

## Prognostic and predictive potential molecular biomarkers in colon cancer

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### Rezumat

#### *Potențiali biomarkeri moleculari pentru prognosticul și predicția cancerului de colon*

Unul dintre obiectivele cercetării medicale actuale este depistarea de noi biomarkeri care să poată detecta tumorile colonice în stadii timpurii și să poată indica cu acuratețe stadiul bolii. Scopul studiului nostru a fost identificarea unui panel de biomarkeri specifici pentru debutul și progresia cancerul colonic. Am evaluat expresia genică a unui panel de 10 gene candidat (MMP-1, MMP-3, MMP-7, DEFA 1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1) prin real time PCR cantitativ pe tripleți ai mucoasei colonice (țesut normal, adenom și țesut tumoral) recoltați de la același pacient pe parcursul intervenției chirurgicale, pentru un lot de 20 pacienți. Adicional am realizat teste de imunohistochimie pentru DEFA1-3 și SPP-1. Am remarcat faptul că DEFA-5 și DEFA-6 sunt factori cheie în formarea adenomului ( $p < 0.05$ ), DEFA-5, MMP-7 are un rol important în tranziția de la stadiul benign la cel malign ( $p < 0.01$ ) și apoi în metastază, fiind un indicator

prognostic al transformării tumorale și al potențialului metastatic. IL8, indiferent de stadiul bolii, prezintă un nivel de expresie al mARN crescut în adenocarcinoame ( $p < 0.05$ ). nivelul expresiei SPP-1 este corelat cu stadiul tumorii. Sugerăm că nivele crescute de DEFA-5, DEFA-6 (elemente cheie în formarea adenoamelor), MMP-7 (marker de debut al bolii și progresiei către metastază), SPP-1 (marker de progresie) și IL-8 ar putea fi utilizate în diagnosticul cancerului de colon în stadii timpurii și în evaluarea prognosticului de progresie a tumorilor colonice. Dacă nivelele de expresie a DEFA-5 și DEFA-6 sunt scăzute dar nivelele MMP-7, SPP-1 și IL-8 sunt crescute, se poate sublinia faptul că tranziția de la stadiul de adenom la cea de adenocarcinom a avut loc. În concluzie, DEFA-5, DEFA-6, MMP-7, IL-8 și SPP-1 alcătuiesc un panel valoros de biomarkeri a căror detecție poate fi utilizată în depistarea în stadii timpurii dar și evolutive ale bolii cât și pentru prognosticul cancerului colonic.

**Cuvinte cheie:** cancer de colon, biomarkeri, qPCR

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### Abstract

An important objective in nowadays research is the discovery of new biomarkers that can detect colon tumours in early stages and indicate with accuracy the status of the disease. The aim of our study was to identify potential biomarkers for colon cancer onset and progression. We assessed gene expression profiles of a list of 10 candidate genes (MMP-1, MMP-3, MMP-7, DEFA 1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1) by quantitative real time PCR in triplets of colonic

mucosa (normal, adenoma, tumoral tissue) collected from the same patient during surgery for a group of 20 patients. Additionally we performed immunohistochemistry for DEFA1-3 and SPP1. We remarked that DEFA5 and DEFA6 are key factors in adenoma formation ( $p < 0.05$ ). MMP7 is important in the transition from a benign to a malignant status ( $p < 0.01$ ) and further in metastasis being a prognostic indicator for tumor transformation and for the metastatic potential of cancer cells. IL8, irrespective of tumor stage, has a high mRNA level in adenocarcinoma ( $p < 0.05$ ). The level of expression for SPP1 is correlated with tumor level. We suggest that high levels of DEFA5, DEFA6 (key elements in adenoma formation), MMP7 (marker of colon cancer onset and progression to metastasis), SPP1 (marker of progression) and IL8 could be used to diagnose an early stage colon cancer and to evaluate the prognostic of progression for colon tumors. Further, if DEFA5 and DEFA6 level of expression are low but MMP7, SPP1 and IL8 level are high we could point out that the transition from adenoma to adenocarcinoma had already occurred. Thus, DEFA5, DEFA6, MMP7, IL8 and SPP1 consist in a valuable panel of biomarkers, whose detection can be used in early detection and progressive disease and also in prognostic of colon cancer.

**Key words:** colon cancer, biomarkers, qPCR

## Introduction

Colon cancer is a frequent malignancy being associated with high mortality and morbidity.

From histological point of view it develops in an orderly manner from normal colon epithelium through an adenoma precursor stage. The transition from adenoma to cancer spans over 10 to 15 years providing an extended opportunity for intervention and cancer prevention (1).

From the molecular point of view it is a multistep process that involves successive genetic alterations.

In early 90s Fearon and Vogelstein proposed a genetic model for colorectal carcinogenesis (2). This simplified model assumes the progressive accumulation of mutations and chromosomal rearrangements involving critical oncogenes or oncosuppressors genes. (3).

A more detailed molecular analysis of colon cancers revealed colon tumors to be heterogeneous with regard to molecular alterations and potentially categorizable into specific tumor phenotypes based on their molecular profiles (4).

Two of these represent genetic instability classes. The majority of sporadic cases (up to 85%) display chromosomal instability (CIN), which manifests as aneuploid and polyploid karyotypes and multiple structural chromosomal changes (5,6,7,8).

This phenotype is thought to arise through defects in a number of processes, including aberrant expression or mutation of mitotic checkpoint genes, microtubule spindle defects, and telomere dysfunction (9).

In contrast, the remaining 15% of sporadic colon cancers demonstrate microsatellite instability (MSI) phenotype, in which tumors display insertion-deletion mutations, most commonly in short tandemly repeated nucleotides (microsatellites) (10,11).

Molecular analysis of colon tumors has unraveled perturbations of genetic networking, depicted genes involved in disease onset and progression and enabled pharmacologist to discover and to deploy new therapies.

Additionally other studies showed that a series of five SNPs are associated with colon and rectal cancer risk (rs6983267, rs4939827, rs3802842, rs4444235, rs10795668) (12) while the presence of the mutation C677T has a high risk for turning colorectal tumors in un-resectable cases (13).

All these progresses made possible to better understand that each individual have its own risk to develop the disease and also have a different response to a specific treatment, all because of his unique genetic profile.

The consequence is that we are facing today a shift from the concept of "one drug fits all" towards a "therapy with the right drug at the right dose in the right patient". This relative new concept is called personalized medicine.

Within this novel approach of medicine an important role is assigned to biomarkers. Biomarkers can be used for the assessment of patients' prognostic regarding the disease or for prediction of patients' response towards a specific treatment.

For example in metastatic colorectal cancer a prediction biomarker is k-ras which is indicative of the response to a monoclonal antibody called cetuximab (Erbix), that targets the epidermal growth-factor receptor. It is now well established that patients with k-ras mutation tumors will not benefit from EGFR-targeted therapy.

So in order to avoid giving useless treatments like cetuximab, which is relatively expensive, would be most cost-effective if it were given to patients with the highest chance of benefiting from it (14). Until now k-ras remains the only molecular biomarker to be implemented to guide clinical practice (15).

Thus to achieve the goals of personalized medicine it is necessary to design and carry out fundamental research and than to translate this new knowledge into clinical practice.

The aim of this study was to define the levels of expression of a panel of 10 candidate genes in triplets of colon mucosa detected by qPCR. This could enlarge the knowledge concerning colon carcinogenesis and could identify novel biomarkers for early detection and progression of the disease.

## Material and Method

### *Patients and tissue specimens*

Based on an existing written consent, and in accordance with the ethical national and international standards, paired normal, adenoma and primary colon cancer specimens were collected from 20 patients that undergo the surgical removal of cancer at the Surgical Department from Fundeni Clinical Institute.

Criteria for selecting patients were: age between 40 and 80, no chemotherapy or radiotherapy administered before surgery and without cancers at first degree relatives.

For each patient, during surgery, one tissue sample, from each tissue type, was taken for histological examination and another was snap frozen in liquid nitrogen and stored at low temperatures for the transcriptomic study.

Samples were histologically confirmed by eosin–haematoxylin staining and were staged according TNM operative staging system. Also, pathological characteristics such as tumour size, tumour grade and differentiation degree were recorded for the tumor and dysplasia degree, diameter and distance from the tumour were recorded for the adenoma (Table 1).

**Table 1.** Clinicopathologic characteristics of the patients included in the study

|                                              |                           |
|----------------------------------------------|---------------------------|
| <b>Clinic characteristics:</b>               |                           |
| Age#                                         | 63.28 ± 9.126             |
| Man, no (%)                                  | 16 (80)                   |
| Women no (%)                                 | 4 (20)                    |
| Smokers, no (%)                              |                           |
| Yes                                          | 3 (15%)                   |
| No                                           | 17 (85%)                  |
| CEA preparatory value                        |                           |
| [0-3.4]                                      | 6 (30%)                   |
| [3.4-10]                                     | 3 (15%)                   |
| > 10                                         | 11 (55%)                  |
| <b>Adenoma histological characteristics:</b> |                           |
| Diagnostic                                   | adenomatous tubulo vilous |
| Dysplasia degree, no (%)                     |                           |
| I                                            | 17 (85)                   |
| I,II                                         | 3 (15)                    |
| Polyp type, no (%)                           |                           |
| Sessile                                      | 5 (25)                    |
| Pedunculatus                                 | 15 (75)                   |
| Polyp diameter (cm)                          | 0.5-2.5                   |
| <b>Tumor histological characteristics:</b>   |                           |
| Diagnostic                                   | adenocarcinoma            |
| Tumor stage (TNM), no (%)                    |                           |
| I                                            | 1 (5)                     |
| II                                           | 6 (30)                    |
| III                                          | 7 (35)                    |
| IV                                           | 6 (30)                    |
| Differentiation degree, no (%)               |                           |
| G1                                           | 12 (60)                   |
| G1-G2                                        | 7 (35)                    |
| G2                                           | 1 (5)                     |
| Tumour size (cm)                             | 3.0-7.0                   |
| Perineural tumoral infiltration, no (%)      |                           |
| Yes                                          | 1(5%)                     |
| No                                           | 19 (95%)                  |
| Vascular tumoral embolisation, no (%)        |                           |
| Yes                                          | 1 (5%)                    |
| No                                           | 19 (95%)                  |
| Tumoral location, no (%)                     |                           |
| Left colon                                   | 12 (60)                   |
| Right colon                                  | 5 (25)                    |
| Transverse colon                             | 3 (15)                    |

# as mean ± SD

## RNA extractions

RNA was extracted from normal, adenoma and tumoral frozen tissues with Triagent, according to manufacturer's protocol (Sigma) and clean up was made on Qiagen columns (Qiagen). Following purification, concentration of RNA samples was asses spectrophotometrically (GeneQuant, Amersham) and the quality was evaluated with Agilent 2100 Bioanalyzer (Agilent Technologies, Ca). Samples with ratio 280/260 nm of 1.8-2 and with RIN greater than 8.0 were used in further studies (Fig. 1).

## Real time PCR

Two-step relative quantification was performed in 7300 Real-time PCR (Applied Biosystems) using TaqMan probe based chemistry. This approach allowed us to determine fold changes within adenoma and tumoral samples compared with paired normal tissue, which was used as calibrator. cDNA was obtained with High Capacity cDNA Archive Kit (Applied Biosystems). PCR amplification was carried out in triplicate for each sample, in a volume of 25 µl at the following conditions: 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 minute and extension at 60°C for 1 minute. The level of each mRNA

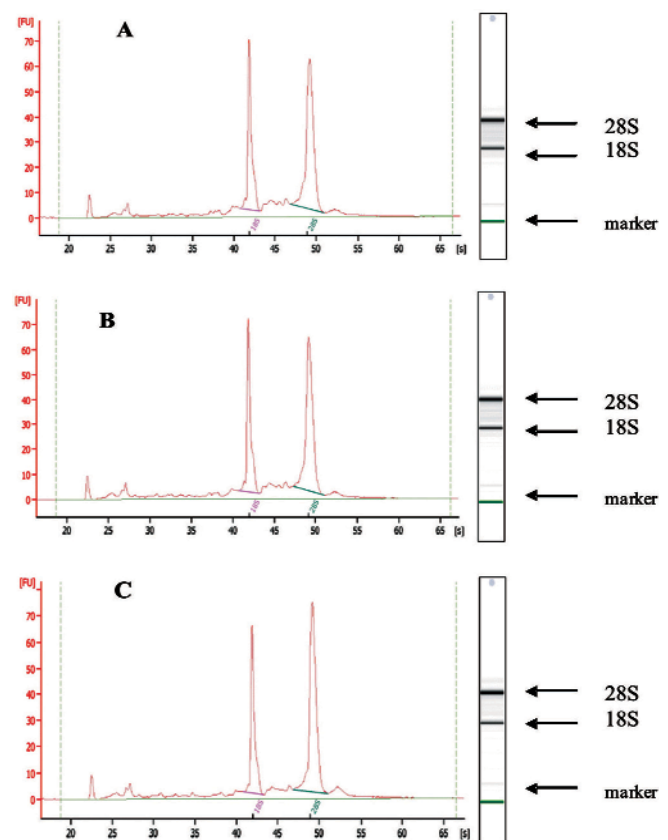


**Figure 1.** RNA quality control on Agilent Bioanalyzer 2100

A. normal tissue, 28S/18S = 1.6, RIN=9.3,

B. adenoma 28S/18S = 1.5, RIN=9.4

C. tumoral tissue, 28S/18S = 1.7, RIN=9

was normalized to 18S. We analyzed data with SDS 1.4 software using comparative CT method [ $2^{-(\Delta\Delta Ct)}$ ]. The candidate genes were: MMP-1, MMP-3, MMP-7, DEFA 1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1.

### Immunohistochemistry

Paraffin embedded tissue samples, from 20 patients (normal, adenoma and tumors) were stained using standard immunohistochemical method. We used the following antibodies: Osteopontin: mouse-anti-human (Abcam), Defensin 1-3: mouse anti-human alpha defensin, Novocastra Lab Vision and goat anti-mouse-FITC conjugated (Santa Cruz).

### Results and discussions

Within our study we investigated, by qPCR, the mRNA level of expression, for a panel of 10 genes involved in colon cancer onset and progression on a cohort of 20 patients with sporadic colon cancer. The clinicopathologic characteristics of the patients in this study are shown in table 1.

Normal, adenoma and tumoral tissues were prelevated from the same patient during surgery and the studied genes were MMP-1, MMP-3, MMP-7, DEFA-1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1.

Using triplets of colon mucosa makes possible to apply a biological filter that enable to avoid the interference of genomic artifact with the analysis, which is often reported because of the use of samples from different patients.

*Defensin 5 and defensin 6 are highly expressed in adenoma compared with tumoral tissue, while defensin 1 is higher expressed both in adenoma and in tumoral tissue*

Our results show that mRNA level of defensin 5 (DEFA-5) and defensin 6 (DEFA-6) are significantly higher expressed in adenoma compared with tumor samples (Fig. 2,  $p$  value  $< 0.05$ ).

*Differential expression of interstitial collagenase (MMP-1), stromelysin 1 (MMP-3) and matrilysin 1 (MMP-7) in colon triplets*

Metallo proteinases are a family of zinc endopeptidases secreted or attached to the membrane with role in extracellular matrix remodeling, wound healing, inflammation, progress of diseases such as atheroma, arthritis, cancer, chronic tissue ulcers.

Related with cancer these enzymes are associated with processes involved in tumor promotion, tumor cell invasion of the basement membrane and stroma, blood vessel penetration, metastasis and metastasis tumor growth. MMP transcripts are generally expressed at low levels, but rise rapidly when tissues undergo remodeling, such as inflammation, wound healing and cancer (16).

MMP-7 (matrilysin 1) is produced abundantly by colorectal cancer cells.

In our study we observed that mRNA levels of MMP-1

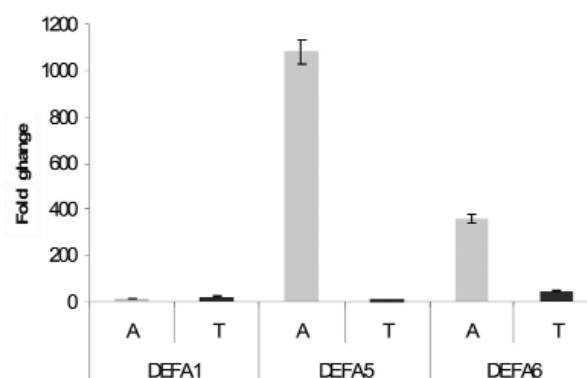


**Figure 2.** Defensin mRNA expression in adenoma and tumoral tissue (compared with paired normal tissue) by real time PCR

and MMP-3 are higher in tumoral tissue than in adenomas (Fig. 3,  $p$  value  $< 0.05$ ). Although it has been reported that there is a significant correlation between the grade of histologic differentiation and MMP1 activity, our results indicate no association between them.

Also, our data show that MMP-7 is expressed in adenoma and in tumoral tissue at almost the same level ( $p$  value  $< 0.01$ ), suggesting its important role both in tumor promotion as well in tumor progression (Fig. 3). MMP-7 level is increased compared with MMP-1 and MMP-3 ( $p$  value  $< 0.05$ ). We observed a direct correlation of MMP-7 level with the presence of hepatic metastasis.

The presence of a mutation in the APC gene results in nuclear accumulation of the beta-Catenin/TCF complex, which serves as a transcriptional factor that upregulates MMP-7 expression. It acts during different stages of malignant transformation, splitting E-cadherin, an intracellular adhesive molecule of epithelia, matrix molecules in the tumor-stroma interface and integrins which link matrix components to epithelial cells. The use of oligonucleotide antisense to the matrilysin-1 drives to a clearer reduction of invading processes,

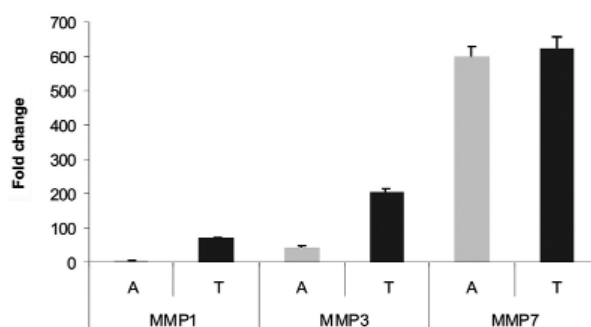


**Figure 3.** Metallo proteinases mRNA expression in adenoma and tumoral tissue (compared with paired normal tissue) by real time PCR

suggesting therefore an important therapeutic potential.

MMP-7, together with MMP-26 (matrilysin 2) is the only one MMP synthesized and exclusively secreted by the tumor cells. (17)

It has been shown to play important roles in the regulation of several biochemical processes such as the activation-degradation of various proteins such as pro-alpha defensin. (17,18)

Also, Kurokawa et al (19) have reported that the expression of MMP-7 by colon and rectal cancer cells predicts metastatic potential of stage I (pT1).

*Osteopontin (SPP-1, OPN) is higher expressed in tumoral tissue than in adenomas*

Osteopontin is a multifunctional, secreted, phosphorylated, acidic glycoprotein; it is expressed in many cell types including epithelial cells. Osteopontin exists as an immobilized form in EMC in mineralized tissues and as a soluble form, a cytokine in body fluids.

Osteopontin is implicated in bone development, immune response modulation and signal transduction pathways; osteopontin molecule contains an integrin-binding RGD sequence and others sequences interacting with CD44v6/7.

Osteopontin was reported as linked with tumorigenesis, invasion and metastasis (20,21,22).

The osteopontin gene is considered a transcriptional target of aberrant Wnt signaling and its expression could predict survival in human colon cancer (23).

Osteopontin is often co-localized with members of matrix metalloproteinase (MMP) family. There was reported that OPN is a novel substrate for two MMPs, MMP-3 (stromelysin1) and MMP-7 (matrilysin) (24). The authors predicted that interaction of MMPs with OPN at tumor and wound healing sites in vivo may be a mechanism of regulation of OPN bioactivity. (24)

In our study the level of expression for SPP-1 (OPN) was shown to be higher in tumoral tissue compared with adenoma (Fig. 4, p value < 0.01). There is also a direct correlation between tumor level and mRNA levels. These results lead to the conclusion that SPP1 can be considered a marker for colon cancer progression.

*Interleukin 8 (IL-8) higher expressed in adenocarcinomas*

Interleukin-8 (IL-8) is a multifunctional cytokine, member of CXC chemokine superfamily. The receptors for IL-8, CXCR1 and CXCR2 are present in many normal and tumoral cells (25). Primary function of IL-8 is the induction of chemotaxis in its cells. By autocrine and paracrine mechanisms IL-8 have an important role in angiogenesis, tumor growth, tumor progression and metastasis (26). It was reported that colonic cells express many chemokines including IL-8. It was also demonstrated that IL-8 induces tumor cells migration and its expression was found correlated with tumour growth and progression, angiogenesis and metastasis. (27,28,29,30,31,32,33,34).

Our data show that IL8 has a high mRNA level in adenomas but higher in adenocarcinomas (Fig. 4, p value < 0.05). Also we observed that there is no connection between

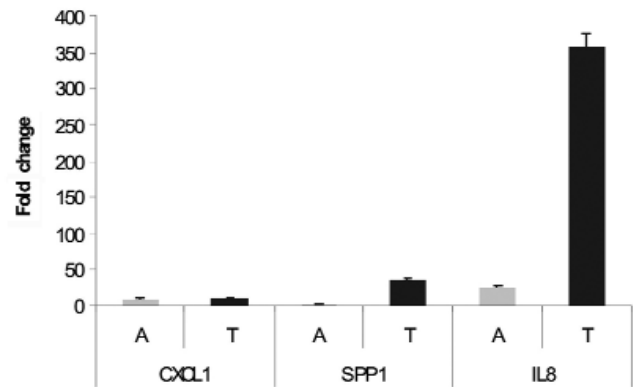


**Figure 4.** Cytokine mRNA expression in adenoma and tumoral tissue (compared with paired normal tissue) by real time PCR

tumor stage and IL-8 expression.

These results imply a direct correlation between IL-8 levels onset and progression of colon cancer. The connection between inflammation and tumorigenesis is well-established and in the last decade has received a great deal of supporting evidence from genetic, pharmacological, and epidemiological data; inflammatory bowel disease being an important risk factor for the development of colon cancer (35).

#### *Chemokine (CXC motif) Ligand 1 CXCL1*

Chemokine (CXC motif) Ligand 1 (CXCL1) or GRO1 or GROalpha (Growth - regulated oncogene) is a cytokine belonging to the CXC chemokine family which was originally detected in human melanoma cells. This ligand binds to CXCR2 (CXC chemokine receptor 2) and through this elicit its neutrophil chemoattractant activity.

Some studies suggested that CXCR2 could promote pre-neoplastic to neoplastic cellular transformation by persistent stimulation with specific chemokine ligands (36) and the involvement of chemokines receptors in metastasis (37). It was determined that for the CXCL1-induced chemotaxis is required the cdc42-PAK1 cascade and that the CXCL1 induction of MEK-ERK1/2 it is not involved. (38)

It was also reported that CXCL1 is involved in tumorigenesis, in angiogenesis (39) and metastasis (40).

Our results show that CXCL1 is expressed at almost the same level in adenoma and in adenocarcinoma (Fig. 4, p value < 0.01).

#### *Collagen Triple Helix Repeat Containing 1 (CTHRC-1) upregulated in tumor samples*

CTHRC-1 (Collagen Triple Helix Repeat Containing 1) is a glycosylated, secreted protein highly conserved in evolution. It was reported as an inhibitor of TNF beta signaling and it is induced transiently, after artery injury, in smooth muscle cells and adventitial fibroblasts in processes of neointima developing; it is not expressed in normal arteries. (41).

Human tumor analysis demonstrated that aberrant

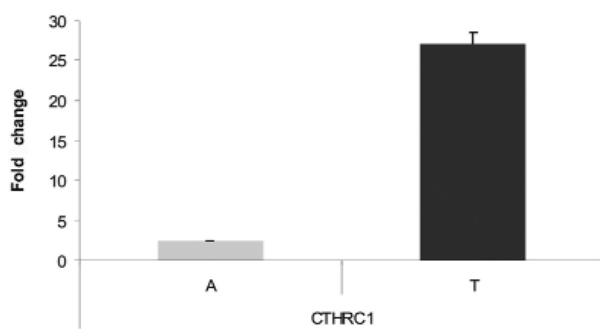


**Figure 5.** *CTHRC1* mRNA expression in adenoma and tumoral tissue (compared with paired normal tissue) by real time PCR

expression of CTHRC-1 is widely present in human solid tumors (42).

CTHRC-1 is considered a co-receptor in the Wnt-PCP pathway: it is a key cofactor for formation of Wnt/Fzd/Ror2 and for activation of the PCP. (43). The Wnt signaling is an important evolutionarily conserved pathway involved in development processes; recent researches suggest the link of Wnt with the cancer development, progression, invasion and metastasis. (44)

CTHRC1 was shown to be upregulated in tumor samples, when compared with normal and with adenoma (Fig. 5,  $p$  value  $< 0.05$ )

Our data are consistent with literature data that pointed out that CTHRC-1 is associated with cancer tissue invasion and metastasis and potentially plays important functional roles in cancer progression, perhaps by increasing cancer cell migration (45).

Taken in consideration our conclusions additional studies to investigate protein expression of these candidate genes should be undertaken.

In that direction, in a first step we determined the clinical utility of the SPP-1 and DEFA 1-3 as potential tumour markers for colon cancer by immunohistochemistry (Fig. 6A-H).

Initially discovered as an inducible, tumor promoter gene, SPP-1 is often overexpressed in human tumor; SPP-1 has been implicated in tumor cell migration and metastasis. Previous studies have shown that SPP-1 is overexpressed in colorectal cancers and could be correlated with tumor progression. SPP-1 is the major phosphoprotein secreted by malignant cells and its serum concentration should be an informative marker for detection of cancer.

Human defensins have been described as cytotoxic peptides, with antibiotic properties, expressed in neutrophils, in epithelial cells of the lung, skin and urogenital tract, in PMNs and intestinal Paneth cells. In addition to their role in host defense against microbial infections, there are studies that report the presence of defensins in tumors.

The expression of SPP-1 and DEFA 1-3 was detected in normal, adenoma and tumoral tissue by immunofluorescence technique.

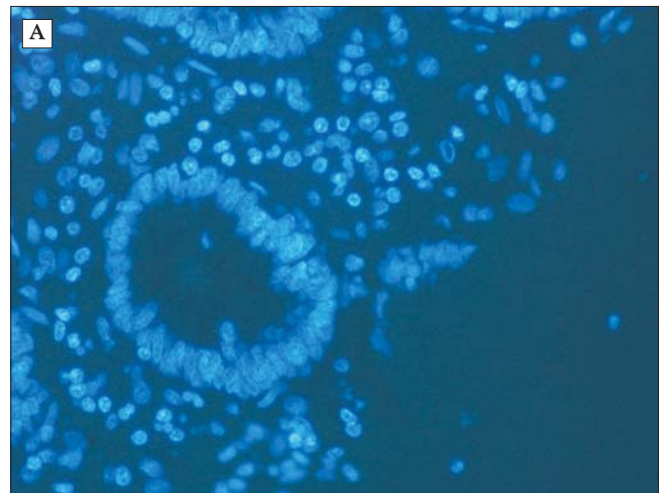


**Figure 6A.** Immunohistochemistry, colon, normal, staining of the nuclei (Hoechst)

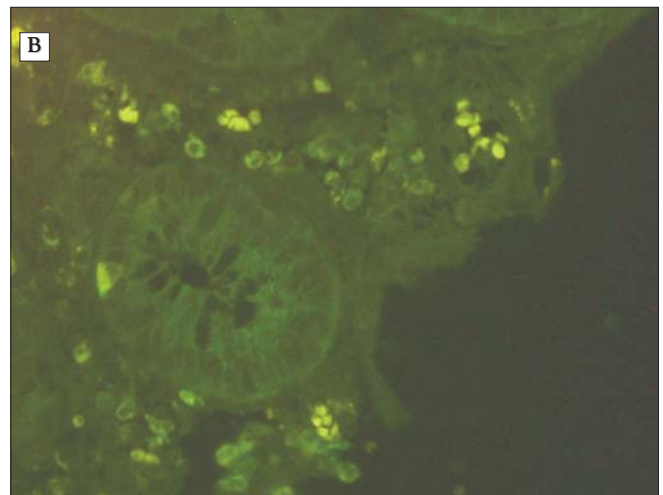


**Figure 6B.** Immunohistochemistry, SPP1, colon, normal (FITC)

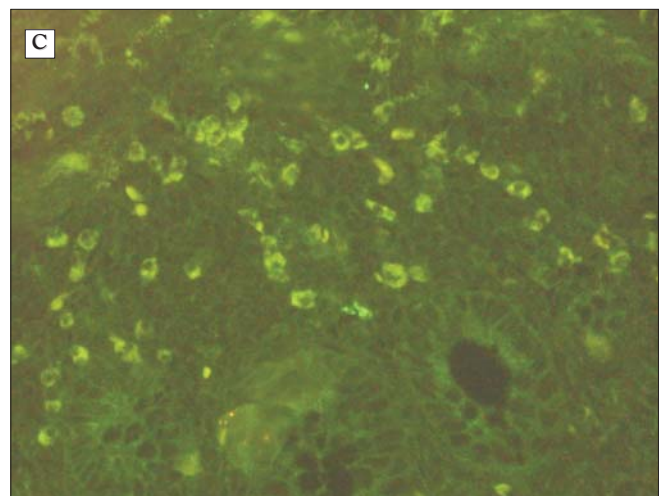


**Figure 6C.** Immunohistochemistry, SPP1, colon, adenoma (FITC)

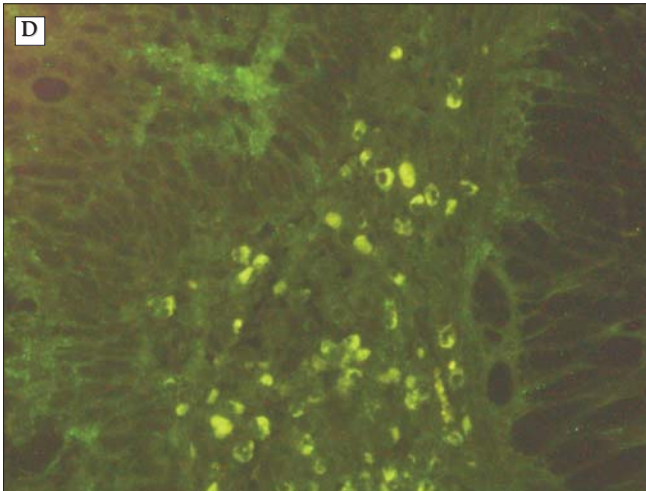


**Figure 6D.** Immunohistochemistry, SPP1, colon, tumor (FITC)

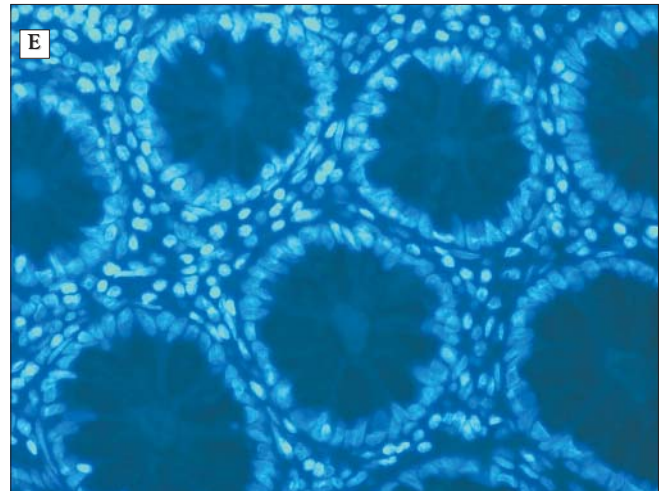


**Figure 6E.** Immunohistochemistry, colon, normal, staining of the nuclei (Hoechst)

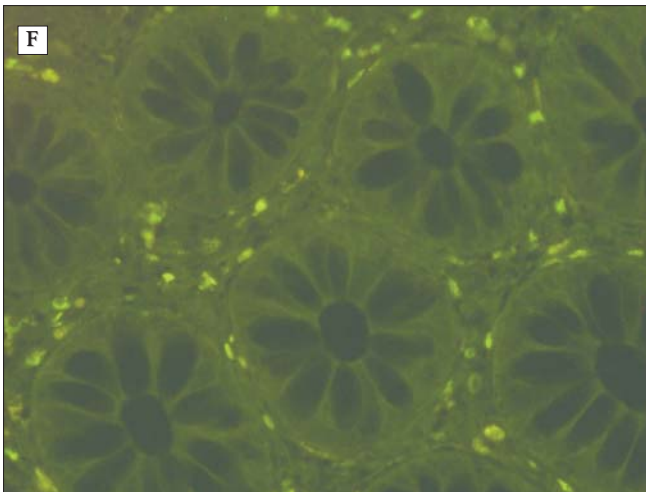


**Figure 6F.** Immunohistochemistry, DEFA 1-3, colon, normal (FITC)

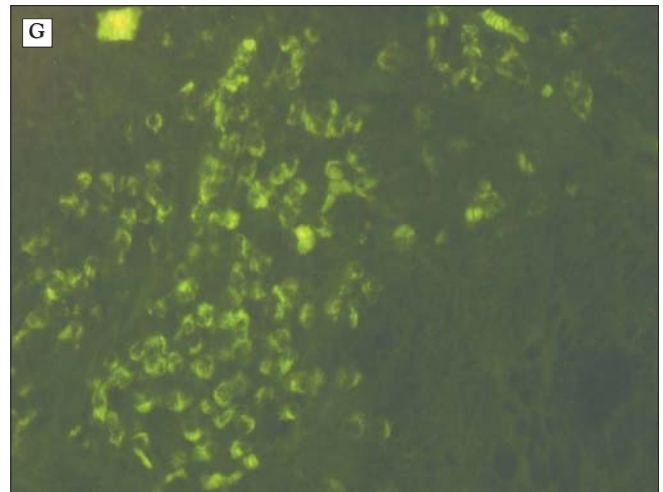


**Figure 6G.** Immunohistochemistry, DEFA 1-3, colon, adenoma (FITC)

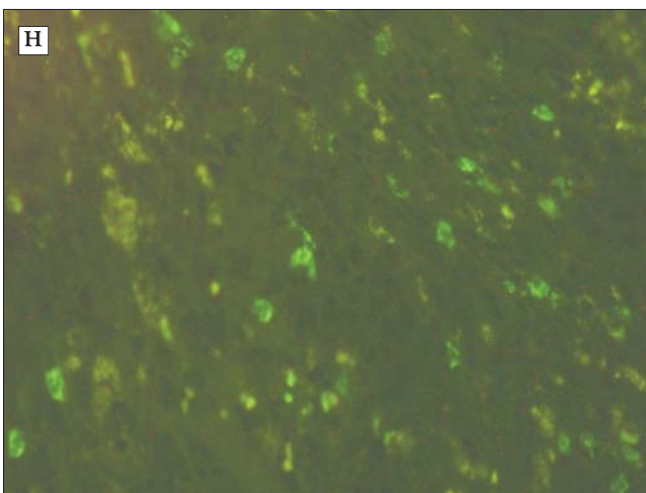


**Figure 6H.** Immunohistochemistry, DEFA 1-3, colon, tumor (FITC)

Immunohistochemistry showed the cytoplasmic localization of SPP-1 and granular cytoplasmic localization of DEFA 1-3. The samples analysis revealed that SPP-1 and DEFA 1-3 expression was higher in all tumors and in some adenomas than in normal tissues.

## Conclusions

Considering that the goal of oncology in the third millennium is to move from a generic way to diagnose and to treat patients to a personalized approach, the existence of accurate biomarkers for early diagnosis and evaluation of disease progression, are imperative.

The direct beneficiary will be the patients because, with the right tools at clinician's hand, they will receive a more precise diagnosis and the right therapy.

The aim of our study was to assess the differences in mRNA levels in triplets of colon mucosa of the same patient:

normal, adenoma and tumoral tissue, by quantitative real time PCR for a list of candidate genes involved in colon cancer onset and progression. Additionally we performed immunohistochemistry for DEFA1-3 and SPP-1.

Our preliminary results show that the panel of genes that were analyzed plays important roles in different steps of colon carcinogenesis. Thus, defensins (DEFA-5 and DEFA-6) can be considered key elements in adenoma formation; osteopontin (SPP-1) can be a marker of progression, IL-8 and CTHRC-1 may be markers of tumor invasion, while MMP-7 is a marker of colon cancer onset and progression to metastasis.

High levels of DEFA-5, DEFA-6, MMP-7, SPP-1 and IL-8 could be used to diagnose an early stage colon cancer, MMP-7 being a prognostic indicator for tumor transformation and for the metastatic potential cancer cells. Further, if DEFA-5 and DEFA-6 level are low but MMP-7, SPP-1 and IL-8 level are high we could point out that the transition from adenoma to adenocarcinoma had already occurred.

Thus, DEFA-5, DEFA-6, MMP-7, IL-8 and SPP-1 consist in a valuable panel of biomarkers, whose detection can be used in early detection and progressive disease and also in prognostic of colon cancer.

Our results show that molecular characterization of tumors, even for each patient, is imperative needed in elaborating personalized protocols for diagnostic and for a better use of already known therapeutics.

Also, the use of this panel of biomarkers, together with the ones already known, may provide a rapid, noninvasive procedure for early diagnosis, risk assessment, establishing prognosis, detecting relapse and choosing the right treatment in accordance with each patients' tumor genomic profile.

## Acknowledgements

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