

# Gene Ontology Analysis of Colorectal Cancer Biomarkers Probed with Affymetrix and Illumina Microarrays

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**Abstract:** Colorectal cancer is the fourth most common cause of death worldwide. Recently, many microarray experiments have been done to investigate the expression of the genes in the colorectal tissues and thus, to find the answers for its occurrence. Previously, we used experiments obtained from both Illumina and Affymetrix microarray platforms to analyze the gene expression in healthy and carcinogenic tissues. As a result we got specific sets of biomarkers that we used to build an accurate Bayesian diagnostic system. The high degree of classifier's sensitivity and specificity intrigued us to proceed with the research of the significant genes we discovered, the biomarkers. Therefore, in this paper we aim towards biomarkers identification and the functional groups they are associated with, i.e., we performed gene ontology analysis. Investigating the genes that control the colorectal carcinogenic tissue development is of central importance to the verification of the biomarkers' revealing method's validity. Moreover, we showed the importance of their participation in the prior distributions modeling, which is the key part for achieving an accurate Bayesian classification, regardless their strict disease and disorder association.

## 1 INTRODUCTION

The cancer incidence, mortality and prevalence were a target of the research which the World Health Organization (WHO) provided in 2008. The results of the GLOBOCAN project showed that the colorectal cancer is the third most common cancer in men, and the second in women with a total incidence of 1,234,000 cases, of which 60% occur in developed regions. The mortality results of 8% of total cancer deaths make this type of cancer to be the fourth most common cause of death from cancer (GLOBOCAN, 2008).

Recently, the scientists provide intensive gene expression profiling experiments in order to compare the malignant to the healthy cells in a particular tissue. The advantage of the microarray technologies enables simultaneous observation of thousands of genes and allows the researchers to derive conclusions whether the disorder is a result of the abnormal expression of a subset of genes.

In our previous researches we assumed that the colorectal cancer occurs as a result of increased and decreases expression levels of a set of significant genes, which we refer to as biomarker genes. Therefore, in (Simjanoska et al., 2013b) and (Simjanoska

et al., 2013a) we used experiments of colorectal carcinogenic and healthy adjacent tissues probed with two widely used microarray technologies, Illumina and Affymetrix. For each platform we developed original methodologies for unveiling the genes that show significant changes in their expression levels in presence of colorectal cancer, both adenomas and adenocarcinomas, and we used the genes' expressions to model binary diagnostic system based on the Bayes' theorem. The outcomes of the classification showed a high precision when diagnosing both carcinogenic and healthy tissues.

The ability of the selected biomarkers to discriminate between colorectal cancer and normal health condition intrigued us to go deeper in the problem and to investigate the biomarkers functions on molecular level, i.e., to perform gene ontology analysis. Analysing the molecular function and the biological processes of the biomarkers will provide answers whether all the significant genes are tightly related to the colorectal cancer phenomena, and whether all of them are necessary for the developed classifier to produce accurate decisions. Conducting this kind of research is of great importance to the Bayesian machine learning classification approach, which we confirmed

to be very accurate, and also very important to verify if the methods used for biomarkers selection are reliable. Furthermore, the results obtained from a research like this can advance the progress of the future personalized cancer treatment (Jain, 2004).

The rest of the paper is organized as follows. In Section 2 we briefly present the work related to our field of interest. The methodology for biomarkers revealing, tissues classification and ontology analysis is explained in Section 3. In Section 4 we present the experiments and the results from the ontology analysis and the additional classification experiments. In the final Section 5 we derive conclusions from the results and we present our plans for future work.

## 2 RELATED WORK

In this section we give a brief review of the recent scientific work that relates to ontology analysis and its importance to various health disorders and diseases. Furthermore, we present some of the most important researches related to the colorectal cancer. Eventually, we exhibit the literature related to the microarray experiments we used in our paper.

In (Ahn et al., 2003) the authors present their research where the gene ontology analysis was used to systematically characterize the global expression profiles at cellular process levels. They showed that potentially significant pathogenetic cellular processes can be identified and showed that the functional profiling has a significant impact on the discovery of pathogenetic pathway in leiomyoma.

Another research is presented in (Holmans et al., 2009) where gene ontology analysis has been used to provide insights into the biology of bipolar disorder.

Avoiding single marker analysis, the authors in (Jia et al., 2010) also incorporated Gene Set Enrichment Analysis (GSEA) and hypergeometric test, and combined them using Fisher's combined method to perform pathway-based analysis in order to detect genes' combined effects on mediating schizophrenia. Interestingly, they found a few pathways to be top ranked and likely associated with schizophrenia, however non of the genes involved in these pathways had been detected by single marker analysis, concluding that this approach may complement the original analysis of genome-wide association studies (GWAS) dataset.

The approach of gene set enrichment analysis for interpreting gene expression data was also discussed in (Subramanian et al., 2005) where the researchers demonstrate how it yields insights into several cancer-related data sets, including leukemia and lung cancer.

They also state that single-gene analysis may miss important effects on pathways. Sometimes an increase of 20% in all genes encoding members of a metabolic pathway may be more important than a 20-fold increase in a single gene. Also another statement that is important to our research is that often the different studies of the same biological system present a list of statistically significant genes that show distressingly little overlap.

Considering the colorectal cancer, we cannot dismiss the Vogelstein's genetic model for colorectal carcinoma which has been proposed as a result of a long term research. In (Kinzler and Vogelstein, 1996) they present the genetic changes associated with colorectal tumorigenesis and distinguish several genes that showed high involvement in colorectal neoplasia. In this paper we will rely on this model, which is a milestone in cancer research (Nature, 2006), to compare our results and to verify our methods for biomarkers selection. However, blindly relying only on Vogelstein's model and not assuming any exceptions is completely wrong. This is confirmed by the research in (Smith et al., 2002) where the authors investigate the mutations in the specific genes introduced by Vogelstein, including adenomatous polyposis coli (APC), Kirsten-ras (K-ras), and p53. According to the results from their research, they come up with the conclusion that multiple alternative genetic pathways to colorectal cancer exist and that the widely accepted genetic model of cancer development is not representative of the majority of colorectal tumors.

Regarding ontology and classification analysis related to colorectal cancer, authors in (Lascorz et al., 2011a) sum up the biomarkers results from 23 different researches. Even though most of them show diversity in the significant genes revealed, the authors in their research take into account the unique biomarkers, which are nearly 1000, and perform ontology analysis using various tools. They mainly hold on to the ontology results analysis of the enriched set of genes, rather than verifying the biomarkers with classification methods so that we can compare our results. Similarly, in (Xu et al., 2013) the researchers use Affymetrix microarray data from 20 patients and a different procedure from the one we presented in 3.1 to reveal significant gene expression, which resulted in 1469 biomarkers. From the ontology analysis they ranked top 10 most important pathways. Comparing our results to theirs, we realized that there is no overlap between ours and their biomarkers sets. Even though they lack a classification analysis, we may include their biomarkers in our future work and test the ability of the Bayesian approach to make an appropriate modelling using different biomarkers reveal-

ing procedure. Since the non overlapping between the biomarkers sets discovered in different scientific papers is very common, a new meta-analysis model of colorectal cancer gene expression profiling studies is proposed in (Chan et al., 2008). As the authors ranked the biomarker genes according to various parameters, the gene CDH3 which we found to play role in the colorectal cancer, is also found by their meta-analysis model. Another interesting approach maintained with classification analysis is presented in (Jiang et al., 2008), where the authors constructed disease-specific gene networks and used them to identify significantly expressed genes. A particular attention is given to five biomarkers, from which one of them, IL8, was also detected by our methodology, but it was not considered important in our research since no specific connection to the colorectal cancer was found in the literature. In order to test the power of the colon cancer-specific gene network biomarkers revealing ability, they use five different classifiers: diagonal linear discriminate analysis (DLDA), 3 nearest neighbours (3NN), nearest centroid (NC), support vector machine (SVM) and Bayesian compound covariate (BCC).

Considering the fact that in this paper we use microarray experiments from Affymetrix and Illumina platforms performed for different purposes, we provide an overview of the work related to these sets of data.

The experiments obtained from the Affymetrix platform were used in several researches. In (Hong et al., 2010) the authors aimed to find a metastasis-prone signature for early stage mismatch-repair proficient sporadic colorectal cancer (CRC) patients for better prognosis and informed use of adjuvant chemotherapy. A transcriptome profile of human colorectal adenomas is given in (Sabates-Bellver et al., 2007) where they characterize the molecular processes underlying the transformation of normal colonic epithelium. One of the data sets has been used in (Watanabe et al., 2006) to clarify the difference between microsatellite instability (MSI) and microsatellite stability (MSS) cancers and, furthermore, to determine distinct characteristics of proximal and distal MSI cancers. A similar research is presented in (Jorissen et al., 2008) where the scientists showed cross-study consistency of MSI-associated gene expression changes in colorectal cancers. The microarray data obtained from the Illumina chip was used in (Hinoue et al., 2012) where the authors performed comprehensive genome-scale DNA methylation profiling of normal and carcinogenic tissues and identified four DNA methylation-based subgroups of CRC using model-based cluster analyses.

### 3 METHODS AND METHODOLOGY

In this section we describe the procedure used for significant genes detection from both widely used types of DNA microarrays, Affymetrix and Illumina. Furthermore, we exhibit the methodology used for building the Bayesian classifier, and finally, we present the gene ontology method that we use in this paper to reveal overrepresented functional groups of genes.

#### 3.1 Biomarkers Detection Methodology

The process for revealing the biomarkers consists of the following steps (Simjanoska et al., 2013b), (Simjanoska et al., 2013a):

- *Quantile normalization.* Since our aim is to unveil the difference in gene expression levels between the carcinogenic and healthy tissues, we proposed the Quantile normalization (QN) as a suitable normalization method (Wu and Aryee, 2010).
- *Low entropy filter.* We used low entropy filter to remove the genes with almost ordered expression levels (Needham et al., 2009), since they lead to wrong conclusions about the genes behaviour.
- *Paired-sample t-test.* Knowing the facts that both carcinogenic and healthy tissues are taken from the same patients, and that the whole-genome gene expression follows normal distribution (Hui et al., 2010), we used a paired-sample t-test.
- *FDR method.* False Discovery Rate (FDR) is a reduction method that usually follows the t-test. FDR solves the problem of false positives, i.e., the genes which are considered statistically significant when in reality there is not any difference in their expression levels.
- *Volcano plot.* Both the t-test and the FDR method identify different expressions in accordance with statistical significance values, and do not consider biological significance. In order to display both statistically and biologically significant genes we used volcano plot visual tool.

#### 3.2 Bayesian Classification

As we discovered the two sets of biomarkers from both microarray chips, we used them in our previous work (Simjanoska et al., 2013b) and (Simjanoska et al., 2013a) to propose a generative approach for building a Bayesian classifier that models the prior distributions at carcinogenic and healthy tissues. Once we modelled the prior distributions for

both classes, carcinogenic and healthy, we were able to use them in the Bayes' theorem and to calculate the a posteriori probability for a given tissue to belong to one of the two classes,  $C_i$ .

Therefore, we calculate the a posteriori probability  $P(C_i|\vec{x})$ , as:

$$p(C_i|\vec{x}) = \frac{p(\vec{x}|C_i) * P(C_i)}{\sum_{i=1}^2 p(\vec{x}|C_i) * P(C_i)} \quad (1)$$

The class-conditional densities, or, the prior distributions,  $p(\vec{x}|C_i)$ , are calculated as the product of the continuous probability distributions of each gene distinctively:

$$p(\vec{x}|C_i) = \prod f_1 f_2 \dots f_n \quad (2)$$

For the prior probabilities  $P(C_i)$ , we defined two test cases:

- Test Case 1: Since we have equal number of tissues into both of the classes, the prior probabilities are also equal  $P(C_1) = P(C_2) = 0.5$ ;
- Test Case 2: The prior probabilities are estimated according to the statistics in (GLOBOCAN, 2008). Therefore,  $P(C_1) = 0.0002$  and  $P(C_2) = 0.9998$ , where  $C_1$  denotes the carcinogenic class, and  $C_2$  denotes the healthy class.

The tissue  $\vec{x}$ , which is an input to the Bayesian classifier, is classified according to the rule of maximizing the a posteriori probability (MAP):

$$C_i = \max p(C_i|\vec{x}) \quad (3)$$

### 3.3 Gene Ontology

The analyses of single markers have been in the focus of the genome-wide association studies. However, it often lacks the power to uncover the relatively small effect sizes conferred by most genetic variants. Therefore, using prior biological knowledge on gene function, pathway-based approaches have been developed with the aim to examine whether a group of related genes in the same functional pathway are jointly associated with a trait of interest (Wang et al., 2010).

The goal of the Gene Ontology Consortium is to produce a dynamic, controlled vocabulary that can be applied to all eukaryotes even as knowledge of gene and protein roles in cells is accumulating and changing (Ashburner et al., 2000). The Gene Ontology (GO) project since 1998 is a collaborative effort to provide consistent descriptors for gene products in different databases and to standardize classifications for sequences and sequence features. The GO project provides ontologies to describe attributes

of gene products in three non-overlapping domains of molecular biology (Harris et al., 2004):

- Molecular Function describes activities, such as catalytic or binding activities, at the molecular level. GO molecular function terms represent activities rather than the entities that perform the actions, and do not specify where, when or in what context the action takes place.
- Biological Process describes biological goals accomplished by one or more ordered assemblies of molecular functions.
- Cellular Component describes locations, at the levels of subcellular structures and macromolecular complexes.

There are many tools based on Gene Ontology resource, however, many of them require local installation and specific platform. Therefore, in this research we use the freely accessible Gene Ontology Enrichment Analysis Software Toolkit, GOEAST. It is a web based tool which applies appropriate statistical methods to identify significantly enriched GO terms among a given list of genes. Beside the other functions, GOEAST supports analysis of probe set IDs from Affymetrix and Illumina microarrays. It provides graphical outputs of enriched GO terms to demonstrate their relationships in the three ontology categories. In order to compare GO enrichment status of multiple experiments, GOEAST supports cross comparisons to identify the correlations and differences among them (Zheng and Wang, 2008).

## 4 EXPERIMENTS AND RESULTS

In this section we present the experiments and the results obtained from the previously defined methodologies.

### 4.1 Microarray Data Analysis

In order to extract significant genes that characterize the colorectal cancer, we used two sets of microarray data. The first was gene expression profiling of 32 colorectal tumors, adenomas, and matched adjacent 32 non-tumor colorectal tissues probed with Affymetrix Human Genome U133 Plus 2.0 Array. It contains 54,675 probes, but the unique genes observed are 21,050. The second is gene expression analysis of 26 colorectal tumors, adenocarcinomas, and matched adjacent non-tumor colorectal tissues, probed with Illumina Human Ref-8 v3.0 whole-genome expression BeadChip. It allows 24,526 transcript probes, but unique genes are 17,853.

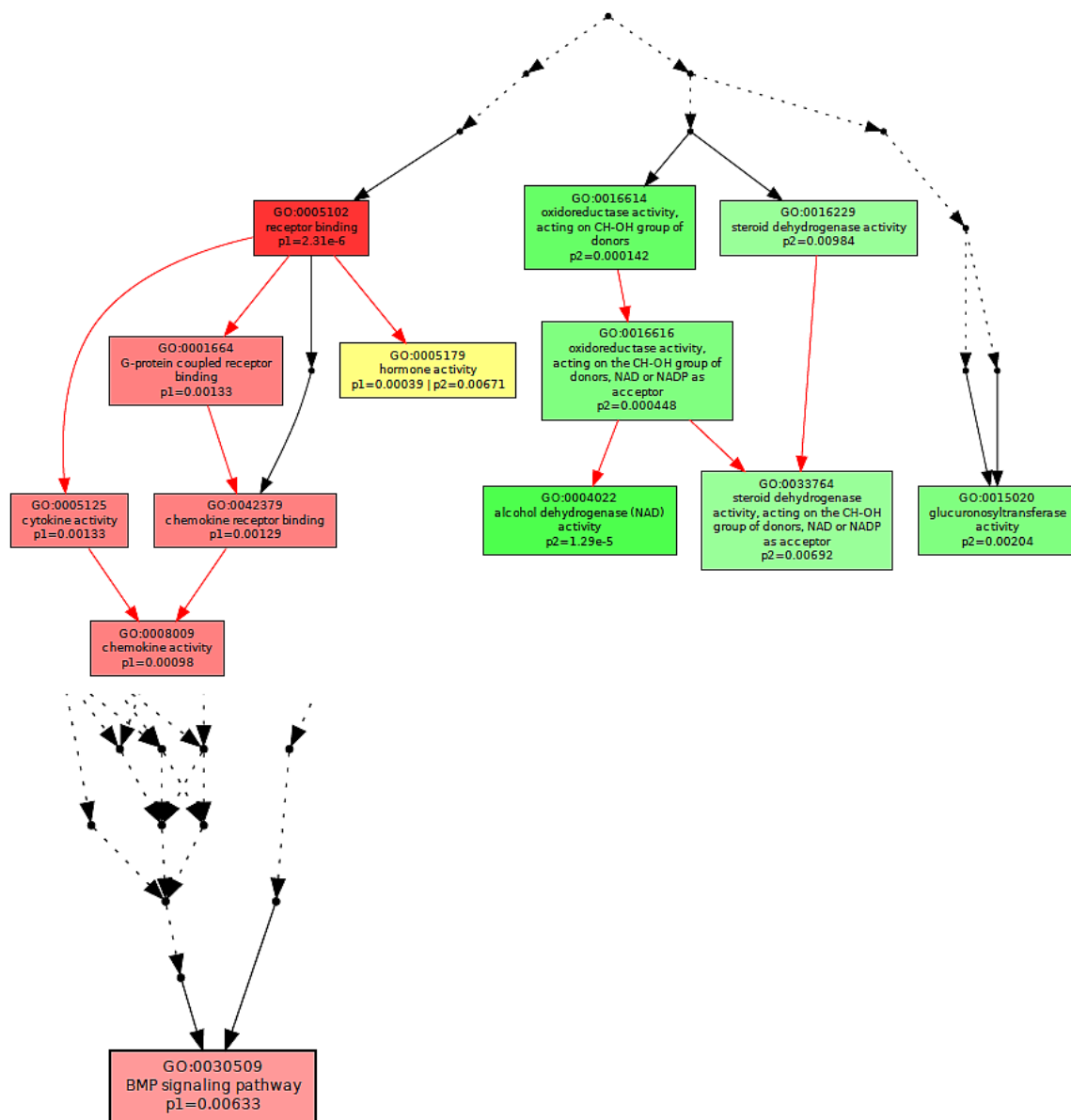


Figure 1: Molecular function.

Both sets of data were preprocessed according to the methodology described in Section 3.1. The results showed 138 significant probes from the tissues probed with Affymetrix microarray, and 213 significant probes from the tissues probed with Illumina microarray.

The significant probes were used in the Bayes' theorem as discussed in Section 3.2. Table 1 presents the results from the tissues classification. Sensitivity refers to the classifier's ability to correctly classify carcinogenic tissues, whereas specificity refers to the classifier's ability to correctly classify healthy sam-

ples. Additionally, we used 239 patients already diagnosed with colorectal cancer, and 12 healthy patients, in order to present the classifier's reliability. We need the results from the Table 1 for comparing the outcomes from the experiments that follow.

## 4.2 Gene Ontology Analysis

Once we revealed biomarker genes that showed excellent ability in distinguishing between the colorectal cancer and the healthy samples, we continued our research in analysing biomarkers functions on molec-

Table 1: Classifier's sensitivity and specificity.

Chip	Performance	Sensitivity	Specificity	Test Cases
Affymetrix	Tissues	1	0.84	Test case 1
		0.94	1	Test case 2
	Patients	0.98	0.92	Test case 1
		0.90	1	Test case 2
Illumina	Tissues	0.96	0.92	Test case 1
		0.81	1	Test case 2

ular level. For this purpose, we used the online available tool GOEAST, previously discussed in Section 3.3. For obtaining reliable results, we chose the Fisher's exact test and a p-value of 0.01. In order to compare both enrichment results, we used the Multi-GOEAST tool and produced the ontologies depicted in figures 1, 2 and 3.

The different colour saturation degrees in the graphs present the enrichment significance of each GO term, defined by the p-value. In the graphical output of Multi-GOEAST results, each set is represented with different colour. Therefore, red and green boxes represent enriched GO terms only found in Affymetrix and Illumina biomarkers, whereas yellow boxes represent commonly enriched GO terms in both experiments.

Figure 1 depicts the molecular function of the differentially expressed genes. The results show that 'hormone activity' is a common molecular function for a subset of the biomarker genes from both microarray platforms. Inspecting the biological processes described in Figure 2, we conclude that there are no processes in common, whereas considering the cellular component analysis in Figure 3 we perceive that some genes from both platforms are found in the extracellular region.

As we examined the genes from the ontology analysis, we derived conclusion that there is a small overlap between the enriched sets from the two platforms. However, as explained in Section 2 this is not an unexpected phenomena.

In order to compare the results, we analyzed another study where the researchers also examined pathways in colorectal cancer development (Lascorz et al., 2011b). They used 242 genes and total of nine tools to detect enrichment of Gene Ontology (GO) categories or Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Among identified the consistently enriched gene categories, we realized that our experiment and their research have the following enriched molecular functions and cellular components in common: *receptor binding, cytokine activity, chemokine activity, hormone activity, oxidoreductase activity, acting on CH-OH group of donors, and oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor.*

Assuming the enriched entities we found are statistically overrepresented among all biomarkers, we performed classification experiments to realize if the Bayesian classifier we developed is able to discriminate between the carcinogenic and the healthy tissues using only the overrepresented genes as a training set. However, the results presented in Table 2 show that the Bayesian classifier's ability to recognize colorectal cancer has decreased.

Table 2: Classifier's sensitivity and specificity for the enriched sets of genes.

Chip	Performance	Sensitivity	Specificity	Test Cases
Affymetrix	Tissues	0.91	0.69	Test case 1
		0.47	1	Test case 2
	Patients	0.96	1	Test case 1
		0.78	1	Test case 2
Illumina	Tissues	0.96	0.88	Test case 1
		0.58	1	Test case 2

Considering the results in tables 1 and 2, we confirmed that the statistical pattern recognition process, i.e. the Bayesian model requires larger amount of data, therefore, it works well only if both enriched and residual genes are taken into account.

Furthermore, the next step is to verify the reliability of the procedure for distinguishing the biomarkers considering their relation to the colorectal cancer.

Using publicly available microarray data profiled on Affymetrix U133A chips, the authors in (Benita et al., 2010) examined gene enrichment profiles from a tissue perspective rather than gene perspective, thereby identifying highly enriched genes within a cell type, which are often key to cellular differentiation and function. To identify genes that are tissue specific, the authors used an enrichment score to benchmark expression levels in one tissue compared to all other tissues. When applying their online available tool Gene Enrichment Profiler on the genes that the ontology tool found to be overrepresented, we noticed that at Affymetrix platform a group of genes are enriched in the Central Nervous System's tissues, whereas the others are enriched in various other tissues. For the overrepresented genes among the Illumina biomarkers, we noticed that the enrichment is also not concentrated in any particular tissue.

This intrigued as to go deeper and investigate every gene involved in the molecular functions and the biological processes from the ontology analysis. Few of them are confirmed to play role in colorectal cancer beside the other related diseases and functions:

- *VIP* - Vasoactive intestinal peptide (VIP) is found in hormone activity and receptor binding molecular functions. Its expression is down regulated at both chips, Affymetrix and Illumina. In (Zhang

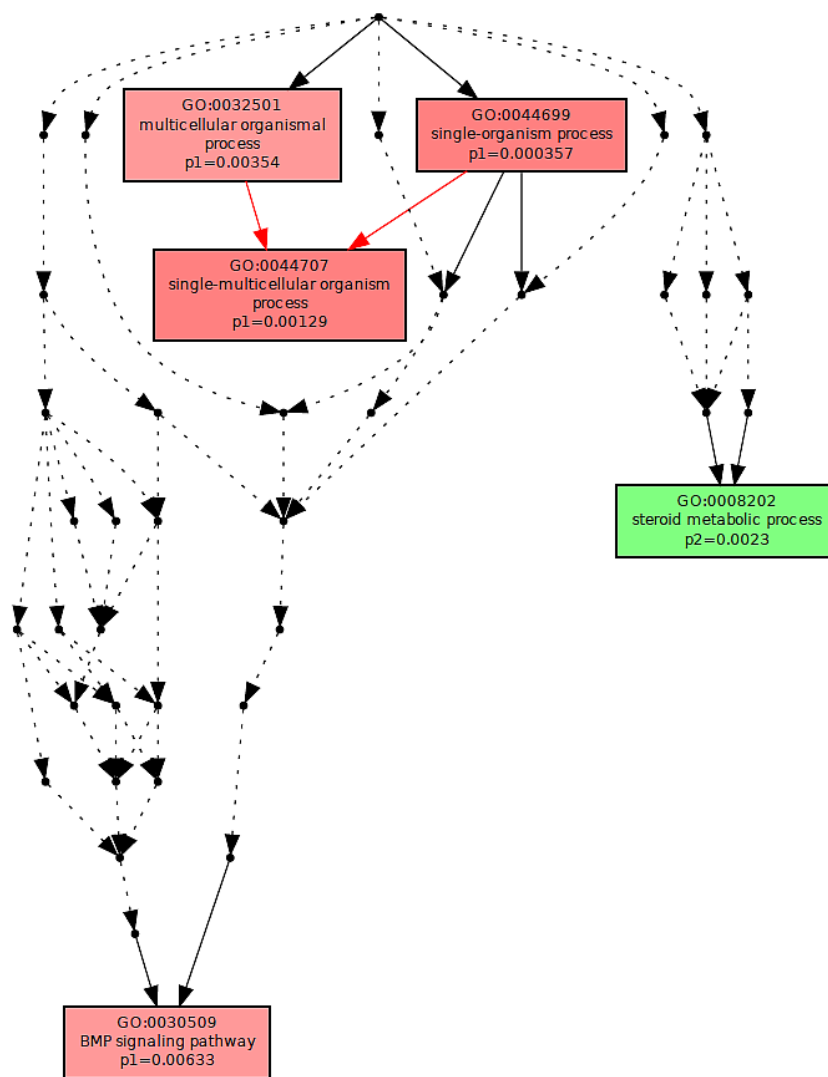


Figure 2: Biological processes.

et al., 1997), the researchers evaluate the expression of VIP receptor in colonic carcinoma and investigate the its role in colon cancer growth.

- *SCG2* - This gene is related to cytokine activity and receptor binding molecular functions. It is found to be significant from the Affymetrix platform and showed decreased expression. The protein encoded by this gene is a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins. Chromogranin genes have been explored in (Pagani et al., 1995) and (Ferro et al., 1995).
- *CHGA* - The protein encoded by this gene is also a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins and is

also down expressed at the Affymetrix chip.

- *GUCA2B* - The expression of this gene is down regulated at Affymetrix chip. It encodes a member of the guanylin family, and is expressed in the stomach and intestine. It may be involved in salt and water secretion into the intestinal lumen as well as the renal tubules, and thus regulate electrolyte homeostasis in these tissues. Its role in the colorectal cancer is discussed in (Li et al., 2009) where the colorectal cancer is observed as a disease of hormone insufficiency. Guanylin correlation is examined in (Camici, 2008) and also new diagnostic and therapeutic approaches for colorectal cancer are discussed.
- *MMP7* - Proteins of the matrix metalloproteinase

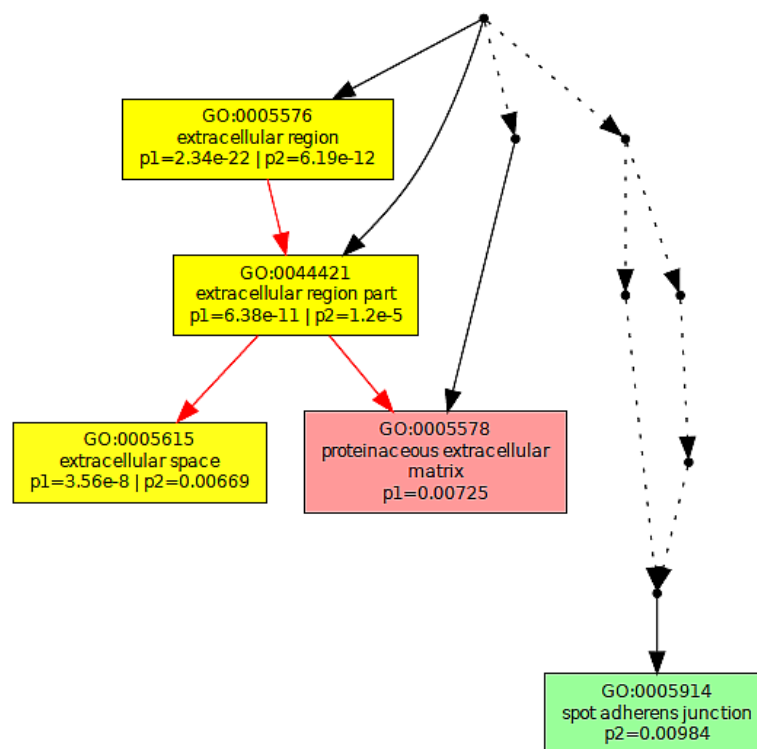


Figure 3: Cellular component.

(MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. This gene showed up expression at the Affymetrix chip. Its association with tumor cell invasion and metastasis is exhibited in (Masaki et al., 2001). Authors in (Mori et al., 1995) find that MMP-7 mRNA is overexpressed in human colorectal carcinomas and that MMP-7 may prove useful as a marker of biologic aggressiveness.

- *MMP3* - MMPs play a central role in cell proliferation, migration, differentiation, angiogenesis, apoptosis and host defences. Dysregulation of MMPs has been implicated in many diseases including arthritis, chronic ulcers, encephalomyelitis and cancer. One of the first steps in metastasis is the degradation of the basement membrane, a process in which MMPs have been implicated. Synthetic or natural inhibitors of MMPs result in inhibition of metastasis, while up-regulation of MMPs led to enhanced cancer cell invasion. This gene has showed up regulation at the Affymetrix chip. Its importance to the colorectal cancer is proved in (Zinzindohoué et al., 2005), (Baba et al., 2004), and (Roeb et al., 2004).

- *CDH3* - This gene is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium-dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. CDH3 is overexpressed in the majority of pancreatic cancer and various other malignancies, including gastric and colorectal cancers (Imai et al., 2008).
- *DHRS9* - This gene is found to be down regulated at the Illumina chip. It is involved in alcohol dehydrogenase (NAD) activity, oxidoreductase activity, acting on CH-OH group of donors, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor, steroid dehydrogenase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor, steroid dehydrogenase activity, and steroid metabolic process. This gene may play a role in the biosynthesis of retinoic acid. The importance of the retinoic acid to the colorectal cancer is explained in (Jette et al., 2004).
- *GUCA2A* - This gene is endogenous activator of intestinal guanylate cyclase. It is highly expressed in ileum and colon. At the Illumina chip it showed down expression. The ontology results showed it is involved in a hormone activity. The possibility



that the loss of guanylin activity leads to, or, is a result of colorectal adenocarcinoma formation is presented in (Cohen et al., 1998).

- *PYY* - This gene shows down expression at Illumina platform. The ontology results showed it is also involved in a hormone activity. *PYY* chemotherapy resistance in colon cancer is discussed in (Kling et al., 1999).
- *HPGD* - This gene encodes an enzyme that function in a variety of physiologic and cellular processes such as inflammation. Inhibits in vivo proliferation of colon cancer cells. It is detected in colon epithelium. According to the ontology, this gene is involved in oxidoreductase activity, acting on CH-OH group of donors and oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor. At the Illumina chip it showed down expression. Its role in the colorectal cancer is researched in (Holla et al., 2008).

However, we found that the set we revealed is able to discriminate between colorectal cancer and healthy tissue. In order to confirm our hypothesis, we used additional biomarkers set, revealed with the *GEO2R* web tool available from the Gene Expression Omnibus database (GEO, 2013). *GEO2R* allows ranking the most significant biomarkers from particular tissues. We used the same data sets as in this paper, and we took into account the top 250 biomarkers. The *GEO2R* biomarkers and the Illumina biomarkers set showed overlap in 84 genes and the retrained classifier with this set of biomarkers showed very high accuracy during the classification, whereas biomarkers from the Affymetrix set showed overlap in only 32 genes and our model was unable to discriminate between the two classes with this retrained classifier.

Very important result is that among the small number of the overlapping genes, we found many of the genes we confirmed to be related to colorectal cancer: *CHGA*, *GUCA2B*, *MMP7*, *CDH3*, *DHRS9*, *GUCA2A*, *PYY* and *HPGD*.

Considering the Vogelstein's model, none of the genes he defined as biomarkers were found in the biomarkers we discovered.

## 5 CONCLUSIONS

The aim of this paper was to show whether the biomarkers revealed by appropriately defined statistical methodology in Section 3.1 and that showed excellent classification ability using the model in Section 3.2, play important biological role in the colorectal cancer development.

For that purpose, we provided gene ontology analysis, and inspected the molecular functions and the biological processes of a particular set of genes that were overrepresented among all biomarkers. Furthermore, using the overrepresented genes, we performed tests over the Bayesian classifier. However, the results showed decreased precision when using only the enriched sets of genes as a training set. This implicated a conclusion that for successful Bayesian modelling, we need larger amount of data which implicates more detailed description of the statistical distribution of the data.

To test the methodology relevance for biomarkers discovery, we used another set of biomarkers, retrieved from the same data sets using the *GEO2R* online tool. Comparing the sets, we perceived that at Illumina microarray data, 84 genes overlap, whereas at Affymetrix microarray data, the number of overlapped genes is 32. In addition, we retrained our Bayesian classifier with the new biomarkers. The results for the Illumina chip were promising, since the overlapping set is larger, whereas the results for the Affymetrix chip were a complete failure. This once again confirmed that our methodology for significant genes revealing produces more biologically significant biomarkers.

Considering the colorectal cancer significance of the biomarker genes, we exhibit few biomarkers that are proved to be related to the disease. This was again supported by the fact that the same significant biomarkers are also found in the intersection between our biomarkers and the *GEO2R* biomarkers.

Therefore, in this paper we confirmed that our previously developed methodology for biomarkers revealing provided successful generative model for tissues and patients recognition, and for the biomarkers involved in this model, we confirmed that they are related to the colorectal cancer using the *GEO2R* online tool.

Further investigations are needed to validate our results and to identify the scientific and applicative potential of the biomarkers for molecular diagnostics, evaluation and prognostic purposes in patients with colorectal cancer.

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