# Chapter 3
# *Streptococcus bovis* and Colorectal Cancer

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**Abstract** The most salient feature of *Streptococcus bovis* (SB) is its clinical association with malignancy of the colon and rectum. The relationship between SB and colorectal cancer (CRC) was already recognized in the 1950s and many case reports and retrospective studies on this association have been published since then. SB is an opportunistic pathogen that normally resides asymptomatically in the human intestinal tract. In compromised individuals, however, this bacterium can cause systemic infections most often presenting as bacterial endocarditis. Investigators reported the presence of colorectal tumours in up to 60% of the cases in which a patient was diagnosed with SB endocarditis or bacteremia. Therefore, these infections are nowadays often regarded as indication for full bowel examination in clinical practice. Importantly, recent studies have indicated that the association between *S. gallolyticus* subsp *gallolyticus* (previously called SB biotype I) with CRC seems much more pronounced than that of other known SB biotypes. Nevertheless, the question whether SB has a causal or predominantly incidental involvement with cancer of the colon remains to be answered. Furthermore, still little is known about the precise molecular mechanisms that determine this specific relationship. This chapter aims to summarize the literature on this subject and to illustrate possible mechanisms behind the association of SB with CRC.

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## Abbreviations

| | |
|---|---|
| CRC | Colorectal cancer |
| ELISA | Enzyme-linked immunosorbent assay |
| HP | *Helicobacter pylori* |
| IBD | Inflammatory bowel disease |
| IC-TOF MS | Immunocapture time-of-flight mass spectrometry |
| MMP | Matrix metalloproteinases |
| NSAID | Non steroidal anti inflammatory drugs |
| OR | Odds ratio |
| PAH | Polycyclic aromatic hydrocarbons |
| SB | *Streptococcus bovis* |
| SIC | *S. infantarius* subsp. *coli* |
| SGG | *S. gallolyticus* subsp. *gallolyticus* |
| SGM | *S. gallolyticus* subsp. *macedonicus* |
| SGP | *S. gallolyticus* subsp. *pasteurianus* |
| SII | *S. infantarius* subsp. *infantarius* |

## 3.1 Colorectal Cancer and Microbial Agents

Colorectal cancer (CRC) is the third most common cancer for men and women in Western society. It is estimated that nearly 150,000 cases were newly diagnosed and 50,000 persons died of this disease in year 2009 in the USA (Horner et al. 2009). The temporal and geographic variations in CRC incidence in US whites and blacks (Horner et al. 2009) and among immigrants (Curado et al. 2007) are best explained by environmental factors rather than genetic predisposition. According to Dr. Parkin's estimate (Parkin 2006), 17.8% of the worldwide cancer incidence is attributable to infectious agents, resulting in approximately 1.9 million cases per year. These include a variety of infectious agents: parasites such as *Schistosoma haematobium* and *Opisthorchis viverrini*, bacteria, such as *Helicobacter pylori* (HP), and, viruses, such as Epstein-Barr virus, Hepatitis virus, and Human herpes, papilloma (HPV), polyma and retro-viruses (IARC 1994; Persing and Prendergast 1999; Del Valle et al. 2002). Several mechanisms have been proposed, including direct effects on host cell proliferation and communication pathways, impairment of host immune system, induction of genomic instability and chronic inflammation (Herrera et al. 2005). Chronic inflammation often accompanies increased host cell turnover, which increases the probability of mutagenic events, and enhanced formation of reactive

oxygen and nitrogen species that damage DNA and induce genomic instability (Coussens and Werb 2002; Blaser 2008; Hussain and Harris 2007; Terzić et al. 2010). Thus, inflammatory responses play decisive roles at different stages of tumour development, including initiation, promotion, malignant conversion, invasion, and metastasis (Grivennikov et al. 2007). Genomic instability may arise from inactivation of DNA mismatch repair (MMR) system (MSH1/2), which leads to the development of a specific molecular subtype of CRC termed microsatellite instability high (MSI-H) (Jass 2007). MSI has been observed frequently in long standing ulcerative colitis mucosa (Ishitsuka et al. 2001) as well as in HP-positive gastric cancer (Li et al. 2005) and MSH2 deficient mice are susceptible to inflammation associated colorectal tumours (Kohonen-Corish et al. 2002). In addition, overexpression of a COX-2 receptor protein has been characterized for MSI-H tumours (Baba et al. 2010). The large bowel is indeed the natural habitat for a large, dynamic and highly competitive bacterial community, which is essential for the control of intestinal epithelial homeostasis and human health. Strikingly, the increase in bacterial colonization from the ileum to the colon (six orders of magnitude; Stone and Papas 1997), is paralleled by a marked difference in cancer incidence (by at least a factor of 30) between the small and large intestines. Although bacterial etiologies in sporadic CRC have never been firmly established in humans, studies in germ free mice suggest that intestinal bacteria are indeed required for colorectal carcinogenesis in model systems (Hope et al. 2005; Sinicrope 2007). Finally, there is good evidence that aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of CRC and its precursor (Rostom et al. 2007; Dubé et al. 2007).

## 3.2 Microbiological Characteristics of *Streptococcus bovis*

*Streptococcus bovis* (SB) is a gram-positive bacterium and lower-grade opportunistic pathogen that can cause systemic infections (endocarditis or bacteraemia) in humans. It is a group D streptococcus with the specific ability to grow in 40% bile (Moellering et al. 1974; Roberts 1992). The classification and identification of SB has been problematic for a long time. Based on phenotypic diversity, three SB biotypes (I, II/1, and II/2) have been reported. Recently, based on biochemical traits, DNA homology and divergence in 16S rRNA sequences, Schlegel et al. (2004) suggested to rename SB I into *S. gallolyticus* subsp. *gallolyticus* (SGG), to divide SB biotype II/1 strains into (i) *S. infantarius* subsp. *coli* (SIC) and (ii) *S. infantarius* subsp. *infantarius* (SII) and to rename SB II/2 into *S. gallolyticus* subsp. *pasteurianus* (SGP; see Table 3.1). In addition, the closely related non-pathogenic strain *Streptococcus macedonicus* was reclassified as *S. gallolyticus* subsp. *macedonicus* (SGM). Earlier studies suggest that SGG and SII are the most commonly isolated pathogens from the SB group, with the former being the more virulent in humans and more often associated with endocarditis (Corredoira et al. 2008a). In a recent reexamination of SB bacteremias in a 20-year period in France, the association of colon tumours with SGG was found to be ~50% versus 11% for SII. Strikingly,

**Table 3.1** Reappraisal of SB nomenclature

| New name | Old name | Association with CRC |
|---|---|---|
| *S. gallolyticus* subsp. *gallolyticus* (SGG) | *S. bovis* biotype I | ++++ |
| *S. infantarius* subsp. *infantarius* (SII) | *S. bovis* biotype II/1 | ++ |
| *S. infantarius* subsp. *coli* (SIC) | *S. bovis* biotype II/1 | ++ |
| *S. gallolyticus* subsp. *pasteurianus* (SGP) | *S. bovis* biotype II/2 | + |
| *S. gallolyticus* subsp. *macedonicus* (SGM) | *S. macedonicus* | – |

however, for non-colonic cancer the association was 6% for SGG versus 57% for SII. Most of the non-colonic cancers associated with SII were of the pancreas and biliary tract (Corredoira et al. 2008b). Because of the lack of unified terminology information in literature, we refer to both SGG and SII as SB in the rest of this chapter.

## 3.3 Association of SB with Colorectal Disease

Although SB is a member of normal gastrointestinal flora in ruminants, e.g., cattle, sheep, horses, pigs, camels and deers (Ghali et al. 2004), it can also found in human feces as well as gastric biopsy materials (Schlegel et al. 2000; Ribeiro et al. 2004). Approximately 10% of healthy individuals have been estimated to carry this bacterium asymptomatically in their digestive tract (Schlegel et al. 2000). While fecal-oral or oral-oral is a possible transmission route between humans, it may be acquired through dietary intake of ruminant-derived foods, such as unpasteurized dairy products (Randazzo et al. 2006), red meat and animal organs (Schlegel et al. 2000). In fact SB is a frequently detected contaminant in commercially available meat (Knudtson and Hartman 1993; Thian and Hartman 1981). The correlation between SB and colonic disease has long been recognized. Besides case-reports for the patients who were diagnosed with asymptomatic colorectal neoplasia simultaneously with SB endocarditis or bacteremia (McMahon et al. 1991; Nielsen et al. 2007; Wentling et al. 2006; Gupta et al. 2010; Kahveci et al. 2010; Kim et al. 2010), investigators have reported increased prevalence of colorectal tumours (cancer and polyps) among patients diagnosed with SB endocarditis or bacteremia. As summarized in the Table 3.2, the prevalence of colorectal tumours ranges from 10% to 60% (Corredoira et al. 2005, 2008a; Murray and Roberts 1978; Klein et al. 1979; Reynolds et al. 1983; Pigrau et al. 1988; Ruoff et al. 1989; Clarridge et al. 2001; Gonzlez-Quintela et al. 2001; Gold et al. 2004; Lee et al. 2003; Zarkin et al. 1990; Jean et al. 2004; Alazmi et al. 2006; Giannitsioti et al. 2007; Beck et al. 2008; Vaska and Faoagali 2009), although these are based on diverse study populations in terms of patient demographics and colorectal surveillance methods. These variations may also be due to the heterogenous definition of the cases, as adenomas have been defined as diseased in some studies but not in others (Boleij et al. 2009b). None of these studies, however, have evaluated their results in comparison with expected frequencies in the general population. The second set of evidence is derived from studies comparing

**Table 3.2** Summary of studies among SB bacteremia patients

| Author (year) | Study location | Patients (n) | Detected colorectal adenomas and carcinomas | |
|---|---|---|---|---|
| | | | n | % |
| Murray and Roberts (1978) | USA | 36 | 4 | 11% |
| Klein et al. 1979 | USA | 29 | 15 | 52% |
| Reynolds et al. (1983) | USA | 19 | 7 | 37% |
| Pigrau et al. (1988) | Spain | 16 | 1 | 6% |
| Ruoff et al. (1989) | USA | 38 | 15 | 39% |
| Zarkin et al. (1990) | USA | 43 | 16 | 37% |
| Clarridge et al. (2001) | USA | 12 | 1 | 8% |
| Gonzlez-Quintela et al. (2001) | Spain | 20 | 6 | 30% |
| Lee et al. (2003) | Hong Kong | 37 | 4 | 11% |
| Gold et al. (2004) | USA | 45 | 17 | 38% |
| Jean et al. (2004) | Taiwan | 19 | 9 | 47% |
| Corredoira et al. (2005) | Spain | 124 | 54 | 44% |
| Alazmi et al. (2006) | USA | 46 | 6 | 13% |
| Giannitsioti et al. (2007)[a] | France | 142 | 70 | 49% |
| Corredoira et al. (2008a) | Spain | 107 | 42 | 40% |
| Beck et al. (2008) | Germany | 15 | 7 | 47% |
| Vaska and Faoagali (2009) | Australia | 20 | 12 | 60% |

[a]Include other benign lesions e.g., diverticulosis and colitis

SB prevalence among various patient groups with or without colonic diseases (Table 3.3) (Klein et al. 1977; Burns et al. 1985; Darjee and Gibb 1993; Dubrow et al. 1991; Potter et al. 1998; Teitelbaum and Triantafyllopoulou 2006; Tjalsma et al. 2006; Abdulamir et al. 2009). While three small studies including 13–46 controls and corresponding 11 CRC, 47 pediatric inflammatory bowel disease (IBD) and 56 polyp patients failed to show any association (Dubrow et al. 1991; Potter et al. 1998; Teitelbaum and Triantafyllopoulou 2006), five other studies found SB carriage (either in stool or antibodies) rates were significantly higher in cancer patients than in controls. Interestingly, three studies also showed that patients with premalignant lesions (IBD or polyps) had intermediate SB carriage rate between cancer cases and controls (Klein et al. 1977; Teitelbaum and Triantafyllopoulou 2006; Tjalsma et al. 2006). For an update, see our recent literature-based meta-analysis on the association between *S. bovis* and CRC (Boleij et al. 2011).

## 3.4 Potential Mechanisms in Carcinogenesis

Despite observations discussed above, implications of SB infection on CRC remain largely elusive. There are several possible interpretations that are not necessarily mutually exclusive. First, it has been hypothesized that colorectal neoplastic sites provide a specific niche for SB resulting in sustained colonization, survival, and the

**Table 3.3** Summary of studies on SB prevalence by colonic disease status

| | No. of subjects | | | | |
|---|---|---|---|---|---|
| Author (year) | Controls | Premalignant | Cancer | SB detection | Significant results |
| Klein et al. (1977) | 105 | 25 (IBD) | 63 | Fecal culture | Cancer>controls |
| Burns et al. (1985) | 216 | 62 (advanced polyps) | 18 | Fecal culture | Cancer>controls |
| Dubrow et al. (1991) | 46 | 56 (polyps) | | Fecal culture | No significant differences |
| Darjee and Gibb (1993) | 16 | – | 16 | Antibody titer | Cancer>controls |
| Potter et al. (1998) | 13 | – | 11 | Fecal culture | No significant differences |
| Teitelbaum and Triantafyllopoulou (2006) | 34 | 47(IBD) | | Fecal culture | No significant differences |
| Tjalsma et al. (2006) | 8 | 4 (polyps) | 12 | Antibody patterns | Cancer/polyps>controls |
| Abdulamir et al. (2009) | 50 | 14 | 60 | Antibody titer | Cancer/adenoma>controls |

establishment of a local tumour-associated (clinically silent) infection. Second, silent SB infection itself possibly promotes colorectal carcinogenesis, which has been supported by several experimental studies. Administration of SB or SB wall extracted antigens in rodents increases the formation of colorectal precursor lesions in a chemical carcinogenesis model (Ellmerich et al. 2000a). This was accompanied by increased expression of proliferative markers and enhanced interleukin IL-8 production in normal colonic mucosa of SB-injected animals. SB wall antigens are capable of adhering to various types of human cells, including GI-epithelial, endothelial and blood cells, as well as to extracellular matrix and induce IL-8 synthesis (Ellmerich et al. 2000b). In fact, increased IL-8 positive cells have been reported in SB seropositive human CRC cases compared with SB-seronegative cases (Abdulamir et al. 2009). IL-8 is a pro-inflammatory cytokine which also possesses mitogenic and angiogenic properties. It increases oxidative/ nitrosative stress and mediate the formation of carcinogenic compounds in gastrointestinal mucosa/ lumen (Federico et al. 2007; Vermeer et al. 2004; Hussain and Harris 2007). IL-8 also leads to cyclooxygenase (COX)-2 overexpression (Biarc et al. 2004). COX-2 driven prostaglandin synthesis stimulates cell proliferation, motility and metastatic potential, promotes angiogenesis, and induces local immunosuppression (Harris 2007; Mutoh et al. 2006). On the other hand, selective and non-selective COX-2 inhibitors reduce the incidence and prevalence of colorectal polyps (Steinbach et al. 2000; Logan et al. 2008). Importantly, increased COX-2 expression has been demonstrated in rodent infectious colorectal carcinogenesis models (Skinn et al. 2006; Newman et al. 2001; Balish and Warner 2002; Wang and Huycke 2007). The induction of COX-2 by SB in colon tissue has been reported for a rat model (Biarc et al. 2004) and may also occur in humans (Fig. 3.1). These enhanced COX-2 activities may also exert synergistic effects with other enzymes sharing substrates (e.g., CYP1 family) in metabolic activation of diet-derived carcinogens, such as polycyclic aromatic hydrocarbons (PAH) found in cooked meat (Wiese et al. 2001; Almahmeed et al. 2004). Such an enzyme, CYP1A1/B1, is indeed overexpressed in CRC and its precursors (McKay et al. 1993; Kumarakulasingham et al. 2005; Chang et al. 2005). This interplay is potentially important because meat consumption is one of the SB acquisition routes and because meat-derived PAH can induce intestinal CYP1A1/ B1 (Lampen et al. 2004). In addition, SB can induce matrix metalloproteinases (MMPs), e.g., MMP2 and MMP9 (Mungall et al. 2001), that play crucial roles in CRC growth and progression (Paduch et al. 2010; Sinnamon et al. 2008; Kim et al. 2009; Miyake et al. 2009). Furthermore, SB may also contribute to intra-colonic formation of potential carcinogens, e.g., nitroso-compounds (McKnight et al. 1999). The human large intestine contains a large amount of nitrogenous residues and nitrosating agents from dietary protein, and enzymatic activities of intestinal bacteria, e.g., streptococci, mediate these reactions (Hughes et al. 2001; Calmels et al. 1996, 1988). Intriguingly, consumption of red meat, a presumed route of SB acquisition, promotes colonic N-nitrosation via increasing supplies of colonic amine, nitrite and arginine (Hughes et al. 2001; Bingham et al. 1996, 2002; Silvester et al. 1997). Notably, large intestinal N-nitrosation does not occur in germ-free animals (Rowland et al. 1991).

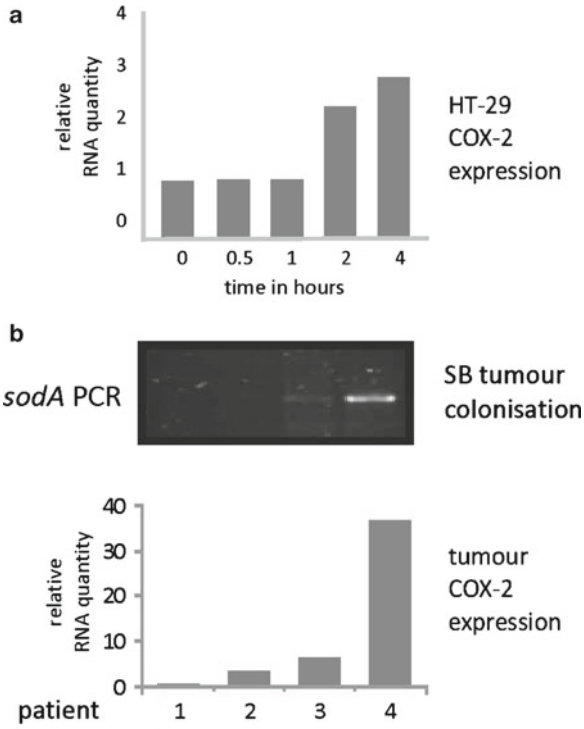


**Fig. 3.1** COX-2 induction by SB. (**a**) The induction of COX-2 by SB was measured in HT-29 colorectal tumour cells *in vitro*. SB and HT-29 cells were co-incubated for 0.5, 1, 2 or 4 h. Subsequently RNA was extracted for real-time PCR procedures. The relative expression of COX-2 was determined by real time PCR using GAPDH as endogenous internal control and considered to be induced at values greater than 1.5. The *bar graph* shows that SB induces the expression of COX-2 after 2 and 4 h which is consistent with previously results in literature (Biarc et al. 2004). (**b**) The correlation of COX-2 expression and the presence of SB in tumour tissue from 4 CRC patients was determined in parallel. The presence of SB was monitored by a nested PCR on the SB *sodA* gene. The results are suggestive for a correlation of SB and COX-2 expression *in vivo*

## 3.5 SB Serology in CRC Patients

Although infections have been recognized as a major preventable cause of human cancer (Kuper et al. 2000), bacterial etiologies in sporadic CRC have not been established in humans. Notably, SB has indeed been recognized as an infectious agent that fulfills the criteria for inferring causality to the highest extent among the four agents evaluated as a potential cause of CRC in a recent review (Burnett-Hartman et al. 2008). However, to our knowledge there have been no epidemiologic studies properly designed to address this issue. The lack of good serological assays for SB infection may have been one of the reasons for scarcity of epidemiologic data. Darjee and Gibb (1993) were the first to monitor increased SB antibody

responses in CRC patients by an ELISA approach. After that, Tjalsma et al. (2006, 2007) established an SB antibody profiling assay exploiting immunocapture time-of-flight mass spectrometry (IC-TOF MS) (Tjalsma et al. 2008), Abdulamir et al. (2009) also developed an ELISA to monitor SB antibodies in CRC patients and controls. As shown in Table 3.3, stronger associations observed by these approaches suggest that antibody assays may be a more powerful tool than fecal culture in assessing the associations between this bacterial infection and colorectal disease. Furthermore, as infectious agents in general induce a more pronounced immune response compared to tumour "self"-antigens, SB antigens could become instrumental in the immunodiagnosis of CRC (Tjalsma 2010).

## 3.6 SB and CRC Risk

To further investigate the exposure to SB in CRC patients, Boleij et al. (2010), developed an ELISA based on SB antigen RpL7/L12, previously assigned as a diagnostic antigen (Tjalsma et al. 2007). This assay was exploited for serological evaluation in Dutch (n = 209) and American (n = 112) populations. These analyses showed that an immune response against this bacterial antigen was increased in polyp patients and stage I/II CRC patients as compared to controls (Odds ratio (OR ) 1.50, 95% Confidence Interval (CI) 0.48–4.62 in the Netherlands; OR 2.75, 95%CI 0.96–7.88 in the US) . Notably, increased anti-RpL7/L12 levels were not or only mildly detected in late stage colorectal cancer patients having lymph node or distant metastasis (Fig. 3.2). Increased anti-RpL7/L12 levels were not paralleled by increased antibody production to endotoxin, an intrinsic cell wall component of the majority of intestinal bacteria, which implicates that the humoral immune response against RpL7/L12 is not a general phenomenon induced by the loss of colonic barrier function. The age-adjusted OR for all colorectal tumours combined was very similar in the US (2.30 95% CI 1.06–5.00) and Netherlands (1.90, 95% CI 0.49–2.84). Even a relatively modest increase should be relevant for the progression of colon adenomas to carcinomas (accumulation of mutations), a process which can take over a decade to take place. In this respect, it is interesting to note that the ORs of 1.5 and 2.8 for early stage CRC were within the range of those calculated for the serological response to a panel of *Helicobacter pylori* antigens in patients with early stage gastric precancerous lesions (ORs ranging from 1 to 9) (Gao et al. 2009). Unfortunately, no data are yet available (August 2010) that correlate SB colonization of tumour tissue with the humoral immune response to SB antigens. Nevertheless, our preliminary studies suggest that tumour tissue provides a niche that allows increased SB colonization (Fig. 3.3). Altogether, these findings suggest that SB constitutes a risk factor for the development and/or progression of pre-malignant lesions into carcinomas. Importantly, cross-sectional and retrospective studies, including the current study and others, are not able to address the temporal relationship between an exposure and a disease outcome directly. Thus, future prospective studies are essential to elucidate the etiological roles of SB in colorectal carcinogenesis.

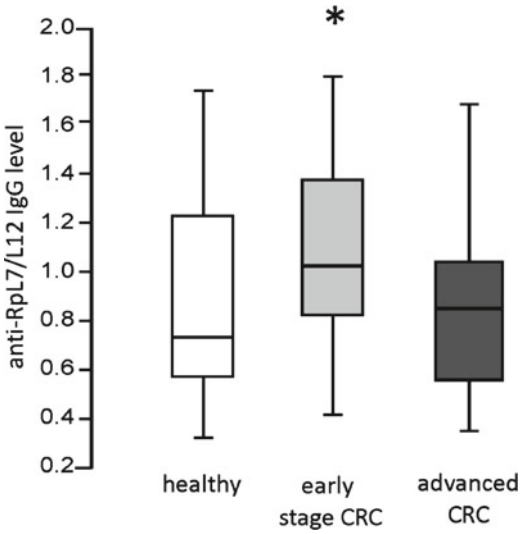


**Fig. 3.2** Humoral immune response against SB antigen RpL7/L12. Serum anti-RpL7/L12 were determined in healthy control subjects (n=60), "early stage CRC" (polyp and local tumours; n=70) and "advanced CRC" (tumours with regional and distant metastases; n=50) by an ELISA (Tjalsma et al. 2007). The results are indicative for a moderate, but significant (*), increased exposure to this antigen during the early stages of CRC. Median levels, second and third quartile (*boxes*), ad ranges (*lines*) are indicated. Relative anti-RpL7/L12 IgG levels were expressed as arbitrary optical density units

## 3.7 Model for the Association of SB with CRC

Based on the current knowledge the following model for the association of SB with CRC can be envisaged (Fig. 3.4). Pre-malignant lesions are initiated by carcinogenic (dietary) factors that diffuse through the colonic mucus layer and induce mutations within the APC or B-catenin genes (Cho and Vogelstein 1992). These thereby immortalized epithelial cells are prone to the accumulation of other mutations and, as a side effect, the aberrant epithelial physiology disturbs the mucus layer covering the epithelial cells (Corfield et al. 2000) and makes it susceptible to bacterial infiltration. Such (pre-) malignant epithelial sites may also provide a selective bacterial microenvironment, for instance by the excretion of specific metabolites, recruitment of immune cells and/or production of selective anti-microbial substances. Bacteria, such as SB, which are unable to effectively colonize the healthy colon may have a competitive advantage in this microenvironment and survive for prolonged periods of time. Tumour infiltration of SB may exert inflammatory factors such as IL-8 and COX-2 and/or lead to increased levels of genotoxins and thereby promote intestinal carcinogenesis. These (pre-) malignant lesions also provide a portal of entry for SB which explains the increased anti-SB antibody titers and increased incidence of SB endocarditis in CRC patients. Late stage tumours entering the metastatic phase may change in such a way that bacterial survival on the tumour surface is diminished or

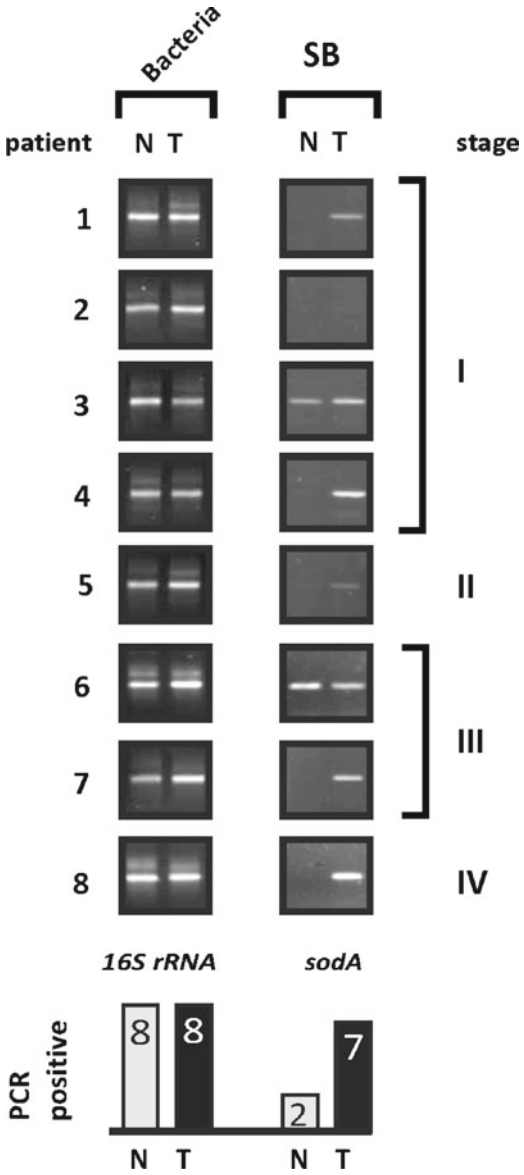


**Fig. 3.3** SB detection in human colonic biopsy samples. The presence of SB in human biopsies from tumour tissue (T) and adjacent non-malignant mucosa (N) was monitored by a nested PCR on the SB *sodA* gene using biopsy-extracted DNA from 8 CRC patients as a template. CRC disease staging is indicated. A broad range 16S rRNA PCR was run in parallel to control for the presence of bacterial DNA and PCR inhibiting substances. The results are suggestive for a preferred colonization of tumour tissue by SB. The identity of the *sodA* PCR fragments was confirmed by nucleotide sequencing, which showed that all products had the highest degree of similarity with SGG (SB biotype I)

antibody expression due to bacterial interaction is reduced. The possibility that tumour progression may drive bacteria out of the cancerous tissue is similar to what has been reported for *H. pylori* during gastric cancer progression (Kang et al. 2006; Brenner et al. 2007). If true, this phenomenon may partly account for a wide range of the prevalence of SB reported for CRC patients that is comprised of various stages of the disease.

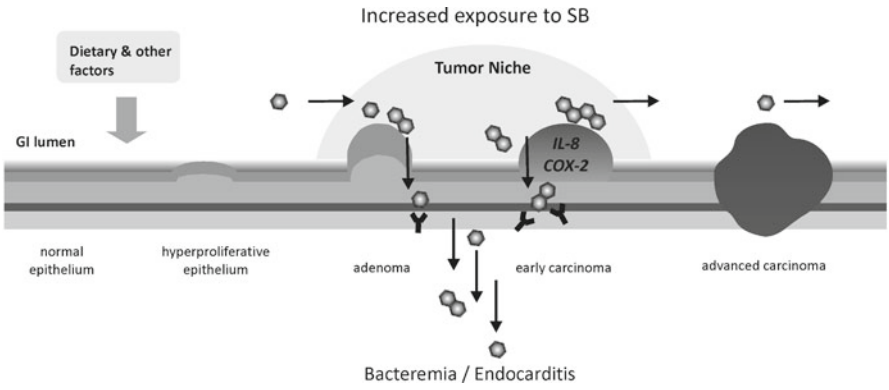


**Fig. 3.4** Model for a temporal association between SB and CRC. The development of colorectal tumours is schematically depicted from *left* (healthy) to *right* (invasive and metastasizing carcinomas). Initiation of carcinogenesis is a multi-factorial process in which dietary factor play an important role. It may be envisaged that adenomas and early carcinomas provide a preferred niche for SB, which leads to subclinical infection and an increased exposure to SB which can be measured by serological assays. Moreover, this could explain the increased incidence of SB bacteremia and endocarditis in CRC patients as these (pre-) malignant lesions can form a portal of entry into the human body. In addition, SB may interfere with colon carcinogenesis for instance by the induction of IL-8 and COX-2, whereas tumour progression may drive SB out of advanced cancerous tissue (see text for details)

## 3.8 Conclusion

The clinical association between SB and CRC is widely acknowledged, and an SB infection is often regarded as an indication for full bowel examination in clinical practice. However, still little is known about the molecular mechanisms behind this association (Boleij et al. 2009a, b). The recent deciphering of the SGG (SB biotype I) genome revealed unique features among streptococci, probably related to its adaptation to the intestinal environment (Rusniok et al. 2010). For instance, SGG has the capacity to use a broad range of carbohydrates of plant origin, in particular to degrade polysaccharides derived from the plant cell wall. Its genome encodes a large repertoire of transporters and catalytic activities, like tannase, phenolic compounds decarboxylase, and bile salt hydrolase, which should contribute to the detoxification of the gut environment. Furthermore, SGG has the potential to synthesize all 20 amino acids and more vitamins than any other sequenced *Streptococcus* species (Rusniok et al. 2010). The surface properties (Fig. 3.5) of this bacterium might be implicated in resistance to innate immunity defenses, and glucan mucopolysaccharides, three types of pili, and collagen binding proteins may play a role in adhesion to tissues in the course of endocarditis. Recent *in vitro* studies revealed that SGG has a unique repertoire of virulence factors that may facilitate infection through (pre-)malignant colonic lesions and subsequently can provide SGG with a

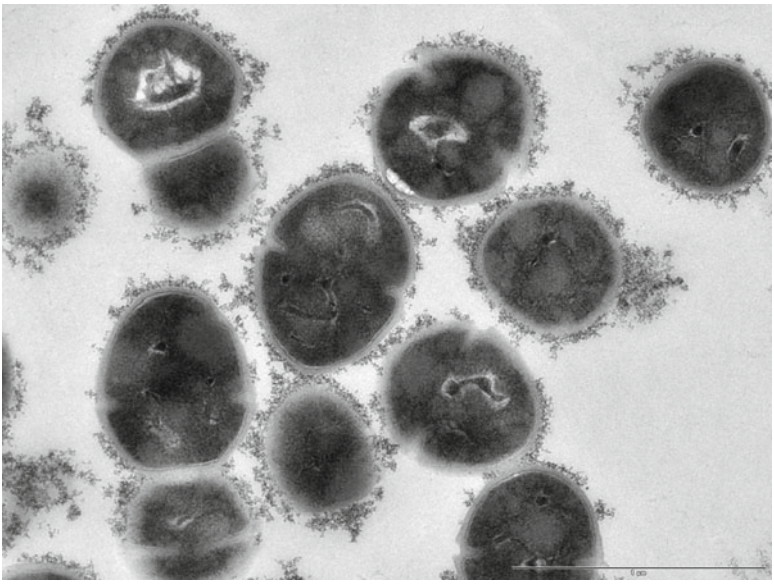

**Fig. 3.5** SB surface structure. Electron microscopy picture of SGG cells (strain UCN34 (Rusniok et al. 2010)) showing the capsule of glucan mucopolysaccharides after polycationic ferritin labelling (Vanrobaeys et al. 1999), which may be important for immune evasion in the course of endocarditis (The picture was kindly provided by Philippe Glaser and Nadège Cayet, Unité de Génomique des Microorganismes Pathogènes, Institute Pasteur, Paris, France)

competitive advantage to evade the innate immune system and to form resistant vegetations at collagen-rich sites in susceptible CRC patients (Boleij et al. 2011a). However, many questions on the relationship between SGG and CRC remain to be answered. Therefore, future studies should answer to which extent polyps and tumours actually provide a niche for SB colonization, and if so, which factors are involved in the adherence to, and/or survival in, the tumour microenvironment and how this increased colonization promotes carcinogenesis. In addition, improved (ELISA) assays are desirable to address the relationship between SB exposure and CRC directly in prospective and retrospective studies. Together, these molecular and epidemiological studies are essential for the full elucidation of the etiological roles of SB in colorectal carcinogenesis.

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