Genome-Wide Expression Analysis using Novel Clustering Methods; Implications for Colon and Skin Cancer

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January 2003

Contents

Chapter 1

Introduction

The DNA microarray technology has received a great deal of attention in the last few years. Advanced computational methods are constantly improving, aiming to analyze and interpret the enormous amount of gene expression data.

In my study, I will apply two advanced clustering methods, developed in our lab, to analyze large sets of gene expression data, obtained from colon cancer clinical samples and skin cultured cell lines.

I will first present in detail the powerful DNA-chip technology with its great advantage in monitoring gene expression of hundreds to thousands of genes in parallel (chapter 2). I will describe the two most common DNA-chip technologies; cDNA and oligonucleotide arrays (introduced by Affymetrix), although only the Affymetrix method was applied in my study.

In chapter 3, I review briefly two clustering techniques; the *SPC* clustering algorithm [1] and the *CTWC* method [2]. The SPC clustering algorithm, based on a physical model of spin magnets, is a hierarchical clustering method that is especially suitable for the analysis of large microarray data sets due to its ability to identify stable clusters and its robustness against noise. The second method is the CTWC method, which provides an efficient way to zero in on subsets of the data and find meaningful partitions that may not be found using conventional clustering algorithms.

I will then present the first part of my study, related to colon cancer (chapter 4). I re-analyze the colon cancer data that was previously analyzed and published by Notterman et al.[3]] The data is comprised of oligonucleotide arrays from human 6500 and 6800 GeneChip of Affymetrix and contains 4 adenoma samples, 18 carcinoma samples and their 22 paired normal colon samples, yielding a total of 44 samples. I give a broad overview of the findings from the paper. In short, Notterman et.al. (NEA) list the genes displaying a 4-fold or more increase or decrease in expression level. In addition, clustering of the NEA data was performed, aiming to classify colon samples in an unsupervised way on the basis of their levels of gene expression.

The results of the Average-Linkage clustering algorithm that was applied exhibit a differentiation of adenomas, carcinomas and their matched normal samples.

I aimed to reproduce the results obtained by NEA, using our advanced clustering methods, and to possibly find new, interesting observations that were not mentioned previously.

I was able to reproduce the main findings of NEA, i.e. partitioning the samples according to the known tumor/normal classifications. I also found many new interesting genes, that were not mentioned by NEA. Some of these genes were found to be related to colon cancer or neoplasia in general. In addition, using the powerful CTWC, I was able to "mine" new partitions within the tumor samples, that were not found previously and may reveal new biological information.

In chapter 5, I focus on the analysis of gene expression data obtained from an experiment on keratinocyte cell lines of the skin, to reveal the underlying genetic differences between Normal Human Epidermal Keratinocytes (NHEK) versus their transformed counterparts, Squamous Carcinoma Cells (SCC). The aim is to gain understanding into the tumorigenesis of epidermal cells into Squanous Carcinoma Cells (SCC). DNA microarray experiment (Affymetrix, ~12000 genes) was performed on cell cultures of NHEK and SCC that were UVB irradiated at various time points. I apply both SPC and CTWC for the analysis of the gene expression data.

The main findings of the analysis indicate that upon UVB irradiation NHEK are protected from cell death by secreted survival factors. These include growth factors, chemokines and other pro-inflammatory mediators, which also promote cell survival and tumor growth. These factors may permit the survival of residual mutant cells that accumulate mutations and may be the seeds for future cancer development.

By using the CTWC method, I was able to partition the normal keratinocytes (NHEK) from their tumor counterparts (SCC), on the basis of small groups of genes and find new, interesting partitions of the samples.

Chapter 2

DNA microarrays

The use of DNA microarrays to analyze gene expression on a global level has received great attention in the last few years and the technology is advancing rapidly.

The DNA-chip method is a powerful, flexible and relatively simple procedure. Unlike traditional methods in molecular biology, which generally work on one or few genes per experiment, the DNA-chip method enables the monitoring of the expression level of hundreds to thousands of genes in parallel [4].

Variation in DNA sequence underlies most of the differences we observe within and between species. Locating, identifying and cataloguing these genotypic differences represents the first steps in investigating the genomic variation among and within living organisms.

Changes in multigene patterns of expression can provide clues about cellular functions and biochemical pathways, as well as discovery of new, interesting genes, which may be potential markers for diagnosis or play a role in drug therapy.

The improvement in DNA-chip technology, together with increasing genome-sequence information for different organisms, including humans, will enable the improvement of the quality and complexity of microarray experiments.

2.1 About the technology

The principle of a microarray experiment, as opposed to the classical northern-blotting analysis, is that mRNA from a given cell line or tissue is used to generate a 'target', which is hybridized in parallel to a large number of DNA sequences (‘probes’), immobilized on a solid surface in an ordered array [5].

The most commonly used systems today can be divided into two groups, according to the arrayed material: complementary DNA (cDNA) and oligonucleotide microarrays.

2.1.1 Complementary DNA (cDNA)

This technology allows comparison of fluorescently labeled cDNA populations from control and experimental tissues, marked by two colors. This technique is flexible in the choice of arrayed elements, particularly in preparation of small, customized arrays for specific investigation.

Probe preparation

Probes for cDNA arrays are double stranded cDNAs (of ~1000bp). Each probe represents a gene and is generated from cDNA libraries or clone collections. The cDNAs are amplified, using the polymerase chain reaction (PCR) and are subsequently printed onto glass slides as spots at defined locations. Each spot contains 10^6-10^7 cDNA molecules and is ~50-100μm in diameter. Using this technique, arrays consisting of more than 30,000 cDNAs can be fitted onto the surface of a glass slide [5] (see figure 2.1).

Target preparation

mRNA from two different tissues or cell populations is extracted using two alternative protocols of extraction; direct extraction of mRNA, using the poly-(A)+ RNA detector, or indirect extraction, by extracting the total RNA and separating the mRNA from the total RNA. mRNA is then converted by RT-PCR to single or double-stranded cDNA, in the presence of nucleotides labeled with two different fluorescent dyes for each tissue or cell population (for example, Cy3 and Cy5). These fluorescent dyes are incorporated into the synthesized cDNA, generating labeled cDNAs. Generally, green label is used for the control and red for the experiment [6]. (see figure 2.1).

Hybridization

Both cDNA samples are mixed in a small volume and hybridized to the array surface, resulting in competitive binding of the differentially labeled cDNAs to the corresponding array elements. Following incubation (for 24h at 45ºc), the microarray is washed, eliminating the target molecules that did not hybridize to the glass surface.

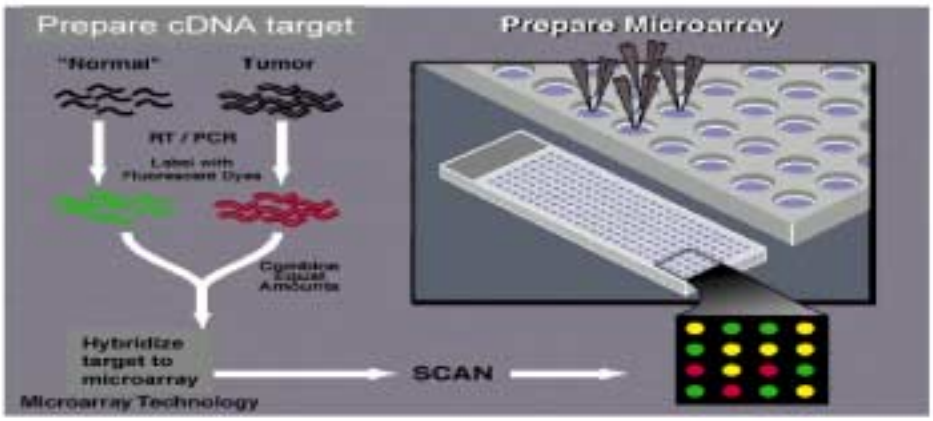

Figure 2.1: cDNA experimental design. For the target preparation, mRNA molecules are extracted from control samples (e.g. chromosome 6 suppressed cells) and experimental samples and are reverse transcribed, to generate fluorescently labeled cDNA targets (Green label is used for the control and red label is used for the experiment). The targets are then hybridized to the microarray. The array is washed and scanned at two different wavelengths to detect the relative abundance for each condition. The red and green colored spots indicate expression of the experiment and control samples respectively, and the yellow and black colored spots indicate co-expression or no expression respectively.

Fluorescence scanning is performed on the array with two different wavelengths, corresponding to the two fluorescent colors that were used. A spot showing no color (black) indicates a gene that is not expressed in either treatment. A green or red color indicates gene expression of the corresponding treatment (red-experiment, or green-control) and a yellow color indicates co-expression of the gene in both cell treatments (see figure 2.1). The ratio of the two fluorescent intensities provides a highly accurate and quantitative measurement of the relative gene expression level in the two cell samples. The calculation of gene expression takes into account background noise that is created as a result of random hybridization around the cDNA spots. The calculation of the gene expression in a certain spot is preformed as follows:

Control $\rightarrow CH1I_{(average\ intensity)} - CH1B_{(background)} = CH1D_{(final\ intensity)}$

Experiment $\rightarrow CH2I_{(average\ intensity)} - CH2B_{(background)} = CH2D_{(final\ intensity)}$

The relative gene expression → $RAT2 = CH2D/CH1D$

2.1.2 Oligonucleotide microarrays (Affymetrix)

This technology was developed by Affymetrix and includes probe preparation (Genechip), instrumentation and software for scanning, collecting and analyzing the results of the microarrays.

Probe preparation

To generate the probes, 20 short oligonucleotide sequences (typically 25mers) are chosen from the mRNA reference sequence of each gene (figure 2.2), often representing the most unique and specific part of the transcript. Each probe cell contains millions of copies of the specific, 25bp long oligonucleotide.

To each probe spot of a related gene that is perfectly complementary to a subsequence of the mRNA reference sequence (referred to as a Perfect Match, PM), there is a paired companion probe that contains identical oligonucleotides, except for a single base difference in a central position (referred to as Mismatch, MM). The mismatched probe of each pair serves as an internal control for hybridization specificity.

Altogether, each gene is represented by 40 probes; 20 pairs of PM and MM of specific oligos comprising a certain gene [7] (see figure 2.2).

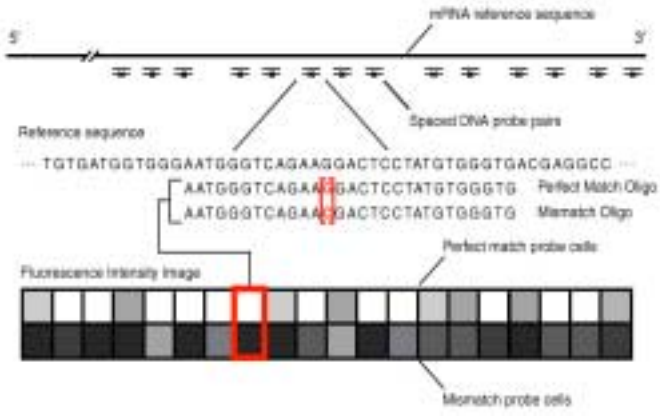

Figure 2.2: The choice of oligonucleotide probes. The probes are short sequences of 25 mer, originally chosen from the mRNA reference sequence. Each gene is represented by 20 pairs of PM and MM probes. The probe cells contain millions of copies of a specific oligonucleotide sequence. There are up to 400,000 different probes per chip.

These probes are synthesized onto silicon wafers (high-density-oligonucleotide array from Affymetrix), using the photolithography method (see figure 2.3).

In DNA photolithography, ultraviolet light is shined through holes in masks in order to direct parallel, stepwise, synthesis of oligonucleotides. At each step in synthesis, light is directed through a mask to deprotect and activate selected sites, and hydroxyl-protected nucleotides couple to the activated sites. The process is repeated, activating different sets of sites and coupling different bases, allowing sequence-defined DNA probes to be constructed at each site [7] (figure 2.3).

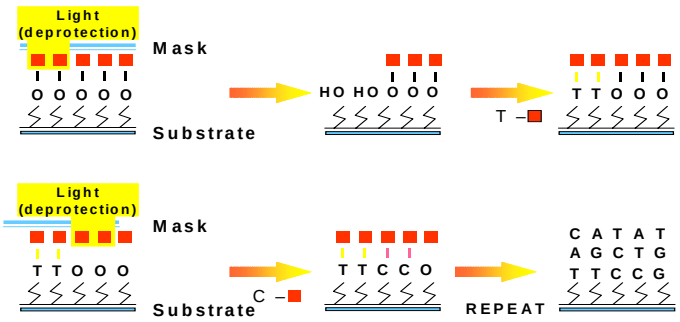

Figure 2.3: synthesis of oligonucleotide arrays using the photolithography method. Light is directed through a mask to deprotect and activate selected sites. Protected nucleotides then couple to the activated sites. This process is repeated, activating different sets of sites and coupling different bases, thus allowing the construction of DNA probes.

Target preparation

mRNA from different tissues or cell populations is extracted (using one of two protocols of extraction mentioned previously) and is reverse transcribed to generate a cDNA strand (figure 2.3). The complementary cDNA strand is then synthesized to generate a DScDNA. During *in vitro* transcription of the cDNA back to cRNA, biotin-labeled nucleotides are incorporated into the synthesized cRNA molecules. This stage results in the labeling and amplification of the RNA molecules which are then fragmented into 50-200bp fragments and hybridized to the array [8].

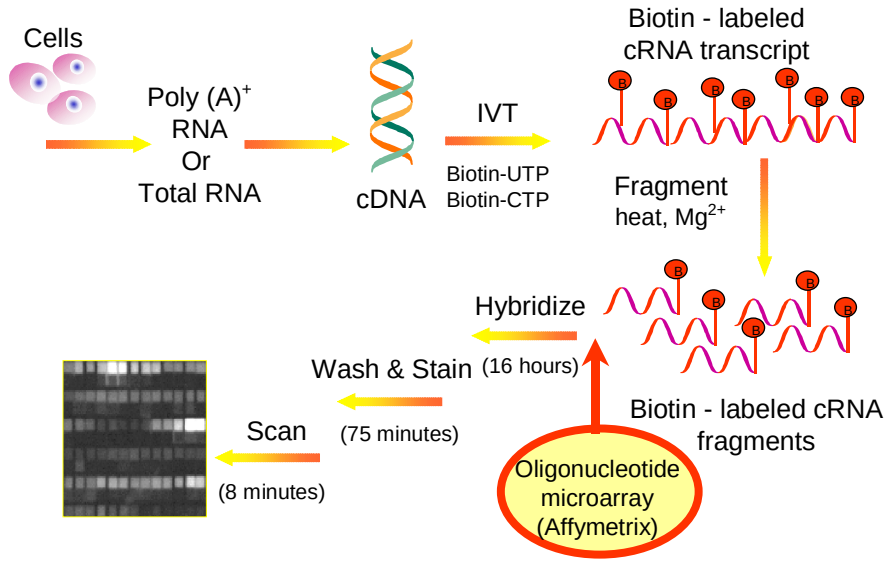

Figure 2.4: The Affymetrix experimental design. In preparing the target, mRNA is extracted from cells, either directly ((using the poly (A)+ RNA detector) or from the total RNA. The mRNA is reverse transcribed to cDNA, which is then transcribed to Biotin-labeled cRNA. The heat fragmented cRNAs are hybridized to the microarray, washed and fluorescently labeled, using the Biotin-binding molecule, Avidin. Fluorescence imaging of the array is then accomplished.

Hybridization

Hybridization of the target samples takes place (at 45ºc for 16h), where each target sample is hybridized to a separate probe array (as opposed to cDNA where targets 'compete' in the attachment to the same probes). The fluorescent labeling procedure takes place through the fluorescently labeled Avidin molecule that binds to the biotin molecules, positioned on the cRNAs nucleotides, yielding a rapid and quantitative measure of each of the individual hybridization reactions.

Calculating gene expression

As was previously shown, each gene is represented by 20 pairs of PM-MM of specific oligos (a total of 40 pixels). For each pair, we calculate the difference in the intensity as follows [9]:

$$Di_{(difference)} = Pmi - Mmi$$

From the 20 *Di* values, we eliminate false measurements (such as PM<MM) and the two most extreme measurements (highest and lowest), remaining with 15-16 *Di* values.

The average of these *Di* values across a set of probes, yields the final value, denoted *Average Difference,* which directly indicates the expression level of the gene:

$$AvgDiff = 1/N \sum Di$$

The use of average differences greatly reduces the contribution of background and cross hybridization and increases the quantitative accuracy and reproducibility of the measurements. However, the drawbacks of using this method are its relative high costs and the fact that only one condition, control or experiment, is measured in each probe array (as opposed to the cDNA method, in which two conditioned samples are co-hybridize to the same probe array).
Of note, in both studies presented in chapters 4 and 5 the analyzed datasets were obtained from the use of the oligonucleotide array method.

Following a typical DNA microarray experiment, we remain with an extremely large amount of measurements. We are then faced with the challenge of locating and extracting meaningful information from these measurements. We turn to various computational methods that challenge this task. Presented in the next chapter are two methods of analysis, developed in our lab and discussed in detail.

Chapter 3

Clustering Methods

The use of the high density DNA microarray technology gave rise to an overwhelming amount of data that needs to be interpreted. In a standard DNA microarray experimental system, expression levels of thousands of genes are monitored over several samples. To asses the quality of the data and to draw significant conclusions, we turn to various statistical and computational methods, among which are various clustering techniques. In this chapter I will present in detail two advanced clustering methods that were developed in our lab, the ***Super-paramagnetic clustering (SPC)*** algorithm [1] and the ***Coupled two-way clustering (CTWC)*** method [2].

3.1 Clustering the data

The large number of measurements obtained from a DNA microarray experimental system contains the expression levels of thousands of genes, denoted *Ng*, over several samples, denoted *Ns*. These measurements together, create an *expression level matrix*, $Ng \times Ns$, where each row corresponds to certain gene and each column to a single sample. We refer to each gene $g = 1, 2, \ldots N$ as a point in a $d = Ns$ dimensional metric space, and each sample is viewed as a point in $d = Ng$ dimensions. The aim is to partition data according to natural classes present in it, assigning data points that are "more similar" to each other to the same "cluster". Clustering analysis is an important technique that is applied in a variety of engineering and scientific disciplines and is frequently used in the analysis of gene expression data. The information gained about a set of data from clustering analysis, using various methods, could suggest a design of new experimental systems and provide new insights into the related subject.

3.2 Super-Paramagnetic Clustering

Super-paramagnetic clustering is a hierarchical clustering method, based on the "Potts model" of magnetic spins. The algorithm assigns a spin to each data point. The spins interact with each other and the overall interactions of the spins with one another is measured in terms of an energy, which is minimal when all the spins are aligned and maximal when each spin points to a different direction. The temperature controls the resolution of the system. At low temperatures, all the spins are aligned and form a single cluster. This is the **Ferromagnetic phase** of the system. As the temperature increases, the system undergoes a sequence of phase transitions. By measuring spin-spin correlations at each T we determine the probability that two data points share the same label and if this probability is high ($C_{ij} > 0.5$), the pair of corresponding data points are placed in the same cluster.

At very high temperatures, the spins are uncorrelated and each data point forms an independent cluster. This corresponds to the **Paramagnetic phase** of the system, where correlations are short ranged. The **Super-paramagnetic phase** is intermediate between these, where large sub-clusters (of the "cluster" of all the points) may emerge. This phase corresponds to spins forming highly correlated domains, where a spin of one domain is uncorrelated with spins from other domains.

A step-by-step procedure

I will demonstrate the ideas behind *Super-paramagnetic* clustering using a data set containing N=5 points. Each data point represents the location of a spin that interacts with its neighbor spins. Pairs of neighbors are connected by edges (figure 2.1).

1) constructing a weighted graph

We use the N points to construct a weighted graph of K neighbors. Points, which are defined as neighbors will be connected by edges. The interaction between neighboring spins is defined as J (we presume no interaction between spins that are not defined as neighbors, $J=0$). The strength of the interaction decreases with increasing distance between the points.

2) Partitioning of the data

The next step is to partition the data in all possible ways (configurations). To data point *i* we assign an integer valued variable $S_i = 1...q$. S_i plays the role of a possible label of data point *i*. In our case *q=4* and the labeling of the data points is performed using 4 different colors. The total number of possible configurations of the points is q^N. In our case there are $4^5 = 1024$ possible configurations of the data. Here are three examples of such configurations:

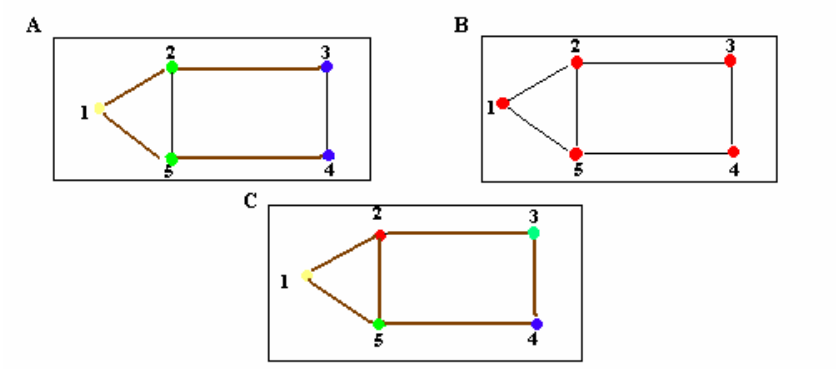

Figure 2.1: Three possible configurations of the data. Each data point is labeled with one of four possible color labels. The brown edges represent weighted edges as a result of placing two neighboring points in different color groups. A; one of 1024 possible configurations, B; At E=0, all points are colored the same. This is a 'low cost' configuration. C; a 'high cost' configuration where neighboring points are labeled with different colors.

Any assignment of the 4 possible colors $\{s\} = \{s_1, s_2, ...s_N\}$, one for each data point, has a cost;

$$H(\{S\}) = \sum_{\langle i,j \rangle} J(1 - \delta(s_i, s_j)) \qquad (1)$$

The function $\delta(s_i, s_j)$ takes the value 1 when points *i, j* are assigned the same color and 0 when they are assigned different colors, meaning that we "pay" a cost J_{ij} for not assigning neighboring points *i, j* to the same color group. The lowest possible cost, $H(\{S\}) = 0$ is obtained when we assign the same color label to all points (this situation corresponds to all data points being assigned to the same group) (see figure 2.1- B). The highest cost, $\sum_{\langle i,j \rangle} J_{ij}$, is obtained when every point is in a different color than its neighbors (if possible; see figure 2.1-C for an example of a high cost configuration). This hierarchical method uses the cost as a parameter to control the resolution.

3) Assigning probabilities to the configurations

SPC generates a statistical ensemble, based on statistical mechanics, in which for a given fixed cost $H(\{S\}) = E$, all the configurations that have the corresponding value of the cost are equally likely. The statistical weight for a given configuration S, is defined as:

$$P(\{S\}) = \frac{1}{Z} e^{\frac{-H(\{S\})}{T}} \qquad \textbf{(2)}$$

H{S} is the cost of the configuration, *Z* is the normalization factor and *T* is the temperature, which controls the resolution of the system. At low temperatures, only the 'low cost' configurations get high probabilities. The 'high cost' configurations can be found with very low probabilities at these temperatures. As the temperature increases, the probabilities of all configurations tend to be equal.

4) Determining correlations between pairs of neighboring points

After calculating the probabilities of each configuration for any given temperature, we can easily determine the correlation between all neighboring spins. We want to determine what is the probability of two neighboring points *i, j* to share the same color label (be in the same group). The correlation is defined as:

$$C_{ij}(T) = \sum_{\{S\}} P(\{S\}) \delta(S_i, S_j) \qquad \textbf{(3)}$$

{S} is the set of all configurations. C_{ij} ranges between 1 and *1/q*. If the correlation of two neighboring points is *C>0.5,* they will be connected by a line, belonging to the same cluster.

Figure 3.2 illustrates the correlation of three pairs of neighboring points, taken from the model, as a function of *T*. The green line, representing the weakest bond (bond 4-5), decreases at a fast rate and reaches the breaking point (*C=0.5*) at *T= 0.06*. The strongest bond (bond 1-2) decreases gradually and breaks at a higher temperature of *T= 0.17*.

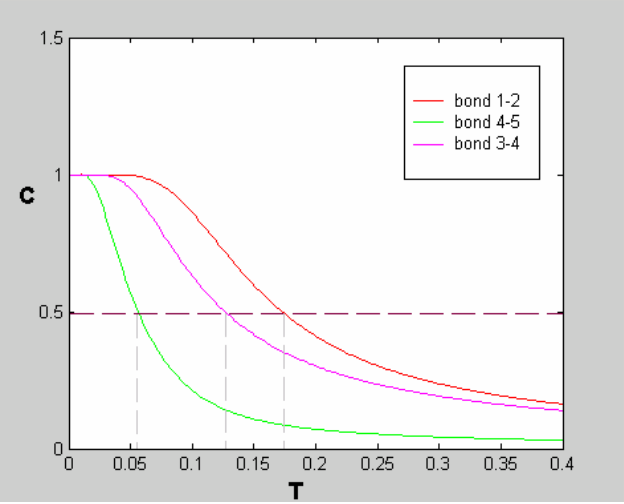

Figure 2.2: The correlation of three bonds as a function of time. The correlation of the weakest interaction, bond 4-5, decreases much faster below *C=0.5* than the other interacting pairs. The strongest interaction, bond 1-2 will be the last to break at *T=0.17*.

The three phases of the magnetic system; **Ferromagnetic, Super-paramagnetic** and **Paramagnetic**, are illustrated in this model. At low temperatures (*T*), the system is in the Ferromagnetic phase where all 5 points are in one cluster and show high correlation to one another. In the model, the **Ferromagnetic phase** is at $T< 0.06$. At higher temperatures, $0.06< T < 0.17$, the system is in the **Super-Paramagnetic phase.** Points within a cluster show high correlation ($C>0.5$), but points that belong to two different clusters are uncorrelated. For $0.06<T<0.13$ only the weak interactions are broken (bonds 4-5 and 2-3) partitioning the points into two clusters. For $0.13<T<0.17$ the cluster (3,4) has broken up, but (1,2,5) is still correlated. At higher temperatures, $T> 0.17$, the system passes to the **Paramagnetic phase**, in which all the pairs of points are uncorrelated ($C<0.5$), each point representing a separate cluster (see Fig 2.3).

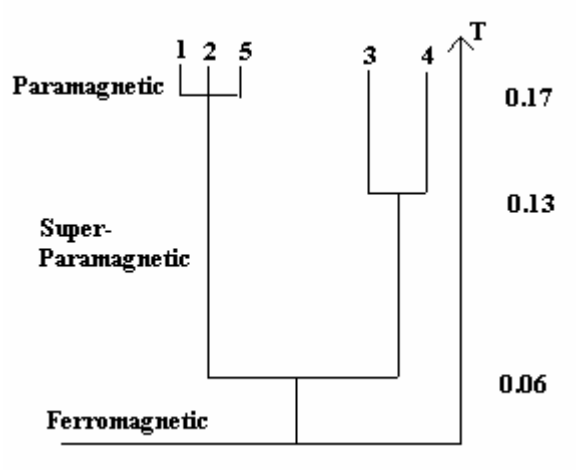

Figure 2.3: The dendrogram, representing the model. The range of temperatures of the three phases are indicated: the Ferromagnetic phase *T<0.06*, the Super-paramagnetic phase *0.06<T< 0.17*, and the Paramagnetic phase, *T>0.17*.

In this example, of 5 spins, the correlations C_{ij} were calculated exactly, enumerating all 4^5 configurations. For large N this is not possible, and one uses a Monte Carlo sampling method to estimate the C_{ij}.

Properties of SPC

SPC is especially suitable for gene microarray data analysis due to its ability to identify stable clusters and its robustness against noise, meaning that eliminating or adding data points will not change the overall structure of the data.

Like other hierarchical clustering methods, SPC generates a dendrogram. By scanning all resolutions of the data (T), SPC represents a sequence of resolutions and not one unique solution. Other useful properties of SPC are that the number of clusters is determined by the algorithm itself and not by the user. This is particularly in our favor in large microarray data sets, where the number of clusters cannot be predicted. In addition, by using the principle of K nearest neighbors, SPC relies on proximity of points and is, therefore, able to identify clusters of irregular, non-spherical shapes.

3.3 Coupled two-way clustering (CTWC)

The *Coupled-Two-Way clustering* algorithm provides an efficient method for producing subsets of the data in an iterative clustering process. The main idea of using this approach for analysis of gene microarray data is to zero in on small subsets of the data. By focusing on small subsets, one can discover partitions and correlations that were masked when the complete dataset was analyzed, using conventional clustering methods. We aim to find highly correlated groups of genes that are possibly related to the same biological process. When such a group of genes is used to cluster the samples, we hope to differentiate them in an unsupervised, biologically meaningful way.

CTWC can be performed using any number of clustering algorithms. However, SPC is especially suitable due to its ability to measure the stability of clusters. By using only stable clusters, we reduce the computation substantially. My results (presented in chapter 4 and 5) were obtained using *CTWC* together with the *super-paramagnetic* clustering algorithm (*SPC*).

Procedure

The expression data is clustered in two ways. G1 refers to the set of all the genes and S1 refers to the set of all samples. Each gene is a point in a S1 dimensional space. The first iteration, denoted G1(S1), clusters all the genes on the basis of their expression levels over all the samples . The reversed operation, denoted S1(G1), is performed, clustering all the samples on the basis of their expression levels over the genes. As a result, stable gene clusters (denoted G2, G3, etc) and stable sample clusters (denoted S2, S3, etc) emerge.

The method scans through all stable clusters of genes, one at a time, and uses them in the next iteration to cluster either all the samples or subsets of samples that were identified. The same procedure is performed on the samples.

This iterative procedure, with each step, zooms further into the data and finds additional subset, that enable new, stable partitions to emerge. For example, S4(G25) is a partition obtained by clustering the subset of samples S4 on the basis of their expression levels over the subset of genes G25.

Finding subsets within the complete dataset that are used to partition the data in new, interesting ways is the great advantage of this method, especially when dealing with gene expression data, since a large amount of the gene expression data constitutes a noisy background which may mask a true effect, provided by small subsets. Furthermore, the underlying assumption is that only a small number of genes participate in a particular biological process. Therefore, when attempting to cluster the full data set, using conventional clustering methods, these sub-partitions may be overlooked.

Chapter 4

Colon Cancer

4.1 Introduction

Colorectal cancer is the second leading cause of cancer-related death after smoking related cancers, for both men and women in the USA and other western countries [10]. An estimated 130,000 new cases are diagnosed annually and currently the lifetime risk in developing this disease is 6% [11].

Colorectal cancer is one of the best studied cancers. It affects the colon and/or rectum, which are both part of the large intestine. It is easily accessible and develops slowly over several years from pre-malignant lesions (adenomatous polyps/adenoma) to invasive cancers. The key molecular events of this multistep process have been characterized [10].

Age is a powerful risk factor in the development of colorectal cancer. At least 50% of the western population develops a colorectal tumor by the age of 70, and in about 1 in 10 of these individuals, progression to malignancy ensues.

Surgical resection remains the only curative treatment, and the likelihood of cure is greater when the disease is detected at an early stage.

Simple lifestyle changes, such as improving diet and implementation of widespread screening can reduce the risk of colorectal cancer.

Colon

The colon and rectum make up the large intestine; the long muscular tube that is part of the digestive system. The colon is the upper five to six feet of the large intestine.

Part of the food, water and fiber that the small intestine is unable to break down is absorbed through the large intestine into the bloodstream.

The colon can be subdivided into five separate sections: the cecum, ascending colon, transverse colon, descending colon, and the sigmoid colon (shown in Fig 4.1)

The rectum is a short muscular tube that goes from the end of the colon to the anus and is the lowest portion of the large intestine. It is the organ that collects and holds the feces until it is ready to be expelled by the body.

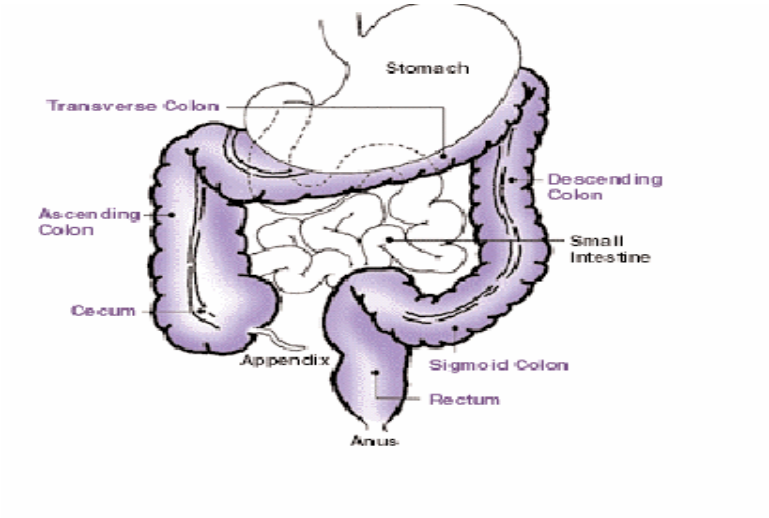

Figure 4.1: Diagram of the colon and rectum

Histologically, the colon is composed of several layers (see figure 4.2). The inner surface of the colon is a mucosal lining, which is composed of an epithelial layer under which is the submucosa and muscles that function in forcing waste materials through the colon into the rectum.

A system of classification is used, which is based on studies of tumorogenic tissue. Clinical stages I through IV are determined, based on size of tumor, growth beyond the colon, and invasion of the lymphatic system. The stages are presented in Figure 4.2.

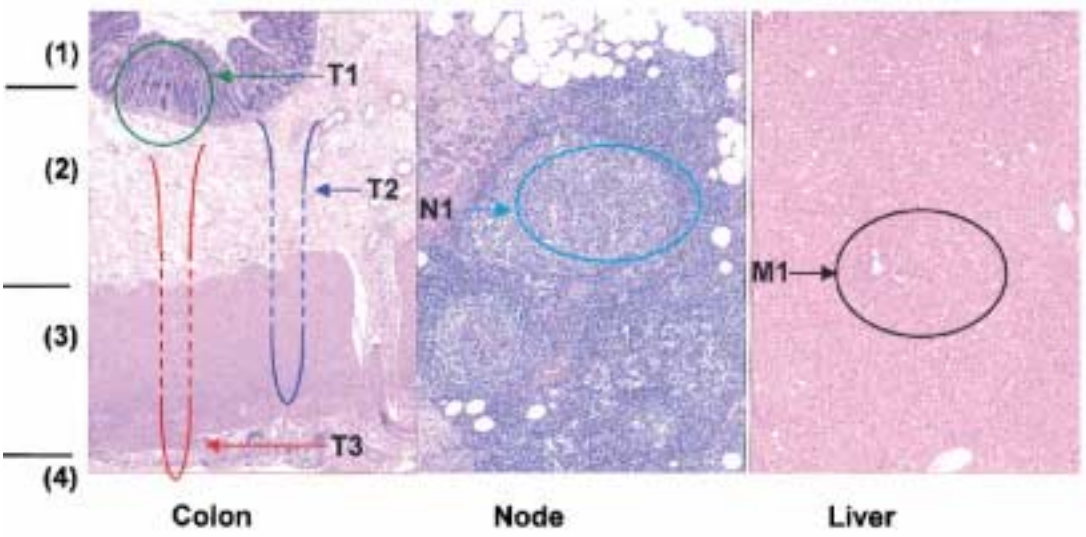

Figure 4.2 Clinical staging of colon cancer relative to the histology of the normal colon. Shown are the principal layers of the colon including the mucosa (1), the submucosa (2), the muscle layer (3), and the subserosa (4). Also shown are micrographs of a lymph node in the colon, and of the distant liver. The outlines show the extent of spread of different potential tumors and their stages. T1 (stage I) cancers are confined to the submucosa. T2 (also stage I) cancers invade the major muscular layer (muscularis propria). T3 (stage II) cancers breach the muscular layer. Cancers metastatic to mesenteric lymph nodes N1 are designated stage III. Cancers metastatic to distant organ sites (M1) such as the liver are designated stage IV.

Colorectal polyps and adenoma

A mucosal protrusion into the lumen of the colon is defined as a colon polyp. Polyps can vary in size, shape and location, they may be single or multiple.

Histologically, colorectal polyps can be classified as neoplastic (adenoma) or nonneoplastic (hyperplastic).

While hyperplastic polyps bear no risk of malignant transformation, adenomatous polyps are premalignant lesions of colorectal cancers and, therefore, need treatment and surveillance. They grow slowly, and the transformation to carcinoma usually takes at least five years.

As people age they are more likely to develop adenomatous polyps; they are found sporadically in approximately 50% of the general population by the age of 70.

Hereditary colon cancer

Epidemiological studies have suggested that up to 15% of colorectal cancers occur in dominantly inherited patterns. The two best defined familial forms are Familial Adenomatous Polyposis (FAP) and Hereditary Non Polyposis Colorectal Cancer (HNPCC). Although these are rare causes of colorectal cancer the genetic bases for both of these syndromes have been discovered, providing further understanding of the molecular events in carcinogenesis.

Familial Adenomatous Polyposis (FAP) is a dominantly inherited disease that affects about 1 in 7000 individuals and accounts for 1% of all colorectal cancers. Patients with FAP typically develop hundreds to thousands of colorectal tumors (called adenomas or adenomatous polyps) during their second and third decades of life and nearly all patients with untreated FAP develop colorectal cancer by the age of 40 [12]. FAP is caused by a germline mutation within the tumor suppressor gene APC (adenomatous polyposis coli), located on chromosome 5 [10]. This gene plays a key role in FAP as well as in the early stages of sporadic colorectal cancer (see more about APC below).

Germline mutations in components of the DNA Mismatch Repair (MMR) complex are the genetic basis of **Hereditary Non-Polyposis Colon Cancer (HNPCC)** [10] [13]. These genes include hMSH2, hMLH1, hPMS1 or hPMS2.

Recent studies show that the great majority of the cases of classic HNPCC arise from mutations in hMSH2 and hMLH1, which encode two required components of the mismatched repair complex [14].

Carriers of these autosomal dominant mutations have an 80% lifetime risk of colon cancer, and an increased risk of gastric and endometrial cancer [12].

Somatic genetics of colorectal cancer

Colorectal cancers not associated with hereditary cancer syndromes are defined as sporadic. The lifetime risk of developing sporadic colorectal cancer after age 50 is approximately 5% for average risk individuals [14].

Vogelstein et. al. have proposed a multi-step model for the genetic events in the progression of sporadic CRC [10] (Fig 4.3). This adenoma-carcinoma model is based on the assumption that in order to undergo full malignant transformation, multiple mutations must occur, including mutational activation of oncogenes and inactivation of tumor suppressor genes.

This multistep process is well illustrated by colorectal cancers, which typically develop over decades and appear to require at least seven genetic events for completion (Fig 4.3). The same genes that are responsible for hereditary CRC (e.g APC and MMR) are also involved in many sporadic CRCs.

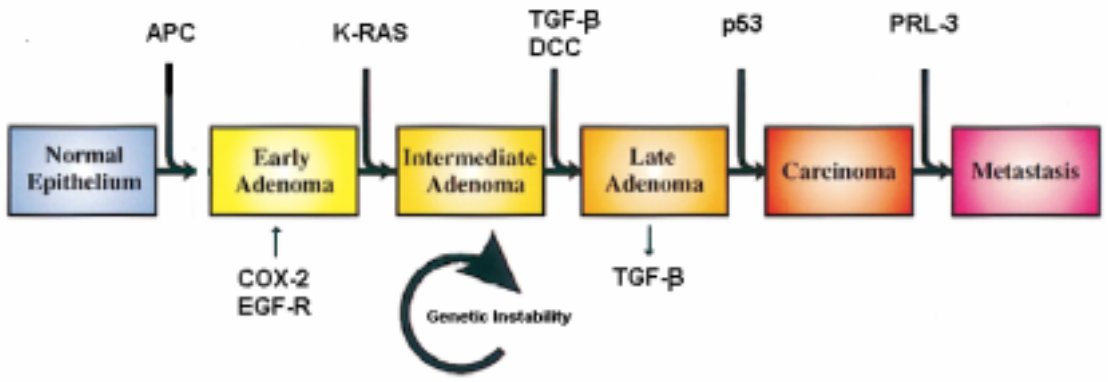

Figure 4.3: The progressive stages of colon neoplasia, starting from normal epithelium to various stages of adenoma, to adenocarcinoma and the final stage of metastasis. Shown above each stage are associated changes in tumor suppressor genes and oncogenes. APC mutations initiate the neoplastic process, and tumor progression results from mutations in the other genes indicated. Shown below each stage are associated changes in growth factor activities. Activation of COX-2 and EGF-R contribute to initiation of colon neoplasia, whereas TGF-β signaling suppresses neoplastic progression. The tumors from patients with hereditary colon cancer go through similar, though not identical series of mutations.

The APC gene was first identified in FAP; however, somatic mutation of APC has also been found in the great majority of sporadic colorectal adenomas and carcinomas and is one of the earliest mutations in colon cancer progression (see FAP in hereditary CRC) [12].

The Wnt multigene family encodes various secreted signaling molecules, activating cell proliferation when binding to their membrane receptors (named Frizzled).

Functionally, the **APC gene** product modulates the oncogenic Wnt signal transduction cascade through its effects on cellular distribution of β-catenin (Fig 4.4). β-catenin is a cell adhesion molecule commonly found in cell membrane tissue.

When APC is mutated, β-catenin accumulates in the cytoplasm and translocates to the nucleus where it associates with members of the Tcf family of transcription factors and modulates the transcription of target genes including *c-MYC*- a classic oncogene that has been shown to promote tumorigenesis, *cyclin D1*, *matrilysin* and *PPAR-δ* [15] (Fig 4.4). In summary, the tumor suppressor APC participates in several cellular processes, from proliferation to apoptosis, in colonic epithelial cells. Some of the functions of APC are attributed to its ability to control β-catenin levels and the transcription of target genes [15].

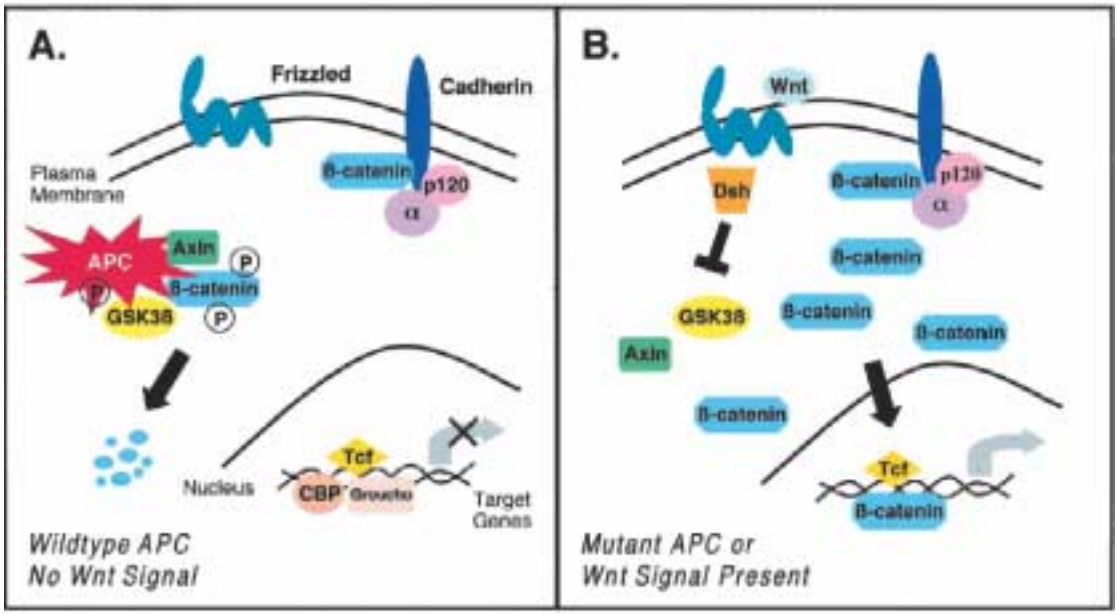

Fig 4.4 The Wnt signaling pathway; APC modulates ß-catenin/Tcf transcriptional activation and Wnt signal transduction. (A) In the presence of APC or in the absence of Wnt ligand, ß-catenin is localized to the adherens junction where it is associated with E-cadherin, α-catenin, p120cas, and indirectly with the cytoskeleton. GSK3ß phosphorylates ß-catenin in a complex that contains ß-catenin, APC, and axin family members, and ß-catenin is rapidly degraded at the proteosome. (B) When *APC* is mutated, ß-catenin accumulates in the cytoplasm and the nucleus. Another way of generating a cytosolic pool of ß-catenin (as when APC is inactivated) is through binding of Wnt ligand to its transmembrane receptor, inactivating the GSK3ß kinase.
ß-catenin associates with members of the Tcf family of transcription factors and modulates the transcription of target genes with Tcf recognition sequences. In some instances, ß-catenin increases transcription of target genes by competing for Tcf binding with co-repressors, such as Groucho and CBP, to relieve transcriptional repression.

The **Ras proteins** are thought to be important for transduction of signals required for proliferation and differentiation. Mutation of Ras results in activated p21-ras, which is associated in a variety of human cancers (in its activated form) including a majority of colorectal cancers. Ras mutations, mainly K-ras, develop in 50% of large adenomas and 50% of colon cancers, and so are early steps in neoplastic progression [16].

Transforming growth factor β (TGF-β) is a small secreted polypeptide hormone that negatively controls colon cell growth. Human colon cancers are in general functionally resistant to TGF-β growth inhibition. In one third of human colon cancers, this resistance is due to mutations of the RII component of the TGF-β receptor complex [13].

Other genes which regulate the signaling of TGF-β and may be mutated are SMAD2 or SMAD4, transcription factors that are phosphorylated by the TGF-β receptor complex [13]. These mutations are temporally coincident with the further progression of colon adenoma to colon carcinoma.

The **DCC gene** on chromosome 18q encodes a protein with significant sequence similarity to neural cell adhesion molecules and other related glycoproteins. Alterations of this gene may interfere with normal cell growth and differentiation by disrupting cell-cell or cell-substrate interactions [17].

Another critical pathway involved in tumor progression involves **p53**, a tumor suppressor gene that is mutated in greater than 50% of colon cancers and is recognized as the most common genetic event in human cancer. Activation of the normal p53 gene inhibits cell growth by blocking the cell cycle or by stimulating apoptosis. P53 is a transcription factor that regulates expression of many genes [18].

Most recently, overexpression of the **PRL-3 tyrosine phosphatase** has been observed in metastatic colon cancers, suggesting that PRL-3 is a direct genetic target contrinbuting to metastasis progression [19].

In addition, a common feature characteristic of cancer is genetic instability. Genetic instability is a necessary molecular catalyst for colon carcinogenesis. Subtle alterations due to mismatch repair deficiency (as in HNPCC syndrome- see hereditary colorectal cancer) occur in a minority of sporadic cases. This mismatch repair (MMR) inactivation speeds up the multistep process of spontaneous gene mutation rates (for both sporadic and genetic HNPCC tumors) [20]. Finally, the oncogenic steps presented in figure 4.3 are characteristic of colorectal cancer, but may be different for other cancer types.

Growth factor pathways are drug targets in colon cancer

Colon adenoma and colon cancers show increased levels of **COX-2** (Fig 4.3), which with COX-1 encodes enzymes that initiate the synthesis of prostaglandins.

Prostaglandins (PGE) and related compounds are collectively known as **eicosanoids**.

They are produced from **arachidonic acid**, a 20-carbon fatty acid (eicosatetraenoic acid) (Fig 4.5). They are formed in most tissues of the body and play a roll in inflammation, fever, regulation of blood pressure, blood clotting and control of reproductive processes and tissue growth.

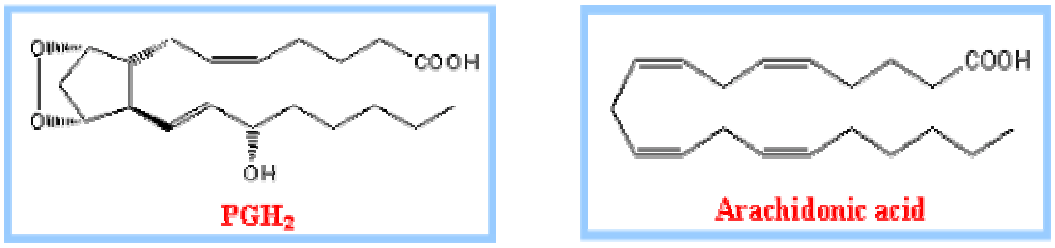

Figure 4.5: right- arachidonic acid, the precursor that is enzymatically converted to the PGE. Left - PGH_2 (prostaglandin H_2), an example of a prostaglandin.

Cox-2 is induced at very early stages of polyp formation and it is believed to play a significant role in tumorigenesis. COX-2 selective inhibitors were shown to induce shrinkage of some colon adenomas in human colorectal cancer and, therefore, can be a novel class of therapeutic agents for colorectal cancers [21]. In addition, chronic use of COX-2 inhibiting drugs, including aspirin, is associated with a decreased risk in colorectal cancers.

Human carcinomas frequently express high levels of **EGF receptor family**, and overexpression of at least two of these receptors, the EGF receptor (EGF-R) and closely related ErbB2, has been associated with a more aggressive clinical behavior. Studies in cell lines suggest that activation of these receptors, which are not amplified in colon cancer, is nonetheless important for growth of these cancer cells, and are hence targets for anti-cancer therapeutics [22]. Small molecules showing promise include inhibitors of EGF-R and inhibitors of the downstream MAP kinase pathway [12]. Antibodies to EGF-R are also promising as anti-colon cancer therapeutics. However, disabling only a single molecular target may not always give a dramatic affect and much work remains to be done in discovering molecular targets for treatment of the disease.

4.2 Clustering analysis of gene expression data of human colon tumors

I analyzed the genome wide expression of colon cancer. I used the data that was previously analyzed and presented in the paper "Transcriptional Gene Expression Profiles of Colorectal Adenoma, Carcinoma, and Normal Tissue Examined by Oligonucleotide Arrays" (by Notterman et.al, NEA) [1][3].

My aim was to identify small groups of genes that are related to the development of colon cancer and to compare my findings to the results of the analysis that was presented in [3].

I used the advanced *Coupled Two Way Clustering (CTWC)* method, which was developed in our lab and is aimed to "mine" gene expression data (see clustering methods). I will first present an overview of NEA and proceed to show the results of my analysis and its conclusions.

An overview of the results and summary of NEA

The main goal of NEA (Notterman et.al) was to classify the colon samples on the basis of their gene expression pattern, in an unsupervised manner.

The use of an oligonucleotide array (from human 6500 and 6800 GeneChip, Affymetrix) to monitor the expression profiles of colon carcinomas, adenomas, and normal tissue was described. The data that was studied contained 4 adenoma samples, 18 carcinoma samples, and their 22 paired normal colon samples, yielding a total of 44 samples.

Relative expression in neoplastic and normal Tissue

At first, for each of the 18 carcinoma paired samples, the relative expression of each gene was compared in normal and neoplastic tissue. The paper lists the genes displaying a 4-fold or more increase or decrease in expression level that was also significant at the $P<0.001$ level [2]. The lists of genes are presented in tables 1 and 2 (see appendix). The tables show that 19 transcripts displayed 4-fold or greater expression intensity in the tumors than in the paired normal tissue (table 1), whereas 47 transcripts displayed

[1] The data is available on the website at: http://microarray.princeton.edu/oncology/.
[2] For the statistical tests, the Student's t test or the Mann-Whitney U test were used.

4-fold or greater exprssion in the normal than in the paired cancer tissue ($P < 0.001$) (table 2)[3]. Some of the genes that appear in the tables 1 and 2 were validated by RT-PCR and are printed in bold letters.

Table 1: the list includes *metalloproteinases* (e.g human *metalloproteinase* and *collagenase*), which are known from the literature to be highly expressed in colonic neoplasia. In addition, the table contains genes which were not previously related to colon cancer but were either linked to other forms of neoplasia or regulation of the cell cycle, such as: *MGSA, GRO-γ, ckshs2, M-phase tyrosine phosphatase*, and *transcription factor IIIA*)

Table 2: the table includes a substantial number of genes that were more highly expressed in normal tissue than in the paired cancer tissue. Several of the genes have been previously shown to be down-regulated in cancer, such as: *guanylin*, a product of colonic epithelial cells, *down-regulated in adenocarcinoma, tetranectin, hevin* and *biliary glycoprotein (BGP1).*

A great number of the genes that appear in these tables will later appear in my clustering analysis, partitioning the different types of tissues and therefore, will be discussed in further detail (see results).

Clustering Analysis

To further probe differences between normal tissue, adenomas and carcinomas, but on a global basis, cluster analysis was performed on all 22 paired adenoma and carcinoma samples.

The second part of the paper presents the clustering analysis that was performed. Since the experiments with paired adenoma and paired carcinoma samples were performed on different GenecChip versions (the experiments with carcinoma and paired normal tissue were preformed with the Human 6500 GeneChip and the experiments with adenomas and their paired normal tissue were performed with the Human 6800 GeneChip), a composite dataset was created, including only the genes common to both versions (approximately 1800 genes). Prior to clustering, the composite dataset was pre-

[3]This table was first comprised of 88 transcripts, however, after removing the 41 transcripts associated with smooth muscle and connective tissue, 47 remained and are presented this way in the paper.(see table 2 – appendix)

processed, including filtering, scaling and normalization (see "materials and methods" in the paper), remaining with 1096 genes and ESTs.

A two-way pairwise Average-Linkage cluster analysis was applied to cluster the 1096 genes on the basis of similarities of their expression profiles over all 44 samples, and vice versa. The resulting expression map was visualized with Treeview and is presented in figure 4.6:

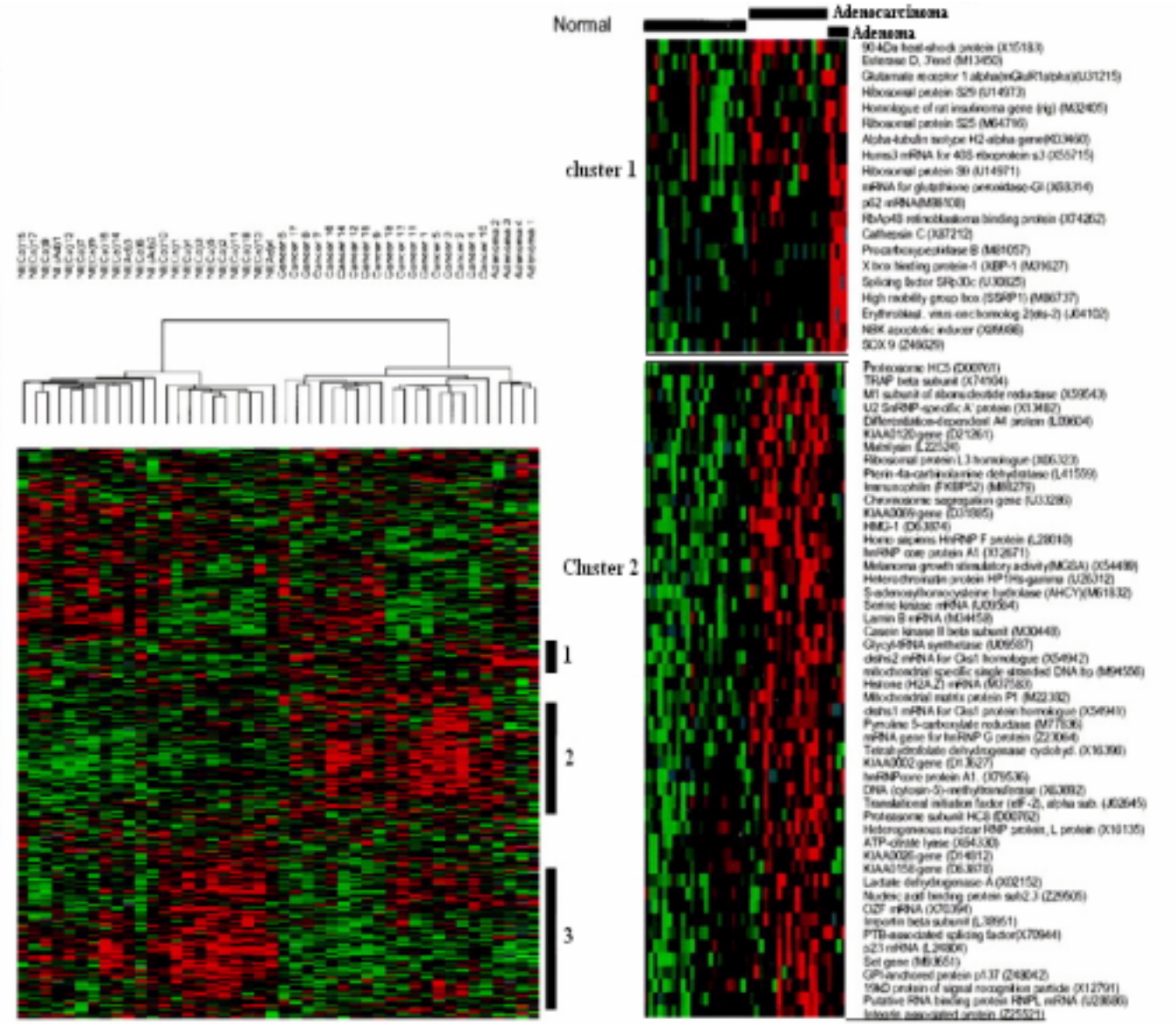

Figure 4.6: Cluster analysis of the combined data set preformed by Notterman et al (see Fig.3 of paper). Right upper panel, cluster 1: a cluster of genes that are more intensely expressed in adenoma than in normal tissue or carcinoma. Right lower panel, cluster 2: A cluster of genes that are more highly expressed in carcinoma than in adenoma or normal tissue.

Referring to the results shown in Figure 4.6, Notterman et.al. described the most striking feature; The three tissue types are well separated from one another.
Looking at the phylogenetic tree (shown in Fig 4.6), one can see that the adenomas and carcinomas are clearly separated from their benign precursors, placed on different trunks, and that the neoplastic tissues are also separated from one another, occupying adjacent branches of the same trunk.
Thus, using the hierarchical clustering algorithm was successful in grouping the three types of colon samples on the basis of their differences in gene expression.

Notterman et.al. marked the three most obvious clusters that appear to differentiate adenomas, carcinomas, and their matched normal samples (Fig 4.6 cluster1-3):

(a) cluster 1: (Fig. 4.6, *right upper panel*) represented a group of genes that were more intensely expressed in adenoma than in either carcinoma or normal tissue.
This group of genes contained several transcription factors, of which some have been implicated as oncogenes (*XBP-1, SSRP1 and SOX-9*), ribosomal proteins, an inducer of apoptosis (*NBK*) and a splicing factor. However it still remains to be shown that these genes play an early role in the transition from adenoma to carcinoma.

(b) cluster 2: (Fig. 4.1, *right lower panel*) contained a group of genes that were more highly expressed in carcinoma than in adenoma or normal tissue. This cluster contains some of the gene products that were also identified as being more highly expressed in colorectal neoplasia than in normal tissue (e.g, *Ckshs2, MGSA, matrilysin*) (see table 1, appendix).

(c) cluster 3: consists of genes that are more highly expressed in the normal samples than in the carcinomas or adenomas. The paper chose not to present this cluster. It only concludes that embedded within this cluster are genes that are known to be repressed in colorectal neoplasms such as: *guanylin* and *colon mucosa antigen*.

4.3 Results

In my work I re-analyze the data that was used by NEA (Notterman et.al.). I apply the SPC clustering algorithm, together with the CTWC method (details about SPC and CTWC methods are given in chapter 2). By using these advanced methods I was able to partition the samples according to the known tumor/normal classification, as was shown by NEA. Many new interesting genes emerged, separating the tumor and normal samples which did not appear in the clusters of NEA. I will focus on these genes, their functional activity and relation to neoplasia.

Furthermore, I show the advantage of CTWC in *mining* new partitions, which have not been found using other clustering methods and may contain relevant biological information.

4.3.1 Prior to clustering

The data set from NEA contains 22 tumor samples; 18 carcinoma and 4 adenoma, and their paired normal samples (see 4.2).

First, following Notterman et.al, I created a composite database that included only accession numbers represented on both GeneChip versions. Values lower than 1 were adjusted to 1. Prior to application of CTWC, I filtered the data using a filtering operation very close to that used by Notterman et.al, remaining with 1592 genes. Data from the two different chips were brought to the same average expression level. The data was then log-transformed, centered about the mean and normalized.

Second, I studied the 18 paired carcinoma samples separately. Of the ~6600 cDNAs and ESTs represented on the array, only genes for which the standard deviation of their log-transformed expression values was greater than 1, were selected. After this filtering process I remain with 768 genes. These values were centered and normalized, prior to application of the CTWC algorithm. The samples were labeled according to additional information about the mRNA extraction protocol used.

4.3.2 Identifying genes that partition the samples according to known classifications

Coupled two way clustering is an iterative procedure (see methods- chapter 3). The first operation, denoted G1(S1), clusters all the genes on the basis of their expression levels over all the samples (note, the complementary operation, denoted S1(G1) is performed, clustering all the samples on the basis of their expression levels over all G1 genes). The result of the G1(S1) iteration yields the dendrogram with the stable clusters that were identified, marked by circles (see figure 4.3.1A). Here, I focus on gene cluster G8 that emerged as a stable cluster in this iteration (see figure 4.3.1A).

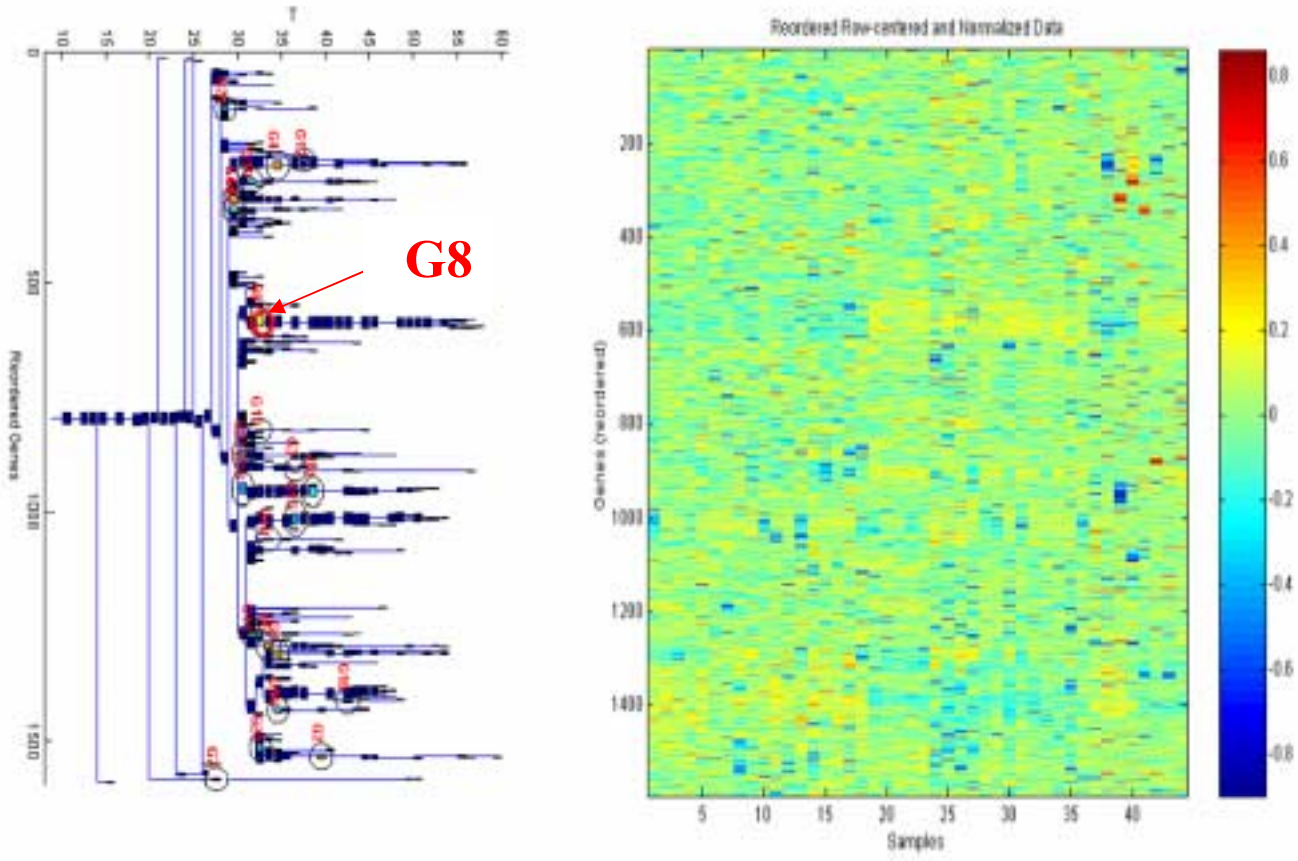

Figure 4.3.1A: The first iteration of CTWC, denoted G1(S1). Left; the resulting dendrogram with the stable gene clusters marked by circles. G8 is one of the stable clusters that emerged and is marked by a red arrow. Right; The expression level matrix of G1(S1). Rows correspond to all the genes (G1), reordered after clustering and the columns correspond to all the 44 samples (S1).

The next step was to cluster all the samples on the basis of the stable gene clusters that were found in the first iteration. Here, I will focus on S1(G8), meaning on the clustering of all the samples (S1) on the basis of gene cluster G8.

S1(G8)

Clustering the 44 samples, using the expression levels of G8, generated a dendrogram (see figure 4.3.1.B- A) which exhibits a clear separation into two large clusters (a and b) and two small ones (c and d). Clusters **c** and **d** contain all the normal samples (both carcinoma and adenoma), **a** the tumor carcinoma samples and **b** the tumor adenoma samples. The colors (see bar on the right hand side of the expression matrix represent the expression levels of the genes in G8, with red denoting high and blue- low values.

Tumor - Normal separation

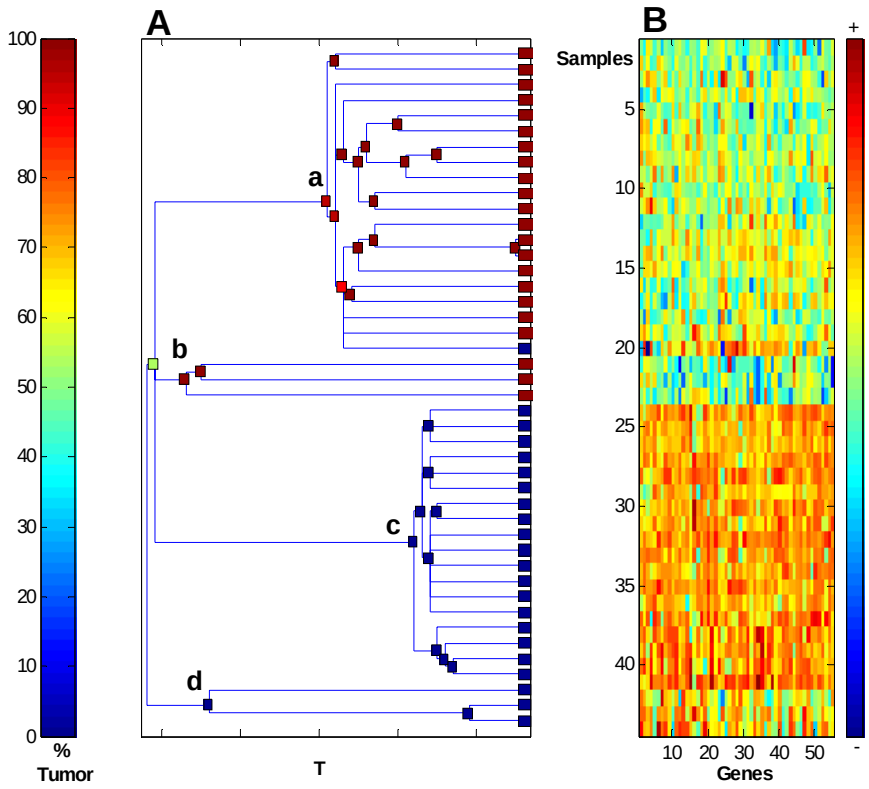

Figure 4.3.1B: S1(G8), A clear separation of the tumor carcinoma and adenoma samples from the normal samples, using the G8 group of genes. (A) The boxes are colored according to the percentage of the tumor samples. (B) The expression level matrix of S1G8. Rows correspond to all the samples and the columns correspond to the genes of cluster G8. The matrix shows high expression levels of the G8 genes in the normal samples compared to the tumor samples.

G8 contains 55 genes, which show *high expression levels in the normal* samples compared to the adenoma and carcinoma samples. The full list of 55 genes can be viewed in the appendix (table 3). Here I show a subset of the genes from cluster G8 which seem interesting. Note that only 12 out of the 55 genes (see full list) were discussed by NEA.

Classification of the interesting genes from cluster G8

Classification	Accesion	Symbol	Name	NEA
Cell adhesion	L34060	CDH8	Cadherin-8	
	M63391	DES	Desmin	Table2
	X06256	VLA5A	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	
	X74295	ITGA7	Integrin, alpha 7B	
	M69066	MSN	Moesin	
	M85289	HSPG2	Heparan sulfate proteoglycan 2	
	X05610	COL4A2	Collagen, type IV, alpha 2	
	X15880	COL6A1	Collagen, type VI, alpha 1	
	X15882	COL6A2	Collagen, type VI, alpha 2	
	X16354	CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1	
	X53416	FLNA	Filamin A, alpha (actin binding proein 280)	
	Z24727	TPM1	Tropomyosin-1	
	D10537	MPZ	Myelin protein zero	
	X64559	TNA	Tetranectin	Table2
	X86693	SPARCL1	Hevin	Table2
Tumor- suppressors and repressors	L07648	MXI1	MAX interacting protein 1	
	M22995	RAP1A	Ras-related protein (krev-1)	Table2
	X57348	SFN	14-3-3 protein sigma	
Phosphatases and kinases	M65254	PPP2R1B	Protein phosphatase 2A-beta	
	X68277	DUSP1	Protein tyrosine phosphatase CL100	
	X07767	PKACA	Protein kinase, catalytic, alpha	
Secretory proteins	M97496	GUCA2	Guanylin	Table2 +cluster
	Z49269	CCL14	Chemokine HCC-1	
G protein coupled receptors	D13626	GPR105	G protein-coupled receptor 105	
	U34038	GPR11	G protein-coupled receptor 11	
Other genes	L02785	DRA	Down regulated in adenoma	Table2 +cluster
	M36634	VIP	Vasoactive intestinal peptide	
	D13168	EDNRB	Endothelin receptor type B	
	J04080	CIS	Complement component C1r	
	X51405	CPE	Carboxypeptidase E	
	L20852	GLVR1	Leukemia receptor virus 1	
	L20859	GLVR2	Leukemia receptor virus 2	

Table 4.1: Classification of the interesting genes from gene cluster G8. The last column indicates if the gene appears in the paper by NEA (see 4.2), either in one of the clusters or in the table 2 of upregulated genes in normal tissues (see table 2, appendix).

The interesting genes from cluster G8 were classified into groups according to their functional activities within the cell and the environment (see table 4.1). An overview from the literature of the gene function and relation to cancer, particularly colon cancer, is presented:

1) Genes related to cell adhesion

The adhesion of cells to each other and to the extracellular matrix is essential for the formation and maintenance of tissues. Many of the adhesion proteins, being positioned on the cell surface, act as receptors, modulating various signaling pathways that mediate apoptosis, cell adhesion, growth, proliferation and migration.

In the progression of tumorigenesis, the ability of uncontrolled growth, migration and invasion of cells into surrounding tissues is often associated with disruption of cell-cell and cell-matrix interactions. Therefore, change of expression of various adhesion proteins, mainly by downregulation, marks the transition from noninvasive growth to a malignant, invasive tumor.

This cluster includes Cadherins, integrins, collagens and more (see table).

Cadherins are a family of membrane proteins that mediate Ca^{2+} - dependent cell-cell adhesion. More than a dozen cadherins have been identified.

These proteins are responsible for maintenance of normal tissue structure in the adult organism, regulated by a variety of extracellular signals. During tumor development, disruption in expression or function of cadherins may cause uncontrolled cell migration and proliferation [23]. The biological role of cadherin-8, which appears in the cluster and is found to be expressed in the brain is not clear at present [24].

Integrins are a large family of receptors, connecting cells to the surrounding ECM. Integrin **VLA5A** is a receptor for fibronectin[4] and increased expression of this receptor was found to suppress cell migration and tumor cell invasion [25].

Moesin (membrane-organizing extension spike protein) function is to link the actin cytoskeleton to the plasma membrane and it also participates in signal transduction pathways. It is involved, together with other members of its family[5] in the migration, adhesion, and structure of cells and was found to be down-regulated in lung cancer [26].

[4] Fibronectin is a multifunctional protein that mediates cell adhesion to the extracellular matrix.
[5] Moesin belongs to a group, known as the ERM family of proteins which includes Ezrin,radixin, moesin and merlin.

Heparan sulfate proteoglycan (HSPG2): heparan sulfate proteoglycans are present on the cell surface or as components of the extracellular matrix (ECM). They mediate both cell-ECM and cell-cell adhesion and are important co-factors in FGFR signaling. Low levels of HSPG correlate with high metastatic activity of many tumors [27].

CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) is a cell adhesion molecule that is a member of the immunoglobulin superfamily. It participates in signaling pathways, interacting with tyrosine kinases and phosphatases [28]. The expression of CEACAM1 was shown to be lower in tumor compared with normal tissue in cancers of the breast, colon and prostate and it is suggested that CEACAM1 may be a tumor suppressor gene [29].

Actin binding proteins in this cluster are **FLNA** and **TPM1. FLNA (filamin A, alpha**, **actin binding protein 280**) links actin filaments to the membrane proteins and interacts with multiple cellular proteins involved in signal transduction [30]. **TPM1 (Tropomyosin-1)** binds to actin proteins and is downregulated in transformed cells [31].

MPZ (myelin protein zero) is a major structural protein in the peripheral and central nervous system (PNS and CNS) that acts as an adhesion molecule of myelin.

It was shown that when expressed in carcinoma cell lines, p0 induced strong intercellular adhesion, although it is not clear how the changes are mediated in carcinoma cells [32].

TNA (tetranectin) is a glycoprotein that is a plasminogen[6] binding protein. It was found to be localized together with plasminogen in the extracellular matrix of colon carcinoma and melanomas, suggesting a role in extracellular proteolysis [33]. The amount of TNA in blood is reduced in cancer, and this may be used as a prognostic marker [34].

Hevin is closely related to the extracellular matrix protein SPARC; it is known to be important for the adhesion and trafficking of cells through the endothelium.

Hevin is down-regulated in many cancers and is a negative regulator of cell growth and proliferation, however neither the mechanism nor the physiological meaning of this down-regulation is known [35].

[6] Plasminogen is the inactive precursor of plasmin; a protease that is responsible for digesting fibrin in the blood.

2) Tumor suppressors and repressors

MXI1 (MAX interacting protein 1) is a member of the c-Myc family of transcription factors and is a transcriptional repressor. While c-myc activates transcription and stimulates cell proliferation, Mxi1 negatively regulates these activities and thus may be a tumor suppressor gene. It was found to be mutated in a few prostate cancers [36] and to inhibit proliferation in malignant glioma cell lines [37].

RAP1A (Ras related protein) is a tumor suppressor gene belonging to the Ras family. It was shown to suppress ras-induced transformation of cells, by interfering with interaction of Ras with its effector [38]. Several reports suggest that Rap1A may also be involved in cell adhesion *via* the regulation of integrins [39] .

SFN (14-3-3 protein sigma) negatively regulates cell cycle progression- it is a p-53 regulated inhibitor of G2/M progression. It binds to a variety of signaling proteins, including kinases, phosphatases, playing an important role in regulatory processes, such as apoptosis and cell cycle control. Overexpression of SFN therefore causes cell cycle arrest [40]. It has also recently been shown that gene expression of SFN is silenced in breast and colon cancer cell lines [41].

3) Phosphatases and Kinases

Protein phosphorylation is one of the most conserved mechanisms for the regulation of cell growth and proliferation. Protein kinases and phosphatases catalyze these phosphorylation reactions. Many kinases promote growth and proliferation signals, whereas phosphatases are negative regulators, which inhibit cell growth signals and proliferation.

PPP2R1B (protein phosphatase 2), regulatory subunit A-beta incodes the beta isoform of the A subunit of the protein phophatase 2A (PP2A). PP2A is one of the major cellular phosphatases that is involved in the regulation of various cellular pathways, including control of cell growth and cell cycle regulation [42].

PPP2R1B gene (the isoform beta of the subunit A) was found to be somaticly altered in human lung and colorectal carcinomas, suggesting a role of PP2A in human tumorigenesis through a mechanism of functional inactivation of the phosphatase activity [42].

DUSP1 (protein tyrosine phosphatase CL100) a phosphatase for ERK2, a proein in the MAP kinase pathway. A steady decrease of this phosphatase was observed in several types of cancers, including colon cancer, with increasing stage of disease [43].

PKACA (protein kinase, cAMP-dependent calalytic, alpha)

The cAMP-dependent protein kinase (PKA) is an intracellular enzyme that plays a role in cell growth and differentiation. PKA has been suggested to regulate transcription through the phophorylation of several different *trans*-activating factors [44].

4) Secretory proteins

Guanylin is a secretory hormone that is expressed in intestinal epithelia**.** Binding of guanylin to its receptor generates osmotic water movement into the intestinal lumen. Guanylin is known to be downregulated in colon cancer and it is therefore speculated to play a role in initiation or progression of colon cancer [45].

HCC-1 is a novel chemokine that induces intracellular ca2+ changes and enzyme release. It is expressed constitutively in several normal tissues, and is present at high concentrations in plasma [46].

5) G protein-coupled receptors

G protein-coupled receptors activate various signal transduction pathways, leading to diverse biological processes, which regulate cell growth and proliferation. The biological function of these two receptors, which appear in this cluster, **GPR105** and **GPR11**, however is still unknown.

6) Other genes

DRA (down-regulated in Adenoma) encodes a sodium independent transporter for sulfate and oxalate. The DRA gene is significantly down-regulated in adenomas and adenocarcinomas of the colon as well as in colon cancer cell lines, although its specific role in colon cancer is not clear [47].

VIP (Vasoactive intestinal peptide) was originally isolated from the intestine where it plays an important role in water and electrolyte secretion. It has been shown to have several biological functions such as vasodilation, smooth muscle relaxation and neurotransmission in the central and peripheral nervous system [48]. VIP has an inhibitory effect on the growth of colon cancer cells [49] whereas it exhibits growth promoting effects on several kinds of tumors such as breast cancer [50].

EDNRB (endothelin receptor type B) is a non-specific receptor for endothelins; a group of peptide hormones released by endothelial cells that have vasoconstrictive and mitogenic properties [51]. Various data suggest that the ET system (endothelins and their receptors) play an important role in proliferation and vascularization of tumor cells. A correlation of EDNRB expression with malignancy has been reported in prostate cancer cells, which show downregulation of EDNRB mRNA [52].

C1S (complement component 1, s subcomponent)- The complement system is a powerful immune effector that can eliminate cells, including virally-infected and cancer cells. Cancer cells are exposed to complement proteins in blood during the process of invasion and metastasis, but some of them are resistant and are thus able to progress. The c1 component in known to initiate the complement cascade.

However, very little information is known on the expression of the complement components in tumor cells or tissues [53].

Summary of gene cluster G8

The genes in cluster G8 show a decrease in the expression levels in the tumor samples. The decrease in expression is mainly of cell adhesion proteins, which correlates with the cells detachment from neighboring cells and migration, in the process of tumor invasion and metastasis.

In addition, a decrease in phosphatases, repressors, and other regulators of cell growth and proliferation is observed, leading to uncontrolled growth of the cell and thus enabling the tumor to rapidly grow and metastasize.

Most of the genes in this cluster were not found by NEA (Notterman et.al.) and some of them are known to be repressed in colon cancer or other forms of neoplasia, such as *protein 14-3-3 sigma, tyrosine phophatase CL100, CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), down regulated in adenoma (DRA) and VIP (vasoactive intestinal peptide)*.

4.3.3

The data set I analyze next contains only the 18 carcinoma samples and their paired normal samples, a total of 36 samples (see materials and methods). I performed the CTWC procedure that yield the partition S1(G25) in the same manner that was shown for S1(G8).

S1(G25)

Clustering the carcinoma samples, using the expression levels of the gene cluster G25, gave rise to a clear partition of the samples into two clusters; one of normal samples **a**, and the other of tumor samples **b**, with *relatively high expression levels of G25 genes in the tumor* cluster (see dendrogram).

Tumor - Normal separation

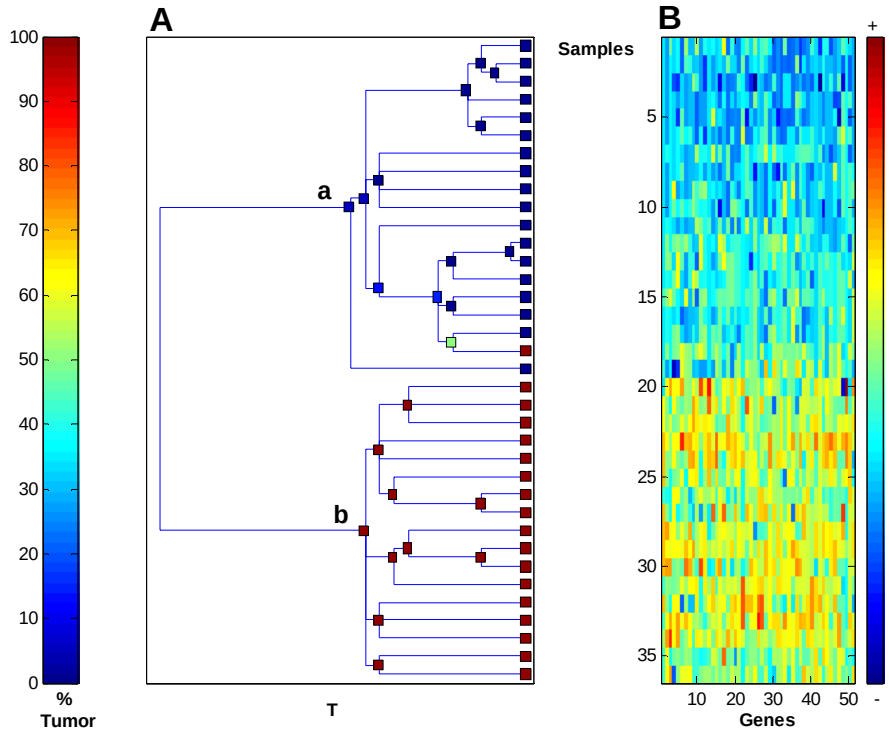

Figure 4.3.2: S1(G25), A clear separation of the tumor carcinoma samples from the normal samples, using the G25 group of genes. (A) The boxes are colored according to the percentage of the tumor samples. (B) The expression level matrix of S1(G25). Rows correspond to all the samples and the columns correspond to the genes of cluster G25. The matrix shows relatively high expression levels of the G25 genes in the tumor samples compared to the normal samples.

Classification of the genes from cluster G25

G25 contains 51 genes, some of which are known to play a role in the crucial events leading to cancer. The full list of genes is shown in the appendix (table 4).

They were classified, based on their functional properties and location in the cell, as follows:

Classification	Accesion	Symbol	Name	NEA
Angiogenic genes	H11084	VEGF-A	Vascular endothelial growth factor A	
	L12350	TSP2	Thrombospondin 2	
Secreted proteins	X54489	MGSA/ Gro -α	Melanoma growth stimulatory activity	Table1+ Cluster
	M36821	Gro-γ	Cytokine Gro-gamma	Table1
	M26383	IL-8	Interleukin 8	
MMP – matrix metalloproteinase	X05231	MMP-1	Collagenase	Table1
	L22524	MMP-7	Matrilysin	Cluster
	Z48481	MMP14/ MT1-MMP	Membrane-type matrix metalloproteinase 1	
	L23808	MMP-12	Metalloproteinase (HME)	Table1
Cell growth and apoptosis	X54942	CKS-2	CDK regulatory subunit 2	Table1+ Cluster
	M96577	E2F-1	E2F-1, pRB-binding protein	
	H24033	Myc	Myc protooncogene	
	M30448	CK2	Casein kinase II, beta subunit	Cluster
	L03840	FGFR4	Fibroblast growth factor receptor 4	
	U33286	CAS	Cellular Apoptosis Susceptibility	Table1+ Cluster
	D21262	P130	Nuclear phosphoprotein p130	Table1
	T51961	PCNA	Proliferating cell nuclear antigen	
Other genes	R88575	TKT	Transketolase	
	R10066	PHB	Prohibitin	
	T52342	TRA1	Tumor rejection antigen 1	
	Z46629	SOX9	Transcription factor SOX-9	Cluster
	R32511	POLK2K	DNA-directed RNA polymerase I, II and III	Table1
	R36977	TFIIIA	Transcription factor IIIA	Table1

Table 4.2: Classification of the interesting genes from gene cluster G25. The last column indicates if the gene appears in the paper by NEA (see 4.2), either in one of the clusters or in table 1, of upregulated genes in carinoma tissues (see table 1 appendix).

The genes were classified to angiogenic genes, secreted proteins, matrix metalloproteinases, genes related to cell growth and apoptosis and others.
A more detailed description of the genes function and relation to neoplasia, namely colorectal cancer (as summerized in the literature) is described.

1) Angiogenic genes

Angiogenesis is a process of the development of new capillary blood vessels, with the result that the oxygen and nutrient supply is improved. Angiogenesis is of particular significance in the growth of tumors and metastatic spread of solid tumors [54] .

Vascular endothelial growth factors (VEGF) is a growth factor which acts specifically on vascular endothelium. VEGF proteins are the most powerful angiogenic agents that increase vascular permeability and endothelial cell proliferation, migration, and differentiation [55]**.** Many studies have demonstrated the overexpression of VEGF-A in various neoplasms, including neoplasms of the colon [56]. Increased expression of VEGF-A within CRCs was associated with lympathic metastasis, and therefore, this member of the VEGF family may be the most important in determining metastatic spread [57]**.** Furthermore, anti-VEGF-neutralizing antibodys were found to exert a potent inhibitory effect on growth of tumor colon cell lines [58].

Thrombospondin-2 (TSP2) is a multifunctional glycoprotein that is an important component of the extracellular matrix and shows inhibitory properties against angiogenesis [59]. Furthermore, TSP2 expression is correlated with inhibition of angiogenesis and metastasis of colon cancer [60].

2) Secreted proteins

Secreted proteins are known to increase the growth and motility of tumor cells.
The secreted proteins **MGSA/GRO- alpha, GRO-gamma** and **IL-8** are members of the chemokine[7] superfamily, playing a major role in inflammation and wound healing. They are potent mitogens, causing re-entry of cells into the cell cycle. Situated on the same chromosomal location, they are similar at both structural and functional levels [61].

[7] Chemokines are small secreted proteins that stimulate chemotaxis; a movement of cells which is affected by the gradient of a diffusible substance.

GRO chemokines (GRO alpha and gamma) play an essential role in tumor progression and their expression in several cell types is similar to IL-8. MGSA/Gro-alpha is known to be elevated in selected colon cancers [61].

Recent studies reported that IL-8 regulates tumor cell growth and metastasis in carcinoma cells of lung, stomach, liver, and prostate cancer [62]. IL-8 up-regulates inflammatory responses, tumor cell proliferation, and migration in colon epithelial cell lines [63]. IL-8 mRNA expression was shown to be significantly low in nonmetastatic colon carcinoma cells and high in metastatic colon carcinoma cells, suggesting an association with metastatic potential [62].

3) MMP- matrix metalloproteinase genes

The marix metalloproteinases (MMPs) are a family of proteolytic enzymes collectively capable of degrading all extracellular matrix components. In cancer, degradation of the extracellular matrix is a key event in tumor cell invasion and metastasis. In CRCs, various members of the MMP family were reported to be upregulated [64].

MMP-7 (Matrilysin) is overexpressed in the malignant epithelium of approximately 90% of human colonic adenocarcinomas and in the majority of human colon tumor cell lines, and is not expressed in most normal tissues [65].

Poor prognosis in colorectal cancer is associated with overexpression of **MMP-1** [66] and **MMP-7** [67].

The literature indicates that production of **MMP-7 (matrilysin)** and **MMP-14** are regulated by the accumulation of beta-catenin in the Wnt signaling pathway (see introduction) [68].

4) Genes related to cell growth and apoptosis

Proliferation and apoptosis are opposing cellular mechanisms. However, the balance between them is crucial for normal development. One of the hallmarks of transformed cells is their ability to escape the normal regulatory mechanisms that control cell growth and division. Thus, the cell cycle regulatory pathway is one of the most frequently mutated in human cancers [69].

CKS-2 (Cyclin dependent kinase regulatory subunit 2) is an essential component of the CDK proteins, but its precise function remains obscure [69].

E2F-1 is a transcription factor. By transactivating genes required for DNA replication (e.g., DNA polymerase alpha) and cell cycle control (e.g. cyclins), E2F-1 regulates transition of the cell cycle from G1 to S phase [70].

Tumors that have acquired several mutations especially, in the Rb pathway, will be further stimulated to grow in the presence of high levels of E2F-1, since Rb binds E2F and suppresses its action [71].

The **c-MYC** oncogene is involved in cell proliferation, and its deregulated expression can contribute to neoplasia promotion and/or progression [72]. It is a transcription target of the beta-catenin/Tcf (see introduction) and is known to act as a transcription factor of growth-related genes. Under certain conditions and in contrast to its activity as an inducer of neoplasic transformation, c-myc was also found to promote apoptosis [72]. Increased expression of the c-myc gene has been identified as an early event of colon carcinogenesis as well as in other types of tumors [73].

Protein kinase CK2 (casein kinase II) is a multifunctional phosphorylating enzyme, which phosphorylates a variety of proteins. Casein kinase II is thought to regulate a broad range of transcription factors and has been implicated in the regulation of many cellular processes, including DNA replication, transcription, and the regulation of cell growth [74].

It is highly expressed in most cancers and this high expression has been correlated with the progression of the cell cycle [75]. A recent study has shown an existence of a complex, consisting of CK2, beta-catenin and Dv1 protein that enhances the Wnt signaling (see introduction) and growth of mammary epithelial cells [74].

Furthermore, the tumor suppressor gene, APC, which is linked to initiation of human colorectal cancer, directly interacts with CK2.

FGFR4 (fibroblast growth factor receptor 4) is a tyrosine kinase receptor for fibroblast growth factors- a large family of mitogenic growth factors.

The FGFR family consists of four members that regulate a multitude of cellular processes, including cell growth, differentiation and migration, and it has been shown that FGF/FGFR system may play a critical role in cancer development due to its angiogenic potential or direct enhancement of tumor growth [76].

Previous studies have demonstrated upregulation or amplification of FGFR4 expression in a variety of human cancers, including pancreatic cancer, breast cancer, and renal carcinoma [77].

CAS (Cellular Apoptosis Susceptibility) is a protein which participates in or regulates nuclear transport of proteins, and is found to play a role in both proliferation and apoptosis [78]. It is amplified in tumor cell lines of the breast, colon and bladder [79]. Increased levels of CAS correlate with tumor progression, suggesting that the evaluation of CAS expression may be a useful diagnostic tool [78].

Nuclear phosphoprotein P130 is a phosphoprotein. It belongs to the Rb family of proteins and is primarily controlled by phosphorylation events. When non-phophorylated, it binds to E2F family of transcription factors and prevents its activity.

PCNA (proliferating cell nuclear antigen) is a commonly used marker for proliferating cells, a 35 kD protein that associates as a trimer and interacts with DNA polymerases δ and ε ; acts as an auxiliary factor for DNA repair and replication. Transcription of PCNA is modulated by p53 [80].

5) Others genes

The group of genes, with detailed explanation about their function and their relation to cancer (particularly colon cancer) are presented below.

Transketolase is a metabolic enzyme which is involved in ribose synthesis, necessary to build nucleic acids for DNA replication, and thus plays an important role in cell proliferation [81].

PHB (Prohibitin) inhibits DNA synthesis and plays a role in proliferation. It may be a tumor suppressor associated with development and/or progression of some sporadic breast cancers [82].

TRA1 (tumor rejection antigen (gp96) 1) is a molecular chaperone that functions in the folding and transport of secreted proteins in the endoplasmic reticulum (E.R). TRA1 belongs to the family of stress proteins, meaning that by acting as a transcription factor, it protects the environment against stress factors, like glucose deprivation and hypoxia. A possible reason for the contribution of stress proteins to tumorigenicity is their protective role against stress in the hostile environment of rapidly growing tumors [83].

Summary of gene cluster G25

G25 contains genes which are up-regulated in the tumor carcinoma samples. During malignant progression of tumor cells, a series of mutations occurs which lead to a deregulated cell cycle, a decreased ability to undergo apoptosis, and an increase ability of cells to invade surrounding tissue and to metastasize.

Many genes in this cluster play a role in these events and therefore, contribute to the progression to the neoplasic state. Some appear in the paper of NEA (Notterman et.al.) as highly up-regulated genes in tumor (see table 4.2 and Table 1 of appendix) and several are known to be overexpressed in colon carcinoma or other forms of cancer such as *VEGF, Cas,* and *tumor rejection antigen 1 (TRA1)*.

4.3.4 New observations- protocols A and B

My next goal was to use the power of the CTWC algorithm to find new classifications that were not observed by NEA (Notterman et.al.).

S1(G3)

Prior to running CTWC, carcinoma samples were labeled according to two experimental protocols that were known to have been used) (see "prior to clustering"); 16 RNA samples (paired samples 3-6, 8-10,11) were extracted directly, using poly-A RNA ('protocol A'), and the other 20 samples (paired samples 12, 27, 28-29, 32-35 39-40) were prepared by extracting mRNA from the total RNA of the cells ('protocol B').

Clustering the 36 carcinoma samples, using the expression levels of the gene cluster G3, exhibits a clear partition of the samples into two clusters, shown in figure 4.3.3. Cluster **b** contains 20 tissues of protocol B, and cluster **a** contains 14 tissues of protocol A. This separation has two mistakes; both samples of patient 9 were labeled A and appear in the cluster of protocol B.

I conclude that by classifying the tissues and using a relatively small subset of 27 genes from the cluster G3 , I was able to identify a clear separation of the tissues according to the mRNA extraction protocol used.

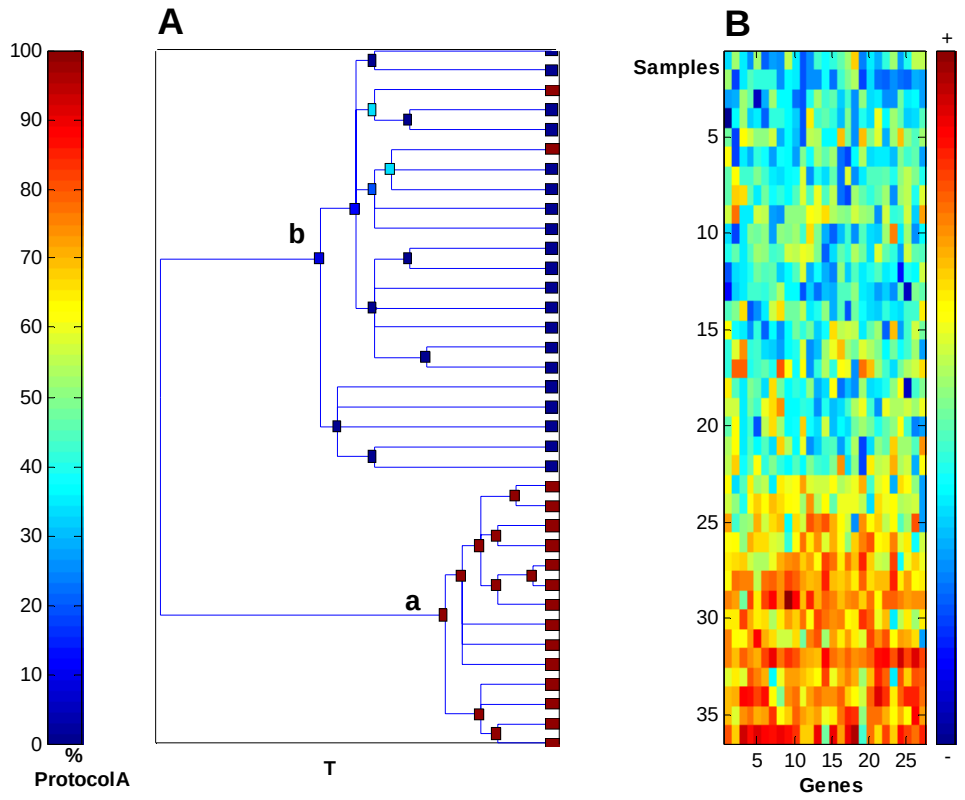

Figure 4.3.3: S1(G3), Separation according to protocol A and B. (A) The boxes are colored according to the percentage of protocol A samples, meaning that the red boxes represent protocol A and the blue samples represent protocol B. (B) The expression level matrix of S1G3. Rows correspond to all the samples and the columns correspond to the genes of cluster G3.

4.3.4 New observations within the tumor samples

S10(G24), S10(G7), S10(G12):

CTWC enables us to find stable clusters within the samples, with no common shared clinical labels. Clustering only the tumor samples (S10, obtained in a previous CTWC iteration) on the basis of their expression over different sets of genes, revealed the following partitions:

Cluster **S10(G24);** S(10) is a cluster of the 18 carcinoma samples (see Figure 4.3.4).

The dendrogram exhibits a clear separation of the tumor samples into 2 clusters. The tumor samples 33, 34, 35, 40 are clustered together (b), and show *high expression levels of the G24 genes* (the list of G24 genes can be viewed in the appendix).

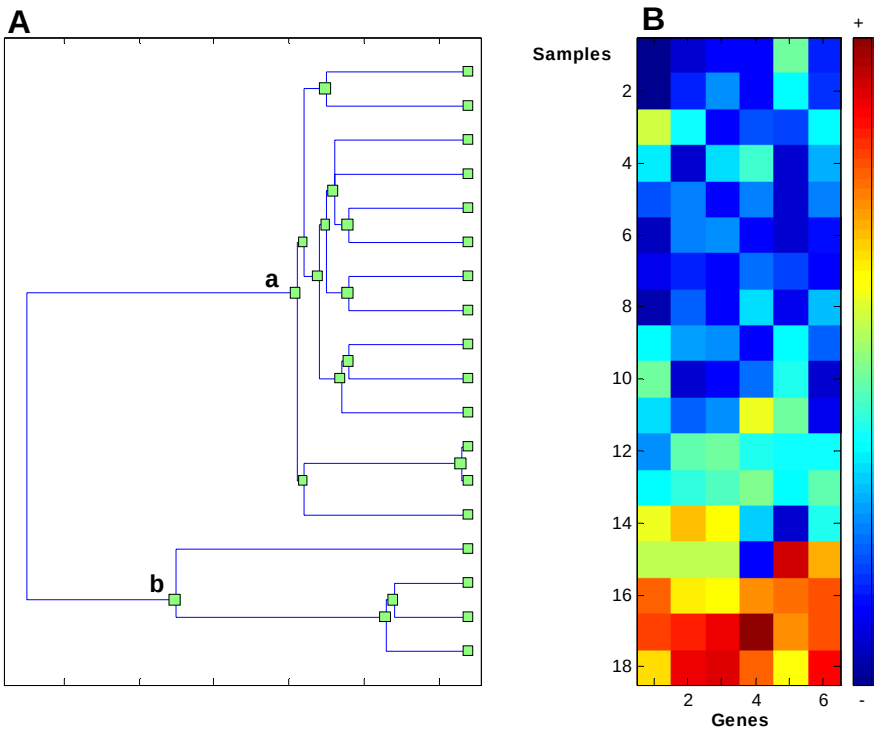

Figure 4.3.4: S10(G24), a separation within the 18 tumor carcinoma samples on the basis of their expression level over gene cluster G24. (A) Note that cluster b contains the tumor samples 33,34,35,40. (B) The expression level matrix of S10(G24). Rows correspond to the 18 tumor samples and the columns correspond to the 6 genes of cluster G24. The matrix shows high expression levels of the tumor samples from cluster b.

The operation **S10(G7)** generated the dendrogram shown in Figure 4.3.5.

Here, the tumor samples 27, 32, 33, 40 are separated from the remaining samples and show *low expression levels of the G7 genes* (the list of G7 genes can be viewed in the appendix).

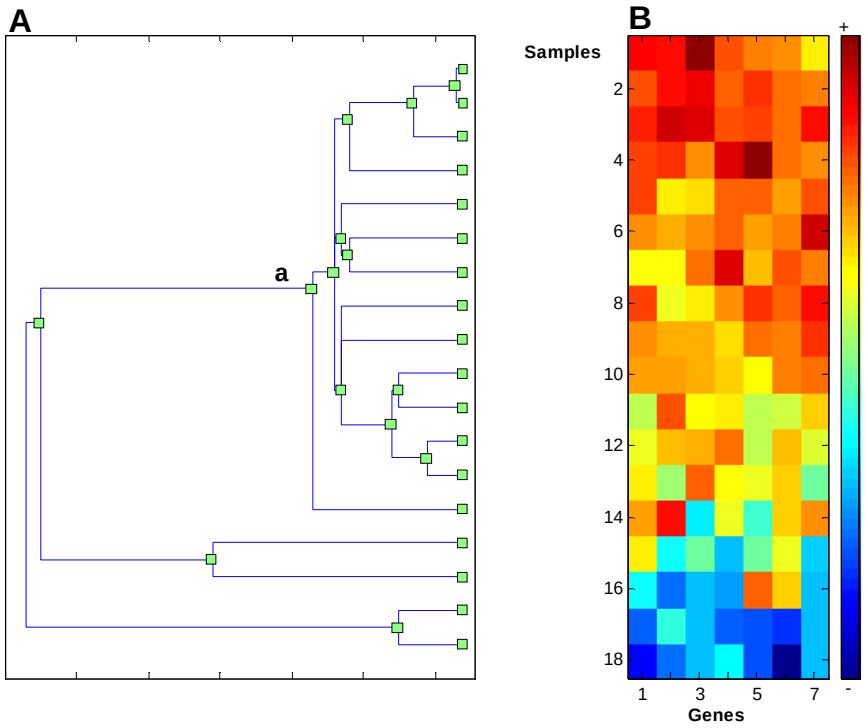

Figure 4.3.5: S10(G7), a separation within the 18 tumor carcinoma samples on the basis of their expression level over gene cluster G7. (A) Note that the samples 27, 32, 33, 40 are separated from the other tumor samples in cluster *a*. (B) The expression level matrix of S10(G7). Rows correspond to the 18 tumor samples and the columns correspond to the 7 genes of cluster G7. The matrix shows high expression levels of the tumor samples from cluster a and low expression levels from the remaining samples.

S10(G12) generated the dendrogram in figure 4.3.6. In this case, the tumor samples 33, 34, 35,12, 40 are clustered together (b) and show *high expression levels of the G12 genes* (the list of G12 genes can be viewed in the appendix).

I conclude that tumor samples 33, 40 and 35 were repeatedly seperated from the remaining tumor samples, which implies that these samples share some common characteristics, perhaps representing a true biological meaning. However, due to lack of additional information about the patients I was unable to determine the biological origin of this separation.

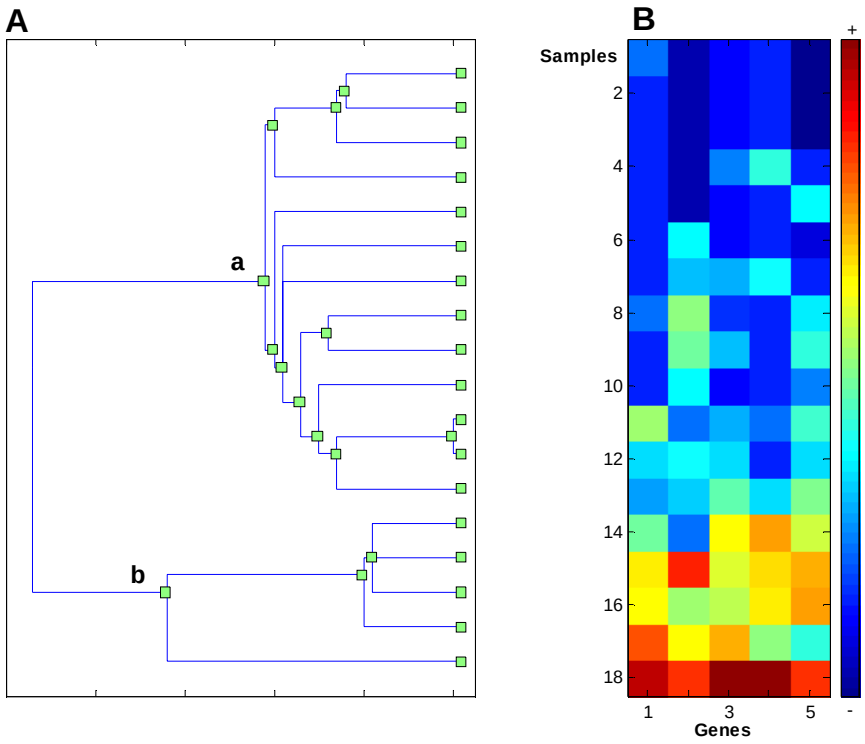

Figure 4.3.6: S10(G12), a separation within the 18 tumor carcinoma samples on the basis of their expression level over gene cluster G12. (A) Cluster b contains the tumor samples 33,34,35,12,40. (B) The expression level matrix shows high expression levels of the genes in the tumor samples from cluster b.

4.3.5 Summary of the results

By using the advanced CTWC method, I was able to obtain a separation of the adenoma, carcinoma, and normal samples on the basis of their differences in gene expression.

Some of the genes that were found, correspond to the genes that were found previously by NEA (Notterman et.al.) and many new interesting genes emerged, some of which were shown by the literature to be biologically meaningful and may be good candidates as markers for classification in the future.

Using the advantage of CTWC in mining gene expression data, I was able to find new classifications of the samples that were not shown by NEA; The samples were classified according to protocol A and B, two distinct protocols for mRNA extraction.

Furthermore, I was able to find stable clusters within the tumor samples that repeatedly separated three tumor samples (33, 35, 40) from the remaining tumor samples, implicating a common feature that has not yet been determined but may be biologically significant.

Chapter 5

Genome-Wide Comparison of Keratinocytes and Squamous Cell Carcinoma Response to UVB Irradiation

5.1 Introduction

The skin is the largest organ in the body and is the primary interface between the environment and the body [84]. The incidence of skin cancer has been increasing over the past decades. It is estimated that in the US alone, about a million new cases occur annually, equivalent to the incidence of all other types of cancer combined. [84]

Although skin cancers are the most common of all malignancies, they are usually not lethal, accounting for less than 0.1% of cancer death. They tend to occur on sun exposed portions of the skin such as the ears, lower lip, nose and hands and are relatively easy to detect and cure by surgery or drugs. [85]

Individuals with light skin (who sunburn easily and suntan poorly), with low degree of protective skin pigmentation, and those living in sunny climates are at greater risk of developing skin cancer [86]. The incidence of skin cancer is expected to rise further, emphasizing the importance of increased prevention and treatment efforts.

The epidermis

The epidermis functions as a barrier, keeping harmful substances out and preventing water and other essential substances from escaping the body. The epidermis is differentiated into five layers all of which are susceptible to sun light-induced cancer (see Figure 5.1). **Keratinocytes** constitute the majority of skin cells and contain the protein keratin. Keratinocytes routinely proliferate, undergo various developmental changes and eventually are shed from the skin (see figure of skin). The three major cell types of the skin are **basal cells** and **squamous cells**, derived from keratinocytes and **melanocytes**. At the base of the epidermis lie the round basal cells, and their progeny towards the surface are flattened, squamous cells. Melanocytes are the cells which produce the protective pigment melanin. They are located in the basal layer and have numerous extensions that reach outward. [86]

The epidermis is a highly compartmentalized, dynamic and homeostatic tissue.
The epidermal keratinocytes are the most susceptible to damage from UV exposure, due to their localization relative to the skin surface, in the epidermis layer. Therefore, most skin cancers in humans arise from the epidermis. [87]

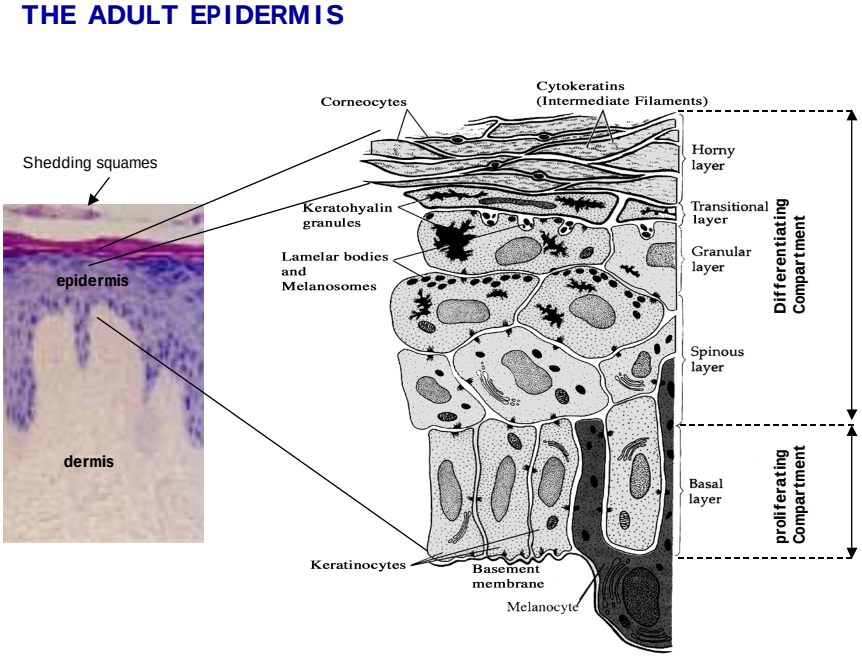

Figure 5.1.1 A view of the epidermis structure. The epidermis is composed of many layers of cells. In the basal layer (the living epidermis), new cells are constantly being reproduced, pushing older cells to the surface. As skin cells move farther away from the basal layer they flatten and shrink, eventually moving out of the basal layer to the horny layer (the dead epidermis). After serving a brief protective function, the keratinocytes are imperceptibly shed off. This process of a living cell's evolution, called keratinization, takes about 4 weeks.

Forms of skin cancer

Skin cancers can be divided into two types corresponding to the major types of skin cells:

1. **Melanoma**: The most deadly, can arise in young adults and involves melanocyte cells only. Little is known about how these tumors are triggered. Fortunately, they are also the least common.

2. **Non- Melanoma**: Basal cell carcinoma (BCC) and Squamous cell carcinoma (SCC), derived from keratinocytes; Actinic Keratosis (AK) and Keratoacanthoma (KA) both of which are precancerous states of squamous cell carcinoma and other types of skin malignant neoplasms.

SCC and BCC usually appear at later stages of life (approx. 50 years or later) and are by far the most common of skin cancers [88]. The pre-cancers Actinic Keratosis (AK) and Keratoacanthoma (KA) are characterized by reddish patches of proliferating and differentiating cells. These pre-cancers usually regress, but can also progress to SCC; a tumor which can progress and metastasize. In contrast, BCCs are much less invasive and rarely metastasize, though they invade locally [86]. They are the most common form of skin cancer and are at least three times more frequent than SCC. [87]

The UV spectrum

The induction of skin cancer is mainly caused by the accumulation of mutations caused by UV radiation.

UV radiation is divided into three sections: UVA (320-400nm), UVB (280-320nm) and UVC (200-280nm) (see figure 5.2). UVC is effectively blocked from reaching the earth's surface through being absorbed by the ozone layer. The less energetic UVA penetrates into the skin: however, it was found to be weakly carcinogenic.

UVB however, is absorbed into the skin, penetrating the epidermis (10% transmission) and is energetic enough to cause DNA damage [87].

Therefore, UVB radiation represent biologically relevant wavelengths to study the cellular responses and their involvement in SCC and BCC.

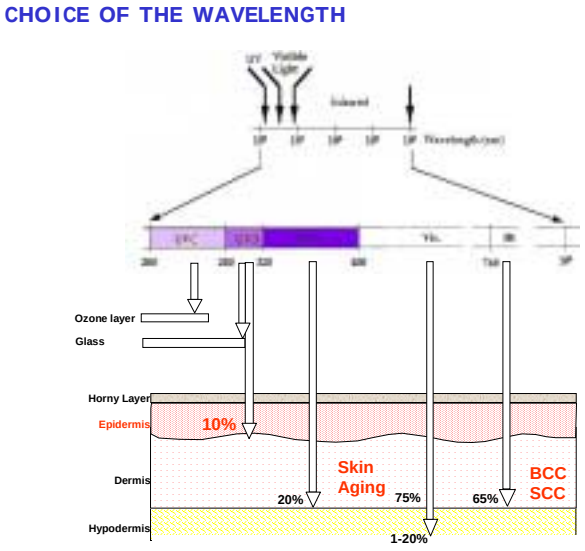

Figure 5.1.2: Interaction of solar radiation with the skin. The longer the wavelength is, the deeper is the penetration into skin. UVC is blocked by the ozone layer. UVA is weakly carcinogenic. Only UVB is energetic enough to cause DNA lesions; it is absorbed into the skin, producing erythema, burns, and eventually skin cancer.

UV "signature" mutations

Ultraviolet light creates mutations where pyrimidine bases (cytosine or thymin) lie adjacent to one another, forming pyrimidine dimers (see figure). The pyrimidine dimers interfere with DNA replication and lead to UV mutations, known as "signature" mutations for UV that can accumulate over time. About two thirds of these mutations are C-to-T substitutions, and about 10 percent of these changes occur at two adjacent Cs, with both bases changing to Ts. [86]

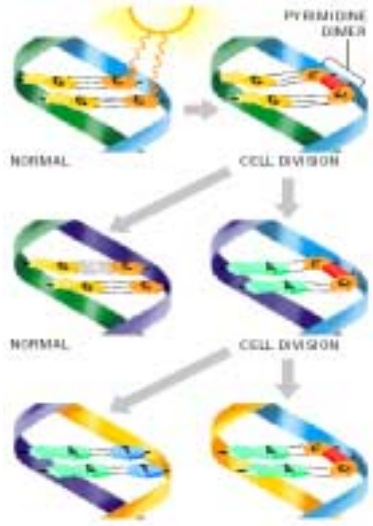

Figure 5.1.3 Mechanism of UVB mutations at dipytimidine sites, e.g. adjacent cytosines. UV breaks down the chemical bonds in adjacent pyrimidine bases. New bonds then form (red) that link the disrupted bases, creating the pyrimidine dimer. Replication will lead to a mismatch mutation in one strand (middle right) and a normal, unaffected strand (middle left). Continued replication will repeat the error (bottom right). On the opposite strand (bottom left), a mutation has been created (C-to-T mutation). The dimer may eventually be eliminated by "excision repair", but the C-to-T mutation is permanent.

UV induction of skin cancer is a multistage process

UV radiation induces genetic alterations in keratinocytes, leading to their neoplastic transformation (see Figure 5.4). Even though cellular mechanisms exist to repair the DNA damage or to induce apoptosis to remove severely damaged cells, the additive effects of mutations in genes involved in these mechanisms, or in control of the cell cycle, can lead to abnormal cell proliferation and thus to tumor development. [89]

The ***p53 tumor suppressor gene*** appears to be one of the key UV-responsive genes, and mutation in this gene is thought to initiate the process of skin carcinogenesis. Mutations were shown to appear in the pre-cancer lesions and were found in over 90% of human SCCs and ~60% of human BCCs. [87]

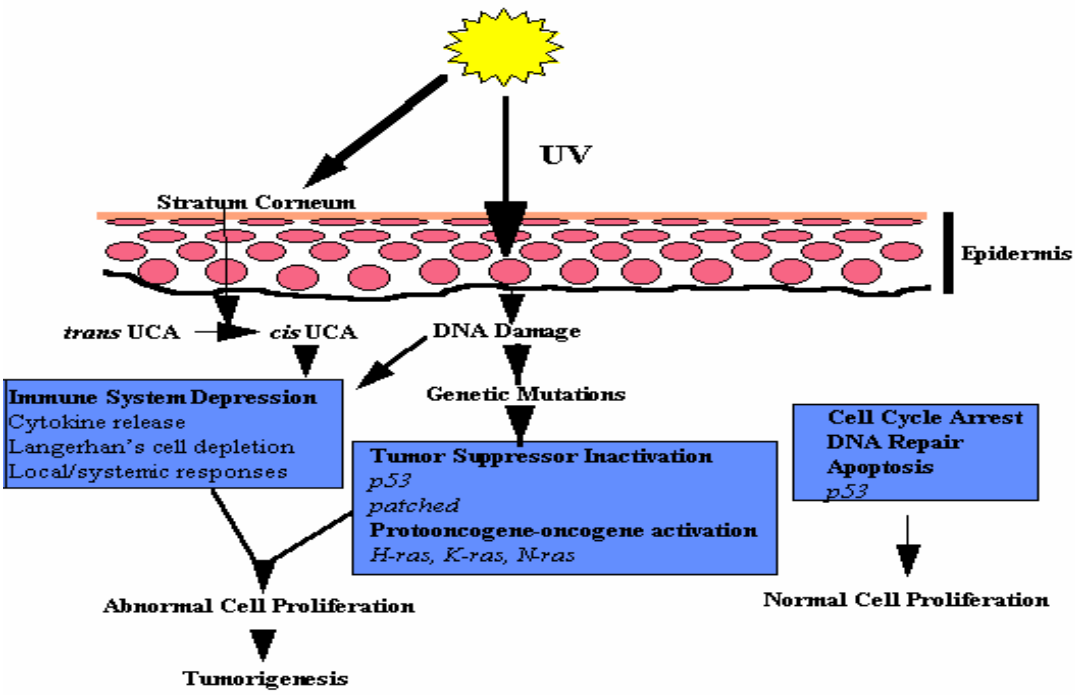

Figure 5.1.4 Pathways involved in skin cancer development.

Cells containing *p53* mutations are relatively more resistant to UV-induced apoptosis, and therefore acquire a growth advantage. Furthermore, the mutated cells expand at the expense of the normal surrounding cells that were damaged and underwent apoptosis, leaving space for p-53mutated cells to grow.

Another tumor suppressor gene known as ***patch***, an integral membrane protein, has been implicated in the development of basal cell carcinoma (BCC) [90].

In addition it was discovered that UV radiation depresses the immune response in the skin, which can permit the growth of emerging tumors produced by UV-induced DNA damage [87]. The suppression of the immune system and prevention of T-cell responses results from induction of suppressor T cells, either by damaged Langerhans cells or inflammatory macrophages that enter the skin following UV exposure. A mechanism for the suppression of the immune system may be the release of cytokines such as IL-10, TNF-alpha. These are known to be secreted by keratinocytes after UV damage [91].

5.2 Comparison of NHEK and SCC response to UVB

This work was done in collaboration with Dr. Jean-Eudes Dazard and Prof. David Givol from the Department of Molecular Cell Biology.

Our aim was to gain insight into the tumorigenesis of normal epidermal cells into Squamous Carcinoma Cells (SCC). We compared the response to UVB of Normal Human Epidermal Keratinocytes (NHEK) vs. their transformed counterpart, using biological and molecular profiling.

We show that NHEK are more resistant than SCC to UVB-induced apoptosis and this resistance is mainly due to the protection from cell death by secreted survival factors, such as DNA repair genes (e.g. GADD45, ERCC1) and cell cycle regulators (e.g. p53, MDM2). The evasion of apoptosis by keratinocytes, subsequent to UV-related DNA damage may potentially lead to undesirable effects by permitting the survival of mutant cells that accumulate mutations and may be the seeds for future cancer development.

DNA microarrays were used in order to compare differential gene expression of the two cell types. For the analysis, I used advanced unsupervised clustering methods that were developed in our lab, namely SPC and CTWC (see methods-chapter 3), to detect gene groups that differentiate the normal samples from the tumor samples.

5.2.1 The experimental system

Keratinocytes were derived from a normal human Caucasian individual and the SCC12B2 cell line was derived from a human facial Squamous Cell Carcinoma (SCC). Cells were rinsed twice with pre warmed PBS^- at 37°C and then subjected to a single UVB exposure at a dose of 400 J/m^2.

RNA was extracted at the following times: 0.5h, 3h, 6h, 12h, 24h post irradiation for NHEK and at 6h, 12h post irradiation for SCC12B2. For NHEK, four non-irradiated controls (0h, 0.5h, 12h, 24h), and for SCC12B2, only one control (0h) was used. cRNA preparation and microarray hybridization were carried out according to the supplier's protocol(Affymetrix®, Santa Clara, CA), using Genechip® HG-U95Av2.

5.3 Results and discussion

In this section I focus on the analysis of the gene expression data obtained from the DNA chip experiments.

Many of the molecular events associated with transformation and metastatic tumor progression in human SCC remain unknown. Therefore, the use of microarrays may reveal underlying genetic differences between normal and carcinoma cells that have not yet been detected in biological study.

The findings of this analysis indicate that NHEK are more resistant than SCC to UVB-induced apoptosis and this resistance is mainly due to the protection from cell death by secreted survival factors, namely growth factors, chemokines and other inflammatory mediators, which play a role in immunologic reactions. These protective factors permit the survival of UVB damaged keratinocytes that are subsequently able to progress and develop into cancer.

5.3.1 the original data sets

The gene expression data was composed of two sets of DNA chips. Each chip contains probes for ~12000 genes.

The first data set contained 9 chips, dedicated to NHEK; 4 control samples (non-irradiated, at 0h, 0.5h, 12h, 24h time points) and 5 UVB irradiated samples (at 0.5h, 3h, 6h, 12h, 24h time points). The second data set contained 3 SCC samples; one control (non-irradiated) and two UVB irradiated samples (6h, 12h). Gene expression values lower than 30 were adjusted to 30 to eliminate noise from the data. Looking at Figure 5.3 we can observe that values above log2(30) show consistent expression at 0h and 24h in the control (i.e. scatter close to the y=x line).

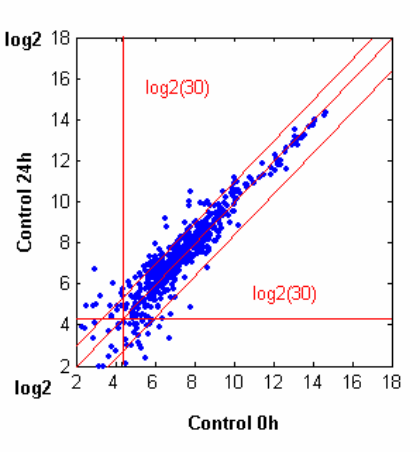

Figure 5.3: Scatter plot of regulated genes, control 0h versus control 24h in NHEK. The threshold value of 30 was chosen since the extent of scatter is greater for values below 30.

Values were then log2 transformed. Expression level ratios were determined for each gene, at each experimental time point, with respect to the average of the four controls in NHEK data set, and to the control at time 0 in the SCC data set.

5.3.2 UVB modulated genes in NHEK and SCC

To select UVB modulated genes, I applied an arbitrary filtering on the ratios of gene expression, of two fold change in at least two time points. 251 and 788 genes passed this filter in NHEK for the up and downregulated genes, respectively. For practical reasons, I downsized the downregulated list to those that showed threefold change of expression and ended up with a list of 322 downregulated genes in NHEK (see Figure 5.3.1A). Similarly, a list of 127 upregulated and 117 downregulated genes were drawn for SCC cells (Figure 5.3.1A). Table I contains selected genes of interest as they appear in the full lists (see Table I at end of chapter 5 and the full lists of genes, Table II and III, in the appendix). The genes from Tables I, II and III are divided into classes according to their biological activity within the cell.

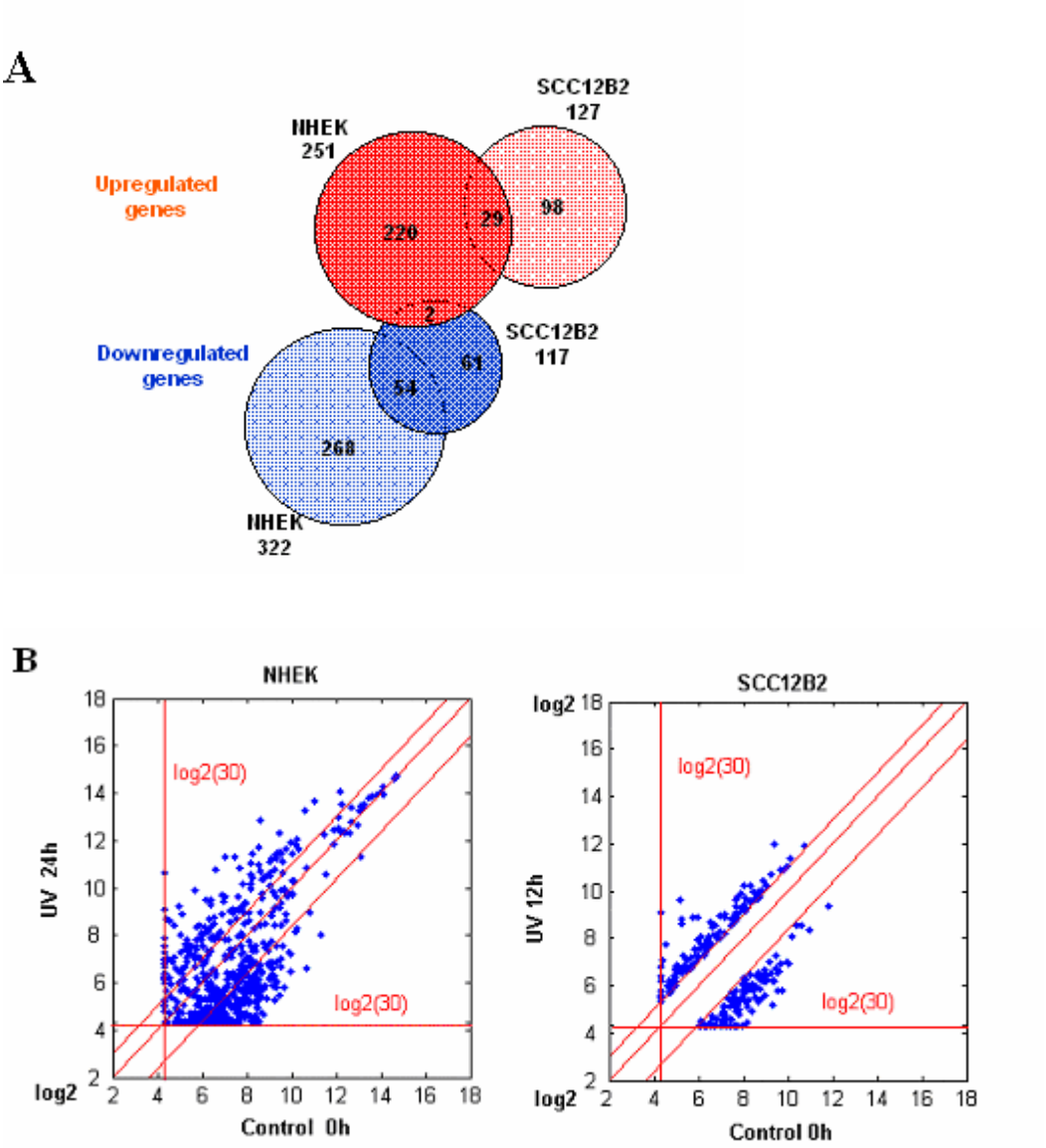

Figure 5.3.1: (A) Venn diagram of up and downregulated genes from NHEK and SCC. (B) Scatter plots of the up and downregulated genes. *Left;* control 0h versus UVB 24h in NHEK. *Right:* control 0h versus UVB 12h in SCC.

Scatter plot of expression values of the genes modulated in NHEK (573) and SCC (244) confirmed the choice of the thresholds (see Figure 5.3.1B). In control cells, when plotting control 24h vs. control 0h, the majority of gene expression levels remained contained between the twofold (Figure 5.3 *upper line*) and threefold (Figure 5.3. *lower line*) borderlines, as opposed to their expression in UV-treated cells (Figure 5.3.1B). This implies that cells undergo a significant change in expression as a result of UV exposure.

Intersection of lists of genes, as visualized by Venn diagrams, showed that 29 out of the 127 upregulated genes in SCC (23%) are common with NHEK, and 54 out of the 117 downregulated genes in SCC (46%) are in common with NHEK. This indicates high level of similarity in the response to UVB of both cell types, reflecting their common origin.

5.3.3 Classification of genes

UVB is the most abundant carcinogen to which we are most exposed. The skin is the target for this carcinogen and in the long run, may develop skin tumors.
Our aim was to learn about the consequence of this exposure in keratinocyte cells.
Functional classification shows the complexity of the transcriptional response in that many classes of genes appear to be regulated, several of each including both up and down regulated genes without an evident pattern of activation or repression (Table I).
However, focusing on specific functional activities (e.g. repair, transcription) illustrates the relationship between UVB and cell response.

repair

The major DNA damage by UVB is the promotion of pyrimidine dimmers, which result in mutations (see introduction). To correct these mutations, the DNA repair machinery is induced. In our list, several genes related to DNA repair are upregulated, including ERCC1 and ERCC2, two enzymes involved in DNA nucleotide- excision repair (NER), a way to remove the damaged bases. Interestingly, in the disease *'Xeroderma Pigmentosa'*, which is due to deficiency in the ERCC family, the exposure to the sun leads to a great damage due to lack of repair.
In addition, upregulation is observed in the multifunctional genes GADD45A and GADD45B, which are known to regulate DNA repair, growth control and apoptosis in keratinocytes following UV exposure and are targets of p53 (class #3, Table I) [92]. Several histones, among which are H1X, H2A's and H2B's, are also found to be upregulated (class #12, Table I). The DNA repair machinery requires chromatin modification and remodeling for repair of DNA lesions. Recently, evidence for a role for histones H2B's has been found during repair of UV-induced DNA damage in yeast [93], and mice lacking *H2AX* were shown to be highly sensitive to radiation [94].

Apoptosis

Another defense mechanism of the skin is to drive to apoptosis those cells which are unworthy of repair or cells which escaped DNA repair (see introduction).

Our results show that UVB induce simultaneously the expression of several pro and anti-apoptotic genes in keratinocytes (class #2, Table I). Caspase-8 is decreased (class #2, Table I) and there is an increase in expression of anti-apoptotic genes such as *BCL-2*, *TNFAIP3*. (class #2, Table I).

Moreover, two Heat Shock Proteins (*HSP70* and *HSP90*) are also induced (class #2, Table I). These HSP are inducible by stressful events, including UVB irradiation in NHEK *in vivo*, and are thought to protect cells from apoptosis [95].

Overall, the balance between the forces that push towards apoptosis and those that push towards the survival of the cell will determine the fate of the cell.

Furthermore, the response of keratinocytes to UVB illustrates the balance between mechanisms of repair and apoptosis. At first, effort is made to repair the mutations by DNA repair (NER) and to activate all the necessary enzymes for this. However, repair is never complete and then the damaged cells are marked for apoptosis, to avoid mutated cells, which may be pro-cancerous.

Survival factors

In addition to DNA repair, keratinocytes activate many genes, which protect cells from death and are known as survival factors. These are secreted growth factors, chemokines and other inflammatory mediators, which play an important role in immunologic reactions (*in vivo*). This group includes CXC chemokines family: IL-8, GRO-1 (alpha), GRO-2 (betta), GRO-3 (gamma), SCYA20, the prostaglandin endoperoxide synthase COX-2, IL-6, IL-1β, HB-EGF, S100A9 and INSL4 (class #6, Table I).

Chemokines (e.g. IL-8, GRO1/2/3, SCYA20) constitute a large superfamily of secreted proteins that cause direct migration of leukocytes (see colon cancer results; S1(G25)). The members of the CXC subfamily contains the first two cysteine residues (at the 5' region), separated by a single nonconserved amino [96].

Altogether, these secreted factors promote tumor development, angiogenesis and metastasis but most importantly they are survival factors, which may participate in the protection of NHEK from apoptosis, after exposure to UVB.

COX-2 is an enzyme which converts arachidonic acid to prostagladins and is believed to play a significant role in tumorigenesis due to its anti-apoptotic effect (see colon cancer introduction).

IL-8, GRO-1 and COX-2 are known together to promote tumor growth, metastasis, and angiogenesis in epithelial cancers [97], with a notable implication in colorectal and squamous cell carcinoma [98].

The transcription of these secreted factors is modulated, in part, by the nuclear factor NF-kB transcription factor (class #11, Table I). NF-kB is known to be involved in the inflammatory immune responses and activation of NF-kB is associated with the transcription activation of secreted factors such as IL6-, IL-8, IL-1 and GRO-1 (alpha) [99].

In addition, UVB irradiation increased the levels of the growth factors: *Insulin-like growth factor 4,* a protein with sequence similarity to insulin that is capable of eliciting the same biological responses, and *HB-EGF,* a ligand of EGF receptor and a cell mitogen that is expressed primarily by interstitial and vascular smooth muscle cells (class #6, Table I), favoring keratinocyte proliferation. Both are anti-apoptotic.

Altogether our results provide indications in favor of a protective response and resistance against apoptosis.

Transcription

In the regulation of transcription, UVB downregulated the expression of numerous transcription factors among which are *c-MYC*, *E2F3* and *E2F5,* that under certain conditions may be pro-apoptotic. In addition, six transcriptional repressors are up-regulated (*ATF3*, *ATF4*, *DRAP1*, *TSC22*, *SAP18*, *ID2*) (class #11, Table I). Overall, this suggests a balance in favor of a general down regulation of the transcriptional machinery. However, UVB irradiation did increase the expression of immediate early transcription factors such as *FOS*, *JUN*, *JUNB* and *JUND*(class #11, Table I).

Other genes

Our data show an increase in *matrix metalloproteinases (MMPs); MMP10* and *MMP1* that are known to be involved in vascular permeability and progression of tumor invasiveness and metastasis (see colon cancer results, S1(G25)).

P63 is a *p53* homolog that is expressed as Δ*Np63* (with an N-terminal deletion) in keratinocytes. This gene is believed to be required for the maintenance of keratinocyte stem cells *in vivo* and *in vitro [100]* and is a marker for these stem cells. Throughout differentiation of keratinocyte stem cells, the expression of *p63* decreases. *p63* is a dominant negative partner that represses *p53* and therefore enables cells to undergo proliferation. Once *p63* is downregulated, *p53* is activated, the proliferation rate decreases as cells undergo differentiation.

Interestingly, the downregulations of *p63* (-18 fold) upon UVB irradiation is similar to its downregulation upon differentiation. This downregulation of *p63* is delayed until 24h post irradiation (class #9, Table I). It is conceivable that its high initial levels of expression, sustained during the first 12h post irradiation, maintained the proliferation of keratinocytes during this period of time.

5.3.4 Cluster analysis

A. SPC clustering analysis

I applied the SPC clustering algorithm (see chapter 3-methods) on the 573 up and downregulated genes from the NHEK data set. Each of the 573 genes is represented by its expression levels at 6 experimental time points (0h, 0.5h, 3h, 6h, 12h, 24h). The resulting data set is an expression matrix A in which each row i ($i = 1,2,...,573$) represents a gene vector, each column j ($j = 1,...,6$), a time point over the time course experiment (sample vector) and where each element A_{ij} of A, is the $\log_2$ transformed signal of the gene i, measured at time point j. Prior to clustering, the genes were centered about the mean and normalized. The dendrogram is presented in figure 5.3.2A. Next, from the reordered expression matrix, after clustering, I subtracted from each component C_{ij} the first component C_{i1} such that each gene vector starts with a zero value in its first component, corresponding to time point 0h, and then removed the 0h column (Figure 5.3.2B).

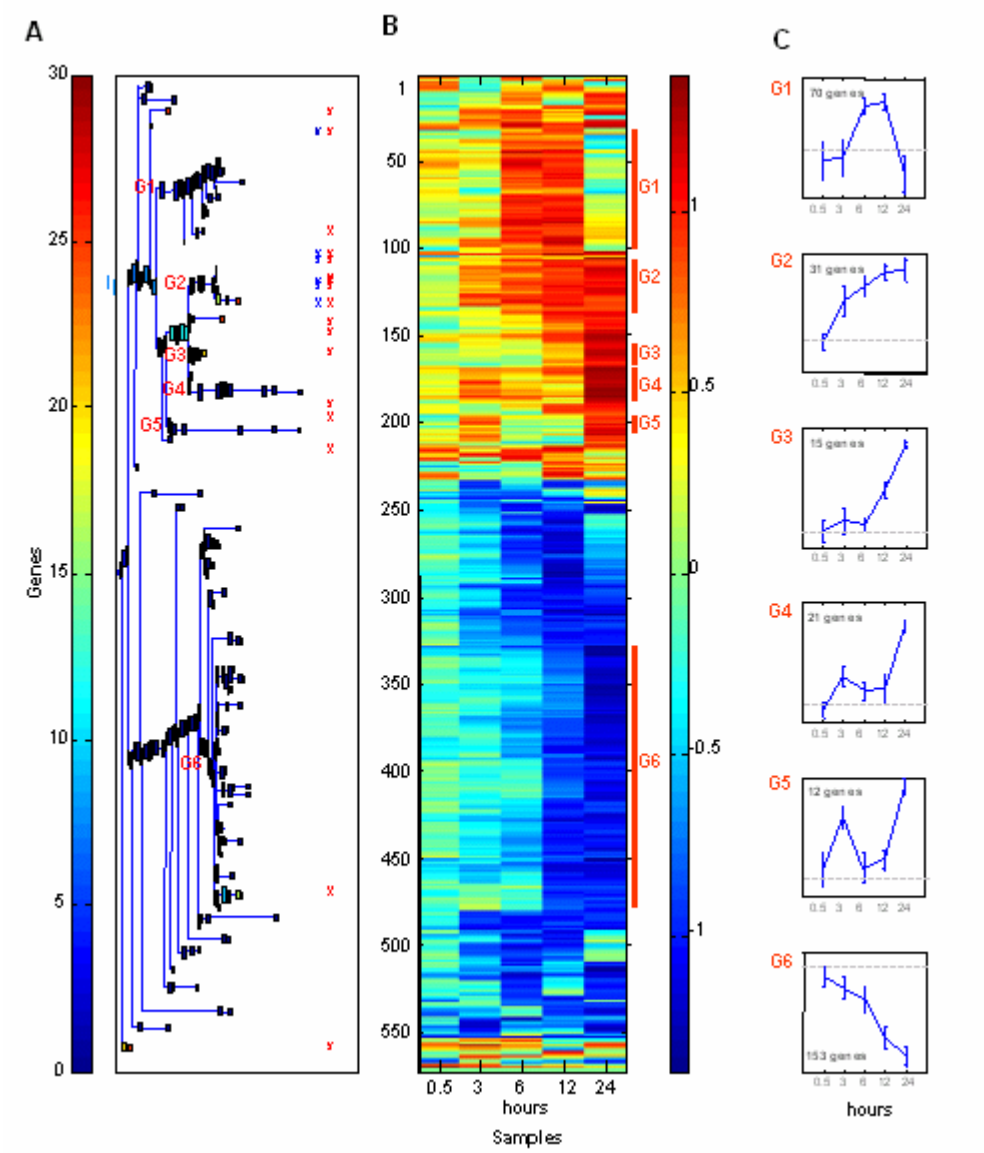

Figure 5.3.2 Clustering results using super-paramagnetic clustering (SPC) for the 573 genes that were up and downregulated from the NHEK data set. (A) Dendrogram with six stable clusters that were identified and marked G1-G6.
Each box is colored according to the percent of genes, belonging to class #6 (Table I and II, 'cytokines and growth factors'). In addition, genes of class #6 are marked by red crosses. Genes belonging to the CXC family members are marked by blue crosses. The color represents induction (red) or repression (blue). (B) The expression matrix after substracting from each gene expression level the first column (control 0h) and subsequently removing the first column. (C) Gene profiles of the six clusters G1-G6.

The clustering operation revealed a clear-cut partitioning between up and downregulated genes (Figure 5.3.2A & B). I identified six stable clusters of genes: one large cluster (G6, of 153 genes) contains downregulated genes, and five contain upregulated genes (clusters G1-G5, Figures 5.3.2A & B). Figure 5.3.2C shows the expression profiles of the genes in each of the clusters G1-G6.

To visualize how certain genes that belong to a particular biological function are distributed among the clusters, I colored each box in the dendrogram according to its proportions of genes belonging to the functional class #6, which includes secreted proteins related to inflammation (labeled '+', Table I) and chemokines of the CXC family. In addition, I marked the genes of class #6 by red crosses (Figure 5.3.2A). Six out of the 31 genes (~ 20%) of cluster G2 are of class #6, 4 of these (*IL-8*, *GRO-2 (beta)*, *GRO-3* (gamma) and *SCYA20*) are members of the CXC family (66% efficiency of the CXC family). Interestingly, additional genes cluster together in G2, among which five are transcription factors, two are major transcription factors of *IL-8* (*JUNB* and *JUND*) (Table I & Figure 5.3.2A), and five are DNA repair-related genes (*GADD45A*, *TOB1*, *H2AA*, *H2BC*, *H2BQ*), suggesting that they may act in concert in response to UVB radiations.

B. *Coupled Two Way Clustering (CTWC)*

I applied the Coupled Two Way Clustering method (CTWC) on the gene expression data sets (see chapter 3 – methods). My aim was to identify groups of genes that partition keratinocytes from SCC and to find other possible partitioning of the data based on various groups of samples, such as partitioning of UVB exposed samples versus non-UVB exposed samples from both SCC and NHEK cell types.

I performed the analysis on the DNA chip data sets taken from all samples of both NHEK and SCC gene expression experiments (see the original data sets). I merged the data sets to generate a complete gene expression matrix. Values less than 30 were rescaled to 30, $\log_2$ transformed, centered and normalized. I selected only genes with a standard deviation (of the transformed data) greater than 1. This yielded an expression matrix of 1269 genes by 12 samples. As for the choice of the optimal K-nearest neighbors parameter for the *APC* clustering algorithm (see chapter 3-methods), I used K=10 for the genes and the value of K = 4 for the samples. The first iteration of CTWC, denoted G1(S1), identified 33 stable gene clusters.

I will focus on 5 partitions that show the most interesting and significant separations of the samples (see Figure 5.3.3)

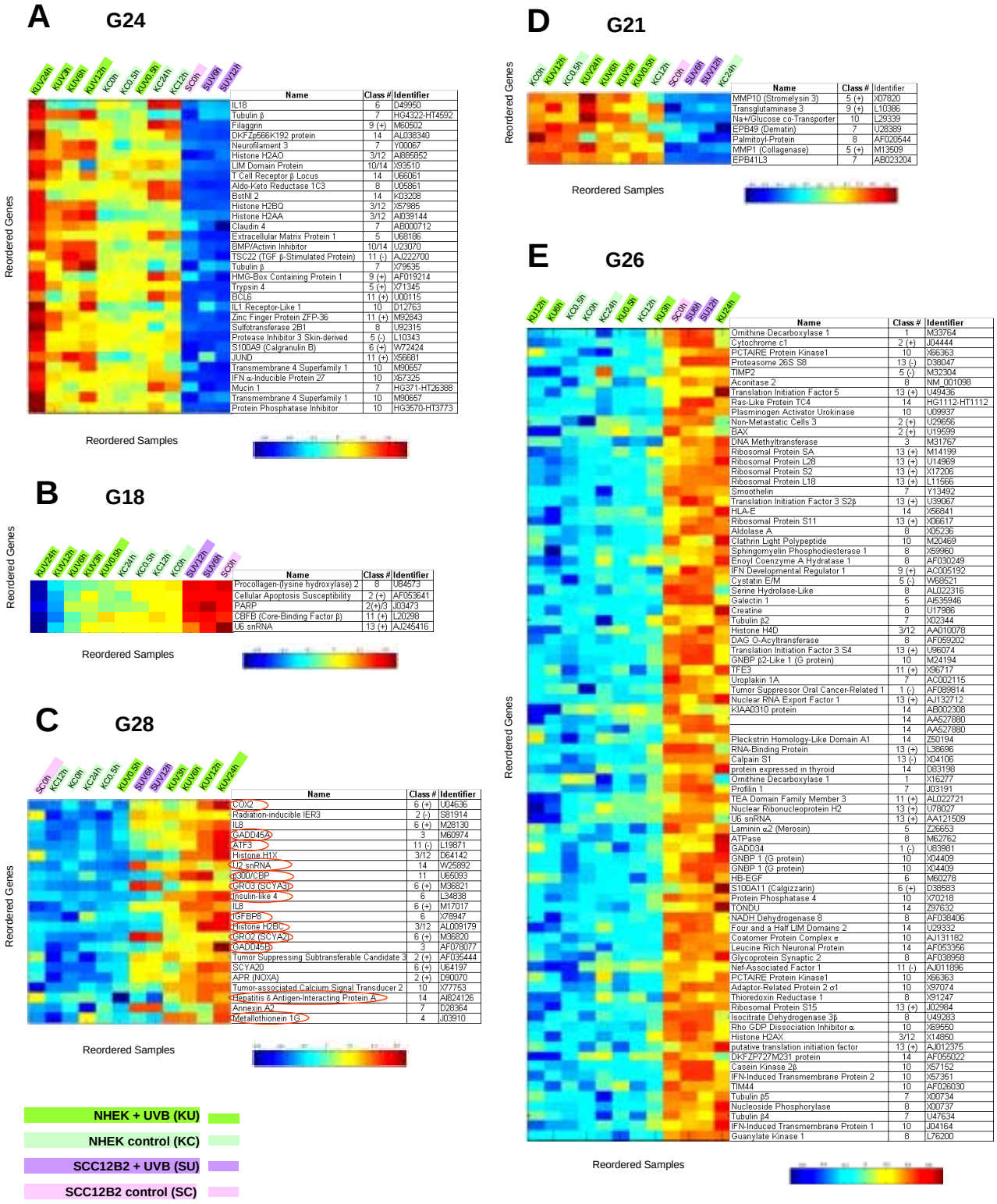

Figure 5.3.3 ***Coupled Two Way Clustering*** **(CTWC) showing partitions of genes and samples of NHEK and SCC. The five most interesting partitions are presented and marked A-E. Circled genes are discussed in the "5.3.3 Classification of genes" section.**

Cluster G24 (figure 5.3.3A) contains 31 genes that show a clear partitioning of the SCC from NHEK. The expression levels of the G24 genes is low in SCC, as opposed to NHEK which show higher expression levels that further increase after UVB irradiation, especially after 24h. This cluster includes genes which play a role in inflammation; a homolog to *IL-1R*, IL-18, a protease that degrades membrane proteins *TRYPSIN-4* which may promote migration of cells, and MUCIN-1, the latter being a notable tumor-associated antigen in breast cancer, and believed to play a role in tumor progression and metastasis.

Cluster G18 (Figure 5.3.3B) contains 5 genes that are gradually downregulated as a result of UVB exposure in NHEK cells, whereas in SCC they exhibit constitutive high level of expression in all the samples. G18 contains two pro-apoptotic genes: *PARP;* an enzyme that modifies various nuclear proteins and has been implicated in various cellular processes, including transcription, neoplastic transformation and apoptosis, and the Cellular Apoptosis Susceptibility gene (*CAS*); a protein which regulates nuclear transport of proteins. This gene is known to correlate with tumor progression and was introduced in the chapter on colon cancer (see chapter 4, results; S1(G25)).

Altogether, this supports our biological observations that SCC are more sensitive to apoptosis and therefore exhibit higher levels of pro-apoptotic genes. This unexpected outcome is precisely the opposite of the common understanding that tumor cells are more resistant to apoptosis. However, this is a particular case of tumor cells that doesn't necessarily apply in other transformed cell lines.

Cluster G28 (Figure 5.3.3C) contains 22 genes that exhibit low expression levels in both SCC and NHEK before irradiation (controls) and upregulated expression after irradiation. This gene cluster is rich in genes of class #6, and to a lower extent, in DNA repair-related proteins (classes #3 & #12).

Looking at the UVB exposed samples only, some of the genes show higher expression levels in NHEK than in SCC samples, particularly in 12h and 24h (these genes are marked in circle). These genes include survival factors, e.g. members of the CXC family; GRO-2 & 3 (GRO-beta & gamma) and IL-8, COX-2, growth factors Insulin-like 4 & IGFBP8, DNA-repair genes GADD45A & GADD45B and transcription factor ATF3, supporting our theory (see sec. 5.3.3) of secretion of survival factors by NHEK as a mechanism to avoid apoptosis.

Cluster G21 (Figure 5.3.3D) contains 7 genes that show low expression levels in SCC and one control sample of NHEK. In contrast, they show high expression levels in NHEK. This cluster contains the metalloproteinases *MMP1* & *MMP10* that mediate tumor invasiveness and enable vascular permeability. In addition, they facilitates the release of other mediators of inflammation (see more about MMPs in results of chapter 4; S1(G25)).

Cluster G26 (Figure 5.3.3E) is a large cluster of 82 genes that separates SCC samples from all the other NHEK samples. However, not entirely, since sample UV24h from NHEK is grouped together with SCC samples. The SCC samples, together with UV24h from NHEK, show a constitutively higher expression level of G26 genes than in NHEK, where they remain low. The underlying assumption is that at longer periods of time after irradiation (24h), NHEK begin to behave like SCC cells. Furthermore, in line with our biological observations that SCC are more prone to undergo apoptosis, we note upregulation of two strong pro-apoptotic genes in this cluster, *CYTOCHROME C* and apoptosis regulator *BAX*.

5.3.5 Summary

In summary, the transcriptional program of normal keratinocyte cells after intermediate UVB doses involves (i) activation of the DNA repair genes, (ii) expression of immediate early transcription factors of the stress response, (iii) transcriptional activation of genes, which are characteristic for an inflammatory response, (iv) maintenance of cell proliferation and (v) enhanced cell survival.
Our proposed model for UV-induction of skin cancer is presented in figure 5.3.4.
The evasion of apoptosis in DNA damaged cells may potentially lead to the undesirable effect of increasing the number of live cells bearing DNA damages and mutations, which may develop later on into tumors.

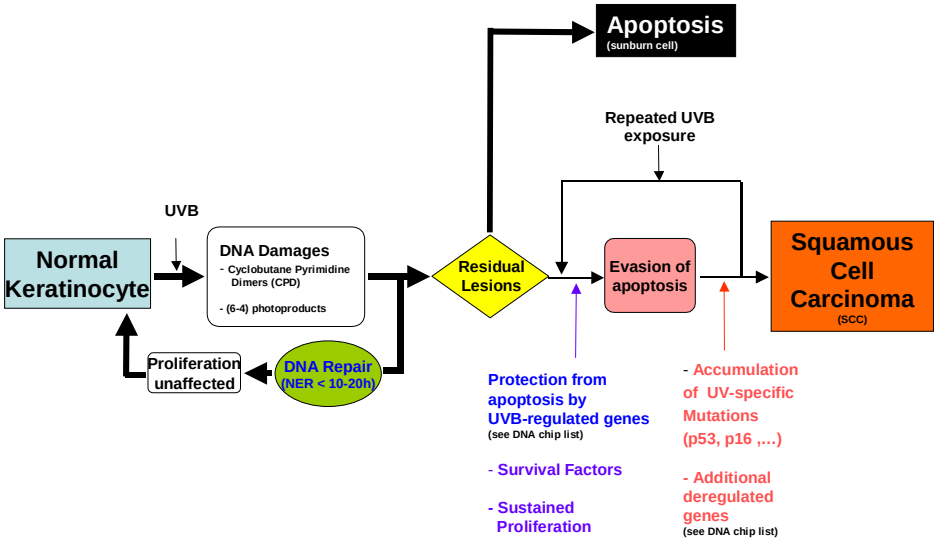

Figure 5.3.4: A model for UV-induction of skin cancer. The scheme illustrates the mutagenic effect of UVB (by creating pyrimidine dimmers), followed by DNA repair (NER). The residual unrepaired cells may be eliminated by apoptosis or retained by survival factors. Some of these cells evade apoptosis in spite of DNA damage and may develop later to cancer.

By using the CTWC method we were able to partition the normal keratinocytes (NHEK) and their tumor counterparts (SCC) into distinct groups, on the basis of small sets of genes. We were able to find new and interesting partitions, not necessarily depending on cell type, but other partitions of samples such as partitioning of UVB exposed samples versus non-UVB exposed samples (e.g. S1(G28)). The partitioning of NHEK and SCC samples reflects variation in the response of NHEK and SCC to UVB.

Table I

		Symbol	Name	Accession Nb.		NORMAL KERATINOCYTES (NHEK) 0.5h	3h	6h	12h	24h		SCC12B2 6h	12h
1 Cell Cycle (+/-) / Oncogene / Tumor Suppressor													
	(+)	CCNA1	Cyclin A1	U66838	↑	**1.2**	**0.8**	**1.1**	**2.1**	**7.4**		1.0	1.0
	(+)	CCNB1	Cyclin B1	M25753	↓	**-1.1**	**-1.1**	**-1.4**	**-3.7**	**-4.3**		0.9	0.6
	(+)	CCNB2	Cyclin B2	AL080146	↓	**-1.6**	**-1.1**	**-1.3**	**-4.0**	**-4.3**		0.8	0.6
	(+)	CCNE1	Cyclin E1	M74093	↓	**-0.9**	**-1.7**	**-3.2**	**-7.2**	**-0.6**		0.6	0.3
	(+)	ODC1	Ornithine Decarboxylase 1	M33764	↑	**1.4**	**3.2**	**1.5**	**1.0**	**3.5**		1.6	1.4
	(+)	HRAS	Ha-ras Oncogene	J00277	↑	**0.9**	**1.3**	**2.3**	**1.7**	**2.6**		0.8	0.6
*	(+)	MDM2	MDM2	M92424		0.9	0.9	1.5	1.9	1.1		1.0	1.0
	(+/-)	SFN	14-3-3 σ (Stratifin)	X57348	↑	**1.4**	**1.5**	**2.2**	**2.0**	**1.1**		0.9	0.9
	(-)	PPP1R15A	GADD34	U83981	↑	**0.8**	**1.6**	**1.4**	**2.2**	**5.1**		0.8	0.9
*	(-)	CDKN1A	p21 CIP1	U03106		1.6	1.6	1.3	1.4	5.0		1.1	0.9
	(-)	CDKN1C	p57 Kip2	D64137	↑	**1.6**	**2.0**	**2.2**	**1.3**	**3.5**	↑	**3.8**	**3.3**
	(-)	CDKN1B	p27 Kip1	AI304854	↓	**-1.7**	**-3.0**	**-1.4**	**-1.3**	**-3.4**		1.0	1.0
	(-)	WEE1	WEE1	X62048	↓	**-1.6**	**-6.7**	**-14.9**	**-18.9**	**-4.5**	↓	**-8.6**	**-9.8**
*	(-)	TP53	p53	X02469		0.7	0.9	0.8	1.4	0.8		1.0	1.2
2 Apoptosis (+/-)													
	(+)	CRADD	RAIDD	U79115	↑	**0.7**	**1.7**	**1.6**	**3.1**	**2.4**		1.1	0.8
	(+)	TSSC3	Tumor Suppressing STF 3	AF035444	↑	**1.5**	**4.1**	**3.4**	**4.1**	**4.4**		1.5	1.4
	(+)	PMAIP1	APR (NOXA)	D90070	↑	**2.0**	**7.0**	**14.7**	**18.1**	**13.0**	↑	**2.8**	**3.3**
	(+)	TNF	TNFα	X02910	↑	**1.5**	**1.4**	**1.8**	**2.2**	**1.4**	↑	**10.5**	**10.4**
	(+)	CASP8	Caspase 8	X98172	↓	**-1.0**	**-1.1**	**-1.2**	**-3.7**	**-7.2**		0.4	0.2
	(-)	BCL2	BCL-2	M14745	↑	**2.0**	**1.0**	**1.0**	**3.6**	**1.0**		1.0	1.0
	(-)	API5L1	Antiapoptosis 5-Like 1	Y15906	↑	**1.5**	**4.0**	**3.2**	**1.1**	**2.4**		0.9	0.4
	(-)	IER3	Radiation-inducible IER3	S81914	↑	**1.5**	**5.2**	**5.1**	**5.4**	**4.5**		1.4	1.2
	(-)	TNFAIP3	TNF α-Induced Protein 3	M59465	↑	**1.0**	**0.7**	**1.2**	**2.4**	**6.8**	↓	**-3.7**	**-4.3**
	(-)	HSPA2	HSP70	L26336	↑	**1.8**	**1.8**	**2.1**	**2.2**	**1.9**		1.0	1.0
	(-)	HSPCB	HSP90	W28616	↑	**1.2**	**1.2**	**2.1**	**2.1**	**0.6**	↑	**2.3**	**2.8**
	(-)	MCL1	Myeloid Cell Leukemia 1	L08246		0.8	0.4	0.4	0.4	1.6	↓	**-7.4**	**-8.9**
	(-)	BAG5	BCL2-Associated Athanogene 5	AB020680		1.0	0.6	0.4	0.5	1.1	↓	**-3.5**	**-5.7**
3 DNA Repair													
		GADD45A	GADD45A	M60974	↑	**1.5**	**3.8**	**4.7**	**7.0**	**9.2**	↑	**2.0**	**2.4**
		GADD45B	GADD45B	AF078077	↑	**1.6**	**2.3**	**2.2**	**2.8**	**4.8**		1.2	1.5
		ERCC1	ERCC1	M13194	↑	**1.2**	**2.0**	**1.4**	**1.1**	**3.9**		1.3	1.3
		ERCC2	ERCC2	AA079018	↑	**1.1**	**1.6**	**2.4**	**2.2**	**1.4**		1.3	1.9
		BTG1	B-cell Translocation Gene 1	X61123	↑	**1.1**	**1.3**	**1.7**	**2.2**	**3.4**		1.1	0.6
		TOB1	Transducer of ErbB-2, 1	D38305	↑	**0.9**	**1.5**	**2.0**	**2.9**	**2.5**	↑	**2.9**	**3.2**
4 Oxydative Stress / ROS Metabolism (+/-)													
	(+)	CYBA	Superoxide-Generating NADPH Oxidase	M21186	↑	**1.3**	**1.0**	**1.7**	**2.5**	**2.8**		0.9	0.7
*	(+)	NOSIIc	Nitric oxide synthase II	U18334		0.6	0.2	1.1	1.9	0.6		1.5	0.6
	(-)	MT1G	Metallothionein 1G	J03910	↑	**1.0**	**3.2**	**3.4**	**2.0**	**3.8**		1.6	1.3
	(-)	MT2A	Metallothionein 2A	AI547258		2.3	0.7	0.8	1.9	0.6	↓	**-4.4**	**-3.6**
	(-)	PRDX3	Peroxiredoxin 3	D49396		0.7	0.9	0.8	0.9	0.7	↑	**2.2**	**2.4**
5 Extra Cellular Matrix / Protease (+/-)													
*	(+)	MMP1	Collagenase	M13509		0.8	1.4	1.3	1.8	3.3		1.5	1.8
	(+)	MMP10	Stromelysin 2	X07820	↑	**1.6**	**2.5**	**2.2**	**2.1**	**4.5**		1.0	1.0
	(+)	PRSS4	Trypsin 4	X71345	↑	**1.4**	**2.2**	**1.4**	**1.9**	**7.0**		1.0	1.0
6 Growth Factor / Chemokine / Cytokine / Inflammation (+/-)													
	(+)	IL8	IL-8 (CXC)	M28130	↑	**2.0**	**10.6**	**36.8**	**48.1**	**71.7**	↑	**12.5**	**11.9**
	(+)	IL6	IL-6	X04430	↑	**1.0**	**1.3**	**4.1**	**3.7**	**1.9**	↑	**4.3**	**4.0**
	(+)	IL1B	IL-1β	X04500	↑	**1.0**	**1.3**	**1.4**	**1.6**	**1.9**	↑	**2.1**	**2.3**
	(+)	GRO1	Small Inducible Cytokine B1 (MGSA) (CXC)	X54489	↑	**2.2**	**1.7**	**2.2**	**3.1**	**3.2**	↑	**2.9**	**2.0**
	(+)	GRO2	Small Inducible Cytokine B2 (CXC)	M36820	↑	**1.4**	**3.9**	**5.8**	**6.0**	**5.1**	↑	**11.6**	**8.9**
	(+)	GRO3	Small Inducible Cytokine B3 (CXC)	M36821	↑	**2.0**	**2.4**	**6.8**	**7.9**	**5.2**	↑	**10.3**	**8.9**
	(+)	PPBP	Small Inducible Cytokine B7 (NAP-2) (CXC)	M54995	↑	**0.8**	**1.2**	**2.2**	**2.6**	**2.3**		1.0	1.0
	(+)	SCYA20	Small Inducible Cytokine A20 (CXC)	U64197	↑	**1.9**	**3.7**	**11.5**	**21.5**	**17.0**	↑	**3.5**	**4.0**
	(+)	PTGS2	Cyclooxygenase (COX-2)	U04636	↑	**0.8**	**2.8**	**4.8**	**13.5**	**24.7**	↑	**5.5**	**7.9**
	(+)	HB-EGF	HB-EGF-Like	M60278	↑	**1.7**	**1.5**	**3.2**	**4.6**	**9.9**		1.3	1.9
	(+)	S100A9	Calgranulin B	W72424	↑	**1.1**	**1.5**	**1.6**	**2.2**	**6.0**		1.3	1.7
		INSL4	Insulin-like 4 (IGF Family)	L34838	↑	**1.0**	**12.0**	**13.4**	**20.2**	**26.1**		0.9	1.9
9 Development / Differentiation (+/-)													
	(+)	TP63	p63 (ΔNp63)	Y16961	↓	**-0.9**	**-1.8**	**-2.0**	**-3.7**	**-18.0**		0.4	0.2
	(+)	PUM1	Pumilio 1	D43951	↓	**-1.6**	**-3.2**	**-6.6**	**-23.7**	**-2.4**	↓	**-6.0**	**-10.3**
	(+)	PUM2	Pumilio 2	D87078	↓	**-1.3**	**-2.1**	**-3.1**	**-5.7**	**-2.8**		0.5	0.4
11 Transcription (+/-) / Replication													
	(+)	FOS	FOS	V01512	↑	**5.4**	**4.7**	**4.7**	**2.1**	**0.6**	↑	**16.1**	**27.8**
	(+)	JUN	JUN	J04111	↑	**2.9**	**7.9**	**5.7**	**2.9**	**1.1**		1.2	0.9
	(+)	JUNB	JUNB	M29039	↑	**2.0**	**4.5**	**3.6**	**4.0**	**3.4**		1.3	0.7
	(+)	JUND	JUND	X56681	↑	**1.3**	**2.2**	**3.7**	**3.0**	**2.6**	↑	**3.7**	**2.0**
	(+)	TLS/CHOP	GADD153	HG2724-HT2820	↑	**2.5**	**3.8**	**1.6**	**0.2**	**1.1**		1.6	1.0
	(+)	MYC	MYC	V00568	↓	**-1.5**	**-3.5**	**-3.8**	**-4.9**	**-4.2**	↓	**-6.9**	**-6.6**
	(+)	E2F3	E2F3	D38550	↓	**-1.4**	**-2.9**	**-3.6**	**-3.5**	**-2.9**	↓	**-3.3**	**-3.3**
	(+)	E2F5	E2F5	U31556	↓	**-1.2**	**-1.6**	**-1.3**	**-3.2**	**-6.3**		0.8	0.9
*	(+)	RELA	NFkB (p65 subunit)	L19067		1.3	1.4	1.6	1.3	1.4		0.8	0.7
	(-)	TSC22	TGF β-Stimulated Protein	AJ222700	↑	**1.2**	**2.3**	**4.0**	**3.1**	**1.6**		0.9	0.7
	(-)	DRAP1	DR1-Associated Protein 1	U41843	↑	**1.3**	**1.7**	**4.2**	**0.7**	**5.3**	↑	**2.6**	**3.7**
	(-)	ATF3	ATF3	L19871	↑	**1.3**	**3.8**	**4.7**	**10.8**	**17.1**	↑	**2.4**	**5.6**
	(-)	ATF4	ATF4	AL022312	↑	**1.2**	**1.7**	**2.1**	**2.2**	**3.1**		1.2	1.0
	(-)	SAP18	Sin3-Associated Polypeptide	W27641	↑	**1.8**	**1.5**	**2.1**	**2.1**	**0.8**		1.3	1.6
	(-)	ID2	Inhibitor of DNA Binding 2	D13891	↑	**1.9**	**4.0**	**3.0**	**2.1**	**2.1**	↑	**14.6**	**22.0**
12 Histone / Chromatin													
		H1FX	Histone H1X	D64142	↑	**0.9**	**1.4**	**3.5**	**3.5**	**4.6**		1.0	0.9
		H2AFA	Histone H2A A	AI039144	↑	**1.1**	**4.8**	**9.4**	**13.4**	**12.4**		1.0	1.0
		H2AFG	Histone H2A G	Z80776	↑	**2.4**	**1.8**	**1.6**	**4.3**	**6.3**		3.9	1.3
		H2AFO	Histone H2A O	L19779	↑	**1.5**	**3.2**	**2.1**	**3.0**	**18.0**		1.9	2.0
		H2AFX	Histone H2A X	X14850	↑	**1.0**	**2.5**	**0.7**	**1.3**	**3.2**		1.2	1.0
		H2BFA	Histone H2B A	AJ223352		1.1	0.9	1.2	1.1	2.4	↑	**3.0**	**2.9**
		H2BFC	Histone H2B C	AL009179	↑	**1.0**	**2.2**	**3.2**	**6.5**	**5.5**		1.0	1.0
		H2BFG	Histone H2B G	Z80779	↑	**1.5**	**2.5**	**1.1**	**1.3**	**4.9**		1.8	2.1
		H2BFQ	Histone H2B Q	X57985	↑	**1.5**	**5.3**	**5.3**	**7.9**	**12.1**		1.1	1.3
		MORF	Histone Acetyltransferase	AB002381	↓	**-0.9**	**-1.6**	**-1.4**	**-5.4**	**-6.7**		0.4	0.6
		ZNF220	Zinc Finger Protein 220	U47742	↓	**-1.3**	**-2.4**	**-3.2**	**-4.1**	**-7.2**		0.4	0.2
		BAZ1A	Br Domain adj. to ZF1A (hist. acetyltransferase)	AL050089	↓	**-1.3**	**-1.9**	**-2.0**	**-3.9**	**-5.1**		0.9	0.6
		BAZ1B	Br Domain adj. to ZF1B (hist. acetyltransferase)	AF072810	↓	**-0.8**	**-1.1**	**-5.5**	**-3.4**	**-2.5**		0.6	0.4
		BAZ2B	Br Domain adj. to ZF2B (hist. acetyltransferase)	AL080173	↓	**-1.2**	**-2.2**	**-2.0**	**-7.8**	**-3.8**		0.5	0.4

Table 1: Selected genes, modulated by UVB in NHEK and SCC

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Appendix

Chapter 4

Table 1

Accession no.	Description	Intensity in tumor	Intensity in normal	Tumor/Normal
X54489	**Human gene for MGSA**	105.1	10.0	10.5
U22055	**Human 100 kDA coactivator mRNA, complete cds**[a]	72.9	10.0	7.3
D14657	Human mRNA for *KIAA0101* gene, complete cds	64.8	10.0	6.5
M61832	Human *S*-adenosylhomocysteine hydrolase (AHCY) mRNA, complete cds	123.1	20.7	6.0
M77836	Human pyrroline 5-carboxylate reductase mRNA, complete cds	95.5	17.9	5.3
D21262	Human mRNA for *KIAA0035* gene, partial cds [? nucleolar phosphoprotein]	55.6	10.8	5.2
M36821	Human cytokine (GRO-γ) mRNA, complete cds	141.2	27.6	5.1
L23808	**Human metalloproteinase (HME) mRNA, complete cds**	71.1	14.0	5.1
R08183	Similar to bovin hs 10-kD protein 1(chaperonin 10)(HSPE1)(NM_002157)	439.4	91.1	4.8
L29254	Human (clone D21-1) L-iditol-2 dehydrogenase gene, exon 9, and complete cds	47.2	10.0	4.7
H50438	**M-phase inducer phosphatase 2 (*Homo sapiens*)**	46.8	10.0	4.7
U33286	Human chromosome segregation gene homolog CAS mRNA, complete cds	98.8	21.2	4.7
X54942	**H. sapiens CKSHS2 mRNA for CKS1 protein homologue**	131.9	30.1	4.4
R32511	*H. sapiens* cDNA clone 135395 3' [RNA POL II subunit]	43.5	10.0	4.3
T87871	*H. sapiens* cDNA clone 115765 3' [myoblast cell surface antigen 24.1 DS]	42.1	10.0	4.2
X05231	Human mRNA for collagenase (identical to metalloproteinase 1)	41.8	10.0	4.2
R36977	Similar to *H. sapiens* general transcription factor IIIA (GTF3A) mRNA	177.5	43.6	4.1
U17899	Human chloride channel regulatory protein mRNA, complete cds	66.3	16.5	4.0
X54942	**H. sapiens CKSHS2 mRNA for CKS1 protein homologue**	171.9	43.1	4.0

Table 1: Transcripts more highly expressed in adenocarcinoma than in paired normal tissue. Intensity values <10 are adjusted to 10. Only transcripts with a 4-fold difference or greater ($P < 0.001$) in expression intensity between tumor and normal are included. Transcripts shown in bold capital letters were confirmed by RT-PCR. Gene descriptions have been edited.

Table 2

Accession no.	Description	Intensity in tumor	Intensity in normal	Normal/Tumor
M83670	Human carbonic anhydrase IV mRNA, complete cds	10.0	378.	37.9
M97496	*H. sapiens* **guanylin mRNA, complete cds**[a]	53.6	1082.9	20.2
X64559	*H. sapiens* **mRNA for tetranectin**	10.0	137.8	13.8
T54547	*H. sapiens* cDNA similar to M84526 complement factor D precursor	10.0	119.9	12.0
M95936	Human protein-serine/threonine (AKT2) mRNA, complete cds	10.0	113.5	11.4
T55200	*H. sapiens* cDNA similar to gb:M10942_cds1 human metallothionein-le gene	10.0	84.0	8.4
T46924	*H. sapiens* cDNA similar to gb:U11863 amiloride-sens amine oxidase	15.1	124.0	8.2
L11708	Human 17 ß-hydroxysteroid dehydrogenase type 2 mRNA, complete cds	16.6	134.6	8.1
T46933	*H. sapiens* cDNA clone 70843 3' [11-ß dehydrogenase]	11.2	84.9	7.6
H54425	*H. sapiens* cDNA similar to gb:M10942_cds1 human metallothionein-le gene	18.2	135.5	7.4
M26393	Human short chain acyl-CoA dehydrogenase mRNA, complete cds	10.0	71.8	7.2
M82962	Human *N*-benzoyl-L-tyrosyl-*p*-amino-benzoic acid hydrolase α subunit mRNA	10.0	71.3	7.1
J03037	Human carbonic anhydrase II mRNA, complete cds	10.0	65.1	6.5
T72257	*H. sapiens* cDNA similar to gb:L07765 liver carboxylesterase	10.0	63.1	6.3
M84526	Human adipsin/complement factor D mRNA, complete cds	43.9	260.4	5.9
T76971	*H. sapiens* cDNA similar to gb:X64177 *H. sapiens* mRNA for metallothionein	38.3	217.7	5.7
H77597	*H. sapiens* cDNA similar to gb:X64177 *H. sapiens* mRNA for metallothionein	57.0	320.5	5.6
T67986	*H. sapiens* cDNA clone 82030 3' similar to gb:X14723 clusterin precursor	35.0	195.7	5.6
R99208	*H. sapiens* cDNA clone 200586 3' similar to gb:X76717 *H. sapiens* MT-1l mRNA	10.0	55.7	5.6
U03749	Human chromogranin A (*CHGA*) gene, exon 8, and complete cds	10.0	55.0	5.5
R93176	Soares 1NFLS *H. sapiens* cDNA similar to gb:M33987 carb. anhydrase Iα	10.0	53.1	5.3

L02785	*H. sapiens* **colon mucosa-associated (DRA), complete cds**[a]	161.0	848.1	5.3
R94967	*H. sapiens* cDNA similar to gb:L11924 hepatocyte growth factor	10.0	52.4	5.2
J03037	Human carbonic anhydrase II mRNA, complete cds	10.0	51.8	5.2
M74509	Human endogenous retrovirus type C oncovirus sequence	15.1	77.9	5.2
L11708	Human 17 ß-hydroxysteroid dehydrogenase type 2 mRNA, complete cds	18.7	96.2	5.2
X77777	*H. sapiens* intestinal VIP receptor related protein mRNA	13.7	70.5	5.1
R69552	*H. sapiens* cDNA clone 155302 3' [glutamate]	10.0	50.6	5.1
R50730	*H. sapiens* cDNA similar to gb:Z19585 thrombospondin 4 precursor	10.0	50.3	5.0
H43887	*H. sapiens* cDNA similar to gb:M84526 complement factor D prec.	84.8	400.4	4.7
U17077	Human BENE mRNA, partial cds	32.5	147.6	4.5
U25138	Human MaxiK potassium channel ß subunit mRNA, complete cds	14.9	67.4	4.5
X86693	*H. sapiens* **mRNA for hevin like protein**	47.0	212.6	4.5
H57136	*H. sapiens* cDNA similar to SP:A40533 A40533 cAMP-DEP protein kinase	10.0	44.5	4.5
X73502	*H. sapiens* mRNA for cytokeratin 20[a]	55.2	245.6	4.5
J03037	Human carbonic anhydrase II mRNA, complete cds	10.0	44.0	4.4
R70806	*H. sapiens* cDNA similar to gb:X62535 diacylglycerol kinase	10.0	43.9	4.4
T51913	*H. sapiens* cDNA similar to gb:S45630 α crystallin B chain	10.0	43.5	4.3
T50678	*H. sapiens* cDNA contains TAR1 repetitive element [α tryptase]	12.5	53.7	4.3
Z50753	*H. sapiens* mRNA for GCAP-II/uroguanylin precursor[a]	42.9	183.7	4.3
M58286	*H. sapiens* tumor necrosis factor receptor mRNA, complete cds	30.5	130.5	4.3
U08854	Human UDP glucuronosyltransferase precursor (UGT2B15) mRNA, complete cds	30.8	131.2	4.3
X52679	Human ASM-2 mRNA for sphingomyelin phosphodiesterase (EC 3.1.4.12)	10.0	42.2	4.2
T71025	*H. sapiens* cDNA similar to gb:J03910_rna1 human	217.8	893.3	4.1
M12272	*H. sapiens* alcohol dehydrogenase class I γ subunit (ADH3) mRNA	42.9	174.8	4.1
M26683	Human IFN-γ treatment inducible mRNA	37.8	152.0	4.0
D90313	**Human mRNA for biliary glycoprotein, BGPI**[a]	12.7	51.0	4.0

Table 2: Transcripts were more highly expressed in paired normal tissue than in adenocarcinoma. Intensity values <10 are adjusted to 10. Only transcripts with a 4-fold difference or greater (P < 0.001) in expression intensity between tumor and normal are included. Transcripts shown in bold capital letters were confirmed by RT-PCR. Gene descriptions have been edited.

Table 3

Cluster G8

1	D10511	Human gene for mitochondrial acetoacetyl-CoA thiolase, exon 12
2	D10523	Human mRNA for 2-oxoglutarate dehydrogenase, complete cds
3	D10537	Human mRNA for myelin protein zero
4	D13168	Human gene for endothelin-B receptor (hET-BR), exon 7
5	D13626	Human mRNA for G protein-coupled receptor 105, complete cds
6	D14662	Human mRNA for KIAA0106 gene, complete cds
7	D16294	Human mRNA for mitochondrial 3-oxoacyl-CoA thiolase, complete cds
8	D42047	Human mRNA for KIAA0089 gene, partial cds
9	J02854	Human 20-kDa myosin light chain (MLC-2) mRNA, complete cds
10	J04080	Human complement component C1r mRNA, complete cds
11	L02785	Homo sapiens colon mucosa-associated (DRA) mRNA, complete cds
12	L07648	Human MXI1 mRNA, complete cds
13	L11708	Human 17 beta hydroxysteroid dehydrogenase type 2 mRNA, complete cds
14	L20852	Human leukemia virus receptor 2 (GLVR2) mRNA, complete cds
15	L20859	Human leukemia virus receptor 1 (GLVR1) mRNA, complete cds
16	L34060	Homo sapiens cadherin-8 mRNA, complete cds
17	L41143	Homo sapiens expressed pseudo TCTA mRNA at t(1;3) translocation site, complete cds
18	M22995	Human ras-related protein RAP1A (Krev-1) mRNA, complete cds
19	M36634	Human vasoactive intestinal peptide (VIP) mRNA, complete cds
20	M54927	Human myelin proteolipid protein mRNA, complete cds
21	M63391	Human desmin gene, complete cds
22	M63603	Human phospholamban mRNA, complete cds
23	M64497	Human apolipoprotein AI regulatory protein (ARP-1) mRNA, complete cds
24	M65254	Protein phosphatase 2A regulatory subunit-beta mRNA, complete cds
25	M69066	Human moesin mRNA, complete cds
26	M74096	Human long chain acyl-CoA dehydrogenase (ACADL) mRNA, complete cds
27	M76378	Human cysteine-rich protein (CRP) gene, exons 5 and 6
28	M82962	Human N-benzoyl-L-tyrosyl-p-amino-benzoic acid hydrolase alpha subunit (PPH alpha) mRNA, complete cds
29	M83186	Human cytochrome c oxidase subunit VIIa (COX7A) muscle isoform mRNA, complete cds
30	M85289	Human heparan sulfate proteoglycan (HSPG2) mRNA, complete cds
31	M95787	Human 22kDa smooth muscle protein (SM22) mRNA, complete cds
32	M97287	Human MAR/SAR DNA binding protein (SATB1) mRNA, complete cds
33	M97496	Homo sapiens guanylin mRNA, complete cds
34	U13395	Human oxidoreductase (HHCMA56) mRNA, complete cds
35	U17077	Human BENE mRNA, partial cds
36	U20938	Human lymphocyte dihydropyrimidine dehydrogenase mRNA
37	U34038	Human G protein-coupled receptor 11 complete cds
38	X05610	Human mRNA for type IV collagen alpha (2) chain
39	X06256	Human mRNA for integrin, alpha 5 (VLA5A) (fibronectin receptor alpha subunit)

40	X07767	Human mRNA for cAMP-dependent protein kinase catalytic subunit type alpha (PKACA)
41	X15880	Human mRNA for collagen VI alpha-1 C-terminal
42	X15882	Human mRNA for collagen VI alpha-2 C-terminal
43	X16354	Human mRNA for CEACAM1 (transmembrane carcinoembryonic antigen BGPa)
44	X51405	Human mRNA for carboxypeptidase E
45	X53416	Human mRNA for actin-binding protein (filamin) (ABP280)
46	X54162	Human mRNA for a 64 Kd autoantigen expressed in thyroid and extra-ocular muscle
47	X57348	H.sapiens mRNA for protein 14-3-3 sigma (strafitin)
48	X64559	H.sapiens mRNA for tetranectin
49	X68277	H.sapiens CL 100 mRNA for protein tyrosine phosphatase
50	X74295	H.sapiens mRNA for alpha 7B integrin
51	X86693	H.sapiens mRNA for hevin like protein
52	X87159	H.sapiens mRNA for beta subunit of epithelial amiloride-sensitive sodium channel
53	Z22535	H.sapiens ALK-3 mRNA
54	Z24727	H.sapiens tropomyosin isoform mRNA, complete CDS
55	Z49269	H.sapiens gene for chemokine HCC-1

The cluster of G8 genes. *Genes marked in yellow*; appear in the table 2 (see appendix) of highly up-regulated genes in normal tissues in the paper of NEA. *Genes marked in green;* appear in both the table and clusters of NEA.

Table 4

Cluster G25

1	D14657	Human mRNA for KIAA0101 gene, complete
2	D21262	Nuclear phosphoprotein p130
3	D25218	Human mRNA for KIAA0112 gene
4	H11084	Vascular endothelial growth factor
5	H24033	similar to gb:K02276 MYC PROTO-ONCOGENE PROTEIN (HUMAN);
6	H30564	Homo sapiens cDNA clone 190395 3'
7	H87456	Homo sapiens cDNA clone 252485 3'
8	X54942	similar to gb:X54942 CYCLIN-DEPENDENT KINASES REGULATORY SUBUNIT 2 (HUMAN);
9	H90495	Homo sapiens cDNA clone 241524 3'
10	L03840	Human fibroblast growth factor receptor 4 (FGFR4) mRNA, complete cds
11	L12350	Human thrombospondin 2 (THBS2) mRNA, complete cds
12	L20298	Homo sapiens transcription factor (CBFB) mRNA, 3' end
13	L22524	Human matrilysin (MMP-7) gene, exon 6 and complete cds
14	L23808	Human metalloproteinase (HME) mRNA, complete cds (MMP-12)
15	L29254	Human (clone D21-1) L-iditol-2 dehydrogenase gene, exon 9 and complete cds
16	L41559	Homo sapiens pterin-4a-carbinolamine dehydratase (PCBD) mRNA, complete cds
17	M26383	Human interleukin-8, complete cds

18	M30448	Human casein kinase II beta subunit mRNA, complete cds
19	M36821	Human cytokine (GRO-gamma) mRNA, complete cds
20	M61832	Human S-adenosylhomocysteine hydrolase (AHCY) mRNA, complete cds
21	M77836	Human pyrroline 5-carboxylate reductase mRNA, complete cds
22	M96577	Homo sapiens (E2F-1) pRB-binding protein mRNA, complete cds
23	R02151	similar to SP:S40468 S40468 PROTEASOME SUBUNIT RC10-II - ;
24	R08183	similar to SP:CH10_BOVIN Q04984 10 KD HEAT SHOCK PROTEIN, MITOCHONDRIAL ;
25	R10066	Homo sapiens cDNA clone 128808 3' similar to gb:S85655 PROHIBITIN (HUMAN);
26	R32511	Polymerase RNA and DNA directed polypeptide
27	R36977	similar to SP:TF3A_XENLA P03001 TRANSCRIPTION FACTOR IIIA ;
28	R44770	yg23a06.s1 Homo sapiens cDNA clone 32991 3'
29	R46716	yg54f12.s1 Homo sapiens cDNA clone 36504 3'
30	R50499	yj60e02.s1 Homo sapiens cDNA clone 153146 3'
31	R61502	yh16a01.s1 Homo sapiens cDNA clone 37679 3'
32	R88575	similar to gb:X67688 TRANSKETOLASE (HUMAN);
33	T50321	yb24c06.s1 Homo sapiens cDNA clone 72106 3'
34	T51961	similar to gb:M15796 PROLIFERATING CELL NUCLEAR ANTIGEN (HUMAN)
35	T52342	Human tumor rejection antigen (TRA1)
36	T60780	similar to gb:X06994 CYTOCHROME C1, HEME PROTEIN PRECURSOR (HUMAN);
37	T70062	yc17d09.s1 Homo sapiens cDNA clone 80945 3'
38	U17899	Human chloride channel regulatory protein mRNA, complete cds
39	U22055	Human 100 kDa coactivator mRNA, complete cds
40	U33286	Human chromosome segregation gene homolog CAS mRNA, complete cds
41	X05231	Human mRNA for collagenase (MMP-1) (E.C. 3.4.24)
42	X12466	Human mRNA for snRNP E protein
43	X16396	Human mRNA for NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase (EC 1.5.1.15)
44	X54489	Human gene for melanoma growth stimulatory activity (MGSA)
45	X54941	H.sapiens ckshs2 mRNA for Cks1 protein homologue
46	X54941	H.sapiens ckshs2 mRNA for Cks1 protein homologue
47	X56597	Human humFib mRNA for fibrillarin
48	X62570	H.sapiens mRNA for IFP53
49	Z11887	H.sapiens PUMP-1 gene encoding PUMP
50	Z46629	Homo sapiens SOX9 mRNA
51	Z48481	H.sapiens mRNA for membrane-type matrix metalloproteinase 1 (MT-MMP)

The cluster of G25 genes. *Genes marked in purple*; also appear in the clusters in the paper of NEA. *Genes marked in yellow*; appear in the table 1 of up-regulated genes in tumor versus normal tissues in the paper of NEA (See appendix- Table 1). *Genes marked in green;* appear in both the table and clusters of NEA.

Cluster G3

1	D13902	Human mRNA for peptide YY, complete cds
2	D30758	Human mRNA for KIAA0050 gene, complete cds
3	D49490	Human mRNA for protein disulfide isomerase-related protein (PDIR), complete cds
4	H19272	yn50d10.s1 Homo sapiens cDNA clone 171859 3'
5	H27202	yl15f10.s1 Homo sapiens cDNA clone 158347 3'
6	H46934	yo15h09.s1 Homo sapiens cDNA clone 178049 3'
7	H70609	similar to contains MER22 repetitive element ;
8	H85835	ys95a03.s1 Homo sapiens cDNA clone 222508 3'
9	L13268	Homo sapiens N-methyl-d-aspartate receptor (NR1-3) mRNA, 3' end
10	M28650	Human excision repair protein (ERCC-1) mRNA, complete cds. cDNA clone pcDE
11	R16153	ya48f08.s1 Homo sapiens cDNA clone 53165 3'
12	R39010	yd08d06.s1 Homo sapiens cDNA clone 25204 3'
13	R41866	yg12f04.s1 Homo sapiens cDNA clone 31854 3'
14	R49565	yg68b12.s1 Homo sapiens cDNA clone 38251 3'
15	R56052	yg91c01.s1 Homo sapiens cDNA clone 40877 3'
16	R61523	similar to gb:U02389 MITOCHONDRIAL LON PROTEASE HOMOLOG PRECURSOR (HUMAN);
17	R72164	yj88b08.s1 Homo sapiens cDNA clone 155799 3'
18	T40653	similar to SP:PLC_HUMAN P01243 LACTOGEN PRECURSOR ,
19	T66307	similar to gb:M11717_rna1 HEAT SHOCK 70 KD PROTEIN 1 (HUMAN);
20	U19261	Human Epstein-Barr virus-induced protein mRNA, complete cds
21	X04500	Human gene for prointerleukin 1 beta
22	X16351	Human testis mRNA for a sex hormone-binding related protein (SHBGgrp)
23	X52229	Human mRNA for transmembrane epithelial tumour mucin antigen
24	X53683	Human LAG-1 mRNA
25	X69550	H.sapiens mRNA for rho GDP-dissociation Inhibitor 1
26	X83535	H.sapiens mRNA for membrane-type matrix metalloproteinase
27	X86779	H.sapiens mRNA for FAST kinase

Cluster G7

1	M28214		Homo sapiens GTP-binding protein (RAB3B) mRNA, complete cds
2	M91670		Human ubiquitin carrier protein (E2-EPF) mRNA, complete cds
3	T96816		ye51a11.s1 Homo sapiens cDNA clone 121244 3' similar to gb:M88468 MEVALONATE KINASE (HUMAN);
4	T99498		ye64h10.s1 Homo sapiens cDNA clone 122563 3'
5	X52426		H.sapiens mRNA for cytokeratin 13
6	X60489		Human mRNA for elongation factor-1-beta
7	Z17240		Homo sapiens for mRNA encoding HMG2B

Cluster G12

1	R00453		ye72d11.s1 Homo sapiens cDNA clone 123285 3'
2	T49945		ya99e09.s1 Homo sapiens cDNA clone 69832 3' similar to gb:X00570 APOLIPOPROTEIN C-I PRECURSOR (HUMAN)
3	X02530		Human mRNA for gamma-interferon inducible early response gene (with homology to platelet proteins)
4	X17668		Human mRNA for indoleamine 2,3-dioxygenase
5	X72755		H.sapiens Humig mRNA

Cluster G24

1	K01144		Human major histocompatibility class II antigen gamma chain mRNA, complete cds
2	M18737		Human Hanukah factor serine protease (HuHF) mRNA, complete cds
3	M18737		Human Hanukah factor serine protease (HuHF) mRNA, complete cds
4	M79463		Human PML-2 mRNA, complete CDS
5	T62558		yc04f07.s1 Homo sapiens cDNA clone 79717 3' similar to gb:X02902 HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DR-1(DW14) BETA (HUMAN);
6	U15085		Human HLA-DMB mRNA, complete cds

Chapter 5

Table II Online Supplemental Data

		Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
1 Cell Cycle (+/-) / Oncogene / Tumor Suppressor											
↑	(+)	CCNA1	Cyclin A1	U66838	G3	1.2	0.8	1.1	2.1	7.4	
↑	(+)	ODC1	Ornithine Decarboxylase 1	M33764		1.4	3.2	1.5	1.0	3.5	
↑	(+)	HRAS	Ha-ras Oncogene	J00277		0.9	1.3	2.3	1.7	2.6	
↑	(+)	RAP1A	Ras Oncogene-Related Protein	M22995		2.0	0.5	2.2	1.4	1.2	
↑	(+)	MNAT1	Menage A Trois 1	X87843		1.9	2.5	2.8	0.8	1.6	
↑	(+/-)	SFN	14-3-3 σ (Stratifin)	X57348	G1	1.4	1.5	2.2	2.0	1.1	
↑	(-)	NF2	Neurofibromatosis 2 Tumor Suppressor	HG3236-HT3413	G1	1.0	1.8	6.9	10.0	1.4	up SCC12B2
↑	(-)	APCL	APC Like	AB012162		2.2	0.5	2.0	3.0	1.7	
↑	(-)	CDKN1C	p57 Kip2	D64137	G4	1.6	2.0	2.2	1.3	3.5	up SCC12B2
↑	(-)	PPP1R15A	GADD34	U83981	G3	0.8	1.6	1.4	2.2	5.1	
↓	(+)	CCNB1	Cyclin B1	M25753	G6	-1.1	-1.1	-1.4	-3.7	-4.3	
↓	(+)	CCNB2	Cyclin B2	AL080146	G6	-1.6	-1.1	-1.3	-4.0	-4.3	
↓	(+)	CCNE1	Cyclin E1	M74093		-0.9	-1.7	-3.2	-7.2	-0.6	
↓	(+)	MKI67	Antigen Ki-67	X65550	G6	-1.0	-1.3	-1.2	-2.9	-5.2	
↓	(+?)	RBBP2	Retinoblastoma-BP2	S66431		-1.1	-2.2	-3.6	-2.9	-3.1	
↓	(+)	BCAR3	Breast Cancer Anti-Estrogen Resistance 3	U92715		-1.0	-2.4	-3.1	-12.1	-3.9	
↓	(+)	FYN	FYN Oncogene	M14333	G6	-1.1	-1.3	-1.4	-4.9	-10.6	
↓	(+)	ABL1	c-abl Oncogene	X16416	G6	-0.9	-1.4	-2.8	-3.8	-8.1	
↓	(+)	ERBB3	c-erb-b2 Oncogene 3	M34309		-1.8	-1.5	-3.2	-3.4	-1.1	
↓	(-)	DMTF1	Cyclin D-Binding Myb-Like	AF052102		-1.2	-1.7	-3.4	-4.6	-1.9	
↓	(-)	WEE1	WEE1	X62048		-1.6	-6.7	-14.9	-18.9	-4.5	down SCC12B2
↓	(-)	CDKN1B	p27 Kip1	AI304854		-1.7	-3.0	-1.4	-1.3	-3.4	
↓	(-)	CUL1	Cullin 1	AF062536	G6	-1.2	-1.3	-2.4	-4.1	-6.4	
↓	(-)	BUB1B	Budding Uninhibited by Benzimidazoles 1b	AF053306	G6	-1.2	-1.5	-1.4	-2.9	-7.3	
↓	(-)	RFP2	Ret Finger Protein 2 (Tumor Suppressor)	AJ224819		-1.1	-2.7	-3.2	-5.0	-1.6	
↓	(-)	FAT	FAT1 (Tumor Suppressor)	X87241	G6	-1.2	-1.8	-2.4	-3.9	-4.0	
↓	(-)	FAT2	FAT2 (Tumor Suppressor)	AB011535	G6	-1.3	-1.6	-3.0	-3.7	-7.9	
2 Apoptosis (+/-)											
↑	(+)	CRADD	RAIDD	U79115		0.7	1.7	1.6	3.1	2.4	
↑	(+)	TSSC3	Tumor Suppressing STF 3	AF035444	G2	1.5	4.1	3.4	4.1	4.4	
↑	(+)	PMAIP1	APR (NOXA)	D90070	G2	2.0	7.0	14.7	18.1	13.0	up SCC12B2
↑	(-)	BCL2	BCL-2	M14745		2.0	1.0	1.0	3.6	1.0	
↑	(-)	API5L1	Antiapoptosis 5-Like 1	Y15906		1.5	4.0	3.2	1.1	2.4	
↑	(-)	IER3	Radiation-inducible IER3	S81914	G2	1.5	5.2	5.1	5.4	4.5	
↑	(-)	TNFAIP3	TNF α-Induced Protein 3	M59465	G3	1.0	0.7	1.2	2.4	6.8	down SCC12B2
↑	(-)	HSPA2	HSP70	L26336		1.8	1.8	2.1	2.2	1.9	
↑	(-)	HSPCB	HSP90	W28616	G1	1.2	1.2	2.1	2.1	0.6	up SCC12B2
↓	(+)	CASP8	Caspase 8	X98172	G6	-1.0	-1.1	-1.2	-3.7	-7.2	
↓	(+)	DATF1	Death Associated Transcription Factor 1	AB002331		-1.2	-3.3	-3.5	-3.0	-1.1	
↓	(-)	BIRC2	cIAP1	U37547	G6	-1.4	-1.2	-1.9	-3.6	-3.2	
3 DNA Repair											
↑		GADD45A	GADD45A	M60974	G2	1.5	3.8	4.7	7.0	9.2	up SCC12B2
↑		GADD45B	GADD45B	AF078077	G4	1.6	2.3	2.2	2.8	4.8	
↑		ERCC1	ERCC1	M13194	G3	1.2	2.0	1.4	1.1	3.9	
↑		ERCC2	ERCC2	AA079018	G1	1.1	1.6	2.4	2.2	1.4	
↑		BTG1	B-cell Translocation Gene 1	X61123		1.1	1.3	1.7	2.2	3.4	
↑		TOB1	Transducer of ErbB-2, 1	D38305	G2	0.9	1.5	2.0	2.9	2.5	up SCC12B2
↓		XPA	Xeroderma Pigmentosum A	D14533	G6	-1.2	-1.0	-1.4	-5.7	-7.2	
↓		REV3L	Catalytic Subunit of DNA Polymerase ζ	AL096744	G6	-2.2	-1.9	-3.5	-7.4	-8.5	
↓		CHD1	Chromodomain Helicase	AF006513		-1.2	-2.3	-4.1	-3.8	-1.0	down SCC12B2
↓		MSH6	G/T Mismatch Binding Protein	U73737		-1.1	-3.8	-2.1	-5.0	-2.7	
↓		POLE2	DNA Polymerase ε2	AF025840	G6	-0.6	-0.8	-1.1	-3.0	-3.0	
↓		PMS1	Postmeiotic Segregation Increased 1	U13695	G6	-1.0	-1.4	-1.0	-3.0	-5.3	
↓		MLH3	MutL Homolog 3	L40399	G6	-1.2	-1.7	-1.3	-3.7	-8.7	
4 Oxydative Stress / ROS Metabolism (+/-)											
↑	(-)	MT1G	Metallothionein 1G	J03910		1.0	3.2	3.4	2.0	3.8	
↑	(+)	CYBA	Superoxide-Generating NADPH Oxidase	M21186		1.3	1.0	1.7	2.5	2.8	
5 Extra Cellular Matrix / Protease (+/-)											
↑	(+)	MMP10	Stromelysin 2	X07820	G4	1.6	2.5	2.2	2.1	4.5	
↑	(+)	PRSS4	Trypsin 4	X71345	G4	1.4	2.2	1.4	1.9	7.0	
↑	(+)	KLK13	Kallikrein 13	AA401397		0.8	1.4	1.6	2.0	3.0	
↑	(-)	SPINK1	Trypsin Inhibitor	AI961220	G3	1.4	1.3	1.0	2.9	8.4	
↑	(-)	KNG	Kininogen	K02566		2.4	0.9	0.9	2.1	1.2	
↑	(-)	CST6	Cystatin E/M	N80906	G1	1.5	1.1	2.2	2.6	1.2	
↑		COL11A1	Collagen XI α1	J04177	G1	1.0	1.0	2.6	2.6	1.6	
↑		COL19A1	Collagen XIX α1	D38163		2.4	1.0	1.0	2.5	1.9	
↑		LAMA2	Laminin α2 (Merosin)	Z26653	G3	1.2	1.2	1.1	2.1	3.5	
↓	(+)	PRSS12	Neurotrypsin (Motopsin)	AI810767		-1.0	-1.0	-3.0	-3.1	-3.1	
↓	(-)	KOC1	IMP-3	U97188	G6	-1.8	-1.6	-2.7	-6.4	-10.2	
6 Growth Factor / Chemokine / Cytokine / Inflammation (+/-)											
↑	(+)	IL8	IL-8 (CXC)	M28130	G2	2.0	10.6	36.8	48.1	71.7	up SCC12B2

		Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
7 Structural Protein / Adhesion / Migration / Junction											
↑		CDH16	Cadherin 16 (KSP-Cadherin)	AF016272		1.7	1.0	2.2	1.8	2.6	
↑			Tubulin α1	HG2259-HT2348	G4	1.6	2.9	1.8	1.9	4.3	
↑		TUBB2	Tubulin β2	X02344	G4	1.6	3.2	1.9	1.8	4.7	
↑		TUBB4	Tubulin β4	U47634	G4	1.7	2.1	1.8	2.3	4.7	
↑		TUBB5	Tubulin β5	X00734	G4	1.3	1.9	1.6	2.1	3.7	
↑		TUB	Tubulin β	HG4322-HT4592	G2	1.4	2.1	2.5	3.0	2.8	
↑		TUBB	Tubulin β Polypeptide	X79535	G1	1.2	1.4	2.6	3.4	2.0	
↑		ANXA2	Annexin A2	D00017	G1	1.6	1.4	2.2	2.2	1.1	up SCC12B2
↑		ANXA2P3	Annexin A2 Pseudogene 3	M62895	G1	1.6	1.3	2.2	2.1	1.1	
↑		CEACAM1	Carcinoembryonic Antigen-Related to CAM 1	S71326		1.0	1.0	2.9	1.9	6.6	
↑		CLDN4	Claudin 4	AB000712	G2	1.4	2.5	3.1	2.7	4.6	
↑		NEF3	Neurofilament 3	Y00067	G2	1.4	2.3	3.1	4.1	4.7	
↑		PFN1	Profilin 1	J03191	G4	1.8	2.7	2.4	2.2	3.9	
↑		MAP1A/1BLC3	Microtubule-associated Proteins	W28807		0.8	0.6	1.1	2.8	2.1	
↑		PIN	Dynein	AI540958	G1	1.2	1.6	2.2	2.4	1.3	
↑		DGCR6	DiGeorge Syndrome Critical Region 6	X96484		1.6	1.7	2.2	2.0	2.2	
↑		GP1BB	Glycoprotein Ib β polypeptide	L20860		1.0	2.1	2.9	1.0	0.9	
↑		DCTN1	Dynactin 1	AF086947		0.8	1.3	3.4	1.5	5.1	
↑		TAGLN	Transgelin	M95787		1.9	2.2	2.1	2.8	2.4	
↑		NP25	Transgelin 3	Z78388		1.9	1.6	2.6	1.9	2.8	up SCC12B2
↑		SPTBN1	Spectrin β Non-Erythrocytic 1	AJ005694	G1	1.7	1.3	2.6	2.6	0.7	
↑		MYL6	Myosin Light Polypeptide 6	M22919	G1	1.5	1.6	2.0	2.1	1.3	
↑		CRYBA4	Crystallin β A4	U59057	G1	1.4	0.9	2.1	2.1	1.7	
	↓	KIF2	Kinesin Heavy Chain Member 2	Y08319	G6	-1.0	-1.9	-3.5	-7.5	-5.2	down SCC12B2
	↓	KNSL1	Kinesin-Like 1	U37426	G6	-2.2	-1.2	-2.3	-3.6	-9.6	
	↓	KNSL5	Kinesin-Like 5	X67155	G6	-1.7	-1.9	-2.1	-3.4	-3.4	
	↓	GCP3	Spindle Pole Body Protein	AF042378	G6	-1.4	-1.3	-1.5	-3.9	-7.7	
	↓	GJB3	Connexin 31	AF099730		-0.8	-0.6	-3.6	-4.2	-0.2	
	↓	LMNB1	Lamin B1	L37747	G6	-0.9	-1.0	-0.8	-3.2	-3.2	
8 Metabolism / Energy											
↑		GAD2	Glutamate Decarboxylase 2	M74826		2.9	0.8	3.2	0.8	0.8	
↑		PKM2	Pyruvate Kinase	W28740	G1	1.4	1.9	3.4	3.7	0.7	
↑		PCK2	Pyruvate Carboxykinase 2	X92720		1.1	1.3	1.4	2.2	2.5	
↑		GUK1	Guanylate Kinase 1	AI971642	G1	2.3	1.8	3.8	2.8	1.3	
↑		SULT2B1	Sulfotransferase 2B1	U92315	G4	1.3	2.4	1.6	1.9	5.5	
↑		ECHS1	Enoyl Coenzyme A Hydratase 1	D13900		1.6	2.1	1.0	0.9	2.8	
↑		NDUFA1	NADH Dehydrogenase 1α1	N47307	G1	1.3	1.4	2.3	2.1	1.3	
↑		NDUFC1	NADH Dehydrogenase 1	AA760866	G1	1.3	1.2	2.1	2.2	0.9	
↑		UQCRB	UQBP	T79616	G1	1.4	1.1	3.1	2.6	1.1	
↑		ATP5I	ATP synthase	AA426364	G1	2.0	1.7	4.4	4.6	1.2	
↑		APRT	Adenine Phosphoribosyltransferase	Y00486	G4	1.3	2.2	1.6	1.9	2.5	
↑		GCHFR	GTP Cyclohydrolase	U78190		1.3	0.9	0.8	2.1	2.6	
↑		GPI	Glucose Phosphate Isomerase	K03515	G5	1.4	2.2	1.2	1.3	2.8	
↑		ACO2	Aconitase 2	NM_001098	G5	0.9	2.3	1.3	1.2	3.1	
↑		CYP11B1	Cytochrome P450 XIB	X55764		1.9	1.3	2.6	3.0	2.3	
↑		CYP2J2	Cytochrome P450 IIJ	U37143		1.7	1.4	2.4	2.3	1.4	
↑		COX17	Cytochrome c Oxidase AP	AA149486	G1	1.2	1.1	2.1	2.6	0.8	
↑		COX7C	Cytochrome c Oxidase VIIc	AI708889	G1	1.0	1.2	2.7	2.2	1.6	
↑		COX7A2	Cytochrome c Oxidase VIIa	AA978033	G1	1.2	1.3	2.1	2.3	1.1	
↑		OAS2	2'-5'-oligoadenylate Synthetase 2	M87284	G1	1.4	1.8	2.7	2.6	1.5	
↑		NEU1	Sialidase 1	AF040958		0.8	1.0	3.8	0.8	7.8	
↑		HMGCS2	HMG-CoA synthase	X83618	G1	1.1	2.4	1.8	2.6	1.1	
↑		SURF1	Surfeit 1	Z35093		1.2	2.1	2.3	1.5	3.2	
↑		TSTA3	P35B	U58766	G2	0.9	1.2	2.2	3.0	3.2	
↑		KYNU	Kynureninase	AI148772		1.2	2.6	2.5	1.4	1.7	up SCC12B2
↑		APOE	Apolipoprotein E	M12529		3.2	1.4	1.7	0.8	3.4	
↑		TK1	Thymidine Kinase 1	M15205	G1	1.8	1.3	2.7	2.1	1.2	
↑		MAOB	Monoamine Oxidase B	M69177		2.6	0.7	1.6	2.5	0.7	
↑		GCB	Glucocerebrosidase	J03060		1.5	1.2	2.8	2.7	2.2	
↑		QPCT	Glutaminyl cyclase	X71125		0.6	1.1	1.4	2.0	2.1	
↑		HEXB	Hexosaminidase B	M23294		2.1	0.8	1.5	2.1	1.1	
	↓	AKR1C3	Aldo-Keto Reductase Family 1C3	D17793		-0.5	-1.4	-1.4	-2.2	-2.5	
	↓	GNE	N-Acetylmannosamine Kinase	AJ238764	G6	-1.2	-1.6	-2.0	-3.6	-5.2	
	↓	GCLC	Glutamate-Cysteine Ligase	M90656		-1.9	-1.6	-4.7	-7.9	-1.1	
	↓	ATP2B1	ATPase	J04027	G6	-0.9	-1.3	-1.2	-3.0	-3.0	
	↓	SLC9A6	Solute Carrier Family 9 Isoform 6	AF030409	G6	-1.0	-1.4	-1.3	-3.0	-4.6	
	↓	ADSL	Adenylosuccinate Lyase	AL022238	G6	-1.0	-1.5	-1.7	-2.9	-8.1	down SCC12B2
	↓	AD-017	Glycosyltransferase	L13435		-1.0	-1.8	-3.5	-1.7	-4.6	
9 Development / Differentiation (+/-)											
↑		(+) TGM3	Transglutaminase 3	L10386		1.2	1.1	1.8	2.7	2.4	
↑		(+) CSRP1	Cysteine Glycine-Rich Protein 1	M33146	G2	1.3	2.1	1.5	2.1	1.8	
↑		(+) KRTHA4	Keratin Hair Acidic 4	Y16790	G4	1.1	2.6	2.9	2.5	11.5	
↑		(+) MYF6	Myogenic Factor 6 (Herculin)	X52011	G1	1.3	1.8	2.4	2.4	0.9	
↑		(+) DLX2	Distal-Less Homeo Box 2	L07919		1.1	2.9	4.4	2.7	0.6	
	↓	(+) TP63	p63 (ΔNp63)	Y16961	G6	-0.9	-1.8	-2.0	-3.7	-18.0	
	↓	(+) PUM1	Pumilio 1	D43951		-1.6	-3.2	-6.6	-23.7	-2.4	down SCC12B2
	↓	(+) PUM2	Pumilio 2	D87078		-1.3	-2.1	-3.1	-5.7	-2.8	
	↓	(+) JMJ	Jumonji	AL021938	G6	-1.2	-1.8	-3.3	-5.5	-9.8	down SCC12B2
	↓	(+) FEM1B	FEM-1	AB007856		-1.7	-5.7	-8.0	-8.0	-6.0	
	↓	(+) EDR2	Early Development Regulator 2	U89278		-1.1	-2.3	-7.7	-7.7	-1.8	down SCC12B2
	↓	(+) FLG	Filaggrin	M60502		-1.8	-2.5	-2.5	-1.2	-0.6	
	↓	(+) PAFAH1B1	Platelet-Activating Factor Acetylhydrolase	L13385	G6	-1.0	-1.1	-2.0	-3.7	-3.8	
	↓	(+) FALZ	Fetal Alzheimer Antigen	U05237		-1.7	-2.3	-1.9	-3.4	-3.0	

	Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
10 Signal Transduction / Intracellular Traffic										
↑	IL1RL1	IL-1 Receptor-Like 1	D12763		1.0	1.7	2.3	5.9	7.9	
↑	CHRNB4	Cholinergic Receptor Nicotinic β4	U48861	G1	1.3	0.9	2.2	2.7	1.3	
↑	CHRNB1	Cholinergic Receptor Nicotinic β1	X14830	G1	2.0	2.4	3.5	2.7	1.3	
↑	PCTK1	PCTAIRE Protein Kinase1	X66363		1.9	1.6	1.8	3.1	4.3	
↑	PRKR	IFN-Inducible dsRNA Dependent Protein Kinase	U50648	G1	0.7	1.7	3.3	3.8	1.1	up SCC12B2
↑	DUSP1	Dual Specificity Phosphatase 1	X68277	G4	1.5	1.7	1.6	2.3	3.0	
↑	PPP2R1A	Protein Phosphatase 2 Regulatory Subunit A	J02902	G4	1.4	2.0	1.7	1.6	3.2	
↑	ITPR1	Inositol 1,4,5-triphosphate Receptor 1	D26070		2.4	2.2	0.9	1.2	0.9	
↑	PIK3R3	PI3-kinase Regulatory Subunit 3	D88532		2.5	1.2	2.3	0.8	0.8	
↑	PPIF	Cyclophilin F	M80254		0.8	1.3	1.6	2.5	4.0	
↑	GPR9	G Protein-Coupled Receptor 9	X95876	G1	1.6	1.3	2.1	2.3	1.2	
↑	GPS2	G Protein Pathway Suppressor 2	U28963		1.5	2.3	1.2	0.7	3.1	
↑	GEM	GTP-Binding Protein	U10550		0.9	0.9	1.0	4.5	2.7	
↑	ARHGDIA	Rho GDP Dissociation Inhibitor α	X69550	G4	1.2	2.3	1.9	1.3	5.0	
↑	TM4SF1	Transmembrane 4 Superfamily 1	M90657		1.2	1.3	1.8	3.3	5.1	
↑	TIM44	TIM44	AF026030		2.3	1.3	0.7	1.3	4.0	
↑	ADM	Adrenomedullin	D14874	G3	1.1	1.5	1.3	2.3	6.8	
↑	HTR4	Serotonin Receptor 4	Y12505	G1	2.5	1.7	1.8	2.8	1.0	
↑	SGK	Serum/Glucocorticoid Regulated Kinase	Y10032	G2	1.5	2.5	3.3	3.3	4.5	
↑	CLTB	Clathrin Light Polypeptide	M20469	G3	1.3	1.5	1.1	2.1	6.6	
↑	C8FW	Mitogenic Regulated Phosphoprotein	AJ000480		1.6	1.0	2.7	2.3	3.6	
↑	LOC55858	TPA Regulated Locus	W28729		7.0	4.4	3.4	2.7	2.0	up SCC12B2
↑	RANBP1	RAN Binding Protein 1	D38076	G1	0.9	1.0	2.1	2.1	0.6	
↑	RANBP9	RAN Binding Protein 9	AA191426	G1	1.5	1.9	2.2	2.1	0.7	
↑	LIM	LIM Protein	AI341656	G1	1.5	1.0	2.7	2.9	1.0	
↑	DSCR1L1	Down Syndrome Gene 1-Like 1	D83407		2.4	1.1	3.0	1.8	1.9	
↑	TACSTD2	Tumor-Associated Calcium Signal Transducer 2	X77753	G2	1.7	3.7	4.1	5.3	5.1	up SCC12B2
↑	GNB2L1	GNBP b2-Like 1 (G protein)	M24194	G5	1.4	2.3	1.1	1.4	4.3	
↑	TCN1	Transcobalamin I	J05068		1.5	1.2	0.7	2.4	2.4	
↑	GPAA1	Anchor Attachment Protein 1	AB002135	G1	1.4	1.8	2.1	2.2	1.3	
↑	TRIM15	Tripartite Motif-Containing 15	U34249		0.9	1.3	0.9	2.7	2.4	
↑	NMA	BMP/Activin Inhibitor	U23070	G2	1.3	2.5	3.9	3.9	4.4	
↑		Phosphoglycerate kinase {alternatively spliced}	S81916	G1	0.9	1.1	2.2	2.3	1.7	up SCC12B2
↓	MAP3K5	MAPKKK 5	U67156	G6	-1.1	-2.1	-3.2	-3.5	-7.1	
↓	MIG2	Mitogen Inducible 2	Z24725	G6	-0.9	-1.5	-3.2	-2.9	-8.0	
↓	PIP5K2B	PI4P5-Kinase IIB	U85245	G6	-1.1	-2.0	-4.0	-5.3	-19.8	
↓	PIK3C3	PI3-Kinase Class 3	Z46973	G6	-1.1	-1.7	-1.1	-3.8	-4.2	
↓	PIK3R4	PI3-Kinase Regulatory Subunit 4	Y08991		-1.6	-0.9	-3.4	-0.9	-3.4	
↓	PRKCBP1	Protein Kinase C Binding Protein 1	W22296	G6	-1.6	-2.4	-2.8	-6.2	-6.3	down SCC12B2
↓	PRKCD	Protein Kinase C δ	D10495		-1.4	-3.8	-1.7	-2.7	-1.0	
↓	STK15	Serine/Threonine Kinase 15	AF011468		-1.1	-1.0	-3.6	-3.7	-5.1	down SCC12B2
↓	STK18	Serine/Threonine Kinase 18	Y13115		-1.5	-1.9	-2.7	-3.5	-3.5	
↓	AKAP2	A kinase Anchor Protein 2	AB023137	G6	-1.0	-1.7	-1.4	-5.7	-5.7	
↓	AKAP9	A kinase Anchor Protein 9	AJ131693		-1.4	-2.6	-2.9	-1.8	-6.2	
↓	AKAP10	A kinase Anchor Protein 10	AA114830		-1.5	-2.4	-4.5	-17.2	-6.7	down SCC12B2
↓	DYRK1A	Dual-Specificity Tyrosine-Kinase 1A	D86550		-1.5	-5.4	-6.4	-10.5	-7.3	down SCC12B2
↓	DYRK2	Dual-Specificity Tyrosine-Kinase 2	Y09216		-1.1	-2.3	-13.8	-7.3	-1.6	
↓	TTK	TTK Protein Kinase	M86699	G6	-1.9	-1.5	-2.4	-6.9	-9.6	
↓	TLK1	Tousled-Like Kinase 1	D50927	G6	-1.6	-1.7	-2.4	-4.6	-17.7	down SCC12B2
↓	TLK2	Tousled-Like Kinase 2	AB004884	G6	-0.9	-1.7	-2.8	-5.2	-6.1	down SCC12B2
↓	PTK2	PTK2 Protein Tyrosine Kinase 2	L13616	G6	-1.3	-1.7	-1.8	-3.5	-11.6	
↓	PPP1R3C	Protein Phosphatase 1 3C	N36638		-1.6	-2.9	-4.9	-2.9	-4.8	
↓	PPP1R12A	Protein Phosphatase 1 12A	D87930	G6	-1.2	-1.9	-2.3	-3.1	-4.8	
↓	PTPN3	Protein Tyrosine Phosphatase 3	M64572		-1.3	-1.7	-1.3	-11.6	-4.2	
↓	PDZ-GEF1	PDZ Guanine Exchange Factor 1 (GEF1)	AF070570		-1.3	-3.9	-22.6	-19.6	-16.1	down SCC12B2
↓	ARHGEF7	Rho Guanine Exchange Factor 7 (GEF7)	D63476	G6	-1.1	-1.6	-2.0	-3.6	-4.4	down SCC12B2
↓	GFR	M-Ras-Regulated GEF for Rap1	D87467		-1.6	-3.2	-4.6	-1.7	-5.0	
↓	IRS1	Insulin Receptor Substrate 1	S62539	G6	-1.2	-1.4	-2.6	-3.7	-5.3	
↓	TGFBR2	TGF β Receptor II	D50683		-1.2	-2.9	-3.3	-6.9	-6.2	down SCC12B2
↓	FGFR2	FGF Receptor 2 (KGF Receptor)	M87770	G6	-1.5	-1.4	-1.7	-5.2	-5.2	
↓		Glucocorticoid Receptor β	HG4582-HT4987	G6	-1.1	-1.7	-2.0	-5.3	-5.3	
↓	BDKRB2	Bradykinin Receptor B2	X86163		-1.5	-3.0	-3.3	-3.1	-2.7	
↓	ADORA2B	Adenosine A2b Receptor	X68487		-1.5	-2.1	-4.8	-5.1	-5.3	down SCC12B2
↓	SMAP	Thyroid Hormone Receptor Coactivating Protein	AW020536		-1.8	-1.8	-5.0	-9.7	-2.8	down SCC12B2
↓	HMG2L1	High-Mobility Group Protein 2-Like 1	AL079310		-1.2	-3.3	-6.9	-11.6	-5.0	
↓	ADRB2	Adrenergic β2 Receptor	M15169		-1.8	-3.2	-3.3	-2.2	-0.8	
↓	CBLB	Cas-Br-M Retroviral Transforming Sequence B	U26710		-1.5	-2.8	-3.5	-5.1	-5.1	
↓	LPP	LIM Preferred Translocation Partner in Lipoma	U49957		-1.5	-4.6	-4.6	-3.5	-4.6	
↓	VRP	Vascular Rab-GAP/TBC-Containing	AB024057	G6	-1.1	-1.9	-3.0	-4.0	-6.3	
↓	RIN2	RAB 5 Interacting Protein 2	AL049538	G6	-1.2	-2.0	-3.0	-5.7	-5.8	
↓	ADCY9	Adenylate Cyclase 9	AF036927	G6	-1.6	-2.2	-2.7	-3.4	-11.5	
↓	EFNB2	Ephrin-B2	AI765533		-1.0	-3.5	-5.7	-2.1	-1.5	
↓	XPO1	Exportin 1	Y08614	G6	-1.3	-1.9	-2.2	-7.8	-10.9	
↓	QKI	Quaking	AL031781	G6	-2.2	-2.1	-2.3	-3.5	-5.2	
↓	APPBP2	Amyloid β Precursor-Binding Protein 2	D86981	G6	-1.5	-1.5	-2.2	-4.2	-4.2	
↓	MTM1	Myotubular Myopathy 1	U46024		-1.5	-1.7	-3.6	-4.3	-4.1	
↓	CBFA2T2	Core-Binding Factor α2	AF069747		-1.3	-3.2	-1.9	-1.9	-3.1	
↓	CTBP2	C-Terminal Binding Protein 2	AF016507	G6	-1.1	-1.8	-1.8	-3.2	-6.6	
↓	SCAP2	src Family Associated Phosphoprotein 2	AF051323		-1.2	-1.4	-3.3	-3.8	-1.8	
↓	LNK	Lymphocyte Adaptor Protein	AF055581		-1.2	-3.0	-1.2	-3.0	-1.9	
↓	C1orf9	Chromosome 1 Open Reading Frame 9	AL035291	G6	-1.3	-1.9	-1.4	-3.6	-3.6	
↓	PSCD1	Cytohesin 1	M85169		-1.1	-1.5	-3.4	-7.5	-1.9	
↓		LDL Receptor	L00352		-1.6	-2.3	-6.3	-8.1	-1.5	
↓	DKK1	Dickkopf 1	AB020315		-1.7	-3.6	-4.1	-1.6	-0.4	

		Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
11 Transcription (+/-) / Replication											
↑		(+) FOS	FOS	V01512		5.4	4.7	4.7	2.1	0.6	up SCC12B2
↑		(+) JUN	JUN	J04111		2.9	7.9	5.7	2.9	1.1	
↑		(+) JUNB	JUNB	M29039	G2	2.0	4.5	3.6	4.0	3.4	
↑		(+) JUND	JUND	X56681	G2	1.3	2.2	3.7	3.0	2.6	up SCC12B2
↑		(+) STAT6	STAT6	U16031	G1	1.3	1.1	2.4	2.4	0.7	
↑		(+) CITED2	p300/CBP	U65093	G2	0.9	3.5	5.5	7.2	2.2	up SCC12B2
↑		(+) TLS/CHOP	GADD153	HG2724-HT2820		2.5	3.8	1.6	0.2	1.1	
↑		(+) NR1D1	Nuclear Receptor Subfamily 1D1	X72631	G3	1.3	1.5	1.8	3.1	9.4	
↑		(+) NR4A2	Nuclear Receptor Subfamily 4A2	X75918	G1	1.4	2.4	2.3	3.5	1.3	
↑		(+) ETR101	Immediate Early Protein (IER2)	M62831		2.0	3.2	2.0	1.5	2.5	
↑		(+) FOXC1	Forkhead Box C1	AF078096		1.0	1.2	1.6	2.9	2.8	
↑		(+) SOX20	SRY (Sex Determining Region Y)-Box 20	AB006867	G4	1.5	1.8	1.8	2.0	3.1	
↑		(+) ZFP36	Zinc Finger Protein ZFP-36	M92843	G4	1.5	2.6	2.0	1.8	4.4	
↑		(+) KIAA0628	Zinc Finger Protein C2H2 (krueppel family)	AB014528	G1	1.6	0.8	3.4	3.8	0.6	
↑		(+) XBP1	X-box Binding Protein 1	Z93930	G4	1.5	2.2	1.9	1.5	3.5	
↑		(+) PAX8	Paired Box 8	X69699		0.8	1.6	3.6	1.2	3.5	
↑		(+) TFE3	TFE3	X96717		1.8	1.7	2.1	1.6	4.3	
↑		(+) TITF1	Thyroid Transcription Factor 1	U43203		2.2	0.9	2.3	1.6	1.2	
↑		(+) CCNT2	Cyclin T2	AF048732	G3	1.0	1.0	1.0	2.5	3.5	
↑		(-) TSC22	TGF β-Stimulated Protein	AJ222700	G1	1.2	2.3	4.0	3.1	1.6	
↑		(-) DRAP1	DR1-Associated Protein 1	U41843		1.3	1.7	4.2	0.7	5.3	up SCC12B2
↑		(-) ATF3	ATF3	L19871	G2	1.3	3.8	4.7	10.8	17.1	up SCC12B2
↑		(-) ATF4	ATF4	AL022312	G2	1.2	1.7	2.1	2.2	3.1	
↑		(-) SAP18	Sin3-Associated Polypeptide	W27641	G1	1.8	1.5	2.1	2.1	0.8	
↑		(-) ID2	Inhibitor of DNA Binding 2	D13891		1.9	4.0	3.0	2.1	2.1	up SCC12B2
	↓	(+) MYC	MYC	V00568		-1.5	-3.5	-3.8	-4.9	-4.2	down SCC12B2
	↓	(+) E2F3	E2F3	D38550		-1.4	-2.9	-3.6	-3.5	-2.9	down SCC12B2
	↓	(+) E2F5	E2F5	U31556	G6	-1.2	-1.6	-1.3	-3.2	-6.3	
	↓	(+) SMAD3	SMAD3	U68019	G6	-1.4	-1.6	-3.3	-10.0	-7.0	
	↓	(+) SMAD5	SMAD5	U59913		-2.1	-3.4	-1.8	-1.8	-3.1	
	↓	(+) SP3	Sp3 Transcription Factor	X68560	G6	-2.0	-2.5	-2.4	-5.7	-5.6	
	↓	(+) NCOA2	Nuclear Receptor Coactivator 2	AI040324		-1.8	-2.8	-5.4	-3.5	-8.2	
	↓	(+) NCOA3	Nuclear Receptor Coactivator 3	AF012108		-2.0	-3.2	-2.4	-3.8	-5.3	
	↓	(+) NRIP1	Nuclear Receptor Interacting Protein 1	X84373		-0.9	-2.6	-4.9	-5.0	-2.9	
	↓	(+) NR3C1	Nuclear Receptor Subfamily 3C1	M10901		-2.2	-2.2	-3.2	-16.2	-7.1	
	↓	(+) CREBBP	CREB Binding Protein	U47741		-1.3	-1.6	-3.0	-3.0	-2.3	
	↓	(+) CEBPD	C/EBP δ (Nuclear factor NF-IL6-β)	M83667	G6	-1.4	-1.4	-1.6	-3.1	-3.8	
	↓	(+) NFYA	CCAAT/BF-B (Nuclear Transcription Factor Y α)	AL031778		-1.1	-1.6	-3.5	-3.5	-2.0	
	↓	(+) HMGIC	High-Mobility Group Protein Isoform IC	X92518	G6	-1.1	-1.8	-3.9	-4.2	-13.1	down SCC12B2
	↓	(+) HIVEP2	HIV I Enhancer-Binding Protein 2	AL023584		-1.6	-2.9	-5.2	-5.2	-5.2	
	↓	(+) ELF1	ETS Domain Transcription Factor 1	M82882		-1.2	-2.6	-3.5	-3.5	-1.7	
	↓	(+) ELF2	ETS Domain Transcription Factor 2	U43189		-0.8	-3.7	-4.5	-5.5	-5.5	
	↓	(+) MLLT2	MLL2 Translocated to 2	L13773		-1.9	-4.0	-3.3	-5.5	-5.1	
	↓	(+) MEIS1	Meis1	U85707	G6	-1.7	-2.2	-1.7	-3.8	-3.8	
	↓	(+) BTEB1	Basic Transcription Element BP1	D31716		-1.2	-3.3	-5.6	-4.4	-1.3	
	↓	(+) FOXO1A	Forkhead Box O1A	AF032885	G6	-1.0	-2.9	-3.0	-12.7	-9.6	
	↓	(+) FOXO3A	Forkhead Box O3A	AF032886	G6	-1.2	-1.9	-2.0	-3.1	-3.9	down SCC12B2
	↓	(+) ZNF44	Zinc Finger Protein 44	X16281		-1.0	-2.9	-3.1	-5.1	-1.7	
	↓	(+) ZNF217	Zinc Finger Protein 217	AF041259		-0.8	-3.6	-3.6	-2.2	-0.9	
	↓	(+) ZNF264	Zinc Finger Protein 264	AB007872		-3.2	-4.2	-4.2	-4.2	-1.6	
	↓	(+) KIAA0441	KIAA0441 protein	AB007901		-0.9	-2.2	-3.4	-3.6	-4.4	
	↓	(+) AHR	Aryl Hydrocarbon Receptor	L19872		-1.1	-3.1	-1.3	-3.4	-2.6	
	↓	(+) ATRX	α Thalassemia	U72936		-1.6	-3.0	-2.9	-2.4	-8.0	
	↓	(+) BACH1	Basic Leucine Zipper Transcription Factor 1	AB002803		-1.6	-2.3	-6.2	-3.3	-0.9	
	↓	(+) KLF7	Kruppel-Like Factor 7	AA478904	G6	-1.3	-2.4	-2.8	-3.0	-7.9	down SCC12B2
	↓	(+) DRIL1	Dead Ringer-Like 1	U88047		-1.3	-1.6	-3.0	-0.8	-3.0	
	↓	(+) TAF5L	TAF5-Like RNA Pol II	AJ009770		-1.0	-1.0	-3.9	-1.9	-3.9	
	↓	(+) TAF11	TAF11 RNA Pol II	X83928		-0.8	-1.4	-3.0	-3.0	-1.9	
	↓	(+) SSBP2	ssDNA-BP2	AL080076	G6	-1.4	-0.7	-1.2	-3.4	-3.4	
	↓	(+) TRIP-Br2	Transcriptional Regulator to PHS-Br2	D50917		-1.0	-1.9	-3.7	-3.0	-1.0	
	↓	(+) AND-1	AND-1 Protein	AJ006266		-2.9	-1.0	-1.3	-1.8	-2.9	
	↓	(+) NFE2L2	Nuclear Factor-Erythroid Like 2	S74017		-1.4	-1.5	-3.6	-5.6	-0.8	
	↓	(-) SHARP	SMART/HDAC1 Associated Repressor Protein	AL096858		-1.8	-2.4	-3.8	-14.1	-5.2	
	↓	(-) PER2	Period Circadian Protein 2	AB002345		-1.8	-3.5	-3.1	-2.7	-3.7	
	↓	(-) ZNF148	Zinc Finger Protein 148	AJ236885		-1.5	-2.2	-3.9	-4.6	-3.5	
	↓	(-) SMAD7	Mothers Against Decapentaplegic 7	AF010193		-0.5	-2.8	-2.8	-0.8	-0.3	
	↓	(-) MNT	MAX Binding Protein	X96401		-0.9	-2.5	-3.5	-4.9	-3.8	
	↓	(-) NR2F2	Nuclear Receptor Subfamily 2F2	M64497		-1.9	-1.8	-3.8	-3.0	-1.3	
	↓	(-) NFKB1	Nuclear Factor κ B1 (p105 inhibitory precursor)	M58603	G6	-0.9	-1.0	-1.4	-3.5	-3.3	
	↓	(-) ID1	Inhibitor of DNA Binding 1	X77956		-0.8	-2.5	-3.4	-5.5	-3.4	down SCC12B2
	↓	(-) CNOT2	CCR4-NOT Transcription Complex 2	AI123426	G6	-1.4	-2.5	-3.2	-3.8	-6.7	down SCC12B2
	↓	(-) CNOT4	CCR4-NOT Transcription Complex 4	U71267	G6	-0.9	-1.3	-1.8	-3.8	-3.1	down SCC12B2
	↓	(-) CTCF	CCCTC-Binding Factor	U25435		-1.3	-1.3	-3.0	-4.9	-2.1	down SCC12B2
	↓	(-) MECP2	Methyl CpG Binding Protein 2	AJ132917	G6	-2.2	-2.5	-3.1	-5.4	-9.5	
	↓	(-) MBD2	Methyl-CpG Binding Domain Protein 2	AF072242	G6	-1.0	-0.8	-1.2	-3.5	-3.1	
	↓	RNF13	Ring Finger Protein 13	AF037204	G6	-1.8	-1.2	-2.2	-3.9	-4.6	
	↓	TRF4	Topoisomerase-Related Function Protein 4-1	AB005754		-1.1	-2.0	-3.0	-3.8	-2.0	down SCC12B2
	↓	POLD3	DNA Polymerase δ3	D26018	G6	-1.1	-2.1	-1.8	-4.2	-9.6	
	↓	ZNF146	Zinc Finger Protein 146	X70394	G6	-1.2	-2.0	-1.2	-3.4	-9.7	down SCC12B2
	↓	ORC2L	Origin Recognition Complex Subunit 2-Like	U27459	G6	-1.6	-0.9	-1.1	-3.3	-5.8	
12 Histone / Chromatin											
↑		H1FX	Histone H1X	D64142	G4	0.9	1.4	3.5	3.5	4.6	
↑		H2AFI	Histone H2A I	AI200373		2.2	1.4	2.4	2.6	4.2	
↑		H2AFA	Histone H2A A	AI039144	G2	1.1	4.8	9.4	13.4	12.4	
↑		H2AFG	Histone H2A G	Z80776		2.4	1.8	1.6	4.3	6.3	
↑		H2AFO	Histone H2A O	L19779	G4	1.5	3.2	2.1	3.0	18.0	
↑		H2AFX	Histone H2A X	X14850	G5	1.0	2.5	0.7	1.3	3.2	
↑		H2BFC	Histone H2B C	AL009179	G2	1.0	2.2	3.2	6.5	5.5	
↑		H2BFG	Histone H2B G	Z80779	G5	1.5	2.5	1.1	1.3	4.9	
↑		H2BFH	Histone H2B H	Z80780		2.2	1.0	1.4	1.6	3.9	
↑		H2BFQ	Histone H2B Q	X57985	G2	1.5	5.3	5.3	7.9	12.1	
↑		H2BFR	Histone H2B R	AI076718		1.4	2.6	2.6	0.6	0.6	
	↓	CENPA	Centromere Protein A	U14518	G6	-1.3	-1.2	-2.3	-7.0	-14.6	down SCC12B2
	↓	CENPC1	Centromere Protein C1	M95724	G6	-1.5	-1.5	-3.0	-4.3	-4.0	
	↓	CENPF	Centromere Protein F	U30872		-1.9	-1.7	-4.1	-2.4	-3.5	
	↓	SMC4L1	Structural Maintenance Chromosomes 4-Like 1	AB019987	G6	-1.7	-1.8	-1.3	-4.8	-6.0	
	↓	SLBP	Stem-Loop (histone) Binding Protein	U75679	G6	-1.4	-1.8	-1.5	-3.6	-3.6	
	↓	MORF	Histone Acetyltransferase	AB002381	G6	-0.9	-1.6	-1.4	-5.4	-6.7	
	↓	ZNF220	Zinc Finger Protein 220	U47742	G6	-1.3	-2.4	-3.2	-4.1	-7.2	
	↓	BAZ1A	Br Domain Adjacent to ZF1A	AL050089	G6	-1.3	-1.9	-2.0	-3.9	-5.1	
	↓	BAZ1B	Br Domain Adjacent to ZF1B	AF072810		-0.8	-1.1	-5.5	-3.4	-2.5	
	↓	BAZ2B	Br Domain Adjacent to ZF2B	AL080173		-1.2	-2.2	-2.0	-7.8	-3.8	

		Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
13 Translation (+/-) / Protein Synthesis (+) / Degradation (-)											
↑	(+)	GC20	Translation Factor Sui1	AF064607		0.7	1.0	1.2	2.3	3.9	
↑	(+)	EIF3S4	Translation Initiation Factor 3 S4	U96074	G5	1.1	2.4	1.1	1.1	4.8	
↑	(+)	EEF1A1	Translation Elongation Factor 1 α1	W28170	G1	1.4	1.4	2.3	2.5	0.8	
↑	(+)	DIM1	Spliceosomal U5 snRNP-Specific Protein	AF023612		2.3	1.6	2.8	2.9	0.9	
↑	(+)	NCBP2	Nuclear Cap Binding Protein Subunit 2	D59253	G1	1.1	1.1	2.2	2.1	1.1	
↑	(+)	RALY	RNA-Binding Protein	L38696	G5	1.0	4.3	1.0	1.1	3.8	
↑	(+)	RNU2	U2 snRNA	W25892		0.9	3.6	4.7	3.0	4.4	up SCC12B2
↑	(+)	RNU17D	RNA U17D Small Nucleolar	AJ006835		0.8	1.5	2.4	1.9	2.5	
↑	(+)	SNRPE	SNRPE	AA733050	G1	1.8	1.5	2.2	2.2	1.0	
↑	(+)	RPP20	Ribonuclease P Protein p20	AA203527		2.6	0.8	2.0	2.6	2.0	
↑	(+)	RPL30	Ribosomal Protein L30	L05095	G1	1.4	1.2	2.0	2.2	1.2	
↑	(+)	RPL31	Ribosomal Protein L31	X15940	G1	1.1	1.2	2.2	2.2	1.2	
↑	(+)	RPL34	Ribosomal Protein L34	L38941	G1	1.3	1.3	2.7	3.2	0.9	
↑	(+)	RPL37	Ribosomal Protein L37	D23661	G1	1.1	1.1	2.3	2.7	1.1	
↑	(+)	RPL37A	Ribosomal Protein L37a	L06499	G1	1.3	1.2	2.2	2.2	1.1	
↑	(+)	RPL38	Ribosomal Protein L38	Z26876	G1	1.6	1.5	2.6	2.2	1.4	
↑	(+)	RPL44	Ribosomal Protein L44	T89651	G1	1.1	1.5	2.3	2.6	1.3	
↑	(+)	LAMR1	Ribosomal Protein SA	M14199		1.7	2.5	0.9	0.9	3.8	
↑	(+)	RPS2	Ribosomal Protein S2	X17206	G5	1.5	2.3	0.9	1.0	6.5	
↑	(+)	RPS17	Ribosomal Protein S17	M13932	G1	1.4	1.2	2.3	2.3	1.1	
↑	(+)	RPS21	Ribosomal Protein S21	X79563		1.6	2.1	1.4	2.1	1.1	
↑	(+)	RPS23	Ribosomal Protein S23	D14530	G1	1.2	1.2	2.2	2.2	1.2	
↑	(+)	RPS27	Ribosomal protein S27	AI557852	G1	1.1	1.2	2.2	2.4	1.2	
↑	(-)	CAPNS1	Calpain S1	X04106	G4	1.5	2.4	1.9	1.5	5.4	
↑	(-)	UBE2M	Ubiquitin-conjugating Enzyme E2M	AF075599		1.7	2.2	3.7	2.6	3.6	
↑	(-)	E2-EPF	Ubiquitin Carrier Protein	M91670	G2	1.7	1.3	2.3	2.3	3.4	
↑	(-)	PSMD8	Proteasome 26S S8	D38047	G2	1.3	1.2	1.6	2.0	2.0	up SCC12B2
↑	(-)		Ubiquitin protein	U49869		1.3	2.1	2.2	1.6	1.1	
↓	(+)	MTIF2	Mitochondrial Translational Initiation Factor 2	L34600		-3.1	-0.8	-2.8	-2.4	-4.5	
↓	(+)	MPHOSPH10	U3 snRNP	X98494		-1.1	-2.2	-2.1	-3.6	-3.5	down SCC12B2
↓	(+)	RRS1	Ribosome Biogenesis Protein 1	D25218	G6	-0.8	-1.2	-1.4	-3.1	-3.9	down SCC12B2
↓	(+)	SFRS2IP	Splicing Factor 2 Interacting Protein	AF030234		-2.7	-2.3	-1.2	-3.3	-5.0	
↓	(+)	PCF11	Pre-mRNA Cleavage Complex 11 protein	AB020631		-2.0	-3.6	-4.7	-1.8	-4.7	
↓	(-)	USP1	Ubiquitin Specific Protease 1	AB014458		-1.6	-1.2	-1.6	-5.0	-3.2	
↓	(-)	USP6	Ubiquitin Specific Protease 6	X63547	G6	-1.7	-2.2	-2.7	-3.6	-13.2	
↓	(-)	USP24	Ubiquitin Specific Protease 24	AB028980	G6	-1.2	-1.7	-2.1	-3.2	-5.2	
↓	(-)	UBE2G1	Ubiquitin-Conjugating Enzyme E2G1	AI701164		-1.5	-2.2	-3.1	-5.5	-1.7	
↓	(-)	UBL3	Ubiquitin-Like 3	AL080177	G6	-1.4	-1.8	-1.4	-3.1	-3.8	
14 Other / Unknown											
↑		GHRH	Growth Hormone-Releasing Factor 5	L00137	G1	1.5	1.9	2.3	2.8	1.3	
↑		CCK	Cholecystokinin	AW043690		0.8	2.4	1.8	4.7	3.3	
↑		NUCB1	Nucleobindin 1	M96824	G5	0.6	2.2	2.0	1.6	3.6	
↑		BRD2	Bromodomain-Containing 2	S78771	G3	0.9	1.7	1.2	2.7	5.8	
↑		DIPA	Hepatitis δ Antigen-Interacting Protein A	AI824126		0.4	1.5	2.5	3.1	2.3	
↑		SEC61G	Sec61 Gamma	AF054184	G1	0.9	1.0	2.8	2.9	1.2	
↑		SDBCAG84	Serologically Defined BRCA 84	AF091085		1.6	2.1	1.3	3.2	2.5	
↑			ESTs	AA203487	G1	1.0	1.6	2.1	2.1	1.4	
↑		AF038169	hypothetical protein	AF038169	G1	1.2	1.3	2.1	2.0	0.7	
↑		HCRT	Hypocretin (Orexin) Neuropeptide Precursor	AF041240		2.2	0.9	1.7	2.6	1.6	
↑		PHLDA1	Pleckstrin Homology-Like Domain A1	Z50194		1.5	1.2	1.1	2.7	2.7	
↑			Libtest16.A02.r	AI541542	G1	1.3	1.2	2.1	2.2	0.9	
↑			seq containing Cytochrome C Oxidase, L31, SRp30c	AA311181		2.2	1.2	1.3	2.9	1.4	
↑			ESTs	AI526078	G1	1.3	1.2	2.1	2.1	1.1	
↑		P311	P311 Protein	U30521	G1	2.1	0.6	2.5	2.5	0.6	
↑		YKT6	SNARE Protein	U95735		1.5	1.6	2.0	2.4	3.3	
↑		ARS	ARS Component B	X99977	G5	0.7	3.1	0.8	0.8	13.3	
↑		GAS	Gastrin	V00511	G3	0.8	2.3	1.5	2.6	6.6	
↑			ESTs	AI971169		1.9	1.3	2.0	2.1	1.4	
↑		HSA6591	Nucleolar Cysteine-Rich Protein	H82458	G1	1.5	0.9	2.1	2.9	0.9	
↑		PRDM1	PR Domain Containing 1	AF084199	G3	2.3	1.3	1.7	3.9	8.4	
↑		TXN	Thioredoxin	AI653621	G1	1.1	1.1	2.1	2.4	1.7	
↑		TRB@	T cell receptor beta locus	U66061	G3	1.0	2.1	1.5	3.0	6.0	
↑		TOM1	Target Of Myb1	AJ006973	G5	1.7	2.4	1.1	1.9	4.4	
↑		PMS2L6	Postmeiotic Segregation Increased 2-Like 6	AI341574		0.8	2.4	3.4	1.8	0.8	
↑			TR5	X15674		0.9	0.9	2.4	0.9	3.4	
↑		KIAA1155	KIAA1155 protein	AF090102		2.6	0.9	3.1	0.9	1.7	
↑		SGNE1	Secretory Granule Neuroendocrine Protein 1	Y00757		3.0	0.9	1.0	2.1	0.7	
↑			Ras Inhibitor Inf	HG511-HT511		1.9	1.4	2.9	2.5	2.3	
↑			Alu repeats 5' to the snRNP polypeptide E	M21259	G1	2.1	0.8	2.1	2.8	0.5	
↑		HSSEXGENE	Sex Gene (Plexin 3)	X87852	G1	1.0	1.8	2.4	2.5	1.0	
↑		KIAA1733	KIAA1733 protein	AL008729	G1	1.6	0.8	2.7	2.4	0.8	
↑		EST00098	hypothetical protein	AI885170		1.4	1.5	1.8	2.1	2.4	
↑		HTN1	Histatin 1	AB021179	G1	0.7	1.7	2.1	2.4	1.0	up SCC12B2
↑		KIAA0542	KIAA0542 protein	AB011114		1.1	2.2	1.8	1.4	2.5	
↑			Major Histocompatibility Complex Class I, G	AL022723	G2	1.5	2.1	2.4	1.5	0.8	
↑		STXBP2	Syntaxin Binding Protein 2	AB002559		0.5	0.8	0.9	2.5	2.4	
↑		LY6E	Lymphocyte Antigen 6 Complex Locus E	U66711	G2	1.3	1.4	2.0	2.3	2.3	
↑		LOC65122	similar to antigen of melanoma	AL022101		1.3	1.0	1.2	2.2	2.2	
↑		SERHL	Serine Hydrolase-Like	AL022316		2.0	2.3	2.2	0.6	2.3	
↑		FCN3	Ficolin 3	D88587		2.3	1.2	2.1	2.2	1.6	
↑		MVP	Major Vault Protein	X79882	G1	1.3	1.2	2.0	2.1	1.4	
↓		KIAA0232	KIAA0232 protein	D86985		-1.4	-2.7	-4.9	-17.8	-5.2	
↓		KIAA0308	KIAA0308 protein	AB002306	G6	-1.5	-3.3	-2.5	-6.7	-16.3	
↓		DKFZp586F122	DKFZp586F1223 protein	AL050204	G6	-2.3	-2.5	-3.0	-5.8	-13.8	
↓			ESTs	W28612		-0.8	-3.3	-3.9	-10.4	-12.5	down SCC12B2
↓		DKFZp564D15	DKFZp564D156 protein	AL049321	G6	-1.9	-2.0	-3.0	-7.9	-13.6	
↓		KIAA0042	KIAA0042 protein	D26361	G6	-2.1	-1.6	-3.5	-6.5	-12.4	
↓		CDYL	Chromodomain Protein Y Chromosome-Like	AL050164	G6	-1.0	-1.7	-3.5	-5.8	-11.8	down SCC12B2
↓			ELISC-1 mRNA	AA975427	G6	-1.9	-3.4	-2.3	-3.9	-9.6	
↓		FLJ20500	hypothetical protein	AA522530		-1.1	-2.1	-6.6	-8.7	-5.8	down SCC12B2
↓			mRNA from chromosome 5q21-22	AB002448		-1.3	-2.4	-8.9	-7.8	-7.7	
↓		KIAA1025	KIAA1025 protein	AB028948		-1.4	-3.3	-4.2	-2.6	-8.9	
↓		KIAA1041	KIAA1041 protein	AB028964		-1.6	-2.4	-5.0	-5.3	-7.5	down SCC12B2
↓		KIAA0442	KIAA0442 protein	AB007902	G6	-1.1	-2.2	-3.9	-7.3	-7.3	
↓		EGFL5	EGF-Like-Domain Multiple 5	AB011542	G6	-1.3	-1.8	-3.2	-3.3	-7.1	
↓		NUP153	Nucleoporin 153kD	Z25535		-1.1	-3.1	-3.3	-6.8	-6.2	down SCC12B2
↓		DKFZP434J214	DKFZP434J214 protein	AL080156		-1.3	-6.7	-5.4	-3.8	-0.5	down SCC12B2
↓		KIAA0172	KIAA0172 protein	D79994		-1.5	-2.0	-4.7	-6.5	-4.8	
↓		DKFZP586C162	DKFZP586C1620 protein	AL050144		-1.1	-2.3	-4.1	-6.4	-3.0	
↓		KIAA0303	KIAA0303 protein	AB002301	G6	-1.0	-1.6	-3.0	-6.4	-4.0	down SCC12B2
↓			cDNA FLJ21243 from clone COL01164	AI768188		-3.2	-5.9	-2.9	-3.4	-5.3	
↓		MGC13033	hypothetical protein	AF070582		-1.3	-2.0	-5.9	-5.9	-5.9	
↓		KIAA0579	KIAA0579 protein	AB011151		-1.1	-4.1	-5.7	-1.5	-4.1	
↓		KIAA0480	KIAA0480 protein	AB007949		-1.4	-3.1	-1.3	-4.5	-5.9	
↓		LOC51580	H-2K Binding Factor-2	D14041	G6	-1.4	-1.6	-3.4	-4.0	-5.9	down SCC12B2
↓		GG2-1	TNF-Induced Protein	AF099935		-1.3	-2.4	-4.3	-5.9	-2.6	down SCC12B2
↓			Cri-du-chat mRNA from clone FBD3	AL080234	G6	-1.5	-0.9	-3.0	-3.1	-5.7	
↓		KIAA0286	KIAA0286 protein	AB006624		-2.9	-3.8	-1.6	-3.9	-5.5	
↓		KIAA0940	KIAA0940 protein	AB023157		-1.7	-3.4	-1.4	-3.8	-5.5	
↓		DKFZP586F242	DKFZp586F2423 protein	AL080209	G6	-2.3	-2.1	-3.2	-3.1	-5.0	
↓		SHOC2	Soc-2 Suppressor of Clear	AB020669	G6	-1.4	-1.9	-3.3	-4.5	-4.9	down SCC12B2
↓		KIAA0470	KIAA0470 protein	AB007939		-3.3	-2.0	-1.3	-3.3	-4.7	
↓		HRIHFB2436	endocrine regulator	AB015342		-2.3	-2.5	-4.1	-3.2	-4.1	
↓		KIAA0947	KIAA0947 protein	AB023164		-1.9	-1.5	-4.5	-4.5	-4.4	
↓		DKFZp586F222	DKFZp586F2224 protein	AI655015		-1.2	-3.4	-4.5	-3.6	-1.1	
↓		ADNP	Activity-Dependent Neuroprotector	AB018327		-1.7	-3.2	-4.5	-3.4	-1.7	down SCC12B2
↓		KIAA0942	KIAA0942 protein	AB023159		-1.3	-2.5	-3.1	-4.3	-4.3	
↓		DKFZP434D19	DKFZP434D193 protein	U79263		-1.6	-2.0	-3.5	-3.0	-4.3	
↓		GS3686	hypothetical protein	AB000115		-4.1	-2.2	-2.8	-3.3	-3.6	
↓		DJ159A19.3	hypothetical protein	U79259		-1.0	-2.1	-3.3	-3.9	-3.8	
↓		KIAA0982	KIAA0982 protein	AB023199		-1.4	-3.6	-2.8	-3.6	-3.6	
↓		FLJ11220	hypothetical protein	AL050064		-1.3	-2.3	-3.3	-3.2	-3.3	
↓		KIAA0922	KIAA0922 protein	AB023139		-0.9	-3.1	-2.4	-3.1	-3.1	
↓		NPAT	Nuclear Protein Ataxia-Telangiectasia Locus	D83243		-3.2	-2.7	-3.2	-3.2	-3.2	

	Symbol	Name	Identifier	Cluster#	0.5h	3h	6h	12h	24h	
↓	IDN3	IDN3 protein	AB019494		-1.5	-1.2	-3.1	-3.1	-3.1	
↓	KIAA0240	KIAA0240 protein	D87077		-1.7	-3.0	-3.0	-3.0	-3.0	
↓	DKFZP564B076	DKFZP564B0769 protein	AL080186		-1.2	-2.9	-3.0	-3.0	-3.0	
↓	KIAA0595	KIAA0595 protein	AB011167		-1.1	-2.6	-3.5	-3.5	-1.0	
↓	KIAA1288	KIAA1288 protein	AL096842	G6	-1.1	-2.0	-2.5	-7.0	-7.1	
↓		Very Low Density Lipoprotein Receptor	D16532	G6	-0.7	-1.2	-2.6	-3.2	-3.3	
↓	DKFZP564I052	DKFZP564I052 protein	AL080063	G6	-0.8	-1.3	-2.2	-5.1	-10.2	
↓	KIAA0244	KIAA0244 protein	D87685	G6	-1.5	-2.5	-2.2	-5.8	-7.8	down SCC12B2
↓	FLRT2	Fibronectin Leucine Rich Transmembrane Protein 2	AB007865	G6	-1.5	-2.1	-2.8	-5.3	-16.9	
↓	KIAA0576	KIAA0576 protein	AB011148	G6	-2.4	-2.6	-2.8	-3.3	-16.8	
↓	KIAA0471	KIAA0471 protein	AB007940	G6	-1.7	-2.1	-2.3	-4.0	-16.5	
↓	KIAA0786	Latrophilin	AB018329	G6	-0.8	-1.5	-1.7	-4.5	-3.2	
↓	KIAA0826	KIAA0826 protein	AB020633	G6	-1.3	-1.8	-2.3	-3.8	-13.3	
↓	KIAA0191	KIAA0191 protein	D83776	G6	-1.7	-2.4	-2.5	-5.6	-11.3	
↓	KIAA0461	KIAA0461 protein	AB007930		-1.4	-1.9	-2.1	-10.2	-3.4	down SCC12B2
↓	KIAA1354	KIAA1354 protein	AA115140	G6	-0.8	-1.1	-2.6	-10.1	-7.7	
↓	DXF68S1E	DNA Segment GS1	M86934	G6	-1.0	-1.4	-2.6	-3.5	-9.9	
↓	KIAA0779	KIAA0779 protein	AB018322		-1.1	-2.9	-3.0	-2.4	-8.8	
↓	MN1	Meningioma 1	X82209	G6	-1.3	-1.4	-2.9	-8.7	-8.8	
↓	KIAA0132	Kelch-like ECH-Associated Protein 1	D50922		-1.2	-1.8	-3.2	-7.5	-2.8	
↓	KIAA0532	KIAA0532 protein	AB011104	G6	-2.5	-2.1	-2.0	-3.8	-7.4	
↓	CLASP1	CLIP-Associating Protein 1	AB014522	G6	-1.7	-1.1	-2.8	-3.1	-6.4	
↓	KIAA1224	KIAA1224 protein	AL049432	G6	-1.1	-1.6	-2.1	-3.7	-6.3	
↓	DKFZp564F112	DKFZp564F112 protein	AL049987		-1.2	-6.2	-2.0	-2.7	-3.5	
↓	KIAA0423	KIAA0423 protein	AB007883	G6	-1.3	-1.7	-2.6	-4.8	-6.1	
↓	KIAA0716	KIAA0716 protein	AB018259	G6	-1.3	-2.4	-2.0	-3.0	-5.6	
↓		α Topoisomerase truncated-form	L47276	G6	-2.1	-1.6	-1.9	-3.0	-5.2	
↓		BRCA2 region sequence CG006	U50535		-1.2	-3.2	-5.2	-2.4	-2.6	
↓	KIAA0261	KIAA0261 protein	D87450	G6	-1.0	-1.8	-2.2	-5.2	-5.2	
↓	DKFZP566C134	DKFZP566C134 protein	AF004292		-1.1	-1.7	-3.1	-5.0	-2.1	down SCC12B2
↓	MICB	MHC Class I Polypeptide-Related B	U65416	G6	-1.8	-1.3	-2.3	-3.7	-4.9	
↓	LOC51141	Insulin Induced 2	AL080184	G6	-1.6	-1.7	-2.2	-4.9	-4.9	
↓	INSIG1	Insulin Induced 1	U96876		-1.7	-3.0	-4.1	-2.2	-2.3	
↓	DKFZp586J072	DKFZp586J0720 protein	AL050151	G6	-1.6	-2.1	-2.4	-4.7	-4.0	down SCC12B2
↓	CHAC	Chorea Acanthocytosis	AB023203		-1.6	-2.5	-1.3	-4.4	-4.0	
↓	KIAA0546	KIAA0546 protein	AB011118		-1.7	-2.5	-3.7	-4.1	-1.7	
↓	DD5	Progestin Induced Protein	AF006010	G6	-2.1	-2.7	-1.7	-4.3	-4.3	
↓	KIAA0993	KIAA0993 protein	AB023210		-1.5	-2.5	-3.5	-2.3	-4.2	
↓	KIAA0637	KIAA0637 protein	AB014537		-1.9	-1.3	-2.9	-4.0	-3.4	
↓	KIAA0054	Helicase KIAA0054	D29677		-1.0	-2.4	-2.1	-3.9	-3.9	
↓	FLJ12443	hypothetical protein	AW024285	G6	-0.9	-1.8	-2.7	-4.0	-3.5	
↓	BCL7A	B-cell CLL/Lymphoma 7A	X89984	G6	-1.0	-1.3	-2.5	-4.0	-3.7	
↓	KIAA0854	KIAA0854 protein	AB020661	G6	-1.3	-1.8	-2.2	-3.4	-3.9	
↓	KIAA0227	KIAA0227 protein	D86980		-1.6	-2.3	-3.1	-2.7	-3.9	
↓	DKFZp586C172	DKFZp586C1723 protein	AL050192	G6	-1.3	-2.3	-1.7	-3.9	-3.9	
↓	MTMR6	Myotubularin Related Protein 6	AF072928		-2.2	-2.0	-1.3	-3.2	-3.7	
↓	FLJ10618	hypothetical protein	AL049246	G6	-1.3	-2.0	-2.1	-3.3	-3.6	
↓	NCK1	NCK Adaptor Protein 1	X17576		-1.3	-1.7	-3.6	-3.5	-2.5	
↓	KIAA0728	KIAA0728 protein	AB018271		-0.9	-1.7	-2.9	-3.4	-2.5	
↓	BANP	BANP homolog, SMAR1 homolog	AL049250		-1.2	-2.6	-3.1	-3.6	-1.8	
↓	MGC9084	hypothetical protein	AL035369		-1.1	-2.1	-3.5	-1.2	-3.5	
↓	MAML1	Mastermind-Like 1	D83785		-1.5	-2.4	-2.0	-3.4	-3.2	
↓	KIAA0433	KIAA0433 protein	AB007893		-1.2	-1.2	-3.0	-3.3	-2.1	
↓	PRO2730	hypothetical protein	AL045811		-2.9	-1.1	-2.7	-3.2	-1.0	
↓	KIAA0952	KIAA0952 protein	AB023169		-1.2	-2.6	-3.1	-2.5	-3.1	
↓	DKFZP434C212	DKFZP434C212 protein	AL080196		-2.9	-1.6	-0.9	-3.0	-3.1	
↓	FLJ23138	hypothetical protein FLJ23138	AI743090	G6	-1.9	-1.3	-1.4	-5.9	-5.1	
↓	SATB1	Special AT-Rich Sequence Binding Protein 1	M97287		-1.2	-1.5	-3.7	-1.5	-3.7	
↓	KIAA0446	KIAA0446 protein	AB007915		-1.0	-1.3	-3.5	-3.3	-1.3	down SCC12B2
↓	KIAA1058	KIAA1058 protein	AB028981	G6	-1.6	-1.1	-1.6	-3.0	-7.1	
↓		mRNA from clone 23763	AF007155		-1.3	-1.2	-3.5	-16.7	-1.3	
↓	KIAA0594	KIAA0594 protein	AB011166	G6	-1.7	-1.8	-2.0	-3.6	-17.0	
↓	SPOP	Speckle-type POZ Protein	AJ000644	G6	-1.8	-1.3	-1.5	-3.9	-9.7	down SCC12B2
↓	KIAA0938	KIAA0938 protein	AB023155	G6	-0.8	-1.0	-1.4	-3.2	-6.3	down SCC12B2
↓	KIAA0981	KIAA0981 protein	AB023198	G6	-1.9	-2.0	-1.9	-3.6	-7.7	
↓	KIAA0179	KIAA0179 protein	D80001	G6	-1.4	-0.8	-1.5	-4.6	-7.5	
↓	SCOP	SCN Circadian Oscillatory Protein	AB011178	G6	-1.0	-1.6	-1.5	-5.4	-6.8	
↓	KIAA1046	KIAA1046 protein	AB028969	G6	-1.0	-1.8	-1.3	-3.2	-6.9	
↓	MOT8	hypothetical protein	AF052143	G6	-1.3	-1.8	-1.7	-3.4	-6.8	
↓	DKFZp564D016	DKFZp564D016 protein	AL050021	G6	-1.6	-1.5	-2.0	-3.2	-6.7	
↓	FLJ20986	hypothetical protein DNA polyA site	Z24724	G6	-1.1	-1.5	-1.7	-3.5	-6.2	down SCC12B2
↓	DKFZp586N012	DKFZp586N012 protein	AL049471		-1.7	-3.0	-5.8	-1.2	-0.6	
↓	KIAA0136	KIAA0136 protein	D50926		-1.1	-1.9	-3.9	-5.6	-1.3	down SCC12B2
↓	KIAA0170	KIAA0170 protein	AL041663		-1.2	-1.1	-5.0	-1.8	-5.4	
↓	KIAA0970	KIAA0970 protein	AB023187	G6	-0.9	-1.3	-1.6	-3.0	-5.4	
↓	BCE-1	B Lymphocyte Gene 1	AF068197	G6	-1.0	-1.4	-1.8	-4.7	-5.2	
↓	KIAA0615	KIAA0615 protein	AB014515		-1.5	-2.0	-5.0	-4.9	-1.2	down SCC12B2
↓	KIAA0869	KIAA0869 protein	AB020676	G6	-1.1	-0.9	-1.6	-3.7	-5.0	
↓	HDCMA18P	HDCMA18P protein	AL049996	G6	-1.7	-0.9	-1.4	-3.7	-4.9	
↓	STARD7	START Domain Containing 7	W27761	G6	-1.3	-1.1	-1.3	-4.7	-4.7	
↓	DKFZp586F101	DKFZp586F1019 protein	AL050102	G6	-1.1	-2.0	-1.9	-4.9	-3.9	
↓	FLJ20275	hypothetical protein	W26023	G6	-1.8	-1.9	-1.6	-3.3	-4.8	
↓	MGC8974	hypothetical protein	AL031427	G6	-1.7	-1.0	-1.7	-4.7	-4.7	
↓	KIAA1064	KIAA1064 protein	AB028987		-1.3	-1.9	-4.4	-3.6	-1.6	
↓	KIAA1117	KIAA1117 protein	AB029040	G6	-1.0	-1.0	-1.0	-4.3	-4.3	
↓	KIAA0156	KIAA0156 protein	AB020880		-1.3	-1.2	-4.2	-4.4	-1.1	
↓	KIAA0182	KIAA0182 protein	D80004		-1.5	-2.0	-3.3	-4.4	-1.7	
↓	KIAA1096	KIAA1096 protein	AI307607	G6	-1.0	-1.5	-1.4	-3.4	-4.4	
↓	NPD009	NPD009 protein	W26407		-1.2	-1.6	-3.9	-1.3	-4.3	
↓	DKFZp434B102	DKFZp434B102 protein	AL080192		-2.0	-3.3	-1.9	-1.7	-4.2	
↓	KIAA0750	KIAA0750 protein	AB018293	G6	-0.5	-1.4	-1.4	-4.1	-3.6	
↓	KIAA0978	KIAA0978 protein	AB023195		-1.1	-2.0	-4.1	-1.9	-3.3	
↓	DKFZP434D133	DKFZP434D1335 protein	AI920820	G6	-1.1	-1.0	-1.4	-3.2	-3.9	
↓	LOC151584	hypothetical protein	AF052174	G6	-1.1	-1.4	-1.3	-3.5	-3.8	
↓	HSRTSBETA	rTS β protein	X67098		-1.4	-3.3	-1.2	-0.9	-3.7	
↓	NP220	Nuclear Protein 220	D83032	G6	-1.1	-1.7	-2.3	-3.6	-3.1	
↓	KIAA1528	KIAA1528 protein	AI138834		-1.2	-1.7	-3.1	-3.5	-1.6	
↓	DJ971N18.2	hypothetical protein	W26226	G6	-1.4	-1.8	-1.7	-3.3	-3.4	
↓	RNTRE	Related to the N Terminus of TRE	D13644		-1.3	-1.1	-2.9	-1.1	-3.2	
↓	KIAA0802	KIAA0802 protein	AB018345	G6	-0.9	-2.0	-1.3	-3.2	-3.2	
↓	SACS	Sacsin	AB018273		-1.6	-1.6	-0.9	-3.0	-3.0	down SCC12B2

Table III Online Supplemental Data

		Symbol	Name	Identifier	6h	12h	
1 Cell Cycle (+/-) / Oncogene / Tumor Suppressor							
↑	(+?)	RBBP4	Retinoblastoma-BP4	X74262	2.3	2.1	
↑	(+)	CDC2	CDC2	D88357	2.1	2.3	
↑	(+)	CSF2	CSF2	M13207	4.6	4.7	
↑	(+)	SPHAR	S-Phase Response Cyclin-Related	X82554	2.5	2.2	
↑	(+)	SKP1A	S-Phase Kinase-associated Protein 1A	U33760	3.1	4.1	
↑	(+)	RALB	c-ral Ras-related Oncogene B	M35416	2.1	2.8	
↑	(-)	CDKN1C	p57 Kip2	U22398	3.8	3.3	up NHEK
↑	(-)	NF2	Neurofibromatosis 2 Tumor Suppressor	HG3236-HT3413	3.9	2.7	up NHEK
↓	(-)	WEE1	WEE1	X62048	-8.6	-9.8	down NHEK
↓	(-)	FUS1	FUS1 (Tumor Suppressor)	AF055479	-3.9	-3.6	
2 Apoptosis (+/-)							
↑	(+)	TNF	TNFα	X02910	10.5	10.4	
↑	(+)	PMAIP1	APR (NOXA)	D90070	2.8	3.3	up NHEK
↑	(-)	HSPCB	HSP90	W28616	2.3	2.8	up NHEK
↓	(+)	FADD	FADD	X84709	-5.0	-5.6	
↓	(+)	TRAF4	TRAF4	X80200	-3.5	-4.5	
↓	(+)	TNFRSF1A	TNFα Receptor I	M58286	-4.9	-6.3	
↓	(-)	TNFAIP3	TNFα-Induced Protein 3	M59465	-3.7	-4.3	up NHEK
↓	(-)	MCL1	Myeloid Cell Leukemia 1	L08246	-7.4	-8.9	
↓	(-)	BAG5	BCL2-Associated Athanogene 5	AB020680	-3.5	-5.7	
3 DNA Repair							
↑		GADD45A	GADD45A	M60974	2.0	2.4	up NHEK
↑		RECQL	RecQ DNA Helicase Q1-like	L36140	2.0	2.3	
↑		RAD51C	RAD51C	AF029670	2.1	2.4	
↑		TOB1	Transducer of ERBB2, 1	D38305	2.9	3.2	up NHEK
↓		CHD1	Chromodomain Helicase DNA BP1	AF006513	-3.4	-5.1	down NHEK
4 Oxydative Stress / ROS Metabolism (+/-)							
↑	(-)	PRDX3	Peroxiredoxin 3	D49396	2.2	2.4	
↓	(-)	MT2A	Metallothionein 2A	AI547258	-4.4	-3.6	
5 Extra Cellular Matrix / Protease (+/-)							
↓		MFAP1	Microfibrillar-Associated Protein 1	U04209	-3.4	-5.3	
6 Growth Factor / Chemokine / Cytokine / Inflammation (+/-)							
↑	(+)	IL-8	IL-8 (CXC)	M28130	12.5	11.9	up NHEK
↑	(+)	IL1B	IL-1β	X04500	2.1	2.3	
↑	(+)	IL6	IL-6	X04430	4.3	4.0	up NHEK
↑	(+)	GRO1	Small Inducible Cytokine B1 (MGSA) (CXC)	X54489	2.9	2.0	up NHEK
↑	(+)	GRO2	Small Inducible Cytokine B2 (CXC)	M36820	11.6	8.9	up NHEK
↑	(+)	GRO3	Small Inducible Cytokine B3 (CXC)	M36821	10.3	8.9	up NHEK
↑	(+)	SCYA20	Small Inducible Cytokine A20 (CXC)	U64197	3.5	4.0	up NHEK
↑	(+)	SCYE1	Small Inducible Cytokine E1	U10117	2.2	2.7	
↑	(+)	PTGS2	Cyclooxygenase (COX2)	U04636	5.5	7.9	up NHEK
↑	(+)	CCBP2	Chemokine Binding Protein 2	U94888	2.7	2.0	
↑		IGFBP7	IGFBP7	L19182	2.1	2.3	
↓		CYR61	IGFBP10	Y11307	-9.8	-6.5	up NHEK
7 Structural Protein / Adhesion / Migration / Junction							
↑		DSG2	Desmoglein 2	Z26317	2.2	2.8	
↑		ANXA2	Annexin A2	D28364	4.3	4.4	up NHEK
↑		ANXA3	Annexin A3	M20560	2.4	2.7	
↑		TPM1	Tropomyosin 1α	M19267	2.4	2.5	
↓			Cadherin (partial cds)	D88799	2.4	2.0	
↓		BAIAP2	BAI1-Associated Protein 2	AB015019	-3.6	-6.8	
↓		KIF2	Kinesin Heavy Chain member 2	Y08319	-4.6	-6.8	down NHEK
↓		KNSL2	Kinesin-Like 2	D14678	-3.5	-4.2	
8 Metabolism / Energy							
↑		GLUD2	Glutamate Dehydrogenase-2	U08997	3.2	3.8	
↑		ME2	NAD(+)-dependent Malic Enzyme 2	M55905	2.1	2.1	
↑		LOC91137	TB1	M74089	2.5	2.4	
↑		CYB5	Cytochrome B5	L39945	2.3	2.8	
↑		APT6M8-9	ATPase	AL049929	2.2	2.2	
↑		ATP5A1	ATP Synthase	D14710	4.1	3.9	
↑		SDHD	Succinate Dehydrogenase	AB006202	2.8	3.0	
↑		KYNU	Kynureninase	AI148772	2.1	2.3	up NHEK
↑		UQCRC2	Ubiquinol-Cytochrome c Reductase	J04973	2.0	2.2	
↑		P5	Protein Disulfide Isomerase-related Protein	D49489	2.0	2.4	
↑		MCAD	Medium-Chain Acyl-CoA Dehydrogenase	M91432	2.9	2.7	
↑		AGL	Amylo-1,6-Glucosidase, 4-α-Glucanotransferase	U84007	2.4	2.8	
↑		SC5DL	Sterol-C5-Desaturase-like	AB016247	2.1	2.7	
↑		ALDH7A1	Aldehyde Dehydrogenase 7, member A1	S74728	2.6	2.7	
↑		HPRT1	Hypoxanthine Phosphoribosyltransferase 1	M31642	2.1	2.4	
↑		SCP2	Sterol Carrier Protein 2	U11313	2.0	2.3	
↓			Spermidine/Spermine N1-Acetyltransferase	HG172-HT3924	2.0	2.2	
↓		HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase	M11058	-4.7	-4.7	
↓		AMD1	S-Adenosylmethionine Decarboxylase 1	M21154	-5.6	-5.1	
↓		DBY	DEAD/H Box Polypeptide Y Chromosome	AF000984	-3.5	-3.7	

		Symbol	Name	Identifier	6h	12h	
9 Development / Differentiation (+/-)							
↑	(+)	TMPO	Thymopoietin	U18271	2.1	2.3	
↓	(+)	PUM1	Pumilio 1	D43951	-6.0	-10.3	down NHEK
↓	(+)	JMJ	Jumonji	AL021938	-3.4	-4.5	down NHEK
↓	(+)	DDEF2	Development Differentiation Enhancing Factor 2	AB007860	-3.1	-3.9	
↓	(+)	EDR2	Early Development Regulator 2	U89278	-5.8	-8.2	down NHEK
↓	(+)	SIAH2	Seven In Absentia 2	U76248	-5.2	-5.2	
10 Signal Transduction / Intracellular Traffic							
↑		MAP2K1	MAPKK1	L05624	2.7	2.6	
↑		STK2	Serine/Threonine Kinase 2	L20321	2.4	2.0	
↑		IL13RA1	IL-13 Receptor α1	Y10659	2.6	2.6	
↑		P23	Unactive Progesterone Receptor	L24804	3.1	3.7	
↑		CD36L2	Thrombospondin Receptor-like 2	D12676	2.1	2.3	
↑		COPB	Coatomer Protein complex, subunit β	X82103	2.1	2.2	
↑		SSR1	Signal Sequence Receptor α	Z12830	2.1	2.1	
↑		LAMP2	Lysosomal-associated Membrane Protein 2	X77196	2.2	2.4	
↑		SLC11A1	Solute Carrier family 11, member 1	D50402	2.1	2.6	
↑		SRI	Sorcin	M32886	2.3	2.6	
↑		SNX3	Sorting Nexin 3	AF034546	2.1	2.1	
↑		TACSTD2	Tumor-associated Calcium Signal Transducer 2	X77753	4.1	3.3	up NHEK
↑		ITM2B	Integral Membrane Protein 2B	AA477898	3.6	4.1	
↑		AP1S2	Adaptor-related Protein complex 1 σ2	AF091077	2.2	2.6	
↑		YWHAZ	14-3-3 ζ Protein	U28964	2.7	3.0	
↑		ARHGDIB	Rho GDP Dissociation Inhibitor (GDI) β	X69549	2.4	2.8	
↑		PRKAR1A	Protein Kinase cAMP-dependent Regulatory Iα	M33336	2.4	2.7	
↑		PRKR	IFN-Inducible dsRNA Dependent Protein Kinase	U50648	3.8	3.8	up NHEK
↑		ADK	Adenosine Kinase	U50196	2.6	2.1	
↑		RSU1	Ras Suppressor Protein 1	L12535	2.3	2.6	
↑		PTK9	Protein Tyrosine Kinase 9	U02680	2.3	2.1	
↑		LOC55858	TPA Regulated Locus	W28729	3.4	3.6	up NHEK
↓		TGFBR2	TGFβ Receptor II	D50683	-4.8	-10.4	down NHEK
↓		PDZ-GEF1	PDZ Guanine Exchange Factor 1 (GEF1)	AF070570	-5.2	-4.8	down NHEK
↓		IL4R	IL-4 Receptor	X52425	-5.5	-6.4	
↓		PGRMC2	Progesterone Receptor Membrane 2	AJ002030	-5.7	-7.1	
↓		ADORA2B	Adenosine A2B Receptor	X68487	-4.1	-6.4	down NHEK
↓		DUSP4	Dual Specificity Phosphatase 4	U48807	-3.5	-8.8	
↓		EXT1	Exostoses 1	S79639	-3.1	-6.7	
↓		FZD2	Frizzled	L37882	-3.5	-6.2	
↓		KIAA0057	TRAM-Like Protein	D31762	-4.3	-10.5	
↓		STK15	Serine/Threonine Kinase 15	AF011468	-5.1	-15.0	down NHEK
↓		DYRK1A	Dual-Specificity Tyrosine-Kinase 1A	D86550	-6.4	-13.1	down NHEK
↓		TLK1	Tousled-Like Kinase 1	D50927	-5.6	-12.8	down NHEK
↓		TLK2	Tousled-Like Kinase 2	AB004884	-3.9	-7.1	down NHEK
↓		AKAP10	A Kinase Anchor Protein 10	AA114830	-4.3	-5.3	down NHEK
↓		PRKCBP1	Protein Kinase C BP1	W22296	-5.2	-4.1	down NHEK
↓		MARK3	MAP/Microtubule Kinase 3	M80359	-3.6	-5.2	
↓		SLK	Ste20 Serine/Threonine Kinase	D86959	-4.3	-7.3	
↓		P85SPR	PAK-Interacting Exchange Factor β	D63476	-4.9	-7.8	down NHEK
11 Transcription (+/-) / Replication							
↑	(+)	FOS	FOS	V01512	16.1	27.8	up NHEK
↑	(+)	JUND	JUND	X56681	3.7	2.0	up NHEK
↑	(+)	SMAD2	SMAD2	U78733	2.6	2.3	
↑	(+)	BTF3L3	Basic Transcription Factor 3-Like 3	M90356	2.4	2.2	
↑	(+)	CITED2	p300/CBP	U65093	3.4	4.1	up NHEK
↑	(+)	DSIPI	Delta Sleep Inducing Peptide Immunoreactor	AI635895	2.2	2.3	
↑	(+)	MYCBP	c-myc BP	AB007191	3.3	3.6	
↑	(+)	TAF2	TAF2 RNA Pol II	AF026445	2.0	2.1	
↑	(+)	SSBP1	ssDNA-BP1	AA768912	2.5	2.8	
↑	(+)	CCNC	Cyclin C	M74091	4.0	3.1	
↑	(-)	DRAP1	DR1-Associated Protein 1	U41843	2.6	3.7	up NHEK
↑	(-)	ATF3	ATF3	L19871	2.4	5.6	up NHEK
↑	(-)	ID2	Inhibitor of DNA Binding 2	D13891	14.6	22.0	up NHEK
↑		RFC5	Replication Factor C5	L07540	2.0	2.1	
↑		RRM2	Ribonucleotide Reductase M2	X59618	3.0	2.6	
↓	(+)	MYC	MYC	V00568	-6.9	-6.6	down NHEK
↓	(+)	E2F3	E2F3	D38550	-3.3	-3.3	down NHEK
↓	(+)	FOXO3A	Forkhead Box O3A	AF032886	-3.7	-4.4	down NHEK
↓	(+)	FOXF2	Forkhead Box F2	U13220	-3.7	-4.7	
↓	(+)	SMAP	Thyroid Hormone Receptor Coactivating Protein	AW020536	-3.9	-4.1	down NHEK
↓	(+)	SOX4	SRY (Sex Determining Region Y)-Box 4	X70683	-3.2	-4.9	
↓	(+)	KLF7	Kruppel-Like Factor 7	AA478904	-3.5	-5.2	down NHEK
↓	(+)	ZNF278	Zinc Finger Protein 278	AL096880	-3.2	-6.8	
↓	(+)	HMGIC	High-Mobility Group Protein	X92518	-3.4	-6.6	down NHEK
↓	(+)	GATA3	GATA-Binding Protein 3	X58072	-3.2	-3.7	
↓	(-)	CNOT2	CCR4-NOT Transcription 2	AI123426	-3.4	-3.6	down NHEK
↓	(-)	CNOT4	CCR4-NOT Transcription 4	U71267	-3.1	-3.2	down NHEK
↓	(-)	TCF8	Transcription Factor 8	D15050	-5.7	-7.6	
↓	(-)	TGIF	TGFB-Induced Factor	X89750	-3.6	-5.0	
↓	(-)	ID1	Inhibitor of DNA Binding 1	X77956	-4.7	-5.5	down NHEK
↓	(-)	ID3	Inhibitor of DNA Binding 3	AL021154	-7.1	-7.7	
↓	(-)	CTCF	CCCTC-Binding Factor	U25435	-3.2	-6.2	down NHEK
↓	(-)	ZNF133	Zinc Finger Protein 133	U09366	-4.7	-4.8	
↓	(-)	ERF	ETS2 Repressor Factor	U15655	-5.0	-5.9	
↓		TRF4	Topoisomerase Related Function Protein 4-1	AB005754	-4.2	-5.1	down NHEK
↓		BRD1	Bromodomain-Containing 1	AL080149	-4.2	-4.2	
↓		ZNF146	Zinc Finger Protein 146	X70394	-3.6	-6.1	down NHEK

		Symbol	Name	Identifier	6h	12h	
12 Histone / Chromatin							
↑		H2BFA	Histone H2B A	AJ223352	3.0	2.9	
↑		NAP1L1	Nucleosome Assembly Protein 1-Like 1	M86667	2.7	2.8	
	↓	CENPA	Centromere Protein A	U14518	-5.3	-10.4	down NHEK
13 Translation (+/-) / Protein Synthesis (+) / Degradation (-)							
↑	(+)	EIF2S3	Translation Initiation Factor 2 S3	L19161	2.2	2.7	
↑	(+)	PAPOLA	Poly(A) Polymerase α	X76770	3.6	3.5	
↑	(+)	FUSIP1	FUS-Interacting Protein 1	AF047448	3.0	2.9	
↑	(+)	SFRS1	Splicing Factor 2	M69040	2.6	2.5	
↑	(+)	RNU2	U2 snRNA	W25892	5.7	3.7	up NHEK
↑	(-)	PSMD8	Proteasome 26S S8	D38047	2.3	2.4	up NHEK
↑	(-)	UBE2D3	Ubiquitin-conjugating Enzyme E2D3	U39318	2.3	2.1	
↑	(-)	UBE2E1	Ubiquitin-conjugating Enzyme E2E1	AI039880	2.3	2.1	
↑	(-)	UK114	Translational Inhibitor Protein	X95384	2.0	2.3	
↓	(+)	RRS1	Ribosome Biogenesis Protein 1	D25218	-3.6	-6.6	down NHEK
↓	(+)	MPHOSPH10	U3 snRNP	X98494	-4.0	-4.8	down NHEK
↓	(+)	RPP38	Ribonuclease P Protein p38	U77664	-4.0	-5.9	
↓	(-)	CSTF1	Cleavage Stimulation Factor 1	L02547	-3.4	-3.4	
↓	(-)	NEDD4L	Ubiquitin-Protein Ligase NEDD4-Like	AB007899	-3.2	-5.4	
14 Other / Unknown							
↑		CORT	Cortistatin	N30625	2.2	2.3	
↑		FSTL1	Follistatin-like 1	D89937	2.6	2.5	
↑		MGC14376	hypothetical protein	AF070569	4.9	6.2	
↑		B2M	β--2-Microglobulin	S82297	4.3	6.1	
↑		FABGL	FabG (β-ketoacyl-Reductase) Like	D82061	3.4	4.3	
↑		HIS1	HMBA-Inducible	AB021179	3.1	3.5	up NHEK
↑			Ras-Like Protein TC21	HG1111-HT1111	2.6	3.3	
↑			Ras-Like Protein TC4	HG1112-HT1112	2.2	2.4	
↑			Phosphoglycerate Kinase	S81916	2.3	3.0	up NHEK
↑		SH3BGRL	SH3 Binding Glutamic Protein like	AF042081	2.7	3.0	
↑		RCN2	E6-BP	X78669	2.3	2.9	
↑		NP25	Neuronal Protein	Z78388	2.5	2.9	up NHEK
↑			GDP/GTP Exchange Protein	HG2036-HT2090	2.9	2.2	
↑		DKFZP564M1416	DKFZP564M1416 protein	AL049934	2.5	2.8	
↑		LOC51014	CGI-109 protein	AB002450	2.7	2.2	
↑		TCTE1L	T-Complex-associated-Testis-Expressed 1-like	U02556	2.7	2.2	
↑		LOC56267	hypothetical protein 669	AF091090	2.5	2.3	
↑		HKE2	HLA class II region Expressed Gene KE2	AI201243	2.2	2.5	
↑		AP4S1	Clathrin-Associated Assembly Adaptor Protein σ4	AB030654	2.1	2.5	
↑		HRB2	HIV-1 Rev Binding Protein 2	U00943	2.4	2.5	
↑			Fk506-Binding Protein	HG1139-HT4910	2.3	2.5	
↑		DKFZP564A033	DKFZP564A033 Protein	AL050006	2.5	2.4	
↑		RA410	Vesicle Transport-related Protein	AB020724	2.4	2.2	
↑		PLSCR1	Phospholipid Scramblase 1	AB006746	2.3	2.1	
↑		DKFZP564M082	DKFZP564M082 Protein	AL080071	2.2	2.3	
↑		UPK1B	Uroplakin 1B	AB015234	2.2	2.1	
↑		BBP	β-amyloid BP Precursor	AI057115	2.2	2.2	
↑		DKFZP586M1523	DKFZP586M1523 Protein	AL050225	2.1	2.2	
↑		RTN4	Reticulon 4	AB020693	2.0	2.1	
	↓	PTHLH	Parathyroid Hormone-Like Hormone	M24351	-7.0	-6.0	
	↓	BLCAP	Bladder Cancer Associated Protein	AL049288	-7.2	-12.8	
	↓	RYBP	RING1 and YY1 Binding Protein	AL049940	-6.4	-10.9	
	↓		ESTs	W28612	-3.1	-10.5	down NHEK
	↓	KIAA0303	KIAA0303 protein	AB002301	-6.0	-9.2	down NHEK
	↓	FLJ20986	hypothetical protein	Z24724	-3.0	-9.1	down NHEK
	↓	ADNP	Activity-Dependent Neuroprotector	AB018327	-9.0	-9.0	down NHEK
	↓	FLJ20505	hypothetical protein	AA418437	-3.3	-8.4	
	↓	NUP153	Nucleoporin 153kD	Z25535	-4.3	-8.4	down NHEK
	↓	KIAA1564	KIAA1564 protein	AI475497	-3.4	-8.2	
	↓	KIAA0174	KIAA0174 protein	D79996	-4.5	-8.0	
	↓	LOC96541	hypothetical protein TL132	AJ012755	-6.6	-7.7	
	↓	KIAA0202	KIAA0202 protein	D86957	-3.0	-7.4	
	↓	SACS	Sacsin	AB018273	-3.2	-7.2	down NHEK
	↓	KIAA0136	KIAA0136 protein	D50926	-4.5	-7.0	down NHEK
	↓	KIAA1041	KIAA1041 protein	AB028964	-3.9	-6.8	down NHEK
	↓	ARL7	ADP-Ribosylation Factor-Like 7	AB016811	-3.2	-6.6	
	↓	SPOP	Speckle-Type POZ Protein	AJ000644	-6.3	-5.1	down NHEK
	↓	KIAA0461	KIAA0461 protein	AB007930	-3.6	-6.1	down NHEK
	↓	KIAA0615	KIAA0615 protein	AB014515	-4.0	-5.9	down NHEK
	↓	PMM2	Phosphomannomutase 2	U85773	-3.3	-5.8	
	↓	DKFZp586J0720	DKFZp586J0720 protein	AL050151	-3.6	-5.8	down NHEK
	↓	CDYL	Chromodomain Protein Y Chromosome-Like	AL050164	-3.6	-5.7	down NHEK
	↓	HSPC111	hypothetical protein	AI553745	-4.0	-5.6	
	↓	ADSL	Adenylosuccinase	AL022238	-5.6	-4.3	down NHEK
	↓	DKFZP564O092	DKFZP564O092 protein	W21827	-4.6	-5.6	
	↓	DKFZP434J214	DKFZP434J214 protein	AL080156	-5.2	-4.3	down NHEK
	↓	SIAH1	Seven In Absentia 1	U76247	-5.2	-5.2	
	↓	DKFZP564A122	DKFZP564A122 protein	W26496	-3.3	-5.1	
	↓	THBD	Thrombomodulin	J02973	-3.1	-4.9	
	↓	FLJ12671	hypothetical protein	AI023044	-3.8	-4.9	
	↓	COIL	Coilin	U06632	-3.3	-4.9	
	↓	DKFZp564E2222	DKFZp564E2222 protein	AL049941	-3.9	-4.8	
	↓	SCHIP1	Schwannomin-Interacting Protein 1	AF070614	-3.7	-4.7	
	↓	GG2-1	TNFα-Induced Protein	AF099935	-4.5	-4.5	down NHEK
	↓	KIAA1039	KIAA1039 protein	AB028962	-3.4	-4.5	
	↓	DKFZp566J2146	DKFZp566J2146 protein	AL050081	-3.6	-4.2	
	↓	FEN1	Flap Structure-Specific Endonuclease 1	HG4074-HT4344	-3.3	-4.2	
	↓		human clone 23589	U79297	-3.1	-4.1	
	↓	DKFZP566C134	DKFZP566C134 protein	AF004292	-3.5	-3.9	down NHEK
	↓	FBXL11	F-Box and Leucine-Rich Protein 11	AB023221	-3.3	-3.5	
	↓	KIAA0938	KIAA0938 protein	AB023155	-3.5	-3.5	down NHEK
	↓	SHOC2	Soc-2 (Suppressor of Clear)	AB020669	-3.1	-3.4	down NHEK
	↓	FLJ20500	hypothetical protein	AA522530	-11.4	-17.1	down NHEK
	↓	KIAA0244	KIAA0244 protein	D87685	-3.5	-3.0	down NHEK
	↓	KIAA0446	KIAA0446 protein	AB007915	-5.0	-3.8	down NHEK
	↓	LOC51580	H-2K Binding Factor-2	D14041	-3.0	-4.7	down NHEK
	↓	M96	Putative DNA Binding Protein	AJ010014	-3.1	-5.2	

יישום והערכה של שיטות אנליזה מתקדמות לחקר ביטוי גנטי בסרטן מעי וסרטן עור

הילה גל

תזה להדרכת מוסמך מוגש למועצה המדעית
של מכון וייצמן למדע

בהדרכת

פרופסור איתן דומני ופרופסור דוד גבעול

ינואר 2003