buds (p=0.0282) had more unfavorable survival time. nTrkB was expressed in low-grade budding cases (p=0.0073), BRAF wild-type tumours (p=0.0519) and expressed infrequently in tumour buds (34/154; p<0.0001).
Conclusions. TrkB appears to be involved in the promotion of tumour budding, particularly in a KRAS wild-type context and highlights a subgroup of aggressive tumour buds at the invasion front of colorectal cancers. These findings underline the potential role of anoikis-resistance in survival of EMT-like tumour budding cells in these patients.

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FP35
CpG promoter hypermethylation of CTNNB1 (β-catenin) and TWIST2 promote a high-grade tumor budding phenotype in colorectal cancer

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Background. Tumor budding in colorectal cancer is an epithelial-mesenchymal transition (EMT)-like process characterized by activation of Wnt signaling underlined by nuclear β-catenin and loss of E-cadherin expression. Here, we determine whether deregulation of WNT-related genes and known repressors of E-cadherin via CpG promoter hypermethylation promotes a pro-tumor budding phenotype.
Methods. Methylation analysis for APC, CDH1 (E-cadherin), CTNNB1 (β-catenin), TWIST, ZEB1 and ZEB2 by pyrosequencing was performed using bisulfite-converted DNA from 209 colorectal cancer patients with full clinical and pathological data. Mutational analysis for KRAS and BRAF was carried out. Tumor budding was assessed according to the 10-in-10 method. Immunohistochemistry for β-catenin, E-cadherin, VEGF and pAKT was performed using a multi-punch tissue microarray.
Results. High-grade budding was observed in 64% of CTNNB1-methylated cancers. 80% of CTNNB1-negative/TWIST-methylated cases were low-grade [p<0.0001; OR: 2.61 (95% CI:1.7–4.0)]. CTNNB1 methylation correlated with loss of E-cadherin expression (p=0.0026), and overexpression of pAKT (p=0.005) and VEGF (p=0.002). Although 66.7% of CTNNB1-methylated cases showed nuclear β-catenin versus 36.4% of CTNNB1-negative cancers, this difference was not significant (p=0.1461).
Conclusions. Hypermethylation of CTNNB1/TWIST may be involved in the process of tumor budding providing for the first time a link between epigenetic changes and a pro-budding (EMT-like) phenotype in colorectal cancer patients.

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FP36
CD8/CD45RO T-cell infiltration in pre-operative biopsies of colorectal cancer predicts histopathologic features of the matched resection specimen and survival outcome

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Background. Reliable prognostic markers based on biopsy specimens of colorectal cancer (CRC) are currently missing. We hypothesize that CD8+ T-effector and CD45RO+ memory T-cell infiltrates in biopsies may be able to predict survival outcome of CRC patients and the histopathological features of the CRC resection specimen in the pre-operative setting. We therefore aimed to develop a prognostic score based on biopsy specimens to allow earlier risk stratification of CRC patients in clinical practice.
Methods. 346 patients treated from 2002–2011 for primary CRC at the Bern University Hospital were included in the study, 185 of which had matched biopsies. Cases were re-reviewed for histopathological features including T, N, M, G, L, V and Pn. Clinical data on age, gender, tumor location, time to progression/metastasis and post-operative therapy was retrieved from patient records. Biopsy specimens were immunostained for pancytokeratin and CD8 or CD45RO. Stromal (s) and intraepithelial (i) CD8+ and CD45RO+ T-cells were assessed in 5 HPF of highest density of infiltration. T-cell infiltrates were assessed for prediction of pTNM-stage of the matched resection specimen and patient survival.
Results. A greater number of total (t) CD8 counts in the preoperative biopsy was significantly associated with more favorable survival outcome (p=0.0139) in patients with primary CRC independently of pT, pN or pM-stage and adjuvant therapy [HR=0.043 (0.26–0.71); p=0.001]. Using Classification and Regression Tree Analysis (CART), full pathological TNM stage including additional histopathologic prognostic parameters of the resection specimen could be predicted by assessment of tCD8 and sCD45RO in the pre-operative biopsy: High numbers of tCD8 in the biopsy predicted earlier T-stage (p<0.0001) in the resection specimen as well as absence of lymphatic invasion (p=0.0165). Infiltration by sCD45RO+ cells was highly predictive of the absence of nodal metastasis (p=0.0243), absence of distant metastasis (p=0.0321) absence of vascular invasion (p=0.0321) and was significantly associated with lower tumor grade (p=0.0156).
Conclusions. Evaluation of CD8+ and CD45RO+ T-cell infiltration in pre-operative biopsy specimens of CRC predicts full TNM-stage of the matched resection specimen and survival outcome. This may allow risk stratification of CRC patients at the time of diagnosis.

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