

Metformin Treatment for Diabetes Mellitus Correlates with Progression and Survival in Colorectal Carcinoma



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Abstract

BACKGROUND: Diabetes mellitus is unfavorably associated with cancer risk. The purpose of this multi-disciplinary project was to evaluate a possible association of diabetes mellitus and other comorbidities and their treatment with progression of colorectal cancer. **PATIENTS AND METHODS:** We investigated the correlation between pathological characteristics and clinical course, including comorbidities in 1004 Czech patients diagnosed and surgically treated for colorectal adenocarcinoma (CRC) between 1999 and 2016. **RESULTS:** In our data set, CRC patients treated with metformin due to coexisting diabetes mellitus type 2 (T2DM) developed fewer distant metastases which clinically correlates with slower CRC progression. Survival in metformin subgroup was longer, particularly in men with CRC. Osteoporosis may be a negative factor of survival in CRC patients. **CONCLUSIONS:** Our findings also indicate that aging, higher tumor grade and TNM stage, coexistence of selected endocrine disorders, and metabolic abnormalities may change the tumor microenvironment and impact survival in colorectal cancer, although mechanism of these observations yet to be explained. Patients with diabetes mellitus type 2 treated with metformin may represent the altered microenvironment with specifically

tuned metabolic molecular responses and with various epigenetic characteristics. More awareness and increased understanding of the mechanisms underlying the positive effect of metformin on patients' survival could offer insight into new treatment methods and permit more individualized treatment plans.

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Introduction

Colorectal cancer (CRC) is frequently diagnosed malignancy worldwide and continues to be a serious cause of morbidity and mortality despite ongoing screening efforts [1]. According to NIH National Cancer Institute statistics, approximately 1 in 22 men and 1 in 24 women will be diagnosed with CRC in their lifetime. Five-year survival 2008–2014 in the United States was 64.5%. CRC affects both sexes most commonly in the 6th decade of life but the proportion of cases diagnosed in individuals younger than 50 years of age increased to 11% in 2013 [2]. In Czech Republic, the incidence follows the worldwide trend [3].

It is known that CRC can develop as a result of accumulated genetic mutations over time similar to other malignancies or silencing tumor suppressor genes in Li-Fraumeni syndrome, although most of CRC cases are the consequence of chromosomal and microsatellite instability and their course and aggressiveness are consequent to epigenetic characteristics [4]. Diabetes mellitus together with obesity and increased level of adipocytokines, fasting hyperglycemia, insulin resistance and hyperinsulinemia, chronic inflammation, and recurrent increased level carcinoembryonic antigen are all thought to play a role in the increased risk for CRC progression and death. All of these characteristics are considered to be negative prognostic factors [5–8].

A number of retrospective studies dealing with CRC in patients with diabetes have been published but were clearly underpowered to detect possible subtle interrelationships between treatment details and patients' survival. It has been proposed that glycemic load adverse effects correlate with recurrence and survival of CRC stage III in recent observational prospective studies [9,10]. Publications based on metaanalyses of large data sets showed up to 44% overall survival benefit in metformin-treated CRC patients with stages I–III [11–13].

Phosphorylated insulin receptors together with increased blood level of IGF1 possibly play a role in development of metastases in CRC which correlates with poor prognosis. The nuclear location of IGF-1R has been observed in chemotherapy resistance [14,15].

The course of CRC and survival may be affected not only by the pathophysiological systemic effects of T2DM but adversely by action of peroral antidiabetics (PAD). Biguanides (metformin, phenformin, and buformin) are derived from the herb *Galega officinalis*. Metformin was developed as therapy for T2DM in the 1950s. It works via inhibition of hepatic gluconeogenesis and increased uptake of glucose in muscle. In malignant cells, metformin action includes LKB1-mediated activation of AMPK, inhibiting mTORC1 signaling, and affects liver homeostasis and metastatic development [16–18]. It has been published that glucose level impacts various cellular morphoproteomic changes (via Sirt1, PIAS1, STAT1, MRP1, mTOR) with adequate consequences for tumor growth as well as metastatic progression [19].

United Kingdom prospective prevention diabetes study suggests that metformin also works by mechanism of insulin resistance in liver, by bile acids metabolism, incretins release, and decrease deposit of

amyloid in metformin users [20]. Some studies observed a 15–30% increased survival of CRC patients treated with metformin compared with patients on other antidiabetic agents [21,22]. Other studies which attempted to avoid the potential confounders of adjuvant therapy, focused more on stratified patients by TNM stage, did not show any effect of metformin on the course of CRC. Recent prospective studies suggested at least a 15% decrease in risk of all-cause mortality in CRC patients with T2DM treated with metformin compared with patients receiving insulin [23–26].

Thyroid hormone substitution in patients with clinical hypothyroidism seems to have protective effects concerning CRC progression [27] when assessing circulating levels of thyroid hormones or receptor THβ1 expression in patients with abnormalities of the thyroid gland [28].

Materials and Methods

Study Population

We identified 1004 Caucasian patients diagnosed with colon and rectal adenocarcinoma in four Czech hospitals between January 1, 1999, and June 30, 2016. In 770 cases, comprehensive clinical data were available. 234 patients were excluded (due to missing lab or clinical data e.g. oncology treatment details, if a statistically insufficient number of CRC patients with specific comorbidity was identified e.g. with neurodegenerative diseases, and because of insulin therapy exclusion criteria). 189 patients were lost to follow-up in one year, and 548 patients were lost to follow-up within five years after CRC diagnosis. The participating institutions were two major hospitals in Prague (General University Hospital, Na Bulovce Hospital) and two regional hospitals, one in the most northern part of the country (Jablonec nad Nisou) and the other in the most southern part of the country (Jindrichuv Hradec). The pathological and clinical data were carefully collected by hand from online records of the healthcare institutions and analyzed retrospectively.

Compliance with Ethical Standards

The study was approved by the Ethics Committee of Na Bulovce Hospital (EK NNB 30.6.2014/7248/EK-Z on September 4, 2014), Hospital Jablonec nad Nisou (LEK 9/2016/St on November 15, 2016), General University Hospital, Prague (158/15 S-IV on March 19, 2015), and Hospital Jindrichuv Hradec (approval was not numbered on February 3, 2015). All procedures involving human participants were in accordance with the ethical standards of the institutional Ethical Committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Definitions

Cases of adenocarcinoma histology C18.0–C20.9 were included. Primary tumors were located in all parts of large intestine: left colon,

transversum (including lial and hepatic flexures), right colon, and rectum; we also recorded rare locations in appendix and Meckel's diverticulum. Five cases of ulcerative colitis and seven cases of Lynch syndrome were found in the data set. The day of surgical resection was considered the day of diagnosis although the disease was always verified by colonoscopy and biopsy a short time prior to resection. The resected specimens were routinely described macroscopically, including tumor location and size. The fat surrounding the large intestine was left overnight in Carnoy's solution to identify all lymphatic nodes. The resection margin was examined. Hematoxylin–eosin staining was used for histologic study and the slides were evaluated using light microscopy by board-certified pathologists. Histopathology evaluation consisted of tumor type, grade, and TNM stage [29].

Collected Data

We recorded the following: a) Age and sex of the patient; b) Location and size of the tumor; c) Histopathology: tumor type; grade; growth pattern; stroma type (desmoplastic, mucinous, fibroproductive, with chronic inflammatory cells); d) Pathological TNM staging combined with clinical data about distant metastases; e) Local recurrence and distant metastases; f) Chemotherapy and radiation data; g) Laboratory values including glucose, creatinine, liver enzymes; h) Last follow-up; i) Death (number of months after diagnosis and the cause related or unrelated to CRC); j) T2DM subgroups according to treatment regimens: diet, insulin, metformin, other oral antidiabetics (gliclazide, glimepiride, glibenclamide); k) Hypothyroidism; l) Other endocrine disorders: struma nodosa and status poststrumectomy, hyperparathyroidism and hyperaldosteronism, adenoma of hypophysis, thyroid, parathyroid, and subrenal glands, adrenal hyperplasia, status postovarectomy, and hormone replacement therapy (HRT) in women, status postorchietomy and prostatectomy, mastopathy in men, abnormal glucose tolerance; m) Autoimmune diseases: chronic atrophic thyroiditis, hepatitis and gastritis, rheumatoid arthritis, psoriasis, and sarcoidosis; n) Metabolic syndrome: obesity, steatohepatitis, hyperlipidemia; o) Osteoporosis (age >65 and clinical statement).

The strengths of this study are consistent data collection that was performed by the same person in all four centers, and multidisciplinary approach. The access to correlating CRC specimens (paraffin blocks) can be advantageous for morphoproteomic studies in the future. On the other hand, some data were not available as some patients were operated on in the university surgical center but they continued to have oncology treatment and follow-up in smaller hospitals out of our reach. Despite our effort, some subgroups of patients are small (including with metformin treatment) and for that reason not all results are statistically significant, which needs to be taken into account when making clinical and scientific judgment on outcomes of this study.

Statistical Methods

The main aim of the study was to explore the impact of different disorders on the overall survival of patients with CRC. Data preprocessing, including cleaning, was applied before analysis. Continuous variable (age) was characterized by the average, minimum, and maximum. Categorical variables were described as frequencies and the relative frequencies of individual variants. In univariate analysis was to compare the characteristics of subjects with and without T2DM, respectively, with and without metformin use, for character-

istics measured on the nominal scale, the Yates chi-squared test was used. The Cox proportional hazards (PH) regression model was used to evaluate the effect of different clinical and pathologic parameters on survival and generate hazard ratios for death. The estimated parameters with the standard error of these parameters, hazard ratios, and corresponding confidence intervals and *p*-values are listed.

In 770 evaluated cases with CRC there were 99 deaths from progression of colorectal carcinoma. Figures display the Kaplan–Meier curves for the compared groups and the *p*-values of the log-rank tests. Kaplan–Meier curves do not go all the way down to zero when the largest observed time (which is around 15 years) is censored. *P*-values less than 0.05 indicate that there is statistical significant difference between the populations (compared groups) in the probability of a death by colorectal carcinoma at any time point.

Results

Our data set consists of data on 483 (62.7%) men and 287 (37.3%) women. The age range was 31–95 years, the average age 68 years. The pathological and clinical findings are summarized in Table 1.

Survival analyses in Table 2.

A survival model for patients with diabetes mellitus was designed to determine the possible effect of metformin use on the survival probability. The estimated parameters with the standard error of these parameters, hazard ratios, and corresponding confidence intervals and *p*-values are shown in Table 4. The estimated hazard rates of dying (statistically significant different from constant 1) are listed as well. The goodness-of-fit for the chosen model was performed by the score log-rank test with significant *p*-value (less than 0.001).

In 118 patients with CRC and T2DM, there were 14 deaths because of progression of colorectal carcinoma. Figures display the Kaplan–Meier curves for the compared groups and the *p*-values of the log-rank tests. Kaplan–Meier curves do not go all the way down to zero when the largest observed time (which is around 11 years for metformin users and around 12 years for patients with diet or other PAD) is censored. *P*-values less than 0.05 indicate that there is statistical significant difference between the populations (compared groups) in the probability of a death by colorectal carcinoma at any time point.

Survival analyses in Table 4.

In concordance with current pathological and clinical experience, survival reflected most significantly aging, higher tumor grade, and TNM stage (Table 1 and Figure 2A). The most frequent comorbidity in CRC in our data set was diabetes mellitus type 2 with prevalence of 16.8% in men and 12.9% in women. T2DM was treated with diet, other PAD, and metformin (Table 3, Figure 3A and B).

The survival analysis showed no difference between men and women with CRC in total pool of data (Figure 1A). Better survival, important for prognosis, is seen in men with CRC, particularly in the subgroup treated with metformin due to T2DM (Figure 1B). Patients treated with metformin also developed fewer distant metastases (5.6%) if CRC progressed later compared with subgroup of CRC patients without metformin therapy (17.2%) and these results were statistically significant (Table 3).

CRC is a highly heterogenous carcinoma and therapies targeting different cellular pathways are expected to have various final effects. Some publications promote rectal adenocarcinoma as a separate category from colonic adenocarcinoma pathogenetically and by progression [30]. Although generally the tumors develop in right and left colon equally, our data suggest most CRC primary tumors were

Table 1. Characteristics of CRC Patients with Coexisting T2DM. Statistical Significance of Differences (Univariate Analysis) Is Shown in Bold.

Characteristics	All Patients (n = 770)	With T2DM (n = 118)	Without T2DM (n = 652)	Chi-square Test (p)
Total Known Deaths	178 (23.1%)	31 (26.3%)	147 (22.5%)	0.445
Gender				
Men	483 (62.7%)	81 (68.6%)	402 (61.7%)	0.178
Women	287 (37.3%)	37 (31.4%)	250 (38.3%)	
Age				
<60	167 (21.7%)	8 (6.8%)	159 (24.4%)	<0.001
>60	603 (78.3%)	110 (93.2%)	493 (75.6%)	
Primary Tumor Location				
Left colon	356 (46.2%)	52 (44.1%)	304 (46.6%)	0.371
Right colon	207 (26.9%)	31 (26.3%)	176 (27%)	
Rectum	177 (23%)	27 (22.9%)	150 (23%)	
Transversum colon	30 (3.9%)	8 (6.8%)	22 (3.4%)	
Histopathology Grade				
Well differentiated	173 (22.5%)	29 (24.6%)	144 (22.1%)	0.076
Moderately differentiated	503 (65.3%)	82 (69.5%)	421 (64.6%)	
Poorly differentiated	94 (12.2%)	7 (5.9%)	87 (13.3%)	
Morphology				
Adenocarcinoma	625 (81.2%)	106 (89.8%)	519 (79.6%)	0.026
Adenocarcinoma with mucinous stroma	137 (17.8%)	12 (10.2%)	125 (19.2%)	
Signet ring cell adenocarcinoma	8 (1%)	—	8 (1.2%)	
pTNM Stage				
In situ	5 (0.6%)	1 (0.8%)	4 (0.6%)	0.724
I	36 (4.7%)	7 (5.9%)	29 (4.4%)	
II	156 (20.3%)	21 (17.8%)	135 (20.7%)	
III	474 (61.6%)	77 (65.3%)	397 (60.9%)	
IV	99 (12.9%)	12 (10.2%)	87 (13.3%)	
Lymphatic Nodes at Time of Resection				
With metastases	331 (43%)	49 (41.5%)	282 (43.3%)	0.805
Without metastases	439 (57%)	69 (58.5%)	370 (56.7%)	
Distant Metastases				
Without metastases	578 (75.1%)	87 (73.7%)	491 (75.3%)	0.861
At least one (at resection time)	99 (12.9%)	17 (14.4%)	82 (12.6%)	
At least one (later)	93 (12.1%)	14 (11.9%)	79 (12.1%)	
Type of CRC Comorbidities				
Osteoporosis	339 (44%)	69 (58.5%)	270 (41.4%)	0.001
Metabolic syndrome	131 (17%)	31 (26.3%)	100 (15.3%)	0.006
Hypothyroidism	70 (9.1%)	13 (11%)	57 (8.7%)	0.537
Endocrinopathy	52 (6.8%)	5 (4.2%)	47 (7.2%)	0.325
Autoimmune diseases	27 (3.5%)	3 (2.5%)	24 (3.7%)	0.729

located in left colon regardless diabetic status. The exception was colon transversum where CRC primary tumor was found in 6.8% with T2DM versus 3.4% in nondiabetic population (Table 1).

Table 2. Fitted Stochastic Cox PH Model for Overall Survival Time of CRC Patients (770 Patients, 99 Deaths for CRC).

Predictors	Coef	SE (Coef)	HR*	95% CI for HR	p
Age	0.022	0.010	1.02	(1.00; 1.04)	0.039
pTNM Stage					
T = <i>In situ</i> , I, II, III; baseline	—	—	—	—	—
T = IV	0.681	0.242	1.98	(1.23; 3.18)	0.005
Histopathology Grade					
Grade I (well differentiated; baseline)	—	—	—	—	—
Grade II (moderately differentiated)	0.333	0.330	1.40	(0.73; 2.67)	0.313
Grade III (poorly differentiated)	0.983	0.390	2.67	(1.25; 5.73)	0.012
Metastases in Lymphatic Nodes					
Without metastases; baseline	—	—	—	—	—
With metastases	1.145	0.248	3.14	(1.93; 5.11)	<0.001
Distant Metastases					
Without metastases; baseline	—	—	—	—	—
At least one metastasis at resection time	1.764	0.267	5.84	(3.46; 9.85)	<0.001
At least one metastasis later	1.215	0.256	3.37	(2.04; 5.57)	<0.001

Score (log-rank overall) test: p < 0.001.

SE, standard error; CI, confidence interval; HR, hazard ratio.

* A positive (negative) coefficient estimate in the Cox PH model corresponds to a higher (lower) risk of death and thus on average a shorter (longer) OS time.

Right-sided tumors showed longest survival regardless any comorbidities—gender—age compared with CRC in left colon, rectum, and colon transversum (Figure 2B). Here anatomy conditions and embryology specifications, blood supply factors, and related biochemical and molecular alterations could make the difference and these factors are worth further investigating.

Adenocarcinoma as most frequent histological type was found in >80%, followed in frequency by adenocarcinoma with mucinous type of stroma with content <50% of mucin in histology (Table 1). Signet ring cell tumor representing intracellular mucin involvement was diagnosed in patients in nondiabetic patients in 1.2% vs. none patients with T2DM (Table 1). Poorly differentiated primary tumors were least frequent in CRC patients without T2DM; however, this group of patients was small (Table 1) and it is difficult to comment on any possible correlation on overall CRC survival in diabetic and nondiabetic population based on tumor morphology features.

Overall survival in CRC patients with osteoporosis was shorter and coexisting other endocrinopathies (Materials and methods/Collected data and Table 1) correlated with longer survival in CRC patients (Figure 4A and B), although the subgroup of patients with these disorders was small and affected the statistical significance. Coexisting osteoporosis was present in 50% of CRC cases with one distant metastasis at time diagnosis and in 30% of CRC cases with at least one metastasis developed later (Figure 5).

Table 3. Characteristics of Patients with T2DM Depending on Coexisting Metformin Treatment. Statistical Significance of Differences (Univariate Analysis) Is Shown in Bold.

Characteristics	Patients with DM2T (n = 118)	Patients with Metformin Treatment (n = 54)	Patients without Metformin Treatment (n = 64)	Chi-square Test (p)
Total Known Deaths by CRC	14 (11.9%)	2 (3.7%)	12 (18.8%)	0.026
Gender				
Men	81 (68.6%)	36 (66.7%)	45 (70.3%)	0.821
Women	37 (31.4%)	18 (33.3%)	19 (29.7%)	
Age				
<60	8 (6.8%)	2 (3.7%)	6 (9.4%)	0.393
>60	110 (93.2%)	52 (96.3%)	58 (90.6%)	
Primary Tumor Location				
Left colon	52 (44.1%)	23 (42.6%)	29 (45.3%)	0.621
Right colon	31 (26.3%)	12 (22.2%)	19 (29.7%)	
Rectum	27 (22.9%)	15 (27.8%)	12 (18.8%)	
Transversum colon	8 (6.8%)	4 (7.4%)	4 (6.3%)	
Histopathology Grade				
Well differentiated	29 (24.6%)	14 (25.9%)	15 (23.4%)	0.632
Moderately differentiated	82 (69.5%)	38 (70.4%)	44 (68.8%)	
Poorly differentiated	7 (5.9%)	2 (3.7%)	5 (7.8%)	
Morphology				
Adenocarcinoma	106 (89.8%)	49 (90.7%)	57 (89.1%)	>0.999
Adenocarcinoma with mucinous stroma	12 (10.2%)	5 (9.3%)	7 (10.9%)	
Signet ring cell adenocarcinoma	—	—	—	
pTNM Stage				
In situ	1 (0.8%)	1 (1.9%)	—	0.405
I	7 (5.9%)	3 (5.6%)	4 (6.3%)	
II	21 (17.8%)	12 (22.2%)	9 (14.1%)	
III	77 (65.3%)	31 (57.4%)	46 (71.9%)	
IV	12 (10.2%)	7 (13%)	5 (7.8%)	
Lymphatic Nodes at Resection				
With metastases	49 (41.5%)	21 (38.9%)	28 (43.8%)	0.729
Without metastases	69 (58.5%)	33 (61.1%)	36 (56.3%)	
Distant Metastases				
Without metastases	87 (73.7%)	45 (83.3%)	42 (65.6%)	0.067
At least one (at resection time)	17 (14.4%)	6 (11.1%)	11 (17.2%)	
At least one (later)	14 (11.9%)	3 (5.6%)	11 (17.2%)	
Type of CRC Comorbidities				
Osteoporosis	69 (58.5%)	30 (55.6%)	39 (60.9%)	0.687
Metabolic syndrome	31 (26.3%)	14 (25.9%)	17 (26.6%)	>0.999
Hypothyreosis	13 (11.0%)	6 (11.1%)	7 (10.9%)	>0.999
Endocrinopathy	5 (4.2%)	3 (5.6%)	2 (3.1%)	0.846
Autoimmune diseases	3 (2.5%)	2 (3.7%)	1 (1.6%)	0.881

Discussion

It is known that clinical outcomes in CRC patients are affected by some comorbidities. The better outcome in patients younger than 60 years of age could be result of a lower incidence of comorbidities compared with older patients, a better physical condition, the ability

to adhere to treatment more consistently, and perhaps stronger support of peers and family. Diabetes mellitus increases the risk for development of cancer [31]. Patients with a self-reported history of T2DM should be also considered at higher risk for cancer [32]. With the increasing worldwide prevalence of diabetes mellitus, a higher incidence of CRC can be expected, along with a less favorable prognosis for those patients on diet only for diabetes, compared with CRC patients without diabetes and treated with PAD, with special attention to subgroup treated exclusively with metformin (Figure 3B).

Diabetes and CRC share some risk factors related to lifestyle, such as lack of exercise and higher body mass index, represented by metabolic syndrome in our data set (obesity, steatohepatitis, and hyperlipidemia). The fact that metabolic syndrome did not worsen survival could result by high level of lipids in blood as well as in liver controlled by therapy for it in most patients in our study. There is evidence in preclinical studies that a high fat diet and low insulin levels are correlated with the inhibition of tumor growth and proliferation in CRC.

Although the pathophysiological mechanism to explain the negative prognostic effect of diabetes has not been elucidated, clinical and epidemiological investigations have suggested that abnormal glucose and lipid metabolism, hyperglycemia, hyperinsulinemia, insulin resistance in peripheral tissues, adipocytokines, long-term chronic inflammation with IL-6, CRP and other cytokines, as well as

Table 4. Fitted Stochastic Cox PH Model for Overall Survival Time of Patients with T2DM and CRC (118 Patients, 14 Deaths for CRC).

Predictors	Coef	SE (Coef)	HR ^a	95% CI for HR	p
Age	0.086	0.041	1.09	(1.01; 1.18)	0.033
Metformin					
Diet or other PAD	—	—	—	—	—
Metformin	-2.367	0.841	-10.64	(-2.02; -55.55)	0.005
Metastases in Lymphatic Nodes					
Without metastases; baseline	—	—	—	—	—
With metastases	1.501	0.714	4.49	(1.11; 18.17)	0.036
Distant Metastases					
Without metastases; baseline	—	—	—	—	—
At least one metastasis at resection time	2.357	0.715	10.56	(2.60; 42.90)	0.001
At least one metastases later	0.153	0.860	1.16	(0.22; 6.29)	0.859

Score (log-rank overall) test: p < 0.001.

SE, standard error; CI, confidence interval; HR, hazard ratio.

^a A positive (negative) coefficient estimate in the Cox PH model corresponds to a higher (lower) risk of death and thus on average a shorter (longer) OS time.

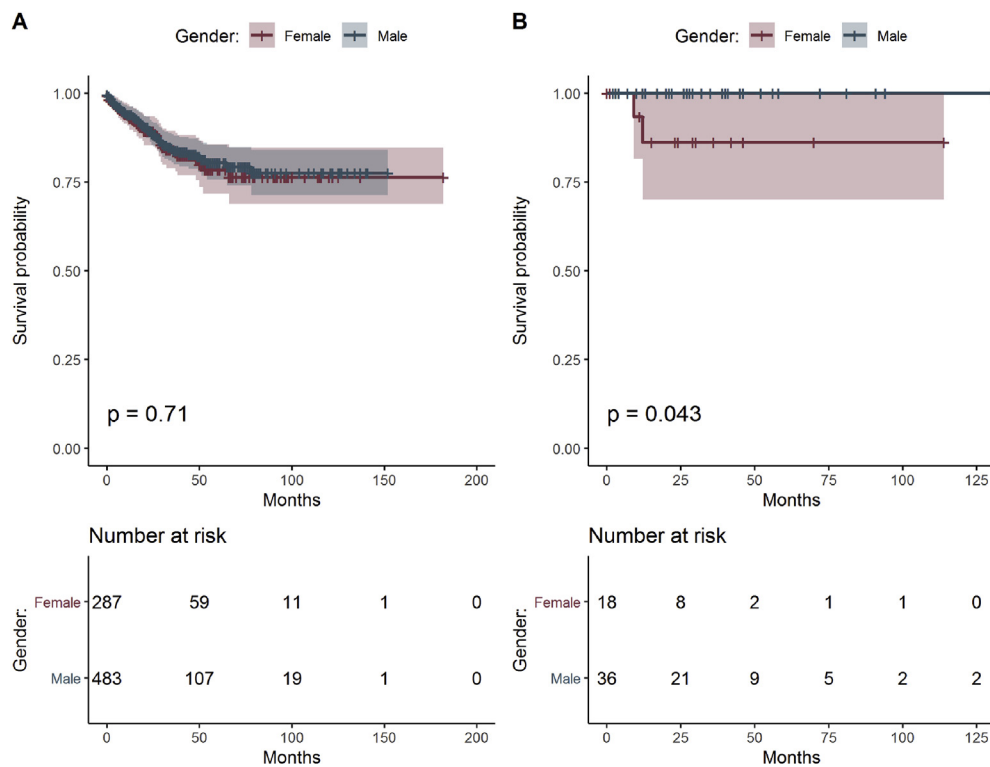


Figure 1. Estimated survival functions for men and women, the p -value of log-rank test comparing survival of both groups and table of frequencies of all patients (A) and patients with T2DM treated with metformin (B).

antiinflammatory chemokines are factors associated with the increased risk of CRC development [33–36].

A poorer survival of CRC patients with T2DM was observed with poorer glycaemia controls correlating with $HbA1c \geq 7.5\%$ and when exogenous insulin was part of diabetic treatment, diabetes developed

at younger age and CRC was more advanced and in the right colon [37]. In literature, high blood glucose level was associated with higher risk of colon cancer in men [38]. Our results indicate longer survival in men with CRC treated with metformin (Figure 1B), although it is not statistically significant due to small number of patients in this

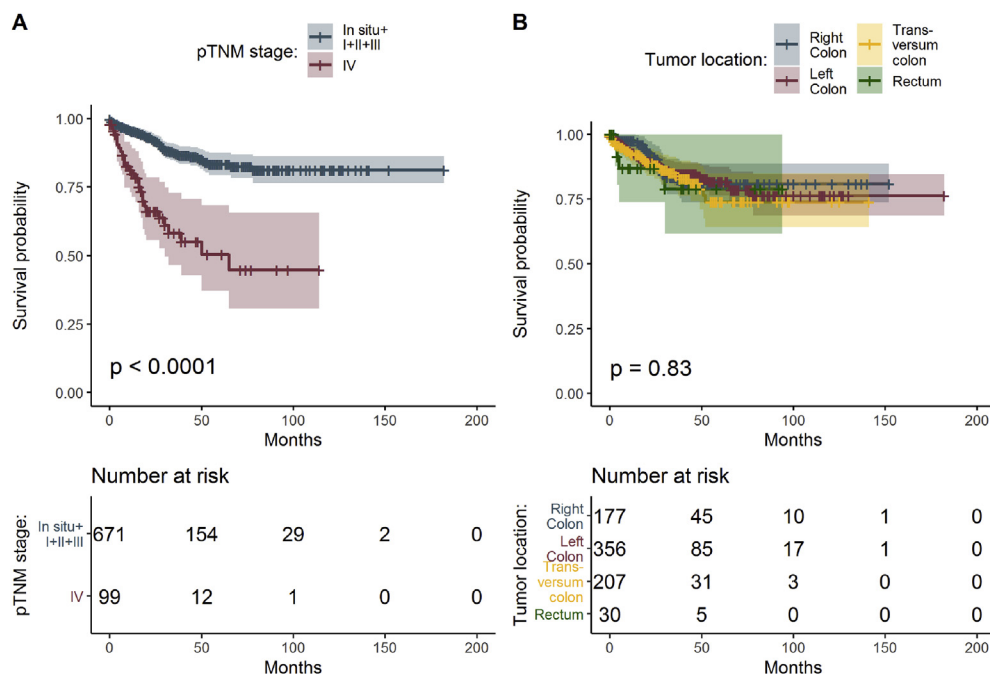


Figure 2. Estimated survival functions for different pTNM classification (A) and carcinoma locations in colon (B), the p -value of log-rank test comparing survival of all groups and table of frequencies of all patients.

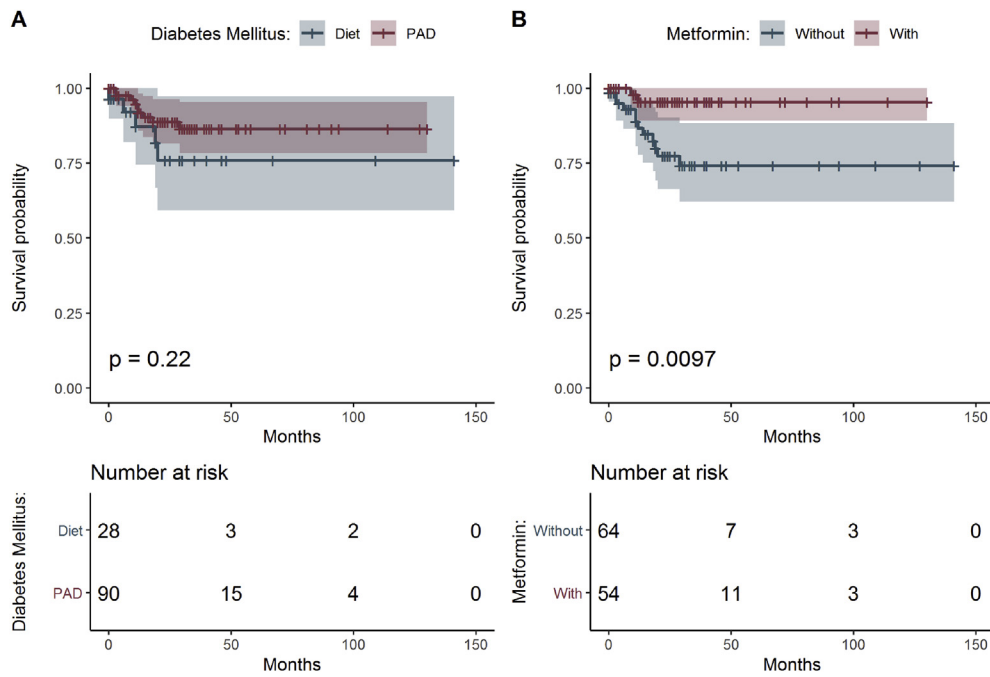


Figure 3. Estimated survival functions for men and women, the p -value of log-rank test comparing survival and table of frequencies of all patients treated by diet and other PAD (A) and patients treated with metformin and without (B).

subgroup. That could correlate with proper control of blood glucose level combined with metformin or an action of this medication in the microenvironment. Evaluating these findings in the context of gender, genetic predisposition, physical activity, nutrition, and hormones might prove a rewarding area for future investigation.

It is known that fibrocytic stromal transition is associated with local invasiveness of CRC but it might be the mucinous mixture in stroma responsible for molecular transition or interactions with tumor

microenvironment that makes diseases to progress and contributes to development of distant organ metastases. Stromal transformation [39,40] and locally hypoxic tumor environment are both possible factors underlying CRC progression at the tissue and cellular level [41].

We identified numerous patients with CRC and with history of long-term treatment for T2DM with specific peroral antidiabetic medication, including metformin. The majority of our patients were

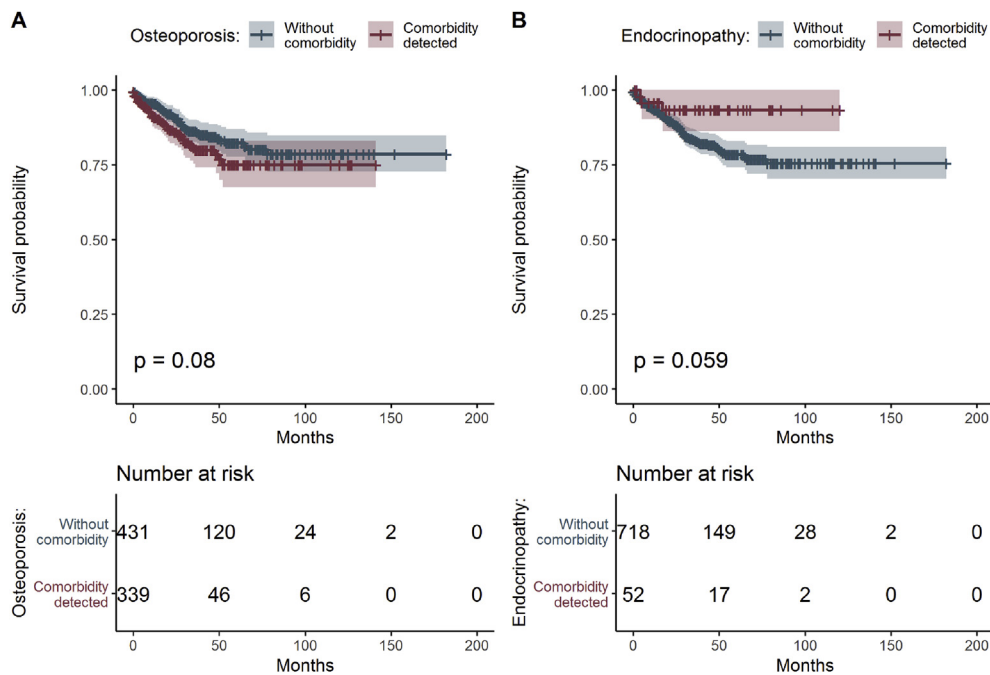


Figure 4. Estimated survival functions for different osteoporosis (A) and other endocrinopathies (B), p -value of log-rank test comparing survival for all groups and table of frequencies of all patients.

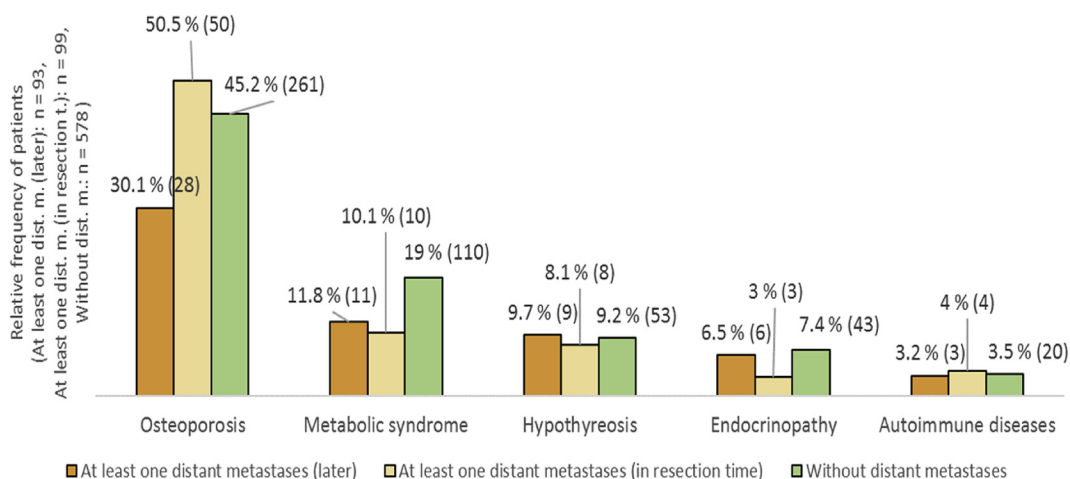


Figure 5. The incidence of endocrine comorbidities among CRC patients according to the distant metastases development.

on metformin for more than one year. The effect of metformin may vary in CRC depending on duration of T2DM. The daily dose of metformin in most patients was 850 mg which could suggest that such a dose is sufficient to improve survival. *In vivo* testing in rats has shown that metformin accumulates in tissues. It is not known if an accumulation of medication could result in a more pronounced effect. Laboratory models show antineoplastic activity of metformin, but metformin concentrations used in many experiments exceed those achieved with conventional doses used for diabetes treatment. The pharmacological effect of metformin includes respiration reduction of cells ATP related [42] and mimics caloric restriction both in cells and systemically, affecting production of metabolic hormones [43]. Decreased blood glucose levels in metformin patients, and possibly a *de novo* synthesis of cholesterol through the intestinal MAPK dependent pathway documented during *in vitro* studies, may lead to tumor cell mitochondrial dysfunction and could explain the systemic protective effect and improved survival in patients with well-controlled diabetes, particularly in the metformin group. Some studies found metformin to potentially promote the immune system response against cancer [44]. Adjuvant therapy with metformin has been observed having positive impact in pancreatic, lung, and liver malignancies, but not in breast and prostate cancer [45,46]. The systematic analysis at biochemical markers and morphology characteristics in correlated CRC specimens (proliferative index, stroma type) might be useful to assess.

Estrogen alone has the proinflammatory effect in colon and can have various effects at tumorigenesis *in vivo* [47]. It has been shown that female hormones, estrogen and medroxyprogesterone acetate, reduced the number of colon cancer in postmenopausal women by 37% compare with placebo in 5-year interval [48,49]. Osteoporosis not only contributes to more advanced local disease in CRC but also negatively advances CRC progression. The impact of osteoporosis in the course of CRC can also relate to estrogen and testosterone. The blood levels of these hormones are known to be reduced by metformin. Recent clinical data showed that hormone replacement therapy (HRT) in women might reduce CRC risk, suggesting possible opportunities for translational research [50]. Growth hormone (GH) therapy and autonomous hyperproduction of GH together with increased level of IGF-1 are associated with a higher risk

for CRC, and close follow-up for these patients is highly advised [51]. Hypothyroidism did not correlate with survival in CRC patients, possibly because of long-term thyroid hormone replacement in all patients and two-thirds of CRC patients being men in whom thyroiditis is much less frequent.

Conclusion

We provide evidence in this large retrospective study from Central Europe that, while diabetes is associated with a higher risk for CRC impacting the incidence, T2DM as comorbidity does not significantly impact survival among CRC patients and contrary, peroral treatment due to T2DM, particularly metformin, demonstrates improved survival in CRC. We emphasize that evaluation of location of primary tumor and histopathologic characteristics is essential for assessment of prognosis for patients with CRC, and it is also of great value to consider the complete clinical profile of each patient, including the medication history, as this may impact the cancer environment. Improved understanding of the effect of metabolic disorders coexisting with cancer and their pharmacologic targeting could lead to better screening methods and updated treatment guidelines to improve outcomes for patients with colorectal cancer.

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Contributions

MKP and PH initiated the study and wrote the first draft of the paper. MKP collected data and completed writing this manuscript. MP, ML and KJ completed the statistical analysis. DC and PD provided the access to most of pathological data and also valuable comments in manuscript. REB contributed to manuscript final review. TG, FT and JG commented on the correlating clinical findings. All authors read and approved the final manuscript.

Competing Interests

The authors declare no competing financial interests.

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References

[1] Bella F, Minicozzi P, Giacomini A, Crocetti E, Federico M, Ponz de Leon M, Fusco M, Tumino R, Mangone L and Giuliani O, et al (2013). Impact of diabetes on overall and cancer-specific mortality in colorectal cancer patients. *Cancer Res Clin Oncol* **139**, 1303–1310.

[2] NIH National Cancer Institute. <https://seer.cancer.gov/> [accessed on 12.12.18].

[3] International Agency on Research on Cancer. <http://gco.iarc.fr/> [accessed on 12.12.18].

[4] Müller MF, Ibrahim A and Arends M (2016). Molecular pathological classification of colorectal cancer. *Virchows Arch* **469**, 125–134. <https://doi.org/10.1007/s00428-016-1956-3>.

[5] Yunusova AV, Kondakova AV, Kolomiets LA, Afanas'ev SG, Kishkina AY and Spirina LV (2018). The role of metabolic syndrome variant in the malignant tumors progression. *Diabetes Metab Syndr* **12**(5), 807–812.

[6] Park H, Cho S, Woo H, Park SK, Shin HR, Chang SH, Yoo KY and Shin A (2017). Fasting glucose and risk of colorectal cancer in the Korean Multi-center Cancer Cohort. *PLoS One* **12**(11), e0188465. <https://doi.org/10.1371/journal.pone.0188465>.

[7] Peeters PJ, Bazellier MT, Leufkens HG, de Vries F and De Bruin ML (2015). The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. *Diabetes Care* **38**(3), 495–502.

[8] Gallagher EJ and LeRoith D (2013). Diabetes, antihyperglycemic medications and cancer risk: smoke or fire? *Curr Opin Endocrinol Diabetes Obes* **20**(5), 485–494.

[9] Meyerhardt JA, Sato K, Niedzwiecki D, Ye C, Saltz LB, Mayer RJ, Mowat RB, Whittom R, Hantel A and Benson A, et al (2012). Dietary glycaemic load and cancer recurrence and survival in patients with stage III colon cancer: findings from CALGB 89803. *J Natl Cancer Inst* **104**(22), 1702–1711.

[10] Mei ZB, Zhang ZJ, Liu CY, Liu Y, Cui A, Liang ZL, Wang GH and Cui L (2014). Survival benefits of metformin for colorectal cancer patients with diabetes: asystematic review and meta-analysis. *PLoS One* **9**(3):e91818. <https://doi.org/10.1371/journal.pone.0091818>.

[11] Garrett CR, Hassabo HM, Bhamdkamkar NA, Wen S, Baladandayuthapani V, Kee BK, Eng C and Hassan MM (2012). Survival advantage observed with the use of metformin in patients with type II diabetes and colorectal cancer. *Br J Cancer* **106**, 1374–1378.

[12] Spillane S, Bennett K, Sharp L and Barron TI (2013). A cohort study of metformin exposure and survival in patients with stage I–III colorectal cancer. *Cancer Epidemiol Biomarkers Prev* **22**(8), 1364–1373.

[13] Weber MM, Fottner CH, Liu SB, Jung MC, Engelhardt D and Baretton GB (2002). Overexpression of the insulin-like growth factor I receptor in human colon carcinomas. *Cancer* **95**(10), 2086–2095.

[14] Wu Y, Yakar S, Zhao L, Henninghausen L and LeRoith D (2002). Circulating insulin-like growth factor-I levels regulate colon cancer growth. *Cancer Res* **62**, 1030–1035.

[15] Codony-Servat J, Cuatrecasas M, Asensio E, Montironi C, Martínez-Cardús A, Marín-Aguilera M, Horndler C, Martínez-Balibrea E, Rubini M

and Jares P, et al (2017). Nuclear IGF-1R predicts chemotherapy and targeted therapy resistance in metastatic colorectal cancer. *Br J Cancer* **17**, 1777–1786.

[16] Dowling RJO, Goodwin PL and Stambolic V (2011). Understanding the benefit of metformin use in cancer treatment. *BMC Med* **9**, 33–39.

[17] Howel J, Hellberg K, Shaw RJ and Manning BD (2014). Mechanism and consequences of hepatic regulation of mTORC1 by metformin. *Cancer Metab* **2**(1), P28.

[18] Pernicova I and Korbonits M (2014). Metformin-mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol* **10**, 143–156.

[19] Schroll MM, Liu X, Herzog SK, Skube SB and Hummon AB (2016). Nutrient restriction of glucose or serum results in similar proteomic expression changes in 3D colon cancer cell cultures. *Nutr Res* **36**(10), 1068–1080.

[20] Evans JMM, Donnelly LA, Emslie-Smith AM, Alessi DR and Morris AD (2005). Metformin and reduced risk of cancer in diabetic patients. *BMJ* **330**(4), 1304–1305.

[21] Ramjessingh R, Orr C, Bricks CS, Hopman WM and Hammad N (2016). A retrospective study on the role of diabetes and metformin in colorectal cancer disease survival. *Curr Oncol* **23**(2), 116–122.

[22] Oh BY, Park YA, Huh JW, Cho YB, Yun SH, Lee WY, Park HC, Choi DH, Park YS and Kim HC (2016). Metformin enhances the response to radiotherapy in diabetic patients with rectal cancer. *J Cancer Res Clin Oncol* **142**(6), 1377–1385.

[23] Chiao EY, Preethi PV and Naik AD (2010). The impact of diabetes process and outcome quality measures on overall survival in patients with co-morbid colorectal cancer. *J Cancer Surviv* **4**, 381–387.

[24] Prieto I, Del Puerto-Navado L, Gonzalez N, Portal-Nuñez S, Zazo S, Corton M, Minguez P, Gomez-Guerrero C, Arce JM and Sanz AB, et al (2017). Colon cancer modulation by a diabetic environment : a single institutional experience. *PLoS One* **12**(3):e0172300doi. <https://doi.org/10.1371/journal.pone.0172300>.

[25] McMenamin UC, Murray LJ, Hughes CM and Cardwell CR (2016). Metformin use and survival after colorectal cancer: a population-based cohort study. *Int J Cancer* **138**, 369–379.

[26] Franggaard T, Thygesen LC and Gögenur I (2018). Association between metformin use after surgery for colorectal cancer and oncological outcomes: a nationwide register-based Study. *Int J Cancer* **143**, 63–72.

[27] Boursi B, Haynes K, Mamtani R and Yang YX (2015). Thyroid dysfunction, thyroid hormone replacement, and colorectal cancer risk. *J Natl Cancer Inst* **107**(6), 1–7. <https://doi.org/10.1093/jnci/djv084>. Print 2015 Jun 7.

[28] Brown AR, Simmen RCM and Simmen FA (2013). The role of thyroid hormone signalling in the prevention of digestive system cancers. *Int J Mol Sci* **14**, 16240–16257.

[29] International Classification of Diseases, 9th revision based on the World Health Organization's Ninth Revision, International Classification of Diseases (ICD-9), <https://www.cdc.gov/nchs/icd/icd9cm.htm>.

[30] Tamas K, Walenkamp AME, de Vries EGE, van Vugt MATM, Beets-Tan RG, van Etten B, de Groot DJA and Hospers GAP (2015). Rectal and colon cancer: not just a different anatomic site. *Cancer Treat Rev* **41**, 671–679.

[31] Orgel E and Mittelman SD (2013). The links between insulin resistance, diabetes, and cancer. *Curr Diab Rep* **13**, 213–222.

[32] Itatani Y, Kawada K, Inamoto S, Yamamoto T, Ogawa R, Taketo MM and Sakai Y (2016). The role of chemokines in promoting colorectal cancer invasion/metastasis. *Int J Mol Sci* **17**(5). <https://doi.org/10.3390/ijms17050643>. E643.

[33] Van Dyke AL, Lang Kuhs KA, Shiels MS, Koshiol J, Trabert B, Loftfield E, Purdue MP, Wentzensen N, Pfeiffer RM and Katki HA, et al (2017). Associations between self-reported diabetes and 78 circulating markers of inflammation, immunity, and metabolism among adults in the United States. *PLoS One* **12**(7):e0182359.

[34] Coghill AE, Newcomb PA, Poole EM, Hutter CM, Makar KW, Duggan D, Potter JD and Ulrich CM (2011). Genetic variation in inflammatory pathways is related to colorectal cancer survival. *Clin Cancer Res* **17**(22), 7139–7147.

[35] Rodriguez AJ, Mastronardi C and Paz-Filho G (2013). Leptin as a risk factor for development of colorectal cancer. *Transl Gastrointest Res* **2**(4), 211–222.

- [36] Tsujimoto T, Kajio H and Sugiyama T (2017). Association between hyperinsulinemia and increased risk of cancer death in nonobese and obese people: a population-based observational study. *Int J Cancer* **141**, 102–111.
- [37] Siddiqui AA, Spechler SJ, Huerta S, Dredar S, Little BB and Cryer B (2008). Elevated HbA1c is an independent predictor of aggressive clinical behavior in patients with colorectal cancer: a case control study. *Dig Dis Sci* **53**, 2486–2494.
- [38] Vulcan A, Manjer J and Ohlsson B (2017). High blood glucose levels are associated with higher risk of colon cancer in men: a cohort study. *BMC Cancer* **17**, 842–849. <https://doi.org/10.1186/s12885-017-3874-4>.
- [39] Conti J and Thomas G (2011). The role of tumour stroma in colorectal cancer invasion and metastasis. *Cancers* **3**, 2160–2168.
- [40] Van Pelt GW, Sandberg TP, Morreau H, Gelderblom H, van Krieken JHJM, Tollenaar RAEM and Mesker WE (2018). The tumor–stroma ratio in colon cancer: the biological role and its prognostic impact. *Histopathology* **73**, 197–206. <https://doi.org/10.1111/his.13489>.
- [41] Andrzejewski S, Gravel SP, Polak M and St-Pierre J (2014 Aug 28). Metformin directly acts on mitochondria to alter cellular bioenergetics. *Cancer Metab* **2**(12). <https://doi.org/10.1186/2049-3002-2-12>. eCollection 2014.
- [42] Hursting SD, Dunlap SM, Ford NA, Hursting MJ and Lashinger LM (2013 Mar 7). Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab* **1**(1), 10. <https://doi.org/10.1186/2049-3002-1-10>.
- [43] Heckman-Stoddard BM, DeCensi A, Sahasrabudhe VV and Ford LG (2017). Repurposing metformin for the prevention and cancer recurrence. *Diabetologia* **60**, 1639–1647.
- [44] Cameron AR, Morrison DL, Levin D, Mohan M, Forteach C, Beall C, McNeilly AD, Balfour DJ, Savinko T and Wong AK, et al (2016). Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ Res* **119**, 652–665.
- [45] Li D, Yeung SC, Hassan MM, Konopleva M and Abbruzzese JL (2009). Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* **137**, 482–488.
- [46] Currie CJ, Poole CD and Gale EA (2009). The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* **52**(9), 1766–1777.
- [47] Heijmans J, Wielenga MC, Rosekrans SL, van Lidth de Jeude JF, Roelofs J, Groothuis P, Ederveen A, de Jonge-Muller ES, Biemond I and Hardwick JC, et al (2014). Oestrogens promote tumorigenesis in a mouse model for colitis-associated cancer. *Gut* **63**, 310–316.
- [48] Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R and Caan B, et al (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *J Am Med Assoc* **291**, 1701–1712.
- [49] Simon MS, Chlebowski RT, Wactawski-Wende J, Johnson KC, Muskovitz A, Kato I, Young A, Hubbell FA and Prentice RL (2012). Estrogen plus progestin and colorectal cancer incidence and mortality. *J Clin Oncol* **30**, 3983–3990.
- [50] Symer MM, Wong NZ, Abelson JS, Milsom JW and Yeo HL (2018). Hormone replacement therapy and colorectal cancer incidence and mortality in the prostate, lung, colorectal, and ovarian cancer screening trial. *Clin Colorectal Cancer* **17**(2), e281–e289. <https://doi.org/10.1016/j.clcc.2018.01.003>. Epub 2018 Jan 12.
- [51] Vilar L, Naves LA, Caldato C and Caldato M (2015). Acromegaly and colorectal cancer. *Transl Gastrointest Cancer* **4**(1), 28–38.