Ph. D. Thesis

The epidemiology and pathogenesis of colorectal cancer (CRC)
The redistribution changes in the localization of the CRC.
The role of calcium-metabolism and calcium sensing receptor and vitamin D receptor (VDR) gene of pathogenesis of CRC.

Dr. Fuszek Péter

Semmelweis University - School of PhD Studies
First Department of Medicine, Semmelweis University,
Ph. D. 2/15
Molecular Genetics, Pathomechanism and Clinical Aspects of Metabolic Disorders

Promoter: Dr. Speer Gábor

Chief of the program: Dr. Lakatos Péter

Examination committee:
Dr. Fehér János D.Sc.
Dr. Fekete György D.Sc.
Dr. Andrikovics Hajnalka Ph.D.
Dr. Juhász Márk Ph.D.
Dr. Demeter Pál Ph.D.

Opponents:
Dr. Herszényi László Ph.D.
Dr. Szentirmai Zoltán Orvostudományok Kandidátusa

2006
SUMMARY

Hungary is among the first in Europe regarding colorectal cancer (CRC) mortality. Epidemiology studies of the last 20 years found a change in CRC location (increasing of the proximal proportion of the CRC). Genetical as well as environmental factors, such as diet have important role in the development of CRC. There are several known genetic variations that may play a role in the development of this disease. Among these are the genetical variation such as a calcium sensing receptor (CaSR) gene A986S and of vitamin D receptor (VDR) BsmI polymorphisms. Our goal was to study the change in the localization in our patient group (n=1738) between 1993 and 2004. In our CRC patients (n=70) we studied their calcium homeostasis, the genetical polymorphism of their CaSR and how these affect each other. We measured the connection between levels of the AFP, CEA, CA19-9 prognostic factors and the genotypes, and patients' calcium-metabolism. Our aim was to record the distribution of the CaSR/VDR polymorphism in patients with rectal tumor. We also studied the connection between the above genotypes and erb-2, EFGR, ras, p53 expression - as prognostic and diagnostic factors of CRC - furthermore with UICC staging for 5 years. Analysis of our data showed an increasing frequency of CRC in both sexes. There was no change in localization, but there were more rectal tumors among distal tumors. In our work we showed that patients with newly discovered CRC had serum calcium and ionized calcium levels below normal. There was no connection between CaSR A986S polymorphism and calcium metabolism or calcium levels. Frequency of CaSR and VDR polymorphism was identical between in the CRC and the control groups. The serum ionic calcium level was inversely proportional with the CA19-9 prognostic marker levels. We showed that in the presence of the VDR gene allel B (i.e. Bb and Bb genotypes) expression of the erb-2 gene is significantly higher, unlike in bb genotype. Among the patients with rectal cancer who died during five year follow-up, AA genotype of the CaSR gene was significantly more frequent compared to survivors at 5 years.

We conclude that it is of paramount importance to consider CRC-as as a priority in Hungary. Various attempts should be made to decrease incidence. Screening should be started. In Hungary it would be indicated to start this at age 50 in males and at age 55 in females. Our work points out the role of Vitamin D/VDR and calcium/CaSR system in the pathogenesis of colorectal cancers. Chemoprevention would be useful; using calcium and Vitamin D. Low level of these may play a role in the pathogenesis of CRC. Normalizing serum calcium level may influence slow malignant transformation in the colon, or would increase local vitamin D synthesis, having a similar effect. We can presume that by reaching the recommended daily calcium intake of 1000-1500 mg for a lifetime, we could decrease the alarming prognosticated frequency of colorectal cancers in our country, and with a very limited cost.

INTRODUCTION

Colorectal adenocarcinoma (CRC) is the fourth most common cause of death in the developed countries. Recently lot of evidences based on earlier research of the past decade at the molecular background of carcinogenesis are known. These data draw attention to the possibility that, the multileveled pathogenic process leading to tumor genesis might be influenced by chemoprevention. Naturally not all factors leading to the CRC are known. Earlier paper indicated that 70% of CRCs involve the left colon, while 30% occur in the proximal colon. Based on epidemiological studies we know that substantial geographical differences exist in the incidence of CRC. Several factors, such as eating habits, race, and differences in the genetic background have been thought to account for those differences. Recent data indicate a redistribution in the localization of the colorectal carcinomas in North
America and Western Europe. Numerous studies reported increase in the incidence of right-sided CRCs, parallel with the decrease of the number of tumors in the distal colon. The proportion of the right colonic segment has risen from 30% to 36%, while that of the rectum fell from 20% to 14%. The background of these changes are not fully known. Possibly the changes and standardization in eating habits occurring in the past decades might be one of the reasons. Our aim was to investigate the change in age at diagnosis, gender, location and cancer stage of CRC cases in the last twelve years in a large number (n=1738) of Hungarian patients. We also know that genetical as well as environmental factors, such as diet have important role in the development of CRC. The 75% of colorectal cancer is sporadic. Presumable the carcinogenesis of sporadic CRC the low penetrance genes and environmental carcinogenic factors have significant role. The western style diet (low in calcium and vitamin D and high in fat) enhances of risk of CRC. There are a lot of evidences that increase of intake of calcium and vitamin D decreases this risk of CRC mentioned above. This processes mostly occurs through the activation of calcium sensing receptor and vitamin D receptor. According to results the calcium and vitamin D influence directly the expression of genes which have role of tumor genesis and it also influence the signalization pathways which in cells control proliferations and differentiations and the apoptosis. There are several known genetic variations that may play role in the development of CRC. Among these there are genetical variation such as a calcium sensing receptor (CaSR) gene A986S and vitamin D receptor gene (VDR) BsmI polymorphisms. The aim of our study is to examine the calcium metabolism in our CRC patients and in control patients focusing on CaSR and VDR gene polymorphism.

AIM

Epidemiological studies (I)

The aim of our study was to investigate the change in age at diagnosis, gender, location and cancer stage of CRC cases in the last 12 years (between 1993-2004) in a large number of Hungarian patients. The aim of our study was to investigate:

1. the incidence of CRC
2. distribution of colorectal cancer patients according to the age at diagnosis
3. distribution of colorectal cancer patients according to the gender
4. the change localization of the colorectal carcinomas
5. histological data
6. analysis of the relationship between location, age and TNM classification

Metabolism of Calcium- and vitamin D, A986S polymorphism of the CaSR gene (II)

The aim of our study was to examine the calcium metabolism and CaSR gene polymorphism of colorectal cancer (CRC) in our patients with colorectal cancer and in control patients. The aim of our study was to investigate:

1. the calcium metabolism in our patients with CRC
2. the genotype frequencies (CASR A986S) of our patients
3. the relationship between genotypes (CASR A986S) and laboratory parameters of calcium homeostasis
4. the possible role of the CaSR A986S polymorphism in the pathogenesis of CRC
5. the relationship between genotypes (CASR A986S) and laboratory parameters of calcium homeostasis and tumor markers (AFP, CEA, CA 19-9)
VDR/CaSR polymorphism of rectum tumors (III)

The aim of our study was to investigate:

1. the VDR BsmI and CaSR A986S polymorphism in patients with rectum tumors
2. the connection between the above mentioned genotypes and erb-2, EFGR, ras, p53 expression – as prognostic and diagnostic factors of CRC – furthermore the connection between UICC staging for 5 years follow-up

PATIENTS AND METHODS (I)

Epidemiological studies (I)

We retrospectively analyzed the histologically confirmed consecutive CRC (n=1738) cases diagnosed at the 1st Department of Medicine and 1st Department of Surgery of Semmelweis University between the 1st of January 1993 and 31st of December 2004.

Statistical analysis: Normality was tested by Shapiro Wilk’S W test. For comparison, D-test and ANOVA test with post hoc Scheffe test were applied. Possible relationships between gender, localization, TNM classification, and age group distribution were assessed using $\chi^2$ and Yates-corrected $\chi^2$ tests. All calculations were performed on Statistica 6.1 (Statsoft Inc, OK, USA).

PATIENTS AND METHODS (II)

Metabolism of Calcium- and vitamin D, A986S polymorphism of the CaSR gene (II)

PATIENTS (II)

Seventy newly diagnosed CRC patients were examined. An age and gender adjusted healthy control group ($n=32$) was selected for comparison of the laboratory data. The CASR genotype frequency was compared to the genotype frequency of our previously determined control group, consisting of 201 healthy adults.

METHODS (II)

Laboratory parameters

To analyze the calcium metabolism of the subjects, serum calcium, phosphate and albumin levels were determined by photometric analysis (Roche, Mannheim, Germany). Ionized calcium levels were also measured in a similar way (Easy- Lite, Bedford, USA). HPLC (Biorad, Hercules, USA) was used to measure the serum 25(OH) vitamin D levels, while serum levels of parathyroid hormone (PTH) were determined by chemiluminescence assay (Elecsys/Roche,Basel, Switzerland). To measure AFP, CEA and CA 19-9, immunoassays (Axxym/Abbott, North Chicago, USA) were utilized.

Sampling and histology

In each case, the diagnosis was established by colonoscopy (Fujinon, Japan), and tissue samples were taken for histological analysis.

Genotyping

The polymorphic region of CaSR gene was amplified by allele specific PCR technique. (Hybaid TouchDown Thermocycler [Hybaid, Teddington, UK]).

Statistics
As the first step, the distribution of continuous parameters was analyzed (Kolmogorov-Smirnov test). Logarithmic transformation was performed as needed. However, results were presented using the original units for easier understanding. The average values for CRC and the control population as well as difference between patients with different genotypes were compared using Student $t$ test with separate variance estimates. $c^2$ test was used to describe the relation between the allele frequency and tumor localization. Finally, we used parametric correlation (Pearson) to describe the relationship between tumor markers and the calcium homeostasis. A $P$ value $<0.05$ was considered statistically significant. All the statistical analyses were performed using SPSS 9.0 for Windows.

PATIENTS AND METHODS (III)

VDR/CaSR polymorphism of rectum tumors (III)

PATIENTS (III)

In our study, 56 patients with rectal cancer consecutively admitted to the Department of Surgery were studied. All patients underwent surgery. The mean follow-up period was 48 months (range 1–60 months). During the follow-up period, 16 patients died. The mean survival period from the time of diagnosis was 19 months (range 1–47 months). In the control group, VDR genotypes of 112 age-matched healthy subjects (two controls for each patient) were determined. The enrolment criteria in the control group included the lack of gastrointestinal pain, family history for colorectal cancer and detection of blood in stool, as well as no pathological finding on abdominal ultrasound (US).

METHODS (III)

Genomic DNA was isolated from the surgically removed rectal cancers and normal mucosa.

Genotyping

The amplified PCR product was digested by using BsmI restriction enzyme (Hybaid-AGS, Teddington, Middlesex, UK, 10 U/ml) for 90 min at 65°C. The BsmI restriction site is missing in the B allele and is present in the b allele. For the PCR reactions, a Hybaid Touchdown thermocycler (Teddington, Middlesex, UK) was used. Electrophoretic separation was carried out in a 2% agarose gel containing 10 mg/ml ethidium bromide.

Immunohistochemistry and protein blotting

The expression of erbB-2 protein was detected. Immunostaining and protein blotting (BioGenex, Mainz, Germany) In the case of erbB-2, tumour samples with identical scoring in both methods were included in the study. To measure AFP, CEA and CA 19-9, immunoassays (Axxym/Abbott, North Chicago, USA) were utilized.

Statistical analysis

Analysis of the relationships among clinical data, and VDR polymorphisms, oncoprotein expression and coexpression was performed by using Chi-square and Yates-corrected Chi-square test where appropriate. A $P$ value $<0.05$ was considered statistically significant. All the statistical analyses were performed using SPSS 9.0 for Windows.

RESULTS

Epidemiological studies (I)
1. Between 1993 and 2004 we detected an increase in the number of CRC cases (both in men and in women).
2. No association was found in location, on the other hand we more frequently diagnosed rectum tumor among distal tumors.
3. We diagnosed: 1694 (97.5%) adenocarcinoma, 15 anaplastic carcinoma, 9 carcinoid, 6 spinocellular cc., 5 GIST tumor, 3 leiomyoma, 2-2 melanoma, 1 lymphoma and shigillocellularis carcinoma. 12 clinical subjects developed synchronous tumors (11 males and 1 female, mean age at diagnosis: 68.8 SD 11.6 years, 6 rectum and transverse colon, and 5 rectum and ascending colon/caecum).
4. Analysis of the relationship between location and TNM classification revealed that rectal tumors were diagnosed slightly earlier (rectum: 64.1±12.1 years vs. left colon: 66.1±12.2 years vs. right colon: 66.0±12.9 years, p=0.03 for all groups, ANOVA, Scheffe post-hoc) and at an earlier stage (T1: 5.6 %, T2: 26.5 %, T3: 61.5 %, T4: 6.4 %) compared to both left- and right-sided CRCs (T1: 4.8 % and 3.1 %, T2: 17.3 % and 15.9 %, T3: 68.0 % and 71.2 %, T4: 9.9 % and 9.8 %, p=0.0008). At the same time no difference was observed in the presence of metastases.

RESULTS

Metabolism of Calcium- and vitamin D, A986S polymorphism of the CaSR gene (II)

1. After examining the calcium metabolism of CRC patients, we found that the serum calcium, ionized and corrected calcium levels of our patients were significantly lower, than those of controls. These calcium levels were at the lower limits of the normal range. Serum phosphate and albumin levels were also determined, which were in the lower end of the normal range. There was no difference in serum 25(OH)-vitamin D and PTH values between patients and controls.
2. There was no relationship between CaSR A986S and calcium metabolism and calcium level in CRC patients.
3. There was no relationship between CaSR A986S and CRC.
4. When examining the correlation between calcium metabolism and CEA, AFP, CA 19-9 tumor markers, we found that the ionized calcium levels of our patients were inversely correlated with the serum level of CA 19-9 tumor marker.
5. There was no significant difference in the CaSR A986S genotype frequency between the healthy population and the CRC patients.

RESULTS

VDR/CaSR polymorphism of rectum tumors (III)

1. There was no significant difference in the VDR BsmI genotype frequency between the control group and the CRC patients.
2. The overexpression of erbB-2 was significantly more frequent in the presence of the B allele of the VDR gene.
3. There was no significant difference in the CaSR A986S genotype frequency between the healthy population and the patients with rectum tumor.
4. Among the patients with rectal cancer who died during five year follow-up, AA genotype of the CaSR gene was significantly more frequent compared to survivors at 5 years. (p<0.05).
5. There was no correlation between CaSR A986S polymorphisms and erbB-2, EGFR, ras and p53 expression.
6. Significant differences were found in the oncoprotein expression pattern of normal and tumor tissues. The erbB-2, EGFR, ras and p53 expression were significantly more frequent in tumor than normal mucosa. The expression of erbB-2, EGFR correlated with prognosis.

CONCLUSIONS

Epidemiological studies (I)

In contrast to Western countries the proportion of proximal CRC did not become higher in Hungary. Still more than two-third of the patients were diagnosed to have distal cancers. The proportion of male patients was higher in this subset of CRC. The high percentage of locally advanced and metastatic cancers supports the need for colorectal screening program in Hungary.

CONCLUSIONS

Metabolism of Calcium- and vitamin D, A986S polymorphism of the CaSR gene (II)

VDR/CaSR polymorphism of rectum tumors (III)

The results of our study support the role of low serum calcium in the pathogenesis of CRC. Contrasting to previous data of others, we found that beside normal vitamin D values the level of calcium and the concentration of serum phosphate were both lower than that of controls suggesting that in CRC the function of vitamin D receptor (VDR) might be damaged. Our study corroborates this hypothesis, we showed that oncogene (HER-2) expression correlated with VDR genotypes in CRC patients. We conclude that it is of paramount importance to consider CRC-as as a priority in Hungary. Various attempts should be made to decrease incidence. Our work points out the role of Vitamin D/VDR and calcium/CaSR system in the pathogenesis of colorectal cancers. Chemoprevention would be useful; using calcium and vitamin D. Low level of these may play a role in the pathogenesis of CRC. Normalizing serum calcium level may influence slow malignant transformation in the colon, or would increase local vitamin D synthesis, having a similar effect. We can presume that by reaching the recommended daily calcium intake of 1000-1500 mg for a lifetime, we could decrease the alarming prognosticated frequency of colorectal cancers in our country, and with a very limited cost.

ACKNOWLEDGMENTS

In many ways this theses of cooperative effort, there have been many friends’ critiques that helped me with completing my work. I do want to special thanks to them for their support an encouragement over the past 3 years. I also would like to express my special thanks to my family for their patience and love.

REFERENCES (Articles in connection with thesis)


   **IF**: 3.318


   **IF**: 1,395


   **IF**: 0,696


   **IF**: 3.318

Other articles


   **IF**: 3.318
3. Peter Laszlo Lakatos, Laszlo Lakatos, Ferenc Szalay, Claudia Willheim-Polli, Christoph osterreicher, Zsolt Tullassay, Tamas Molnar, Walter Reinisch, Janos Papp, Gyula Mozsik, Hungarian IBD Study Group, Peter Ferenci **Hungarian IBD Study Group:** Semmelweis University, 1st Department of Medicine, Budapest: Peter Fuszek et al. Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: Phenotype-genotype correlations. World J Gastroenterol 2005 March 14;11(10):1489-1495 IF: 3.318

4. G. Speer, P. Szenthe, J.P. Kósa, Á.G. Tabák, A. Folhoffer, P. Fuszek, K. Cseh, P. Lakatos: Myocardial infarction is associated with the S allelic variant of the Sp1 binding site polymorphism of collagen type 1A1 gene. Acta Cardiologica (közlésre elfogadva) IF: 0.52

**ABSTRACTS**


8. **P. Fuszek, G. Speer, Zs. Nagy, P. Vargha, J. Papp, P. Lakatos. Polymorphisms of calcium-sensing receptor (CaSR) in colorectal cancer.** Z Gastroenterol 2001; 39


**CHAPTERS found in different books**


