PABP), thereby inhibiting translation initiation.93 Two of the most recognized RNA targets of MSI1 are the genes encoding the Notch antagonist Numb and p21, an inhibitor of cyclin-dependent kinases.94,95 MSI1 was also found to negatively regulate APC translation in human cultured colonocytes.96 Interestingly, reduced APC expression leads to increased levels of MSI1, suggesting that MSI1 itself is a target of the Wnt signaling pathway,96 consistent with an earlier study describing a TCF/LEF binding site on the Msi1 promoter.97 This positive feedback loop might be important for regulating homeostasis of translation initiation.93 Two of the most recognized RNA targets of MSI1 are the genes encoding the Notch antagonist Numb and p21, an inhibitor of cyclin-dependent kinases.94,95 MSI1 was also found to negatively regulate APC translation in human cultured colonocytes.96

Intestinal epithelium cells overexpressing Msi1 increase proliferation and acquire tumorigenic features in xenografts.97 In accordance, knockdown of MSI1 in human colon cancer cells leads to inhibition of proliferation and reduced migratory potential.50 Moreover, knockdown of MSI1 in xenografts results in tumor growth arrest, suggesting that MSI1 may play a role in tumor progression.51 Studies investigating the implications of MSI1 in human CRC are listed in Table 3. The level of MSI1 mRNA expression has been reported to be significantly increased in human colorectal adenocarcinomas, and the expression level varies in normal, adenoma, and carcinoma of colon tissues.50-52 MSI1 expressing tumor cells of the colon predominantly also, although not exclusively, express MSI1 in the cytoplasm.52 MSI1 is often focally expressed in adenomas, whereas the expression pattern in carcinomas is more diffuse.52 Moreover, MSI1 overexpression is significantly associated with the proliferation marker Ki-67, advanced cancer stage, and a more aggressive disease phenotype.50-52 When adjusted for American Joint Committee on Cancer stage, vessel infiltration, histologic type, and grade, MSI1 appears as an independent prognostic marker for prediction of poor outcome in stage III and IV disease (but not stage I and II disease).50 Furthermore, positive MSI1 expression in the primary tumor is associated with a nearly 5.4-fold increased risk of distant metastasis.50 This poor outcome in patients with stage III and IV cancers, who generally receive adjuvant chemotherapy, may be explained by a study in mice showing that MSI1 positive cells are insensitive to 5-FU.98

These studies suggest that MSI1 might be of relevance as both a negative prognostic marker and a predictive marker.

### Table 3 Implications of MSI1 in CRC Patients

<table>
<thead>
<tr>
<th>Main Findings</th>
<th>Study Cohort (No. of Specimens)</th>
<th>AJCC Stage (No. of Patients Analyzed)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI1 is an independent prognostic marker to predict poor outcome.</td>
<td>Tumor (203) Paired healthy (203)</td>
<td>I (24) II (39) III (80) IV (18)</td>
<td>Li et al., 201150</td>
</tr>
<tr>
<td>&gt;2-fold increase in MSI1 mRNA expression in majority of CRC.</td>
<td>Tumor (15) Paired healthy (15)</td>
<td>NS</td>
<td>Sureban et al., 200851</td>
</tr>
<tr>
<td>MSI1 expression correlates to TNM stage.</td>
<td>Tumor (69) Unpaired healthy (8)</td>
<td>I + II (39) III + IV (30)</td>
<td>Fan et al., 201052</td>
</tr>
<tr>
<td>MSI1 mRNA expression differs in normal, adenoma, and carcinoma.</td>
<td>Tumor (31) Unpaired healthy (10)</td>
<td>NS</td>
<td>Fan et al., 201052</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC = American Joint Committee on Cancer; CRC = colorectal cancer; MSI1 = Musashi1; NS = not specified; Paired healthy = healthy colon mucosa from same individual as investigated tumor; TNM = tumor, node, metastasis classification system.
Several studies have also described increased expression of SOX9 at both mRNA and protein level in human CRC specimens and cell lines compared to healthy colon epithelia. Only one small study \((n=10)\) has described a decrease in SOX9 expression in colorectal adenocarcinomas. There is no significant difference in SOX9 expression when comparing adenomatous expression and cancerous expression. SOX9 is expressed in a random heterogeneous manner throughout colorectal tumors. Moreover, a strong expression of SOX9 is more common in non-mucin-producing CRC than mucinous or signet ring carcinomas. One study describes that SOX9 overexpression correlates with vascular invasion in the primary tumor. Others find that high SOX9 expression correlates with age, female sex, and MSI tumors, especially MSI-high tumors. In contrast, correlation between down-regulated SOX9 expression and MSI relative to microsatellite stable tumors has also been described. Table 4 lists the studies on SOX9 in human CRC.

Correlation between SOX9 expression levels and patient survival is inconsistent. When stratified for American Joint

### Table 4 Implications of SOX9 in CRC Patients

<table>
<thead>
<tr>
<th>Main Findings</th>
<th>(No. of Specimens)</th>
<th>AJCC Stage (No. of Patients Analyzed)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX9 is frequently mutated in nonhypermutated tumors.</td>
<td>Tumor (224)</td>
<td>NS</td>
<td>Cancer Genome Atlas Network, 2012</td>
</tr>
<tr>
<td>Heterogeneous expression of SOX9 in tumors.</td>
<td>Paired healthy/blood (224)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOX9 is up-regulated in CRC.</td>
<td>Tumor (3)</td>
<td>NS</td>
<td>Ramalingam et al., 2012</td>
</tr>
<tr>
<td>SOX9 mRNA is up-regulated in CRC and associated with advanced tumor stage.</td>
<td>Tumor (110)</td>
<td>NS</td>
<td>Matheu et al., 2012</td>
</tr>
<tr>
<td>SOX9 overexpression correlates with poorer survival in 5-FU-treated stage III patients.</td>
<td>Tumor (79)</td>
<td>NS</td>
<td>Matheu et al., 2012</td>
</tr>
<tr>
<td>Strong SOX9 expression is most common in non-mucin-producing CRC.</td>
<td>Tumor (441)</td>
<td>II (280) III (161)</td>
<td>Candy et al., 2013</td>
</tr>
<tr>
<td>Strong SOX9 expression correlated with lower overall survival.</td>
<td>Tumor (188)</td>
<td>I/I (97) II (86)</td>
<td>Lü et al., 2008</td>
</tr>
<tr>
<td>SOX9 is overexpressed in CRC.</td>
<td>Tumor (27)</td>
<td>I (6) II (12) III (5) IV (1) NS (3)</td>
<td>Abdel-Samad et al., 2011</td>
</tr>
<tr>
<td>A truncated variant of SOX9 is overexpressed in CRC.</td>
<td>Tumor (17)</td>
<td>I (5) II (8) III (2) IV (1) NS (1)</td>
<td>Abdel-Samad et al., 2011</td>
</tr>
<tr>
<td>SOX9 is up-regulated in CRC.</td>
<td>Tumor (10)</td>
<td>NS</td>
<td>Lü et al., 2006</td>
</tr>
<tr>
<td>Increased SOX9 gene expression in CRC.</td>
<td>Tumor (77)</td>
<td>I/II/III (39) IV/IV (38)</td>
<td>Huang et al., 2013</td>
</tr>
<tr>
<td>High levels of SOX9 associated with age and MSI. No significant decrease in survival for the patients with high SOX9 expression.</td>
<td>Tumor (31)</td>
<td>Dukes A (1) Dukes B (11) Dukes C (19)</td>
<td>Panza et al., 2013</td>
</tr>
<tr>
<td>SOX9 is up-regulated in CRC. SOX9 is down-regulated in MSI relative to MSS tumors.</td>
<td>Tumor (424)</td>
<td>I (23) II (540) III (49) IV (12)</td>
<td>Andersen et al., 2009</td>
</tr>
<tr>
<td>SOX9 was down-regulated in CRC.</td>
<td>Tumor (10)</td>
<td>NS</td>
<td>Chen et al., 2008</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC = American Joint Committee on Cancer; CRC = colorectal cancer; 5-FU = 5-fluorouracil; MSI = microsatellite instable; MSS = microsatellite stable; NS = not specified; Paired healthy = healthy colon mucosa from same individual as investigated tumor; SOX9 = sex-determining region Y-box 9.
Committee on Cancer stage. SOX9 protein overexpression is associated with a lower survival in 5-FU—treated stage III cancers.\(^{54}\) This is not the case for 5-FU—untreated stage III or stage II CRC patients,\(^{54}\) suggesting that SOX9 may be a prognostic indicator in patients receiving 5-FU adjuvant chemotherapy. It should be noted, however, that the study did not adjust for MMR deficiency, which may have both a prognostic value and a predictive value with regard to 5-FU resistance.\(^{106,107}\)

**Discussion**

It is evident that all the 4 stem cell markers are overexpressed at the protein and mRNA levels in primary tumors of CRC patients compared to normal mucosa and that this may have prognostic significance. The exact mechanisms and functions of the increased expression of the intestinal stem cell markers remain to be elucidated, as studies are contradictory with respect to the oncogenic or tumor-suppressive functions. Apparently the proteins play important roles in essential signaling pathways, such as Rb, p53, Notch, and Wnt signaling, in which deregulation of these often are involved in the carcinogenic process. Most studies identifying and investigating adult stem cells of the intestines focus on the small intestine, with little attention paid to the actual colon stem cells. The evidence of Lgr5 marking both colon and small intestine stem cells is convincing.\(^{18}\) However, this is less established for the other 3 markers, with Sox9 studies suggesting that the stem cell populations of the small intestine and the colon might differ completely with respect to the Sox9 expression level.\(^{47,20}\) This could to some extent explain the somewhat different carcinogenic functions observed in CRC cell lines using small intestinal stem cell markers. In addition, pooling right-sided and left-sided colon tumors, and rectum tumors with different mutational profiles in the same investigations might further add to the diversity of expression signatures.\(^{108}\)

Another potential mechanism to the opposing results could be an alternating expression along the carcinogenic progression such that the stem cell—associated pathways or intestinal stem cell proteins are silenced during certain stages of progression and reexpressed at other stages. It has also been suggested that a more primitive stem cell program than the intestinal stem cell signatures might play a role in the progression of CRC.\(^{41}\)

Variants of the different stem cell proteins might also add to the contradicting results of the intestinal stem cell markers’ implications in cancer development and progression. Few studies indicate that variants and mutations of the stem cell genes might be of importance from a prognostic perspective.\(^{3,39,40,53,56}\) Thus, further studies on the functional role and clinical significance of these variants and mutations are needed. LGR5, MSI1, SOX9, and BMI1 expression correlates to various clinicopathologic features of primary colorectal tumors. However, there are some discrepancies between the studies. This could be due to the relatively rare event of some of the features and the relatively small numbers of included patients in some of the studies. However, common to all stem cell markers is that their expression level has been correlated to more advanced disease stage. Furthermore, tumors with MMR deficiency have low expression levels of LGR5 and SOX9, which is in accordance with the favorable prognosis of patients with MMR defect tumors\(^{106,107}\) and the poor prognosis associated with an increased LGR5 or SOX9 expression in some studies.\(^{17,60}\)

A hallmark of cancer stem cells is their potential resistance to chemotherapeutic drugs. Interestingly, low expression of LGR5 in primary tumors from CRC patients correlates with improved response to 5-FU—based chemotherapy, and SOX9 overexpression correlates with short survival in stage III 5-FU—treated CRC patients, suggesting that these markers might be relevant for predicting chemotherapy resistance.\(^{33,54}\) However, larger patient studies are needed to clarify these indications. Another strategy in trying to improve the prognostic and predictive value could be to combine several of the stem cell markers in a panel rather than using only one stem cell marker, as is seen in other studies focusing on other genes and proteins.\(^{48,109}\)

Furthermore, adjusting for known predictive and prognostic factors, such as MMR deficiency, are necessary to further clarify the significance of the stem markers as prognostic and predictive biomarkers.

The expression of the 4 stem cell markers has been described as heterogeneous within tumor tissue. Interestingly, the location of LGR5 expressing tumor cells within the tumor might be relevant in relation to disease stage.\(^{51}\) Thus, it can be speculated that increased expression of stem cell—like cells at the invasive front of the tumor might be associated with a more aggressive cancer phenotype. Several studies have thus implied a role of the stem cell markers in metastasis. However, a heterogeneous expression pattern potentially compromises the use of tissue microarrays, an emerging technique for large-scale patient studies. This technique has been used by several of the studies. If the intestinal stem cell markers are introduced to routine settings, a low-cost, simple analysis using full slides—eg, immunohistochemistry with clearly defined cutoff values—would be preferred to tissue microarray. Another challenge is the current lack of proper antibodies targeting LGR5, which precludes a proper investigation of LGR5 in human tissue.\(^{85}\)

In conclusion, the intestinal stem cell markers LGR5, BMI1, MSI1, and SOX9 are overexpressed in human CRC. The high expression of these stem cell markers might have a prognostic significance and may be associated with chemotherapy resistance. However, further extensive studies are needed to elucidate whether these intestinal stem cell markers can be used as predictive and prognostic biomarkers in a clinical setting.

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