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COLORECTAL CARCINOMA

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Colorectal carcinomas (CRC) belong to the most frequently observed solid tumours known in man. Specifically, in Germany 35/100,000 individuals contract the disease every year. In Croatia, 14% of all cancers are colorectal carcinomas making it the third leading cancer entity. Across genders, CRC can be considered the most common cancer in western industrialized societies, while it is rare in developing countries. For example, in the USA the incidence is approximately 36/100,000, while in Senegal it is only 1.9/100,000 inhabitants. During the last years, morbidity has particularly increased in males. Also, there appears to be a shift from older to younger ages with respect to onset in the affected individuals.

Since the clinical symptoms are very unspecific or may even be absent for a long time, approximately 50% of the patients that finally come to see their doctor with clinical signs, already bear a CRC of the Dukes C stage with metastasis into regional lymph nodes. Like in the other epithelial malignant tumours, the prognosis is dismal, once metastatic spread has occurred. Despite developments in chemotherapy, the overall survival has not changed over the last 25 years. Specifically, the worldwide sales for chemotherapeutic agents have increased from 5.93 Billion US$ since 1996 to approximately 16 Billion US$ in 2004, while the prognosis of the major cancers (lung, prostate, breast, colorectal) has not improved since 1978 (acc. to the news magazine DER SPIEGEL, Nr. 41/4.10.04, Oct. 4th 2004, pg 160 ff).

These figures emphasize two things:
1) curing CRC is not possible up to now and does not seem to be so in the foreseeable future, and
2) mechanisms of tumour development have to be detected in order to develop preventive strategies in preneoplastic stages rather than therapeutic approaches of full-fledged CRC.

11.1 Molecular mechanisms of CRC development

Analysis of the genetic defects found in the rare hereditary cancer forms (FAP and HNPCC) has taught us many important aspects about cancer development in the common sporadic forms i.e. the CRCs with no apparent Mendelian inheritance. In general, two major forms of CRC have been recognized using this approach of genetic analysis. The most common form is the polyposis CRC characterized by a defect in the pathway of the adenomatous polyposis coli (APC) gene, a gene involved in the wnt signaling pathway. APC has first been identified as the molecular defect underlying the rare familial adenomatous polyposis coli (FAP syndrome, less than 1% of the cases). Mutations in the APC gene are observed in approximately 60-70% of all polyposis CRC. In addition, up to 15% of the polyposis CRC carry dominant mutations in the gene CTNNB1 coding for ß-catenin, a protein that is regulated by APC and is involved in signal transductions within the wnt signaling pathway. Finally, it appears that the remaining percent of cases may be caused through epigenetic silencing by methylation of the APC promotor resulting in a loss of APC function.

Altogether, this would suggest that the molecular defects have been entirely identified for the polyposis CRC. The second most common form of CRC (13-15% of the cases) results from defects in the human genes MSH2 and MLH1 coding for molecules of the DNA mismatch repair complex. These defects lead to microsatellite instability and have been first identified in the rare hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) that accounts for 2-4% of all CRC cases. Taken together, there is a strong background of specific genetic aberrations in CRC indicating that cancer as a "disease of genes". In 1990, Bert Vogelstein and colleagues proposed their model of a multistep carcinogenesis using CRC as a model. The Vogelstein hypothesis states that cancers develop in a tissue by an accumulation of genetic defects. To start the process of malignant (i.e. neoplastic transformation) a defect in a so-called gatekeeper gene (like APC) is instrumental. In addition, defects have to occur in a certain order in order to be permissive. For example, while mutations in the p53 or the k-ras genes play a role are important in the progression of neoplastic tumours to cancers, they do not play a role in the early stages during neoplastic transformation.

In contrast, APC defects are very common and remarkably constant throughout all stages of colorectal neoplasia, and functional studies as well as experimental animal models implicate APC as the molecular gatekeeper, whose inactivation initiates – either by genetic or epigenetic defects - neoplastic growth and chromosomal instability (CIN) that eventually leads to polyposis CRC.

However, there are a number of questions that need to be resolved in the current multistep carcinogenesis. Specifically, it is known that a bi-allelic loss of APC function is necessary for colorectal tumour development, since dominant-negative effects of mutant APC have not been convincingly shown. These independent de-novo APC

defects on both alleles have to occur before the crypt cells differentiate and lose their ability to replicate. Specifically, there are several million colonic crypts per colon, each containing only a limited number of stem cells in the bottom compartment. Thus, the required two independent gene mutations/chromosomal alterations have to occur within 3-4 days (i.e. 5-6 cell divisions) to eliminate the APC gatekeeper function.

FAP patients having inherited one defective allele of APC develop up to few thousand neoplastic tumours suggesting that the spontaneous mutation rate of APC alleles is low. For normal individuals carrying two intact alleles the probability to contract these two independent mutational events therefore may be considered extremely low. To account for the frequency of colorectal cancers one would expect the first APC mutation to reside within the replicating cell compartment of the colon crypt, thus marking this crypt. However, recent data clearly demonstrates that APC mutations are absent in the zone of replicating cells. Also, only the upper half of the crypt shows increased and neoplastic proliferative activity. It may be concluded that APC defects mark the step of neoplastic transformation, but are not the initiating event of colorectal tumourigenesis. This would suggest the existence of an important earlier step in tumour development.

11.2 Role of APC in early tumourigenesis

While all evidence points to the fact that defects in the APC tumour-suppressor pathway are sufficient to cause the neoplastic transformation and initiate colorectal carcinogenesis, it is unclear if molecular defects, that are important for tumour development, exist prior to the loss of function of the APC pathway.

Hyperplastic polyps and hyperplastic aberrant crypt foci (ACF), microscopic lesions encompassing only a few colonic crypts are found very frequently in the colon. In contrast to neoplasia, hyperplasia represents a tumour entity characterized by a lack of apoptosis rather than an increased proliferation. The numbers of hyperplastic lesions do not correlate with the number of clinically observed neoplastic tumours, thus supporting the classical concept that they are harmless. Also, defects in the APC pathway are virtually absent from all hyperplastic tumour forms. However, recent evidence has suggested that hyperplastic ACF already represent a continuum of lesions with different dignity. Specifically, hyperplastic ACF and polyps have been shown to harbor focal losses of the DNA mismatch repair complex and chromosomal aberrations or show mutations of CTNNB1 and a concomitant nuclear accumulation of ß-catenin. Importantly, direct histological evidence for an early cancerous lesion developing within a hyperplastic polyp has been recently demonstrated. Finally, animal models investigating the natural history of hyperplastic ACF in the rat colon have demonstrated that, although the vast majority of hyperplastic ACF will regress, the relative risk to develop a dysplastic tumour from a hyperplastic lesion was enhanced 17-fold compared with the normal epithelium. These data support the concept that hyperplastic tumours may, in principle qualify as precursors for neoplasia. However, up to now no marker had been identified showing a direct mechanistic or pathobiochemical link between hyperplasia and neoplasia in the colorectal multistep tumourigenesis.

11.3 Consistent early molecular defects in colorectal tumours

Recent evidence has shown the effects of methylation of the APC pathway in in-vitro cell culture systems. Specifically, the epigenetic silencing of the WNT-inhibitory SFRP genes can lead to ß-catenin accumulation similarly to the APC gene defect itself. It has been speculated that epigenetic defects will sensitize the affected cells for the downstream "real" genetic defects of APC or CTNNB1. It is far too early for a final assessment of the importance of epigenetic changes for colon tumour development. Possibly, they are important to promote the very early events prior to definitive genetic changes. In any case, it appears that the puristic genetic models are more suited to explain cancer progression than tumour initiation.
We have in the past shown for the first time that downregulation or loss of expression of the human Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM 1, formerly designated BGP or CD66a) occurs in approximately 85-90% percent of all CRC. Indeed, others have confirmed by serial analysis of gene expression in colorectal cancer cells that underexpression of CEACAM1 is among the most frequent events in CRC. Subsequently, we have shown that early neoplastic tumours also have lost expression in a near identical frequency. Most recently, we found that CEACAM1 expression is lost in the great majority of hyperplastic tumours i.e. both ACF and hyperplastic polyps. In contrast, APC defects were only observed in neoplastic but not in the hyperplastic tumours. In early adenomas with loss of CEACAM1 expression, APC mutations were detected in 70% of the cases. This represents the overall frequency of APC mutations reported for colorectal neoplastic tumours and demonstrates that loss of CEACAM1 is not an epiphenomenon of APC pathway defects, but occurs as an independent event with even higher frequency. This has prompted us to propose that loss of CEACAM1 may precede APC pathway defects during tumourigenesis. In the hyperplastic lesions we were able to confirm a loss of apoptosis in frequencies comparable to that of CEACAM1 loss. No increased proliferation was noted in the hyperplastic lesions as measured by Ki-67 score in contrast to the adenomas and carcinomas investigated. Moreover, we found a significant positive correlation between CEACAM1 expression and the rate of apoptosis in the hyperplastic tumours suggesting a functional link between CEACAM1 expression and apoptosis. As shown by Nittka et al., we were able to establish that CEACAM1 specifically mediates apoptosis in both CEACAM1-transfected reporter cells as well as CEACAM1-induced HT29 cells. This CEACAM1-mediated effect has to be appreciated considering the known relative apoptosis resistance of HT29 and fact that programmed cell death could not be provoked in HT29 cells expressing CEACAM1 at very low levels. At present, the mechanism by which CEACAM1 mediates this effect is not known. However, first evidence shows that the cytoplasmic domain serves as a caspase-3 substrate upon CEACAM1 signalling. Possibly, CEACAM1 expression serves to regulate tissue homeostasis in the colon mucosa. Very recently, we have also shown that interaction with the EGF receptor - expressed in all CRC - results in changes in proliferation. Together, these results help to explain the tumour suppressor functions that have been noted for CEACAM1 over the years.

11.4 The role of differentiation antigens in tumour development

How do we see the biological role of CEACAM1 in the colon? It is firmly established that the cell surface expression of CEACAM1 and other members of the CEACAM family commences in the lower half

of the colonic crypt and becomes more prominent in the glycocalix as these cells differentiate and migrate towards the gut lumen. Within the glycocalix coat the CEACAM molecules form a dense network via homo- and heterophilic adhesions, a process simulated by our in-vitro crosslinking models. We believe that the maturing and migrating crypt cells receive a pro-apoptotic signal through the increasing number of CEACAM1 molecules crosslinked by molecular adhesion between the CEACAMs on the cellular surface. It is noteworthy that CEACAM1 is the only member in this gene family equipped with a transmembrane and a cytoplasmic domain. Indeed, signal transduction, through CEACAM1, has been shown by us and by others. The failure to express CEACAM1 is therefore likely to reduce apoptosis and may thus directly contribute to hyperplastic tumour formation. At this point, the reasons for the frequent loss of CEACAM1 expression are unclear, but may in part be due to epigenetic phenomena.

How can the loss of CEACAM1 contribute to the current multistep model of colon carcinogenesis? We believe that the failure to express the pro-apoptotic CEACAM1 directly contributes to the generation of hyperplastic lesions. The hyperplasia is characterized by broadening of the proliferative zone of the colonic crypt. The morphology of the crypt architecture appears to be altered with the crypt luminae being exposed to the faecal contents within the colon. This may facilitate various genetic or epigenetic alterations to occur within the non-apoptotic cells of the hyperplastic lesion. Most of the subsequent genetic alterations will not be permissive for a sustained tumour growth or the initiation of neoplasia, but lead to spontaneous regression of the lesion. However, if cells within the hyperplastic lesion suffer a gatekeeper defect, they would take the "Vogelstein exit" to neoplastic tumour growth. It is possible in our opinion that such a hypothetical link between hyperplasia and neoplasia may have been overlooked, since the gatekeeper defects in hyperplasias occur with a very low frequency.

On the other hand, the proposition that hyperplastic lesions may represent the initial, although very inefficient precursors of neoplastic tumour development also allows for a molecular explanation of the high frequency of APC defects in neoplastic tumours.

The broadened proliferation zones and decreased apoptosis in the CEACAM1-negative hyperplastic lesions are consistent with these data and the view that hyperplasia represents a predisposing event preceding APC defects and neoplasia in these crypts. Support for this model comes from a very recent study by Michor et al (31). Using a mathematical model for the cellular dynamics in the colon crypt and the colon cancer initiation these authors conclude that chromosomal changes are very likely to precede APC mutations in colon carcinogenesis.
Taken together, our data allow an extension to the Vogelstein paradigm, thus adding a pathobiochemical/pathophysiological model for the events occurring in the earliest tumour lesions that have not yet contracted gatekeeper defects. Indeed, changes like the one observed for CEACAM1 may be instrumental for subsequent genetic defects. In this regard, the current paradigm of genetic cause of carcinogenesis may fulfill the criteria of a progression model rather than an initiation model. In this case, preventive measures to reduce hyperplasia and thus downstream mutational events may prove to be an attractive alternative pre-emptive measure compared to non-effective chemotherapeutic strategies of progressed malignant colorectal tumours.

Further studies are now needed to unravel the gene regulatory pathways governing CEACAM1 expression to detect the cause for the failure of crypt cells in starting the expression program and the molecular mechanisms by which CEACAM1-expressing cells are susceptible to apoptotic stimuli.

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