Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 4, 2011, pp. 681-685

COLORECTAL CANCER RISK ASSESSMENT USING MOLECULAR BIOLOGY TECHNIQUES

Aurel ARDELEAN
"Vasile Goldis" Western University Arad, Romania
Academy of Romanian Scientists

ABSTRACT
Colorectal cancer is a major health burden, despite advances in surgical treatment and chemotherapy, being the most common cancer and second leading cause of cancer death in both sexes in European Union. In colorectal cancer, various molecular biology techniques are extensively used to identify valuable biomarkers for predicting the clinical outcome and response to treatment. The aim of our study was to evaluate the role of different molecular biology methods in assessing colorectal cancer risk. We included in this study patients diagnosed with colorectal cancer (n=286) and healthy controls (n=190). All diagnoses were pathologically confirmed. Written consent for participation was obtained from all study subjects. DNA and mRNA were obtained from blood and tissue sample-sets. We investigated the risk of colorectal cancer by different techniques addressing multiple targets. ANKRD17 mRNA, VEGF +936 C/T and -634 G/C polymorphisms, mutations in codon 12 of the K-ras gene and Gly120Ala in p53 gene appear to be the most sensitive targets and may have a potential value as additional markers for the risk assessment and prognostic of colorectal cancer.
KEYWORDS: colorectal cancer, molecular targets, K-ras, p53

INTRODUCTION

Over time, molecular biology outlined assumptions, theories, concepts (Pasarin, 2010; Petrescu-Mag, 2009), but it also had serious practical implications, especially in medicine (Kataria et al., 2010) and food industry (Mihociu et al., 2010).

Colorectal cancer is a major health burden (Chirila et al., 2011; Kinzler and Vogelstein, 1996), despite advances in surgical treatment and chemotherapy, being the most common cancer and second leading cause of cancer death in both sexes in European Union.

In colorectal cancer, various molecular biology techniques are extensively used to identify valuable biomarkers for predicting the clinical outcome and response to treatment (Srivastva et al., 2001; Douillard et al., 2000).

The aim of our study was to evaluate the role of different molecular biology methods in assessing colorectal cancer risk.

MATERIALS AND METHODS

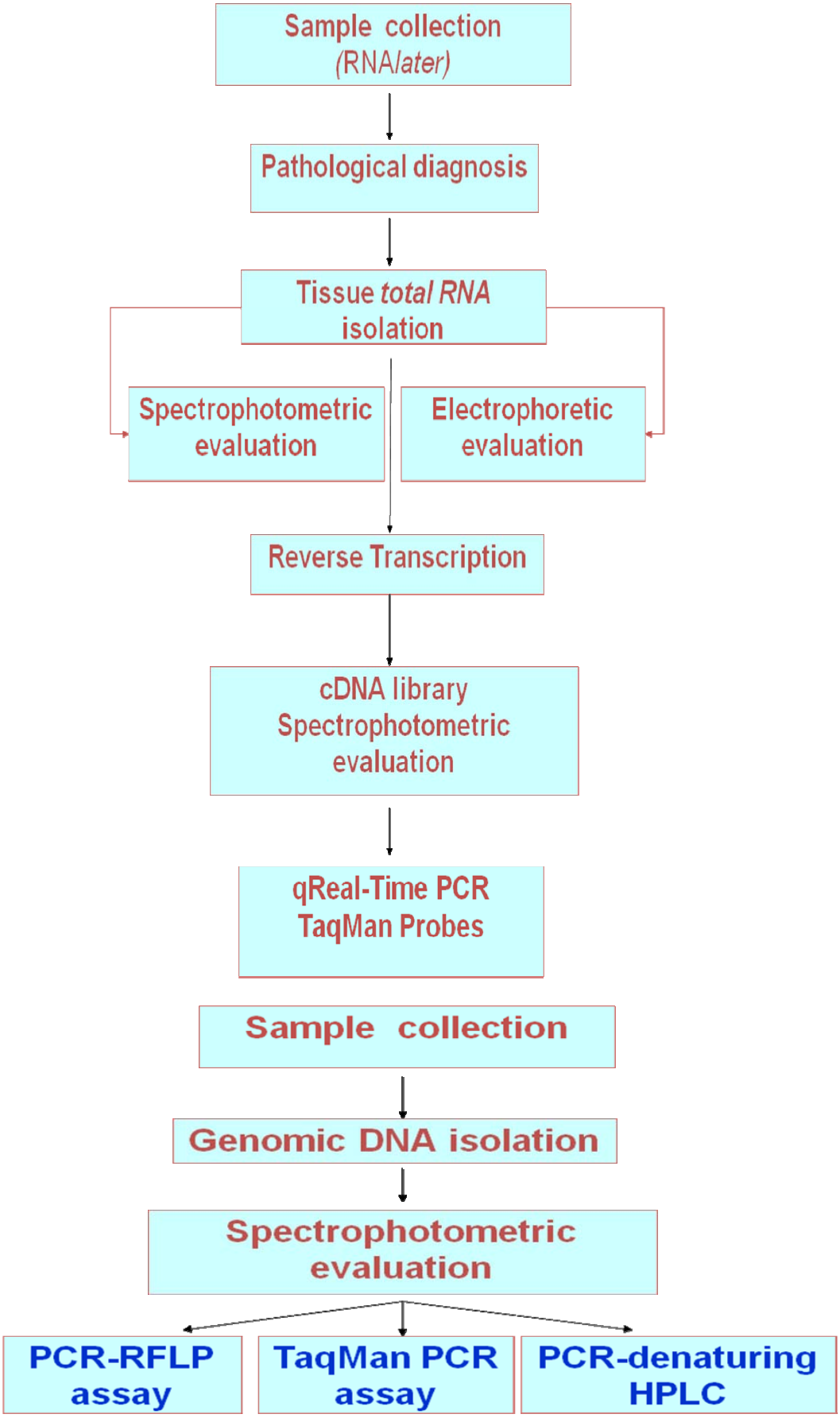
Subjects and samples

We included in this study patients diagnosed with colorectal cancer (n=286) and healthy controls (n=190). All diagnoses were pathologically confirmed. Written consent for participation was obtained from all study subjects.

DNA and mRNA were obtained from blood and tissue sample-sets. We investigated the risk of colorectal cancer by different techniques addressing multiple targets:

i) Real-Time quantitative Reverse Transcription PCR (qRT-PCR) with TaqMan probes specific to measure MMR genes level transcripts;
ii) PCR-RFLP method to identify K-ras and p53 mutations;
iii) TaqMan PCR assay using predesigned TaqMan SNP Genotyping Assays to investigate p53 mutations and polymorphisms;
iv) PCR/denaturing high-performance liquid chromatography assay to determine VEGF polymorphisms.

CORRESPONDENCE: *Aurel Ardelean, "Vasile Goldis" Western University Arad, Romania, e-mail: rectorat@uvvg.ro*

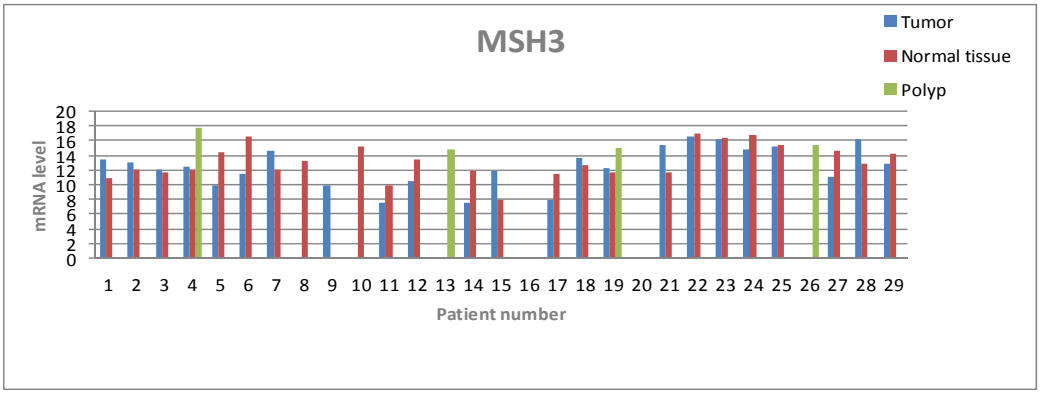


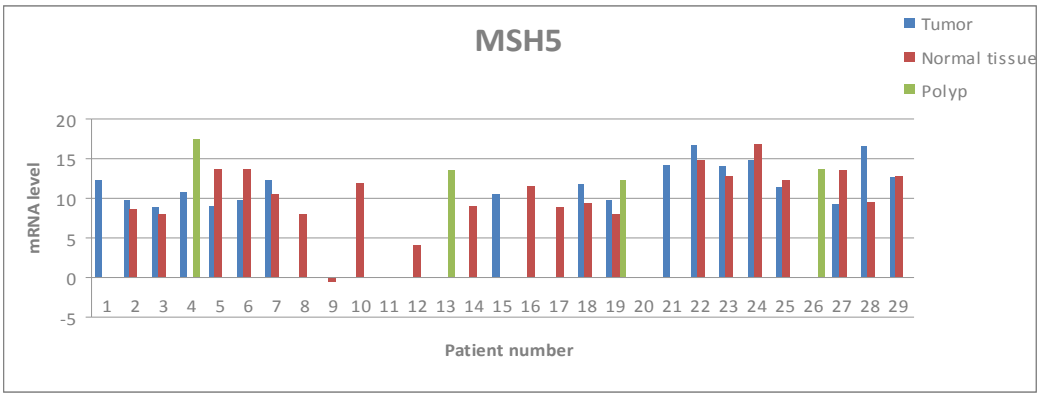
Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 4, 2011, pp. 681-685

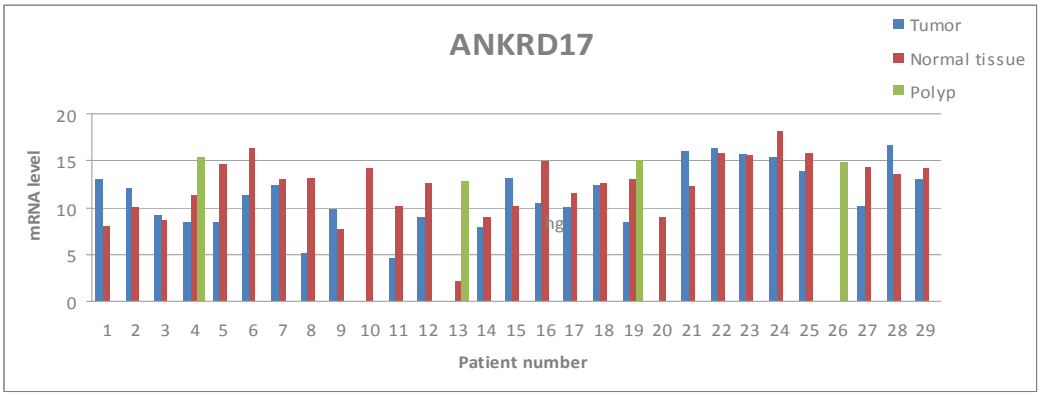
RESULTS AND DISCUSSIONS

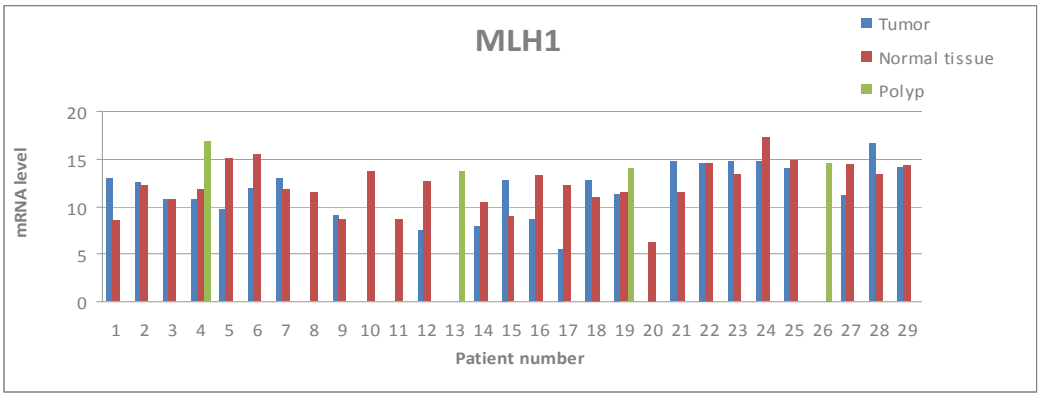
qReal-Time PCR

MMRmRNA levels for the significant correlated genes







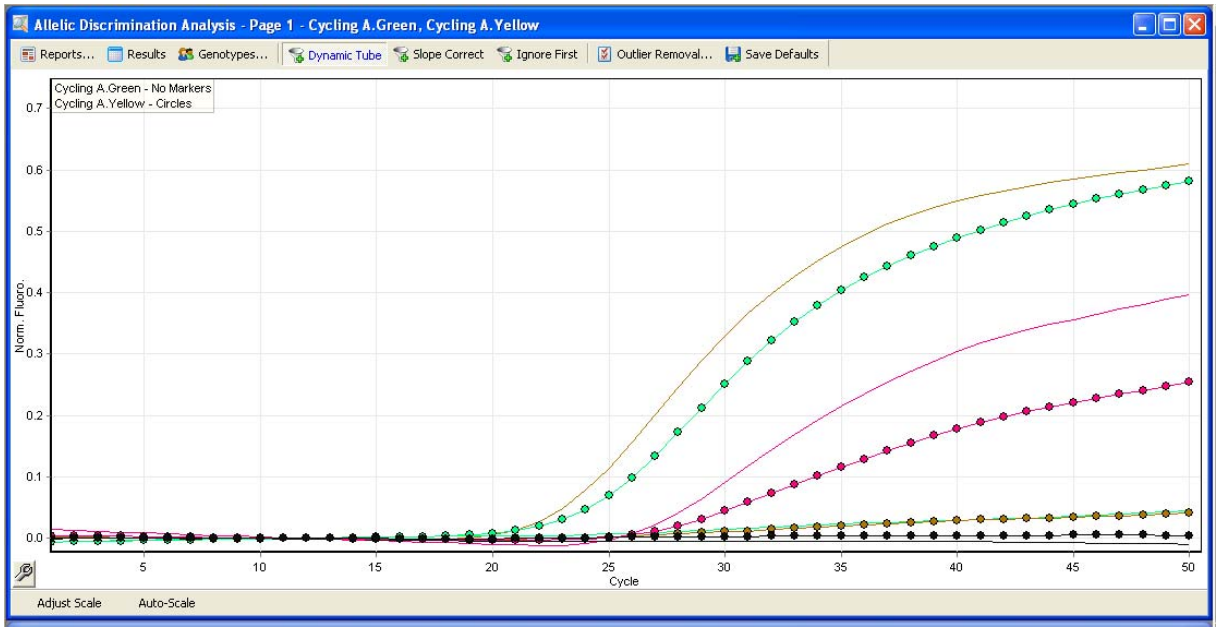


Biostatistics analysis of the gene expression pattern showed a strong correlation (P>0.9) for ANKRD17, MLH1, MSH3, MSH5. mRNA levels of MMR genes were lower in tumor samples when compared to normal tissue, ANKRD17 mRNA being the strongest associated gene.

TaqMan PCR

Genomic DNA was amplified using a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems, Foster City, CA), which contain primers and probes specific for each allelic variant.

We detected by TaqMan PCR a mutant phenotype in p53 gene for Gly120Ala polymorphism in 10% from cases, but not for Val217Met polymorphism.

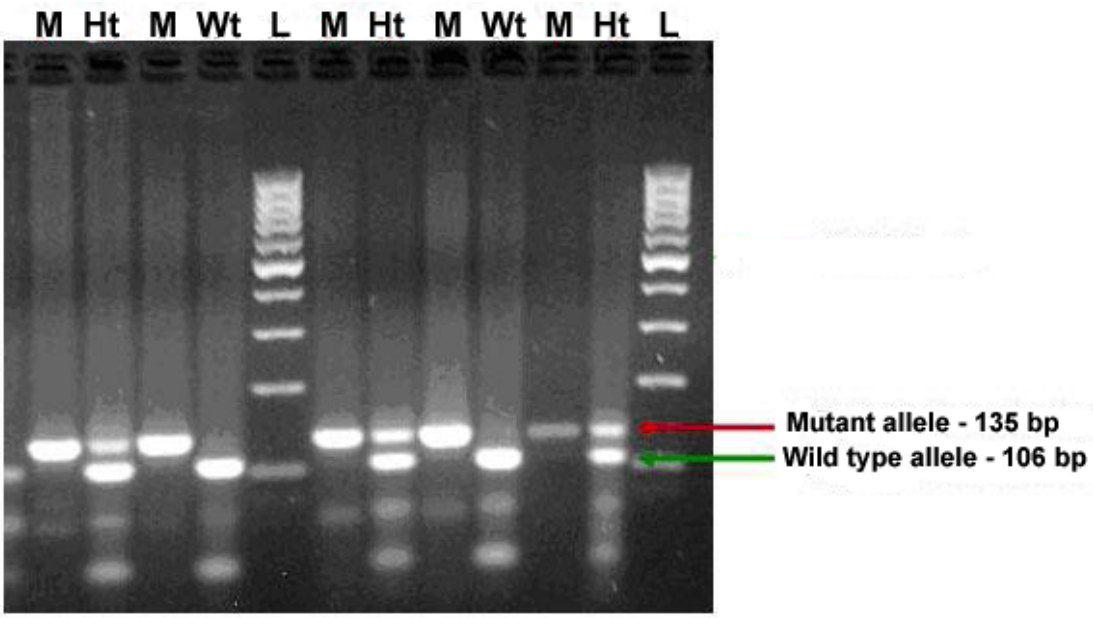


PCR -RFLP

The K-ras mutation rates in both codon 12 and 13 were evaluated by PCR-RFLP using restriction enzymes: Mva I (codon 12) and Hae III (codon 13).

The K-ras mutations rate in codon 12 was higher than controls, mainly for tumors on proximal colon. We not observed mutations in codon 13 of K-ras gene.

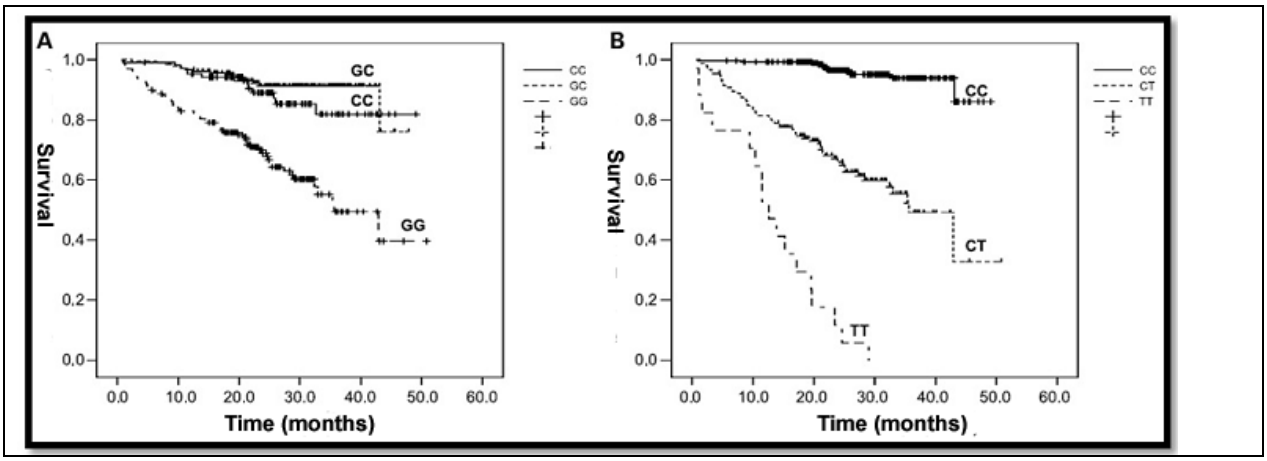
PCR-RFLP was performed by digestion of the PCR product using the restriction enzyme Mva 1 (K-ras mutation in codon 12). This enzyme cleaves only the wild-type but not the mutant alleles of K-ras (M-mutant homozygous, Ht-heterozygous, Wt-wild-type homozygous, L-ladder).



Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 4, 2011, pp. 681-685

PCR –denaturing HPLC

The carriers of -634 G/C and C/C showed a better survival than -634 G/G, whereas the +936 C/T or T/T genotype was associated with a worse survival compared with the +936 C/C genotype.



CONCLUSIONS

ANKRD17 mRNA, VEGF +936 C/T and -634 G/C polymorphisms, mutations in codon 12 of the K-ras gene and Gly120Ala in p53 gene appear to be the most sensitive targets and may have a potential value as additional markers for the risk assessment and prognostic of colorectal cancer.

REFERENCES

Chirila D. N., Pop T. R., Turdeanu N. A., Tubular carcinoid tumor of the appendix – a clinical case, HVM Bioflux, 3(1), pp.1-4, 2011

Douillard J-.Y., Cunningham D., Roth A. D., et al., Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomized trial, Lancet, 355, pp. 1041–1047, 2000.

Kataria N., Kataria A. K., Pandey N., Gupta P., Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries, HVM Bioflux, 2(2), pp.55-60, 2010

Kinzler K. W., Wogelstein B., Lessons from hereditary colorectal cancer, Cell, 87, pp.159-170

Mihociu T., Belc N., Iorga E., Rusu L., Metaxa I., The use of PCR applications for assessment of Clostridium botulinum presence in the culture environment and fish samples in the conditions of open system production from Romanian fisheries, AACL Bioflux, 3(4), pp.289-298, 2010

Pasarin B., The Pricopian «gene theory of sexuality» is just a hypothesis, but good enough to explain the sex determination in fish, AACL Bioflux, 3(2), pp.141-150, 2010

Petrescu-Mag I. V., Winge's sex-linked color patterns and SDL in the guppy: genes or gene complexes?, AACL Bioflux, 2(1), pp.71-80, 2009

Srivastava S., Verma M., Henson D. E., Biomarkers for early detection of colon cancer, Clin Cancer Res, 7, pp.1118-1126, 2001