

## CHAPTER 15

### FROM GENOME TO PROTEOME IN TUMOR PROFILING: MOLECULAR EVENTS IN COLORECTAL CANCER GENESIS

JENS K. HABERMANN<sup>1,2</sup>, UWE J. ROBLICK<sup>1,3</sup>, MADHVI UPENDER<sup>2</sup>,  
THOMAS RIED<sup>2</sup>, AND GERT AUER<sup>3</sup>

<sup>1</sup>Laboratory for Surgical Research, Department of Surgery, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; <sup>2</sup>Genetics Department, National Cancer Institute, NIH, Bethesda, MD, USA; <sup>3</sup>Unit of Cancer Proteomics, Cancer Center Karolinska, Karolinska University Hospital Solna, Stockholm, Sweden

**Abstract:** Biomedical research has advanced rapidly in recent years with the sequencing of the human genome and the availability of technologies such as global gene and protein expression profiling using different chip platforms. However, this progress has not yet been transferred to the bedside. While detection of cancer at early stages is critical for curative treatment interventions, efficient diagnostic and therapeutic markers for the majority of malignancies still seem to be lacking. Comprehensive tumor profiling has therefore become a field of intensive research aiming at identifying biomarkers relevant for improved diagnostics and therapeutics. This chapter will demonstrate a genomic and proteomic approach while focusing on tumor profiling during colorectal cancer development.

#### 1. COLORECTAL CANCER

Colorectal cancer is one of the most common malignancies in the world.<sup>1</sup> While the 5-year disease-free survival rate for early stage tumors (UICC stage I) exceeds 90%, this percentage is reduced to 63% in advanced stage carcinomas (UICC stage III).<sup>2</sup> Therefore, detection of cancer at an early stage is critical for curative treatment interventions and utilization or application of tools and methodologies for early cancer detection can directly result in improving patient survival rates. In current clinical practice, screening for cancer and preinvasive polyps of the colorectum is based on clinical examination, detection of fecal occult blood (FOBT), and sigmoidoscopy or colonoscopy.<sup>3</sup> The successful implementation of these screening procedures has contributed to a reduction in

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the mortality of colorectal carcinomas.<sup>4</sup> However, despite these screening programs, about 70% of carcinomas are detected at advanced tumor stages (UICC III/IV) presenting poor patient prognosis.

The lifetime risk for the development of colorectal carcinomas is considerably increased in patients with ulcerative colitis (UC).<sup>5</sup> Ulcerative colitis can therefore be considered a bona fide premalignant condition leading to the recommendation that patients with UC should participate in surveillance programs to screen for early signs of malignancy.<sup>6</sup> However, reliable surveillance is difficult and 50% of the detected malignancies are already at an advanced tumor stage.<sup>7-9</sup>

## 2. GENOMICS

### 2.1. Genomic aneuploidy and its role in tumorigenesis

When the first quantitative measurements of the DNA content of cancer cells were performed, aneuploidy was defined as a variation in nuclear DNA content in the population of cancer cells within a tumor.<sup>10</sup> Since then, aneuploidy has been observed as a consistent genetic alteration of the cancer genome of different tumor entities.<sup>11-13</sup> In addition, aneuploidy seems to precede the manifestation of malignancy: Löfberg et al. reported aneuploid biopsies in 25% of ulcerative colitis patients with a high risk for colorectal cancer development at least once during 10 years of observation.<sup>14</sup> In other studies, aneuploidy has been repeatedly observed by flow cytometry even in nondysplastic mucosa of ulcerative colitis patients.<sup>15</sup> By means of image cytometry, we could detect highly aneuploid epithelial cell populations scattered over the colon and rectum in premalignant biopsies of eight patients with an ulcerative colitis-associated colorectal carcinoma (UCC).<sup>16</sup> These aneuploid lesions could be observed up to 11 years prior to the final cancer diagnosis (average 7.8 years). They were found in macro- and microscopically unsuspecting mucosa, could even be detected in regenerative epithelium, and were not related to dysplasia. This DNA aneuploidy occurred more frequently in biopsies of patients with a subsequent carcinoma (75%) than compared with biopsies from ulcerative colitis patients without a subsequent colorectal carcinoma (14%,  $p = 0.006$ ). The carcinoma samples of the eight UCC patients also exhibited highly aneuploid DNA distribution patterns. The common conclusion of these studies strongly supports the hypothesis that genomic instability, represented by nuclear DNA aneuploidy, could initiate the process of malignant transformation in colitis as an early event. DNA aneuploidy would therefore indicate an increased risk of progression to invasive properties in genetically unstable cells. However, aneuploidy may be reversible over time once cells are not longer exposed to the inducing agent or carcinogen.<sup>17, 18</sup> Thus, it is reasonable to suggest that the genomic instability reflected by aneuploidy has to be followed by multiple cellular alterations in order to reach malignant properties. One of the decisive steps in this transformational process is the ability of genomically altered cells to proliferate,

which is compulsory for clonal expansion.<sup>19</sup> Interestingly, immunohistochemical expression of the proliferation marker Cyclin A was significantly correlated to aneuploidy in biopsies of the patients with a subsequent carcinoma ( $r = 0.791$ ).

## 2.2. Chromosomal aneuploidies as tumor-specific patterns

With increased resolution of cytogenetic techniques, such as chromosome banding, comparative genomic hybridization (CGH), spectral karyotyping (SKY), and multicolor fluorescence in situ hybridization (FISH), it has become clear that in addition to nuclear aneuploidy, specific nonrandom chromosomal imbalances (heretofore referred to as chromosomal aneuploidy) exist.<sup>20-23</sup> Indeed, despite genetic instability in cancer genomes, cancer cell populations as a whole display a surprisingly conserved, tumor-specific pattern of genomic imbalances.<sup>13, 24, 25</sup> At early steps in the sequence of malignant transformation during human tumorigenesis, e.g., in preinvasive dysplastic lesions, chromosomal aneuploidies can be the first detectable genetic aberration found.<sup>26-29</sup> This suggests that there is both an initial requirement for the acquisition of specific chromosomal aneuploidies and a requirement for the maintenance of these imbalances despite genomic and chromosomal instability. This would be consistent with continuous selective pressure to retain a specific pattern of chromosomal copy number changes in the majority of tumor cells.<sup>13, 30-32</sup> Additionally, in cell culture model systems in which cells are exposed to different carcinogens, chromosomal aneuploidy is the earliest detectable genomic aberration.<sup>33, 34</sup> The conservation of these tumor-specific patterns of chromosomal aneuploidies suggests that they play a fundamental biological role in tumorigenesis.

The progression of colorectal cancer is defined by the sequential acquisition of genetic alterations.<sup>35</sup> At the cytogenetic level, many of these aberrations can be visualized as specific chromosomal gains and losses. These aneuploidies result in a recurrent pattern of genomic imbalances, which is specific and conserved for these tumors.<sup>36</sup> For instance, one of the earliest acquired genetic abnormalities during colorectal tumorigenesis are copy number gains of chromosome 7.<sup>32</sup> These trisomies can already be observed in benign polyps, and can emerge in otherwise stable, diploid genomes. At later stages, e.g., in high-grade adenomas or in invasive carcinomas, additional specific cytogenetic abnormalities become common, such as gains of chromosome and chromosome arms 8q, 13, and 20q, and losses that map to 8p, 17p, and 18q. For a comprehensive summary see the "Mitelman Database of Chromosome Aberrations in Cancer" at <http://cgap.nci.nih.gov/Chromosomes/Mitelman>. These chromosomal aneuploidies are accompanied by specific mutations in oncogenes and tumor suppressor genes, including *ras*, *APC*, and *p53*.<sup>37</sup> It is therefore well established that both, chromosomal aneuploidies and specific gene mutations, are required for tumorigenesis.

Detection of aneuploid lesions in ulcerative colitis patients seems to indicate imminent carcinogenesis with faithful progression to UCC. Recent reports

have provided evidence that genomic aneuploidy in UCC is associated with chromosomal aneuploidies.<sup>38, 39, 40, 41, 42</sup> Unlike sporadic colorectal tumors, UCCs do not follow the adenoma–carcinoma sequence, and the sequential acquisition of chromosomal aneuploidy and gene mutations is less well established. It was therefore interesting to investigate whether the pattern of chromosomal gains and losses in UCC are similar to that described in sporadic carcinomas. This would indicate that the final distribution of genomic imbalances is the product of continuous selection, and that this distribution is independent of whether a carcinoma occurs spontaneously or as a result of, for example, chronic inflammation. We therefore determined the degree of genomic instability by DNA image cytometry and CGH for 23 UCC specimens.<sup>43</sup> All 23 UCC specimens revealed highly aneuploid DNA distribution patterns of the nuclear DNA content, independent of the tumor stage. CGH analysis could identify chromosomal imbalances as follows: the most common DNA gains were mapped to chromosomes or chromosome arms 20q (84% of all cases), 7 (74%), 8q (74%), 13q (74%), 11p and 12 (both 42%), 5p and 18p (both 37%), and 17q (31%). Recurrent losses occurred on 8p (58%), 18q (47%), and 5q (26%).<sup>43</sup> These results show that chromosomal imbalances observed in UCC mainly cluster on the same chromosomes as described for sporadic colorectal cancer. For instance, Ried et al. reported DNA gains that frequently mapped to chromosomes or chromosome arms 7, 8q, 13q, and 20 in sporadic colorectal carcinomas.<sup>36</sup> However, it also becomes clear that sporadic colorectal carcinomas have fewer genomic imbalances than UCCs (Figure 15.1). Additional significant differences exist that characterize UCCs in contrast to sporadic carcinomas: our previous analyses of sporadic colorectal carcinomas revealed an average number of DNA copy alterations (ANCA), calculated as the number of chromosomal copy number changes divided by the number of cases, of 5.6, which was elevated to 13.3 in UCC. This number exceeds that observed in primary liver metastases from colorectal carcinomas, for which the ANCA had been determined to be 11.7.<sup>44</sup> This high degree of genomic instability is also supported by measurements of the nuclear DNA content, which invariably revealed nuclear aneuploidy. We also observed a large number of localized high-level copy number increases (amplifications). Amplifications have been described as a reflection of advanced disease and poor prognosis in other malignancies.<sup>45</sup> Some of the amplifications occurred in regions known to be affected in colorectal carcinomas, such as chromosome arms 6p, 8q, 13q, 17q, and 20q, and for which the target genes are either known or likely candidates have been identified ([http://www.helsinki.fi/cm/cgh\\_data.html](http://www.helsinki.fi/cm/cgh_data.html)). For instance, the frequent gain of chromosome 8 and amplifications that map to band 8q24 target the *MYC* oncogene.

The CGH profile for UCC as presented here, dominated by overall gains and numerous amplifications, is in concordance with the relatively high ANCA value and severe aneuploidy observed in the majority of all 23 UCCs. In comparison, sporadic colon carcinomas show aneuploidy in only 70–80% of the cases, combined with an overall lower ANCA value. The surprisingly high level



remains less clear how genomic aneuploidy and chromosomal imbalances impact on the transcriptome. One could postulate that expression levels of all transcriptional active genes on trisomic chromosomes would increase in accordance with the chromosome copy number. Alternatively, changing the expression level of only one or a few genes residing on that chromosome through tumor-specific chromosomal aneuploidies may be the selective advantage necessary for tumorigenesis. This would require the permanent transcriptional silencing of most of the resident genes. Another formal possibility that must be entertained is that chromosomal copy number changes are either neutral or inversely correlated with respect to gene expression levels. This would mean that gains or losses of chromosomes are a byproduct of specific gene mutations and may not offer any selective advantage. Because of the many chromosomal aberrations usually found in cancer cells, it is difficult, if not impossible, to identify the consequences of specific trisomies, independent from other coexisting genomic imbalances, gene mutations, or epigenetic alterations.<sup>48</sup>

Methodology to analyze the consequences of chromosomal imbalances in tumor genomes has become available through the development of microarray-based gene expression profiling. This method has been first described by Schena et al. and enables one the simultaneous analysis of thousands of genes for their gene expression.<sup>49</sup> Despite the exponential increase in the number of publications describing microarray experiments, only a few reports have attempted to specifically address the question regarding the immediate consequences of chromosomal aneuploidies vis-a-vis the dysregulation of the cellular transcriptome. These reports came to quite different conclusions and none of them attempt to address this question in the clinical setting of colorectal carcinogenesis.<sup>44, 50-53</sup> In addition, a comprehensive exploration of how global alterations of the cellular transcriptome might correlate with sequential steps of cellular transformation from normal mucosa by adenoma and carcinoma up to distant metastases has not been described. Such analyses, however, could reveal potential candidate genes for improved prognostics, diagnostics and therapeutics.

### 3.1. Immediate consequences of genomic imbalances on the transcriptome

Chromosomal aneuploidies are not only observed in sporadic and UCCs but in essentially all sporadic carcinomas. These aneuploidies result in tumor-specific patterns of genomic imbalances that are acquired early during tumorigenesis.<sup>13, 24, 54</sup> For instance, one of the earliest genetic abnormalities observed in the development of sporadic colorectal tumors is trisomy of chromosome 7.<sup>32</sup> Usually, once acquired, these specific imbalances are maintained despite ongoing chromosomal instability.<sup>55</sup> It is therefore reasonable to assume that continuous selective pressure for the maintenance of established genomic imbalances exists in cancer genomes. It is not known how genomic imbalances affect chromosome-specific gene expression patterns in particular and how chromosomal aneuploidy dysregulates the genetic equilibrium of cells in general. To model specific

chromosomal imbalances that expression levels of all genes would increase in accordance with the imbalance. Alternatively, changing the expression of a particular chromosome through selective advantage necessitates transcriptional silencing of genes on that chromosome, a possibility that must be considered. Genes are either neutral or deleterious. This would mean that specific gene mutations and many chromosomal aberrations are not impossible, to identify them from other coexisting mutations.<sup>48</sup>

Chromosomal imbalances in the form of microarray data were first described by Schena and colleagues in thousands of genes for their increased number of publications. Reports have attempted to elucidate consequences of chromosomal imbalances on the cellular transcriptome. None of them attempt to model the consequences of chromosomal imbalances on cellular carcinogenesis.<sup>44, 50-53</sup> In addition, the cellular transcriptome of distant metastases has been analyzed to reveal potential candidate genes for therapeutic targeting.

#### Consequences on the transcriptome

Chromosomal imbalances in sporadic and UCCs but in general result in tumor-specific changes during tumorigenesis.<sup>13</sup> Aberrations observed in the development of chromosome 7.<sup>32</sup> Usually, despite ongoing chromosomal imbalances that continuous selection exists in chromosomal imbalances affect chromosome-specific genes. However, chromosomal aneuploidy is general. To model specific

chromosomal aneuploidies in cancer cells and dissect the immediate consequences of genomic imbalances on the transcriptome, we set up an experimental model system in which the only genetic alteration between parental and derived cell lines is an extra copy of a single chromosome: we generated derivatives of the diploid yet mismatch repair-deficient colorectal cancer cell line, DLD1, and immortalized cytogenetically normal human mammary epithelial cell line (hTERT-HME) using microcell-mediated chromosome transfer to introduce extra copies of neomycin-tagged chromosomes 3, 7, and 13.<sup>56</sup> FISH with chromosome-specific probes confirmed the maintenance of extra copies of these chromosomes under neomycin-selective cell culture conditions. In addition, SKY was performed to determine whether the chromosome transfer process induced secondary karyotypic changes. With the exception of loss of the Y chromosome in all DLD1 + 3 cells analyzed and in a small percentage of DLD1 + 7 cells, all four derivative lines maintained the diploid karyotype of the parental cell line and contained the additional copy of the introduced chromosome. These results were also confirmed by CGH. The global consequences on gene expression levels were analyzed using cDNA arrays. Our results show that regardless of chromosome or cell type, chromosomal trisomies resulted in a significant increase in the average transcriptional activity of the trisomic chromosome ( $p < 0.001$ ) (Figure 15.2).

Several important conclusions regarding the impact of chromosomal aneuploidy on cellular transcription levels can be drawn from our analysis. First, alterations in the copy number of whole chromosomes resulted in an increase in average gene expression for that chromosome. The average increase in gene expression (1.21) however, was lower than the average increase of genomic copy number (1.44). These results were consistent with results from similar analyses of aneuploid colorectal, pancreatic, and renal cancer derived cell lines in which we observed a trend, indicating that indeed chromosomal aneuploidies correlate with global transcription levels.<sup>25, 44, 51</sup> Second, chromosomes not observed to be aneuploid in particular tumor types (i.e., chromosome 3 in colorectal tumors) also had increased transcriptional activity when placed into that cellular environment. Thus, their presence is not neutral with respect to the transcriptome. Third, aneuploidy not only affects gene expression levels on the chromosomes present in increased copy numbers, but a substantial number of genes residing on other chromosomes significantly increased or decreased apparently in a stochastic manner. The influence of chromosomal aneuploidy on the expression level of individual genes was examined by considering only those genes whose expression ratios were  $>2.0$  (upregulated) or  $<0.5$  (downregulated) when compared with the parental cell line. Strikingly, none of the genes were affected in common among any of the four cell lines. This observation is of course consistent with known mechanisms of gene regulation (e.g., activator and suppressor proteins, signaling pathways) and the fact that genes residing in a given pathway are for the most part distributed throughout the genome on different chromosomes. Three groups have analyzed the consequences of constitutional chromosomal trisomies on

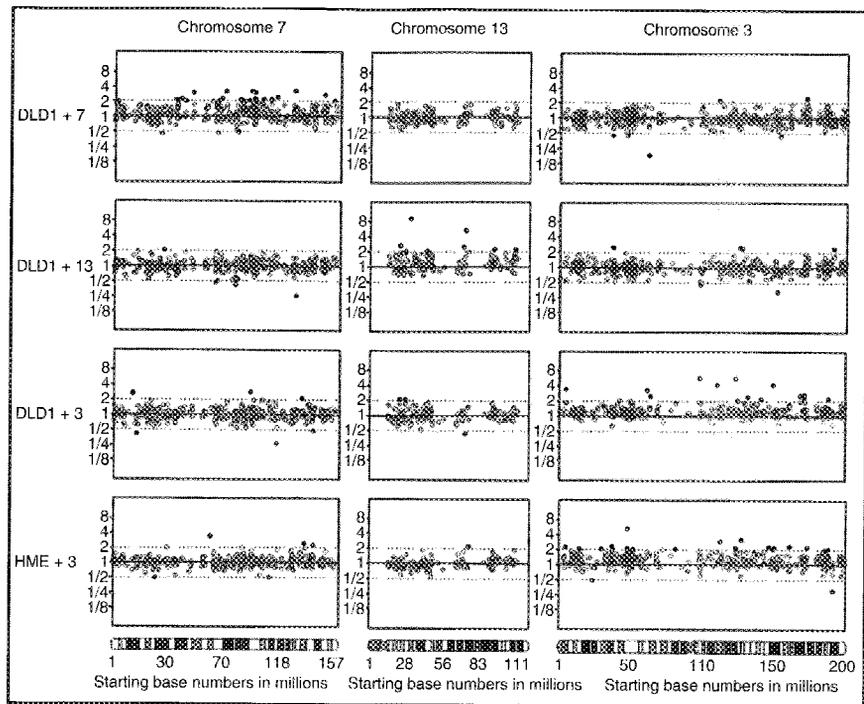
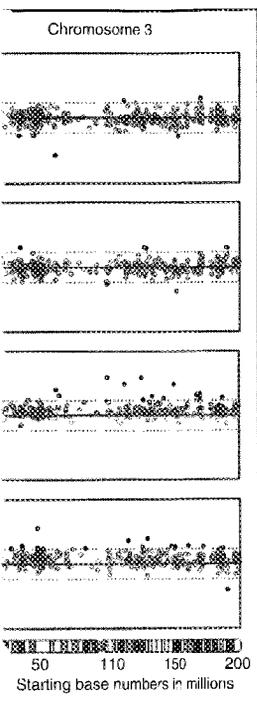


Figure 15.2. Global gene expression profiles. Each scatter plot displays all genes and their corresponding normalized ratio values along the length of each chromosome. Values in open circles represent ratio values between 0.5 and 2.0. Dark dots represent expression ratios  $\geq 2.0$  or  $\leq 0.5$ .

transcriptional activity in noncancerous fetal cells and in a mouse model of human trisomy 21, and attained similar conclusions as us.<sup>57, 58, 59</sup> These studies concluded that the average gene expression of trisomy chromosomes is clearly increased, although this was not due to high-level upregulation of only a few specific genes. However, expression levels of multiple genes throughout the genome were dysregulated. Normal cells with constitutional chromosomal aneuploidies (or segmental duplications) cannot tolerate trisomy of  $>4.3\%$  of the genome.<sup>60, 61</sup> However, this limit is not merely a reflection of the DNA content because multiple copies of an inactivated X-chromosome can be tolerated. Therefore, this limit is more likely to be imposed by global disturbance of the transcriptome as a consequence of genomic imbalances. This hypothesis is supported by the recent identification of differential average expression levels of specific chromosomes. For instance, the average gene expression levels and gene density of chromosomes 13, 18, and 21 are lower than those of smaller chromosomes, yet trisomy of only these chromosomes is compatible with life in noncancerous cells.<sup>61</sup> It is interesting to speculate that one of the specific features of emerging cancer cells, which would differ from nontransformed cells that carry constitutional trisomies, would be the



displays all genes and their corresponding expression ratios  $\geq 2.0$  or  $\leq 0.5$ .

and in a mouse model of as us.<sup>57, 58, 59</sup> These studies of specific chromosomes is clearly a dysregulation of only a few genes throughout the genome. Chromosomal aneuploidies  $>4.3\%$  of the genome.<sup>60, 61</sup> DNA content because multi-erated. Therefore, this limit of the transcriptome as a is supported by the recent of specific chromosomes. The density of chromosomes, yet trisomy of only rous cells.<sup>61</sup> It is interesting g cancer cells, which would nal trisomies, would be the

ability to exceed this transcriptional threshold during the multiple steps required for tumorigenesis. This global dysregulation of the transcriptome of cancers of epithelial origin may also reflect on our ability for therapeutic intervention: although the consequences of a simple chromosomal translocation, such as the *BCR/ABL* fusion in chronic myelogenous leukemia, can be successfully targeted with an inhibitor of the resulting tyrosine kinase activity such as Gleevec, the normalization of the complex dysregulation of transcriptional activity in carcinomas requires a more general, less specific, and hence more complex interference.<sup>62</sup>

#### 4. PROTEOMICS

The term proteome was first defined in 1994 and denotes the entirety of proteins expressed by the genome. Proteomics is thus understood as the consecutive step following genomics. Proteomics techniques have rapidly evolved and are now widely applied to monitor disease-specific alterations.<sup>63</sup>

##### 4.1. Two-dimensional gel electrophoresis

In proteome research, two-dimensional gel electrophoresis (2-DE) is still the cornerstone separation technique for complex protein mixtures<sup>64</sup>. The 2-DE approach allows large-scale screening of the protein components of normal and disease cells<sup>64</sup>. We used quantitative 2-D SDS PAGE (pH 4–7) to analyze protein-based expression profiles for sporadic and hereditary, i.e., familial adenomatous polyposis (FAP), colorectal cancer samples. The 2-D gels were stained using silver, coomassie, or sypro ruby, images were scanned and digitally compared using PDQuest analysis software version 7.3. This is a powerful software offering automated spot detecting and matching function and integrates a statistical software package. Using this technique it is possible to highlight proteins that are differentially abundant in one state versus another (e.g., tumor vs normal).<sup>65</sup>

##### 4.2. Matrix-assisted laser desorption ionization mass spectrometry

Within the matrix-assisted laser desorption ionization (MALDI) technique, matrix and sample are cocrystallized on the MALDI plate and irradiated with a laser pulse.<sup>66</sup> The matrix absorbs the energy and acts as an intermediary for the codesorption and ionization of sample and matrix. The ions are accelerated in an electrical field and enter a field free drift tube. The mass-related time of flight is detected and the analog signal converted and digitalized. The experimentally generated masses are compared with a set of mass profiles in a protein database, e.g., SwissProt, ExPASy, or UniProt. The most similar pattern determines the protein "hit." The tighter the mass tolerance, the more stringent is the identification.

The subsequent application of the 2-DE and mass spectrometry technique could show that tumor-specific quantitative or qualitative changes of protein patterns are indeed discernable. We performed a detailed analysis to identify

sequential alterations of the proteome that define the transformation of normal epithelium and the progression from adenomas to invasive disease. We have analyzed tissue samples from 15 patients, including the mucosa-adenoma-carcinoma sequence from individual patients.<sup>67</sup> We determined the degree of genomic instability during carcinogenesis by measuring DNA contents and assessed protein expression levels by means of 2-DE and subsequent mass spectrometry. The 2-DE revealed a total of 112 polypeptide spots that showed an at least twofold differential expression between the four stages of carcinogenesis.<sup>67</sup> A total of 72 of these polypeptides could be characterized by mass spectrometry and 46 of those were exclusively overexpressed in tumors and metastases. Unsupervised principal component analysis allowed separation of adenomas, carcinomas, and metastases based on protein expression profiles. Interestingly, two dysplastic polyp samples did not conformingly cluster in their cohort and were closer located to the malignant samples (Figure 15.3). Both polyps revealed aneuploid DNA distribution patterns, indicating an increased malignancy potential.

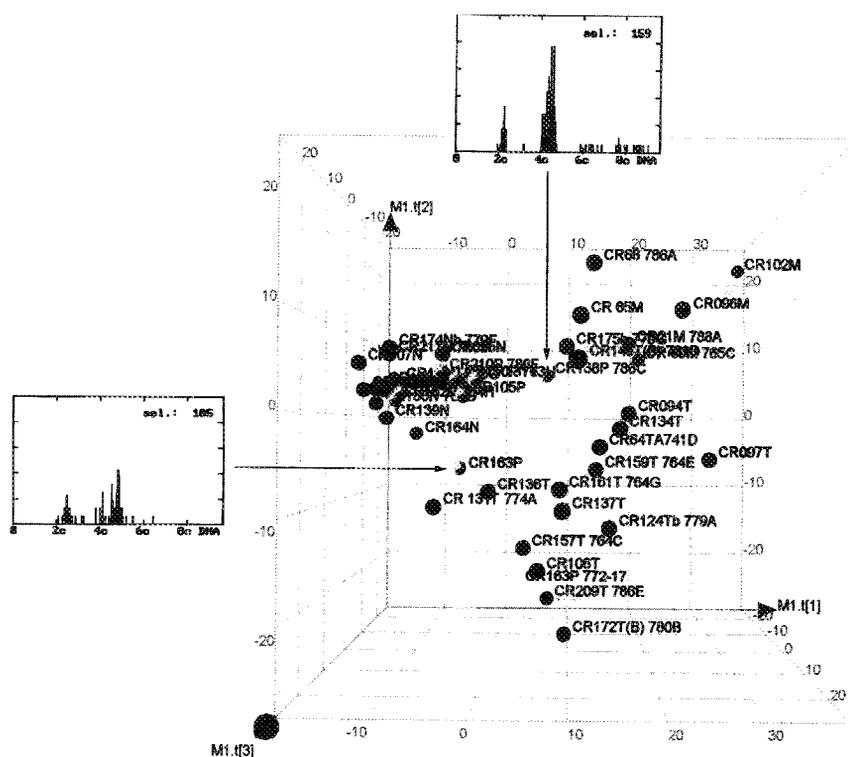
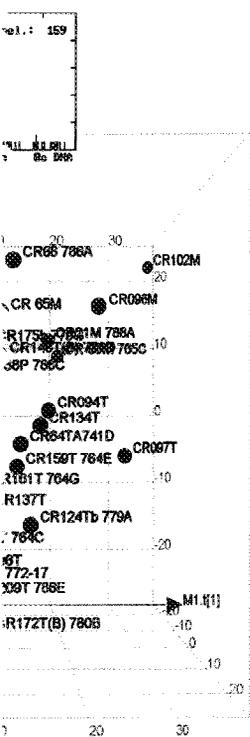


Figure 15.3. PCA plot of the protein expression data of all match set members, with the normal cohort (green), polyps (yellow), tumors (blue), and metastases (red). The arrows highlight two polyps that were outliers, showed aneuploidy in the DNA measurement (shown in the histograms) and clustered closer to the tumors in the three-dimensional space.

transformation of normal invasive disease. We have determined the degree of varying DNA contents and subsequent mass spectrometry spots that showed an at stages of carcinogenesis.<sup>67</sup> analyzed by mass spectrometry in tumors and metastases. separation of adenomas, expression profiles. Interestingly, cluster in their cohort and (5.3). Both polyps revealed an increased malignancy



each set members, with the normal (red). The arrows highlight two elements (shown in the histograms)

FAP also termed adenomatous polyposis coli (APC) is an autosomal dominant inherited disease with a germ line mutation of the APC gene on 5q21. In spite of this specific genetic alteration early diagnosis in young patients without polyposis onset and lack of a family history can be difficult and finally lethal. Thus, there is a need for a better understanding of the disease process on the molecular level in order to be able to introduce more sensitive diagnostic procedures. Proteomics is a multifaceted approach to study various aspects of protein expression. While DNA constitutes the "information archive of the genome," proteins actually serve as the functional effectors of cellular processes. We analyzed protein expression to elucidate pathways and networks involved in the pathogenesis of FAP coli, its associated carcinomas, and in comparison with the sporadic form of the disease.

Protein measurements were performed on 47 samples gained from 15 different patients. Proteins were separated by 2-DE revealing 1950 separated proteins. Qualitative and quantitative differences in expression levels between normal epithelium, adenoma, and carcinoma both in FAP and sporadic colon cancer were compared and statistically evaluated. In addition, collecting "triplets" from the same patient (normal, adenoma, and carcinoma) made also an intra- and inter-patient comparison possible. We found 17 proteins that showed quantitative changes between normal mucosa in FAP and sporadic normal mucosa with a false discovery rate (FDR) less than 10%. Furthermore, qualitative analysis discovered 47 proteins present in all FAP mucosa specimens and absent in the sporadic normal mucosa. Comparing FAP polyps with sporadic colonic polyps we found 49 polypeptides that are present in the FAP samples and absent in all sporadic polyps. One protein was found to be present in the sporadic polyps only. We also found 66 proteins whose absence/presence pattern coincides with the FAP/sporadic cancer grouping. The data obtained on the protein expression level make it possible to diagnose the FAP disease already in the "normally" appearing colorectal mucosa.

#### 4.3. Surface-enhanced Laser Desorption Ionization

One particularly intriguing possibility develops if tumor-specific changes could be detected with noninvasive, cost-efficient formats, for instance, by detection of disease-specific markers in the peripheral blood. However, the use of single serum markers, e.g., carcinoembryonic antigen, has so far failed to deliver markers of high sensitivity and specificity for colon cancer and most other tumors.<sup>68, 69</sup> Comprehensive serum proteome profiling for such tumor-specific markers has therefore become a field of intensive research.<sup>70</sup> A particular promising technique for serum proteome screening is based on surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) mass spectrometry. A major advantage of surface-enhanced laser desorption ionization (SELDI) is that complex protein mixtures can be directly analyzed by mass spectrometry without

any prior separation and purification. SELDI-TOF utilizes chromatographic surfaces that retain proteins from a complex sample mixture according to their specific properties (e.g., hydrophobicity and charge), with the molecular weights of the retained proteins then being measured by TOF mass spectrometry.<sup>70</sup> Microliter quantities of serum are directly applied to chips and the bound proteins are treated and analyzed by mass spectrometry. The mass spectra patterns obtained for different samples reflect the protein and peptide contents of these samples. Protein identification itself needs to be performed in an additional analysis step.<sup>71-73</sup> The reliability and reproducibility have been proven even if variation coefficients of 8–10% indicate the need for technical repeats.<sup>72, 73</sup> SELDI-TOF mass spectrometry is particularly well suited to evaluate low-molecular proteins (0.525 kDa) and is, as such, complementary to the 2-DE approach.

The identification of SELDI-based protein profiles and the subsequent protein identification of features that allow the distinction between malignancy-related and normal sera would be highly beneficial.

## 5. SUMMARY

Genomic aneuploidy occurs early and is commonly found in precancerous biopsies of ulcerative colitis patients who subsequently develop a UCC. The assessment of DNA ploidy could therefore become a basic element in future surveillance programs in ulcerative colitis. The complementary detection of increased cyclin A expression in aneuploid lesions — indicating clonal expansion — seems to be the most powerful combination to predict imminent malignant transformation for an individual patient. Moreover, genomic aneuploidy in UCC tumors correlates with specific chromosomal gains and losses, which, in turn, are associated with increased cyclin A levels. The overall pattern of specific chromosomal aberrations in UCC tumors is similar to that seen in sporadic colorectal carcinomas. The predominance of specific chromosomal aneuploidies in colorectal cancers also affects the transcriptome of cancer cells. In a well-defined model system we could observe that the introduction of an extra copy of a given chromosome increases very specifically the overall average expression of genes on the trisomic chromosomes. Additionally, a large number of genes on diploid chromosomes were also significantly increased, revealing a more complex global transcriptional dysregulation. In addition, increasing genomic instability and a recurrent pattern of chromosomal aberrations are accompanied by distinct protein expression patterns that correlate with subsequent stages of colorectal cancer progression. The identified proteins underwent extensive posttranslational modifications, thus multiplying the transcriptional dysregulation. Analyzing protein-based expression profiles for sporadic and hereditary colorectal cancer samples allowed the detection of a distinct protein expression pattern that seems to be characteristic for FAP-diseased tissue.

## 6. FUTURE PERSPECTIVES

The evolving technique of array CGH allows the identification of DNA copy number changes of, ideally, individual genes and thus enables an increasing resolution compared with conventional CGH, which is performed on more or less condensed chromosomes. One particular application, however, could be the analysis of amplicons identified in sporadic and UCCs. Custom designed arrays that contain genes located on distinct amplified chromosome segments would enable to select for individual genes rather than chromosomal segments that are highly amplified. Such genes could be used as marker genes for colorectal malignancy. Their diagnostic and prognostic potential could be tested with gene-specific probes by means of multicolor FISH in premalignant lesions (e.g., ulcerative colitis biopsies and adenomas). Thus, the combined analysis of DNA ploidy measurements and colorectal cancer-specific Multi-FISH probes in premalignant colorectal lesions could profoundly improve individual risk assessment for imminent colorectal cancer development. However, the design of customized arrays that contain the amplified genes might provide a more rapid and high throughput screening approach compared with Multi-FISH. The application of array CGH would also allow a direct correlation how single gene copy number changes influence the transcriptional equilibrium. The employment of comprehensive gene and protein expression profiling in subsequent stages of colorectal cancer progression allowed the identification of genes and proteins that now warrant further validation by RNA interference (RNAi) experiments, in order to prove their potential for gene and protein expression tailored individualized diagnostic, prognostic, and therapeutic approaches. The combined evaluation of ploidy status, amplification of disease and stage-specific gene probes, and gene and protein expression patterns in clinical tissue samples should be utilized in prospective studies to corroborate their value for improved diagnostics, prognostication, and identification of therapeutic targets. Proteomics-based antibody panels, ELISA tests and strategies combining surface-mediated protein enrichment with direct mass spectrometric quantification and identification of putative markers are thus promising ways for future cancer diagnosis. The field of proteomics promises accurate staging and, it is hoped, individualized prognosis and treatment tailoring in the not-so-distant future. The detection of malignancy related proteins in the serum might provide a rapid, sensitive, and specific screening method for colorectal malignancies even for early disease stages.

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