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Ulcerative Colitis and Colorectal Cancer: Aneuploidy and Implications for Improved Screening

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1. Introduction

Patients with ulcerative colitis (UC) have a significantly increased lifetime risk for the development of colorectal carcinomas. Ulcerative colitis can therefore be considered a bona fide premalignant condition. It is therefore recommended that patients with UC participate in surveillance programs to screen for early signs of malignancy. However, reliable endoscopic sampling and histopathological evaluation is difficult. The diagnostic dilemma is underlined by the fact that despite screening programs about half of the patients with an ulcerative colitis-associated carcinoma (UCC) are diagnosed at an already advanced tumor stage reflecting poor prognosis. For these reasons, it should be obvious that additional markers with high prognostic impact in the individual risk assessment are of high clinical demand.

While genetic and genomic changes during carcinogenesis have been thoroughly studied in sporadic colorectal cancers, less is known about the development of UCCs. This chapter will therefore focus on the role of genomic instability during colitis-associated carcinogenesis and how ploidy assessment might help to improve individual risk stratification regarding imminent colorectal cancer risk and survival prognosis.

2. Pathogenesis of sporadic and ulcerative colitis-associated colorectal cancer

The normal colonic epithelium (mucosa) is a highly dynamic system: Stem cells are located at the basis of epithelial crypts (Wright 2000). They are a source of constantly proliferating cell populations that – while differentiating - migrate to the surface of the colonic crypts from where they are shed into the lumen. The intestinal epithelium is thus renewed every five to six days. Mucosal cells are prone to genetic damage due to the highly toxic and mechanically stressful intra-luminal environment. The rapid clearance of mucosal cells however prevents these cells from being a source of malignant transformation. However, a

high proliferative rate in a toxic environment could also easily accelerate malignant transformation once regulatory mechanisms for cell homeostasis are bypassed. Most colorectal tumors are caused by acquired genetic lesions of single mucosal cells that harbour a growth advantage and – through clonal expansion – rise to invasive carcinomas. Whether these genetic aberrations occur predominantly in stem cells, migrating cells, or mucosal cells at the crypt surface has not been conclusively clarified (Shih et al. 2001; Lamprecht and Lipkin 2002; Bach, Renehan, and Potten 2000). Genetic aberrations can become evident either on the subchromosomal or chromosomal level and target regulatory mechanisms required for the genetic equilibrium such as cell cycle regulation, cellular signalling pathways, proliferation, differentiation, growth inhibition, and apoptosis signalling. Vogelstein and colleagues defined a model of colorectal carcinogenesis in which a non-random accumulation of genetic aberrations can be correlated with morphologic changes of the colon epithelium: the transition from normal mucosa via adenomatous polyp to colorectal cancer and eventually distant metastasis (Fearon and Vogelstein 1990). However, in ulcerative colitis macroscopically visible pre-malignant lesions such as adenomas are missing and the detection of dysplastic lesions and/or dysplasia associated lesion or mass (DALM) are difficult to identify clinically (Riddell 1998).

2.1 Colorectal cancer risk in ulcerative colitis

Three of ten ulcerative colitis patients will eventually develop cancer after a longstanding colitis (Bernstein et al. 2001). Ulcerative colitis can therefore be considered a bona fide premalignant condition. Therefore, it is recommended that patients with UC participate in surveillance programs in order to screen for early signs of malignancy (Daperno et al. 2004). Ulcerative colitis-associated colorectal carcinomas (UCC) do not develop through the adenoma-carcinoma-sequence (Willenbucher 1996). Instead, epithelial dysplasias have been defined as precursor lesions and are meant to be the most predictive feature of intensive and expensive surveillance programs today (Collins, Feldman, and Fordtran 1987). However, reliable endoscopic sampling and histopathological evaluation is difficult (Eaden et al. 2001; Riddell 1998; Lynch et al. 1993). Additionally, a review of 12 surveillance studies with 92 detected carcinomas in 1,916 patients revealed that about half of them were advanced Dukes´ C and D malignancies and only 12% were early stage carcinomas (Lynch et al. 1993).

2.1.1 Tumor dissemination

Metastases are one of the hallmarks of solid tumor malignancy. Once a primary tumor has been detected and surgically removed, the survival of the patient greatly depends on the occurrence of local or distant metastases. Rather than the primary carcinoma itself, it is mainly the metastatic disease that leads to death. The ability of tumor cells to metastasize depends on the acquisition of certain characteristics that allow local or distant spread via the lymphatic or venous system. Early detection of metastasis is important for treatment interventions, however, has proved to be difficult (Calaluce, Miedema, and Yesus 1998).

2.1.2 Local invasion

One of the necessary characteristics of metastasizing tumor cells is the ability to invade the basement membrane. Laminins are major components of the basement membranes that

belong to a family of heterotrimeric glycoproteins. They are composed of at least α, β and γ subunits that can form 12 or more isoforms (Iivanainen, Morita, and Tryggvason 1999). The various isoforms have different tissue specific biological functions, such as cell adhesion, migration, proliferation, as well as growth and differentiation (Tryggvason 1993). The laminin-5 isoform (α3: β3: γ2), also known as kalinin, nicein, epiligrin, and ladsin, plays an important role for epithelial cell adhesion to the basement membrane (Carter, Ryan, and Gahr 1991). In order to invade surrounding tissue, tumor cells attach to the basement membrane by binding to laminin receptors from laminin implemented in the basement membrane. This mimics a physiological process: for example, non-neoplastic cells such as inflammatory and endothelial cells regularly cross the basement membrane. These processes are controlled by regulatory mechanisms and it remains unclear how tumor cells can bypass those mechanisms. One possible mechanism is the ability of tumor cells to express laminin themselves. That would enable the attachment to the basement membrane independent from available receptors of the basement membrane laminin. Interestingly, there is much evidence that shows increased expression of the laminin-5 γ2 gene has been found in invasively growing malignant cells at the epithelial-stromal junction, i. e., at the invasion front of different tumors and colorectal cancers (Pyke et al. 1994; Pyke et al. 1995; Sordat et al. 1998).

3. Aneuploidy and cancer risk stratification in ulcerative colitis

Aneuploidy is a consistent genetic alteration of the cancer genome (Duesberg et al. 1998; Lengauer, Kinzler, and Vogelstein 1998; Ried et al. 1999). 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 (Caspersson 1979). With increased resolution of cytogenetic techniques, such as chromosome banding, comparative genomic hybridization (CGH), spectral karyotyping (SKY), and multicolor fluorescence *in situ* hybridization, it has become clear that in addition to nuclear aneuploidy, specific non-random chromosomal imbalances (heretofore referred to as chromosomal aneuploidy) exist (Caspersson et al. 1970; Kallioniemi et al. 1992; Schrock et al. 1996; Speicher, Gwyn Ballard, and Ward 1996). Indeed, despite genetic instability in cancer genomes, cancer cell populations as a whole display a surprisingly conserved, tumor-specific pattern of genomic imbalances (Ried et al. 1999; Knuutila et al. 1998; Forozan et al. 1997). At early steps in the sequence of malignant transformation during human tumorigenesis, *e.g.,* in pre-invasive dysplastic lesions, chromosomal aneuploidy can be the first detectable genetic aberration found (Hittelman 2001; Hopman et al. 1988; Heselmeyer et al. 1996; Solinas-Toldo et al. 1996). This suggests that there is both an initial requirement for the acquisition of specific chromosomal aneuploidy 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 (Bomme et al. 1994; Ried et al. 1999; Nowak et al. 2002; Desper et al. 2000). Additionally, in cell culture model systems in which cells are exposed to different carcinogens, chromosomal aneuploidy is the earliest detectable genomic aberration (Barrett et al. 1985; Oshimura and Barrett 1986). The conservation of these tumor specific patterns of chromosomal aneuploidy suggests that they play a fundamental biological role in tumorigenesis.

3.1 Chromosomal aneuploidy in sporadic colorectal cancer

The progression of colorectal cancer is defined by the sequential acquisition of genetic alterations (Fearon and Vogelstein 1990). 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 (Ried et al. 1996). For instance, one of the earliest acquired genetic abnormalities during colorectal tumorigenesis are copy number gains of chromosome 7 (Bomme et al. 1994). 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. This chromosomal aneuploidy is accompanied by specific mutations in oncogenes and tumor suppressor genes, including e.g. APC and TP53 (Vogelstein and Kinzler 2004). It is therefore well established that both, chromosomal aneuploidy and specific gene mutations, are required for tumorigenesis.

3.2 Chromosomal aneuploidy in ulcerative colitis-associated colorectal cancer

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 questioned if the pattern of chromosomal gains and losses in UCC are similar to that described for sporadic carcinomas. A similar pattern 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. Recent reports suggested that in general, genomic imbalances observed in UCC cluster on the same chromosomes as those in sporadic colorectal carcinomas (Kern et al. 1994; Holzmann et al. 2001; Willenbucher et al. 1997; Loeb and Loeb 1999; Aust et al. 2000). Our analyses comprises the largest sample collection of UCCs from one clinical center and supports these findings: all 19 UCC specimens showed chromosomal imbalances by comparative genomic hybridization (CGH) 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%) (Habermann et al. 2003). 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 (Ried et al. 1996). However, it also becomes clear that sporadic colorectal carcinomas have fewer genomic imbalances than UCCs (**Figure 1**). 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 (Platzer et al. 2002). This high degree of genomic instability is also supported by measurements of the nuclear DNA content, which invariably revealed gross 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 (Blegen et al. 2001). 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/cmg/cgh_data.html). For instance, the frequent gain of chromosome 8 and amplifications that map to band 8q24 target the *MYC* oncogene. Candidates on chromosome 20 include the nuclear co-receptor activator gene *NCOA3* and a member of the aurora kinase family. Another correlation is the coincidental overexpression of laminin-5 and gain of chromosome band 1q25-q31, the map position of the *LAMC2* gene (laminin-5). Laminin-5 plays a crucial role for invasive capacities of metastasizing cells but it has not been elucidated how increased expression levels are produced. Genomic amplification could be one molecular mechanism leading to laminin-5 overexpression.

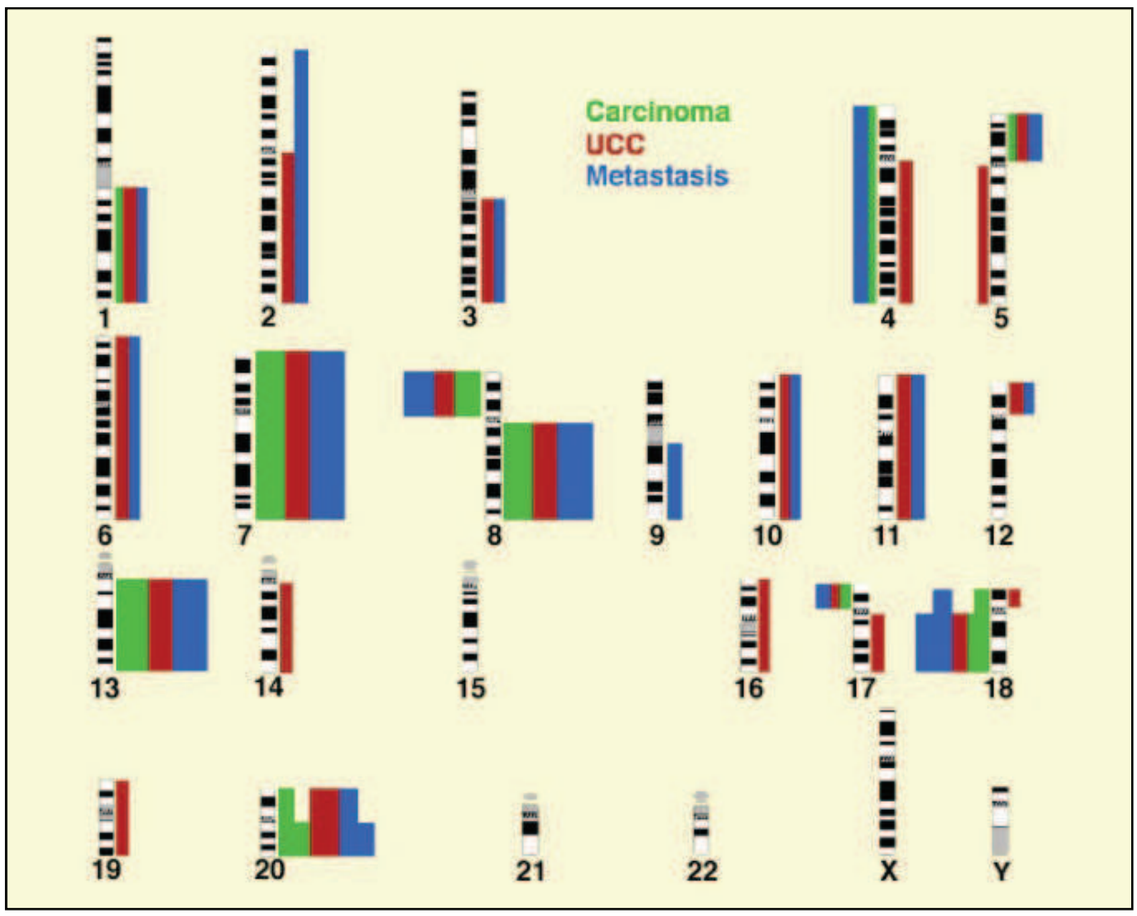

Fig. 1. Comparison of genomic imbalances in sporadic colorectal carcinoma (SCC), ulcerative colitis-associated colorectal cancer (UCC), and liver metastasis of SCC. Bars on the left side of the chromosome ideogram denote a loss of sequence in the tumor genome, bars on the right side a gain of sequence in the tumor genome. The number of alterations per chromosome is normalized to 10 cases for each tumor type. Only ratios greater than 2 have been considered. Figure modified from (Habermann et al. 2003)

The CGH profile for UCC analyzed in our cohort is in concordance with the relatively high ANCA value and severe aneuploidy observed. 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 of ANCA values in UCC could be a reflection of a generally increased genetic instability in UCC, due to the long latency of inflammatory disease before overt tumors develop; however, the data presented here and in the literature clearly indicate that the tumor cell population as an entity selects for a distribution of genomic imbalances that is similar to sporadic carcinomas. Therefore, the tissue origin of the tumor cell, and not the mode of tumor induction, seems to define the similarity between sporadic colorectal cancers and UCC. This is in striking contrast to hereditary colorectal carcinomas arising in the background of mismatch repair deficiency, where neither aneuploidy nor specific chromosomal imbalances are observed (Ghadimi et al. 2000; Schlegel et al. 1995).

3.3 Nuclear aneuploidy and prognosis in sporadic and UC-associated colorectal cancer

The strikingly conserved pattern of chromosomal aneuploidy in sporadic and UC-associated colorectal carcinomas can be reflected by nuclear DNA aneuploidy. Hereby, flow and/or image cytometry might serve as reliable tools with excellent clinical applicability also for high-throughput clinical diagnostics. Interestingly, reported frequencies of aneuploidy in UCCs vary inconsistently between 28.6% and 100% (Holzmann et al. 1998; Fozard et al. 1986). This rather astonishing range could be due to different ploidy assessment techniques, e.g., flow cytometry versus image cytometry, different standardization methodologies, as well as varying definitions of the terms “diploidy” and “aneuploidy” (Holzmann et al. 1998; Fozard et al. 1986; Klump et al. 1997; Clausen et al. 2001; Levine et al. 1991). In addition, a major drawback of the above mentioned studies might be the overall low number of UCC cases analyzed, varying from single case studies up to 17 individual UCC patients (Clausen et al. 2001; Makiyama et al. 1995; Burmer, Rabinovitch, and Loeb 1991). The latter study by Burmer et al found aneuploidy in 88% of cases investigated, however, did not distinguish between carcinomas and nonmalignant dysplastic lesions within these 17 UCC patients (Burmer, Rabinovitch, and Loeb 1991). Against this background, we had compiled 31 UCCs in order to evaluate the frequency of aneuploidy and its association to clinical parameters and survival and in comparison to 257 sporadic colorectal carcinomas. We performed nuclear DNA ploidy measurement by means of image cytometry which allows for simultaneous assessment of histomorphology and/or cytopathology. Histograms were classified according to Auer (**Figure 2**) (Auer, Caspersson, and Wallgren 1980).

UCCs presented aneuploidy at significantly higher frequency than sporadic colorectal carcinomas (100% versus 74.6%; $P < 0.0006$) (Gerling et al. 2010). In addition, we performed a logistic regression analysis comprising age, sex, UICC stage, T- and N-status, histologic tumor grading, underlying inflammation, and DNA ploidy status. Out of these features, logistic regression yielded two parameters to be of significant prognostic value for 5-year survival subsequent to operation for colorectal cancer. Those two significant parameters were age and DNA ploidy status indicating that patients of higher age at diagnosis and patients with aneuploid tumor cell populations have a poor survival prognosis. Additional logistic regression analysis comprising these two significant parameters only, confirmed age (odds ratio [OR], 1.05; 95% CI, 1.02–1.09; $P = 0.003$) and DNA ploidy (OR, 4.07; 95% CI, 1.46

–11.36; P = 0.007) to be independent prognostic parameters. According to the OR of 4.07, DNA ploidy seemed to be the main influencing feature. This was further supported by Kaplan-Meier-Plots showing that diploid SCCs had a more favorable 5-year survival (88.2%) than aneuploid SCCs (69.0%) and UCCs (73.1%) (P = 0.074). Thus, aneuploidy proved to be the strongest independent prognostic marker for R0-resected colorectal cancer patients overall (Gerling et al. 2010).

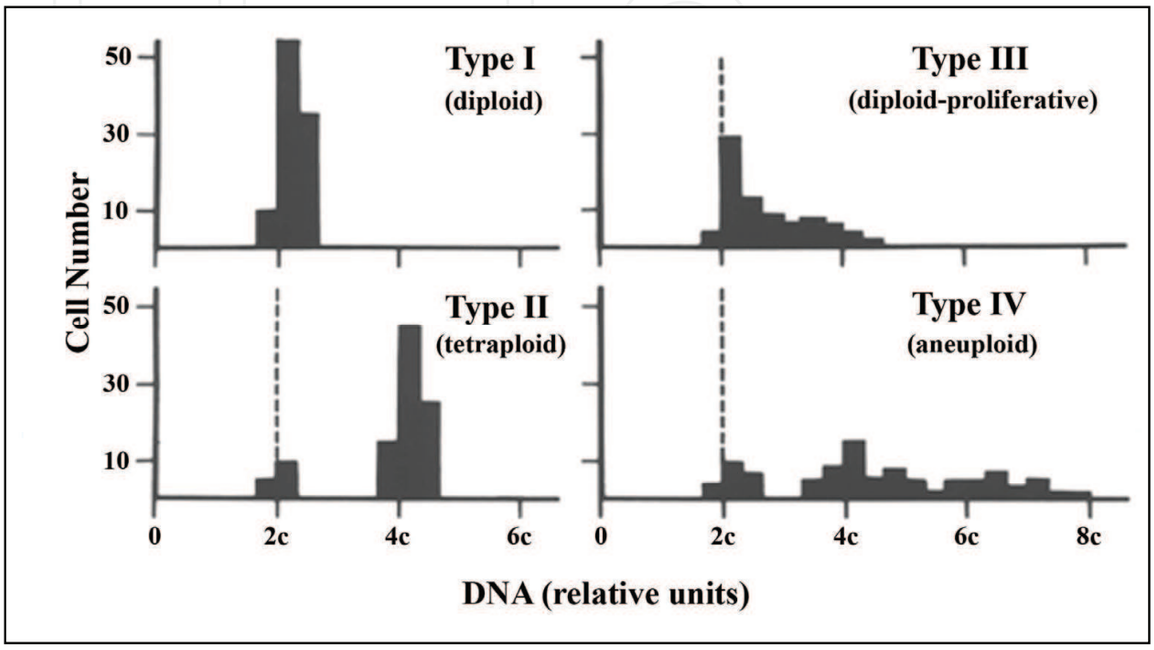

Fig. 2. DNA Histogram types according to Auer. Histograms characterized by a single peak in the diploid or near-diploid region (1.5–2.5 c) were classified as type I. The total number of cells with DNA values exceeding the diploid region (>2.5 c) was <10%. Type II histograms showed a single peak in the tetraploid region (3.5– 4.5 c) or peaks in both the diploid and tetraploid regions (>90% of the total cell population). The number of cells with DNA values between the diploid and tetraploid region and those exceeding the tetraploid region (>4.5 c) was <10%. Type III histograms represented highly proliferating near-diploid cell populations and were characterized by DNA values ranging between the diploid and the tetraploid regions. Only a few cells (<5%) showed more than 4.5 c. The DNA histograms of types I, II, and III thus characterize euploid cell populations. Type IV histograms showed increased (>5%) and/or distinctly scattered DNA values exceeding the tetraploid region (>4.5 c). These histograms reflect aneuploid populations of colon mucosa nuclei with decreased genomic stability

Furthermore, we also showed that diploid tumors at advanced stages (UICC stage III/IV) do present similar survival as compared with aneuploid sporadic and UC-associated carcinomas at early tumor stages (**Figure 3**). This finding might point to the conclusion that the presence of aneuploid tumor cell populations might influence patient's prognosis more dominantly than does tumor stage. This is in line with the finding that the most pronounced difference in prognosis can be observed between diploid SCCs at early stages and aneuploid colorectal carcinomas at advanced stages. Furthermore, UCCs at advanced

stages show a prognosis inferior to that of their sporadic counterpart at the same tumor stage.

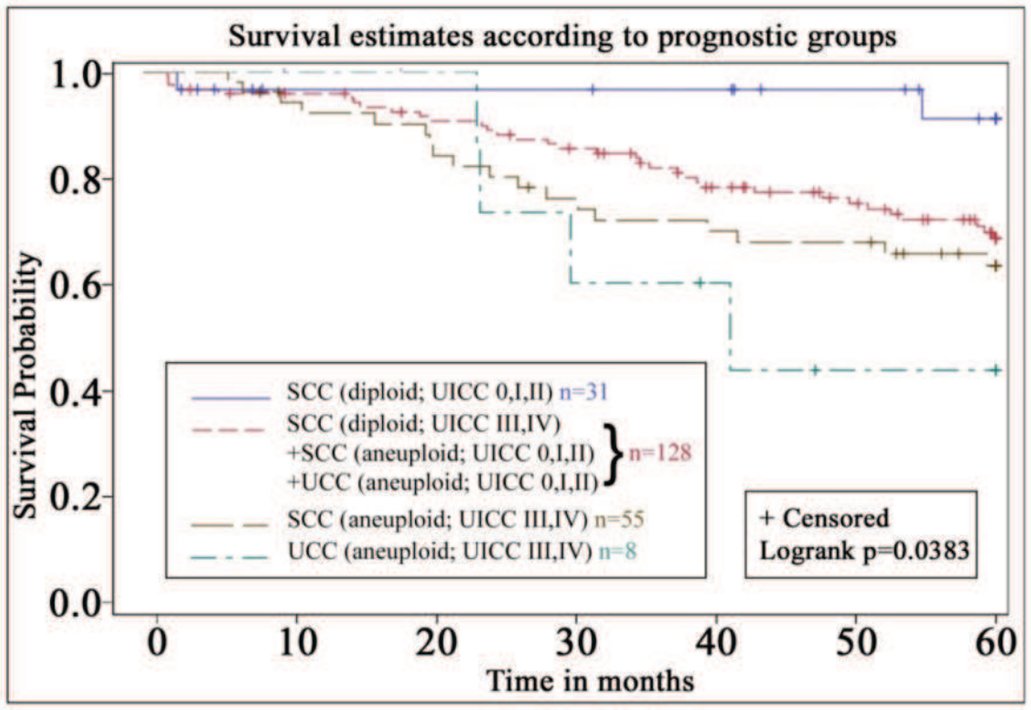

Fig. 3. Kaplan-Meier survival estimates of significant prognostic groups according to UICC stage, ploidy status, and underlying inflammatory disease. SCC, sporadic colorectal cancer; UCC, ulcerative colitis-associated colorectal cancer. Modified from (Gerling et al. 2010)

3.4 Aneuploidy and colorectal cancer risk in ulcerative colitis

The significantly higher frequency of aneuploidy in UCCs than in sporadic colorectal cancer might indicate the dominance of genomic instability not only at the time when malignancy is overt but also for the development of malignant properties. In order to evaluate aneuploidy as a potential predictive marker in patient risk assessment, we analyzed two groups (Habermann et al. 2001): eight patients with ulcerative colitis-associated colorectal carcinomas (UCC), and 16 ulcerative colitis (UC) patients without malignancy but comparable risk factors (duration of disease, extent of inflammation, epithelial dysplasia). A total of 683 paraffin-embedded mucosal biopsies were retrospectively evaluated for inflammatory activity, grade of dysplasia, ploidy status, laminin-5 γ2 chain and cyclin A expression. In all biopsies, mild or moderate inflammatory activity was present in 78% while low-grade or high-grade dysplasia was found in 5.5%. There was, however, no difference in inflammatory activity and dysplasia between patient groups (Habermann et al. 2001).

It is a known fact that dysplasia is absent in 20% - 30% of colectomy specimens containing UCC (Dobbins 1977). A review of 12 surveillance studies with 92 detected carcinomas in 1,916 patients revealed that only 12% were early carcinomas detected by surveillance (Lynch et al. 1993). In our study, only two cancer patients had a distinct high-grade dysplastic

lesion prior to the final diagnosis. One of them was underestimated in the original routine histopathological diagnosis. The tumor stages of the eight UCCs were as follows: one Dukes' A, three Dukes' B, two Dukes' C and two Dukes' D. One of the most important findings of this study was the detection of highly aneuploid epithelial cell populations scattered over the colon and rectum in premalignant biopsies of all eight UCC patients (**Figure 4**). These 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 unsuspicious mucosa, could even be detected in regenerative epithelium, and were not related to dysplasia. This DNA aneuploidy occurred more frequently in biopsies (75%) of UC patients with a subsequent UCC than in those without subsequent malignancy (14%, $p = 0.006$). The carcinoma samples of the eight UCC patients also exhibited highly aneuploid DNA distribution patterns. Löfberg et al. reported aneuploid biopsies in 25% of high-risk patients at least once during 10 years of observation (Lofberg et al. 1992). In other studies, aneuploidy has been repeatedly observed by flow-cytometry in non-dysplastic mucosa of high-risk patients (Rubin et al. 1992). The results of our study support the above-mentioned observations. Genomic instability, represented by DNA aneuploidy, could initiate the process of malignant transformation in colitis as an early event.

Little is known about laminin-5 γ2 chain immunoreactivity in precancerous lesions of malignant human tumors. Previous data from our group had shown various degrees of cytoplasmic laminin-5 γ2 chain immunoreactivity in 96% of primary colon carcinomas, whereas staining was absent in stromal cells and adjacent normal colonic mucosa. There was also an interrelationship between a strong staining pattern and a worse clinical outcome (Lenander et al. 2001). In the present study, seven of the eight UCC specimens had a moderate or strong γ2 chain expression, which was observed predominantly in malignant cells at the invasive front. An interesting finding in patient group A was the detection of immunoreactivity in biopsy specimens up to 13 years prior to the subsequent carcinoma (average 8 years). The overall expression of laminin-5 was significantly more frequent throughout the entire observation period in UC biopsies of patients with a subsequent UCC (20%) than in those without subsequent UCC (5%, $p = 0.002$). Laminin-5 γ2 chain positive cells were distributed over the whole colon and rectum and were not correlated with dysplasia ($r = 0.357$) or inflammatory activity ($r = 0.142$), but interestingly, related to aneuploidy: in the UCC group as many as 26 of 37 of the laminin-5 immunopositive biopsies were aneuploid. In the non-malignancy group, only 2 of 8 immunopositive biopsies showed aneuploidy and the rarely occurring laminin-5 positive cells were generally localized close to the basement membrane, mainly in flat, regenerative epithelium. Thus, the observation of laminin-5 immunoreactivity years prior to a UCC might not just represent the ability of cells to invade but also to wound healing. In fact, strong laminin-5 expression has also been observed in migrating keratinocytes in healing skin wounds (Larjava et al. 1993). The observed phenomenon could thus be affected by the underlying inflammatory disease (Haapasalmi et al. 1995; Thorup et al. 1998). Laminin-5 γ2 chain overexpression in repeated biopsies might be related to upregulated regenerative processes in ulcerative colitis. However, since normal regenerative processes and wound healing generally occur in diploid cell populations, the combined analysis of ploidy and laminin-5 γ2 chain expression may allow identification of premalignant populations with invasive capacity. The present data strongly indicate an increased risk of progression to invasive properties in genetically unstable cells.

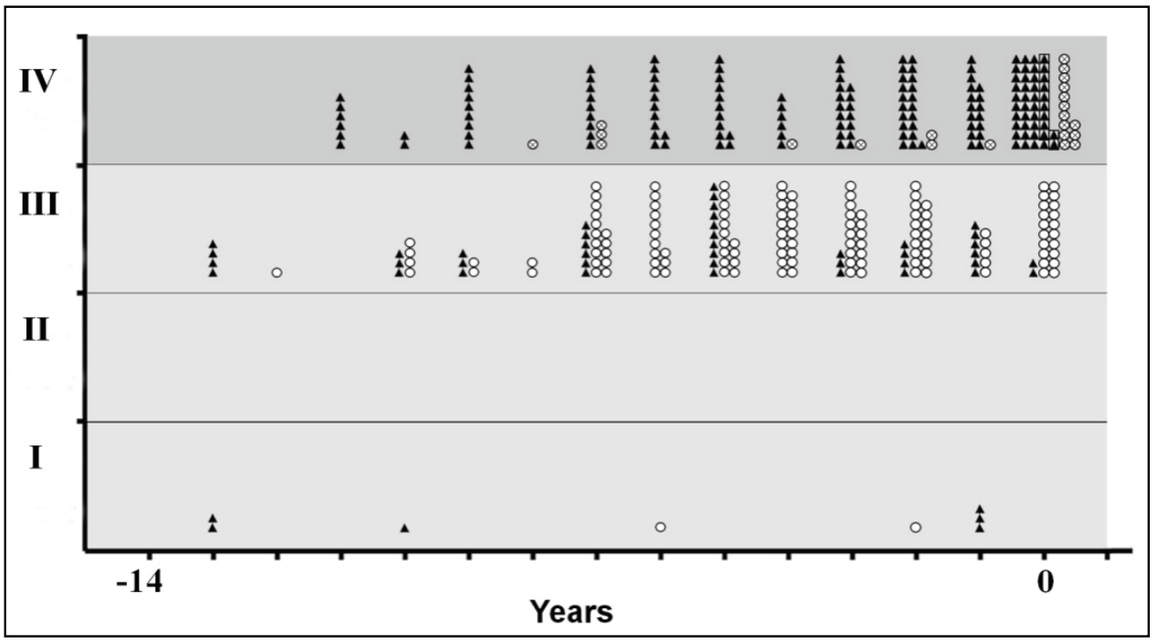

Fig. 4. DNA histogram types in biopsies of 8 patients with (▲) and 16 patients without UCC (O). Aneuploid biopsies defined six patients without UCC to be at increased UCC risk (⊗). The carcinoma specimens are framed ([▲]). The endpoint of the study is represented by year 0 on the x-achsis. Modified from (Habermann et al. 2001)

However, aneuploidy may be reversible over time once cells are not longer exposed to the inducing agent or carcinogen (Auer et al. 1982; Ono et al. 1984). 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 (Wang et al. 2002). In the present study, six out of eight UCC specimens exhibited an increased cyclin A expression pattern. In addition, cyclin A expression was found in 98% of all biopsies, with a higher number of immunopositive cells in biopsies of the UCC group ($p = 0.014$), as well as being mainly observed in aneuploid populations: in the UCC group as many as 12 of 13 biopsies with increased cyclin A staining were aneuploid, whereas in the non-malignancy group only one of four biopsies with similar staining intensity showed aneuploidy. Thus in the UCC group, increased cyclin A expression was significantly correlated to aneuploidy ($r = 0.791$). However, there was no significant correlation with inflammatory activity ($r = 0.178$), grade of dysplasia ($r = 0.485$) or laminin-5 immunopositivity ($r = 0.140$). Since cyclin A expression indicates whether a cell is committed to pass through the cell cycle and divide, i.e. participate in clonal expansion, the fraction of cyclin A positive cells may be used to estimate the risk of aneuploid populations to progress to malignancy. At the study's conclusion and establishment of a risk profile, based on the three parameters discussed above, it was discovered that six out of the 16 patients in group B could be identified as high-risk patients. Of these six patients, one patient developed an invasive carcinoma after the endpoint of the study. By fitting a logistic regression model, DNA-cytometry, laminin-5 positivity and increased cyclin A expression were confirmed as significant predictors for malignant transformation.

4. Conclusions

Genomic aneuploidy occurs early and is commonly found in precancerous biopsies of ulcerative colitis patients who subsequently develop an ulcerative colitis-associated colorectal carcinoma (UCC). The assessment of DNA ploidy could therefore become a basic element in future surveillance programs in ulcerative colitis. The complementary detection of laminin-5 positivity and increased cyclin A expression in aneuploid lesions - indicating invasive potential and 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 are similar to that seen in sporadic colorectal carcinomas. However, the frequency of aneuploidy in UCC is significantly higher than in the sporadic counterpart and is an indpendent poor prognosis factor for the affected patients.

5. Future perspectives

The analysis of DNA ploidy measurements in premalignant ulcerative colitis lesions could profoundly improve individual risk assessment for imminent colorectal cancer development. However, large multicentric prospective studies are warranted to corroborate the value for improved screening and prognostication in ulcerative colitis by means of ploidy assessment. For this purpose we have initiated the *North German Tumor Biobank of Colorectal Cancer* (Acronym: ColoNet; www.northgermantumorbankcrc.de) that currently comprises the catchment areas of the universities and clinics of Hamburg, Lübeck, Rostock, Greifswald, Bad Oldesloe, Berlin-Buch and associated private practices in northern Germany. Within this network, we will investigate the potential benefit of ploidy measures for individual risk and prognosis assessment in ulcerative colitis and associated colorectal carcinomas.

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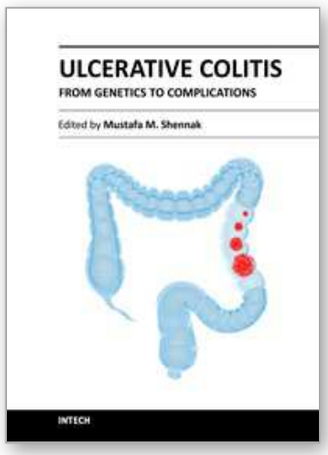

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Ulcerative Colitis (UC) is a rapidly evolving medical field, and will continue to be very exiting in the next few decades. Although the underlying cause of this disease is still unknown, results in research dealing with various issues related to this disease are published every day. Chapters included in this book review the most recent literature on related advancements in regard to this chronic disease, which is controllable but not curable. Aspects like epidemiology, pathophysiology, genetics, incriminated etiologies, clinical aspects, complications, and disease management, including advancements in the diagnostic and therapeutic options, were documented by well known clinicians, researchers, and world wide authorities in their fields. This book on UC will be a valuable addition to each doctor's library interested in this subject, or for physicians dealing with patients suffering from this disease. Authors have also included figures and diagrams to depict their point, and to easily reach the minds of the readers in the simplest way.

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