CHAPTER 7

THE ADENOMA HUNT IN COLORECTAL CANCER SCREENING: DEFINING THE TARGET

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Abstract

Colorectal adenomas are precursor lesions of colorectal cancer. Different biological and metabolic processes contribute to adenomagenesis. Subsequent progression to carcinoma occurs in only about 5% of the cases. Detection and removal of all adenomas would reduce CRC incidence and mortality, but at the cost of major over-treatment. Classical morphological characteristics fail to accurately discriminate between adenomas that will become malignant and those that will not. Understanding the biology of cancer development will help to better characterize adenomas at high risk of progression, and subsequently establish triage tests that allow to safely reserve colonoscopy only for individuals at high probability of having truly high-risk colorectal adenomas. Screening tests based on genomic changes that affect relevant biological and metabolic processes hold most promise in this respect.
Introduction

With over 600,000 deaths annually worldwide, colorectal cancer (CRC) is a major healthcare problem (www.who.int). In the industrialized world it is the second cause of cancer death [1]. Major efforts in drug development, radiation therapy and surgical innovations have reduced local recurrence rates and prolong survival in patients with metastatic disease, but have limited effect on overall mortality. Therefore, detection and removal of colorectal cancer at an early stage, or even a precursor stage, ie adenoma, is commonly recognized as the most realistic approach to reduce CRC mortality rates.

The optimal target lesion to screen for is still uncertain. While US guidelines recommend colonoscopy screening with an emphasis on preventing CRC by removing preferably all adenomas, European guidelines are based on more cost-effective approaches for reducing mortality from CRC on a populationwide scale, using eg fecal immuno-tests (FIT) [2,3]. These different approaches not only reflect different attitudes towards public health, but also emphasize an underlying lack of understanding of the mechanisms that drive progression of adenomas towards cancer.

Colorectal cancer arises in epithelial cells lining the interior of the large intestine, and is caused by changes in gene function - usually acquired during life rather than being inherited - that disrupt the normal functions of these cells. During the process of neoplasia, these cells gradually acquire cancer cell characteristics, such as the potential for unregulated growth, invasion and destruction of surrounding tissues, and metastasis. In the course of this process there is a protracted phase during which the cells exhibit autonomous growth but are, as yet, incapable of invasive growth and metastasis, the adenoma phase.

Autopsy studies have shown that adenomas of the colon are quite common, occurring in about 30% of individuals over 60 years of age [4]. It is estimated that only 5% of all adenomas actually progress to malignancy [5] and that this process generally takes many years. Theoretically, the removal of this 5% of adenomas would be sufficient to prevent colorectal cancer, but unfortunately it is hard to identify this subgroup. In order to avoid over-treatment, it would be preferable to reserve colonoscopy only for those individuals with a high chance of harbouring high-risk lesions. This would require a screening test that is sensitive and specific enough for colonoscopy to be safely reserved for test positives only, and be applied as an intervention rather than a diagnostic procedure. The key question is how to translate the large body of knowledge on colorectal neoplasia into sensible screening strategies with a balanced trade-off between reducing risk of dying from CRC and risk of over-treatment.

Colorectal cancer development: adenoma-carcinoma sequence

The phenotypic concept of the colorectal adenoma-carcinoma sequence was first described by Morson et al in 1975 [6] and was provided with a molecular basis by the work of Vogelstein et al [7]. Since then this model has developed into the textbook paradigm of multi-step carcinogenesis. The phenotypic and molecular annotation of the adenoma-carcinoma sequence in humans, however, is almost exclusively based on extrapolating cross-sectional observations, because hardly any longitudinal data about the natural course of individual lesions exist. This is because once detected, adenomas are completely removed. Moreover, it is important to realize that this gap in the formal evidence
available on this process will not change because the optimal study for this purpose, ie leaving adenomas in situ and sampling them regularly for phenotypic and molecular features until they become malignant, is unethical. As a consequence, only indirect data exist on the average and range of the duration of the adenoma-carcinoma sequence, and on the risk of progression of different adenoma phenotypes (e.g., size, histological type, polypoid, flat or serrated appearance). Nevertheless, the literature is full of firm statements that the adenoma-carcinoma sequence takes 10-20 years and that the risk of progression is determined by increasing size, villous histology and grade of dysplasia [6]. However, small adenomas can also give rise to cancers and strong indications for rapid progression have been documented in population studies [8,9]. In fact, for most of the >1,000,000 new colorectal cancers that annually occur worldwide, it is simply not known whether these cancers have progressed from small or large adenomas, tubular or villous adenomas, nor from flat or polypoid adenomas. Also, the adenoma-carcinoma sequence model has started to live a life of its own, and the perception that many basic researchers and clinicians have of the model has overtaken real-life observations.

One of the main points often overlooked is that the majority of adenomas ‘never’ progress to cancer, ie certainly not within time-frames covered by current screening and surveillance programmes, and that this step is associated with particular biological alterations that provide the malignant phenotype of these tumours. This Perspective contribution will emphasize the biological characteristics that contribute to adenomagenesis and subsequent progression to carcinoma, because understanding the biological characteristics associated with these steps may serve to optimize screening strategies for CRC.

**Biology of colorectal cancer development**

Cancer development from normal tissue requires a complete reprogramming of multiple critical biological processes, such that tightly regulated normal epithelial cells change into cancer cells. Cell growth becomes uncontrolled and limitless, apoptosis is avoided, angiogenesis is acquired, adjacent tissue is invaded and metastases develop [10]. Meanwhile, tumour cells circumvent the activation of immune responses. These biological processes are paralleled by cellular metabolic adaptations, providing proliferating cells with the energy and macromolecules needed for replication of all cellular content [11].

**Mechanisms underlying genetic reprogramming**

Functional changes are caused by multiple genetic (e.g., DNA mutations and copy number aberrations), epigenetic (e.g., DNA methylation and chromatin modifications) and post-transcriptional (e.g., regulation of mRNA stability by miRNAs) alterations [12]. Genetic alterations are facilitated by genomic instability, either by failing DNA mismatch repair leading to microsatellite instability (MSI) or by chromosomal instability (CIN), the cause of which remains to be resolved. CIN is the main type of genomic instability in CRC, occurring in about 85% of colorectal tumours. CIN is characterized by chromosomal gains and losses that lead to gene dosage effects on tumour suppressor genes, oncogenes and miRNAs [13-15].
Defining the target for CRC screening

**Processes reprogrammed during adenoma development and genomic changes involved**

Activation of Wnt disturbs the delicate balance between proliferation and differentiation and is the key step in colorectal adenomagenesis [16]. This is well illustrated by the familial adenomatous polyposis (FAP) syndrome, which is caused by germline mutations of the major Wnt signalling pathway component APC. FAP patients develop hundreds to thousands of adenomas, of which in the natural course of the disease indeed only very few progress to cancer [6].

One way of pinpointing the biological processes involved in adenomagenesis and carcinomagenesis is pathway analysis, based on gene expression microarray data. Such data exist comparing normal colon mucosa to adenocarcinomas and adenomas to carcinomas. Subtracting the latter from the former provides some insights in adenomagenesis pathways. Of the cancer-associated pathways, those involved in regulating self-renewal of the epithelium are disrupted in adenomagenesis, eg proliferation, differentiation and apoptosis. Next to alterations in carcinogenic pathways, substantial metabolic changes occur in adenomas. For instance, glycolysis and associated pathways (oxidative phosphorylation and pyruvate metabolism) appear to be particularly important during adenoma development (Table 1). High levels of glycolysis help proliferating adenoma cells to fulfill their need for energy and macromolecules [11]. LDHA expression is representative for increased pyruvate metabolism, which is associated with enhanced glycolysis. In line with the observed increase in expression of glycolysis and associated pathways is an apparent increase in \( LDHA \) expression in adenomas, while no change is observed during malignant transformation [17,18]. Hypoxia is one of the mechanisms that regulate transcriptional changes to support these metabolic alterations [19]. Altered expression of hypoxia regulated genes is already found in adenomas (Table 1). Development and progression of adenoma lesions is arrested by cellular senescence. Continuously proliferating cells provoke senescence, either through oncogenic stimuli that cause a proliferation block or by telomere shortening [20]. Cellular senescence has been reported to be induced in premalignant tumours (eg adenomas) [21].

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Processes reprogrammed during adenoma-to-carcinoma progression and genomic changes involved

Senescence is an important barrier that halts proliferation and consequently the acquisition of new genetic alterations necessary to gain malignant features. Overcoming senescence is therefore a crucial progression step in malignant transformation, during which stabilization of telomeres (mainly by activation of hTERT), a defective DNA damage response (caused by mutations in genes such as ATM, and due to activity loss of the Rb and p53 pathway effectors) and interference with several signal transduction pathways (eg IL-6 signalling) play a role [20,22]. In addition, progression is marked by a substantial increase in the level of genomic instability, either as MSI or, more frequently, as CIN [23,24]. CIN leads to altered expression of relevant cancer-related genes, such as AURKA and TPX2, located on chromosome 20q. DNA copy number gain of 20q is almost completely absent in adenomas, while present in more than 60% of CRCs [18].

Invasion - tumour cells crossing the basement membrane and entering the underlying stroma - is one of the principle features of malignancy. It is accompanied by major changes in the tumour microenvironment that resemble wound healing, reflected by activation of tumour-specific stroma and angiogenesis [23,25]. Genomic alterations representative for expression changes in these processes are listed in Table 1. Important alterations involve PLK1 and CCNF, which influence proliferation, while SPARC and PDGFRB affect the invasion process and AURKA and TPX2 contribute to chromosomal instability. At the metabolic level, expression of the fatty acid metabolism pathway is altered in adenomas when compared with CRCs. In particular, genes involved in β-oxidation of fatty acids - contributing to energy production - are lower expressed in CRCs relative to adenomas. Proliferating cells require fatty acids for membrane synthesis and modification of membrane-targeted proteins. Growth factor signalling pathways, such as the phosphatidylinositol 3-kinase (PI3K) pathway, therefore suppress fatty acid β-oxidation to maximize lipid synthesis [11]. Alterations in this pathway, such as PIK3CA mutations, also contribute to adenoma-to-carcinoma progression [26].

CRC development and progression model

A model of CRC development and progression, constructed based on the described alterations occurring in carcinogenic and metabolic pathways, is presented in Figure 1. From the above it is evident that adenoma-to-carcinoma progression is not simply an intensification of the processes involved in adenoma development. In addition to, for example, overcoming senescence and altered fatty acid metabolism, chromosomal instability, including amplification of 20q, is a major driver of colorectal adenoma-to-carcinoma progression [18]. This implies that different subgroups of adenomas exist of which some (eg adenomas with 20q gain) have already gained part of the features needed for malignant transformation. These subgroups of adenomas have an increased risk of malignant transformation and can thus be considered high-risk adenomas. The combination of alterations that need to be acquired to gain a transformed tumour phenotype forms a barrier between adenomas and CRCs that is not easily broken. Colorectal adenomas are therefore not simply half-way to becoming cancer, but distinct lesions from CRC which lack important characteristics necessary for malignancy.
Defining the target for CRC screening

Figure 1 CRC development from normal colon epithelium starts with constitutive activation of the Wnt signalling pathway, resulting in adenoma formation. During this adenoma formation, self-renewal of the epithelium is thrown out of balance by changes in proliferation, differentiation and apoptosis. To fulfil their need for energy and macromolecules, proliferating adenoma cells demonstrate high levels of glycolysis, which is associated with alterations in glycolysis-associated metabolic processes, such as oxidative phosphorylation and pyruvate metabolism. Hypoxia, occurring in growing neoplasia, is one of the mechanisms that regulate transcriptional changes to support these metabolic alterations. Senescence limits continuously proliferating adenoma cells by inducing a state of irreversible growth arrest. The majority of adenomas appear unable to overcome senescence, which is mandatory for the accumulation of genetic alterations that drive carcinogenesis. Carcinomas have succeeded in crossing the senescence barrier by disrupting this process, allowing even higher rates of proliferation than initially observed in adenomas and further deregulation of differentiation. The processes of invasion, angiogenesis and stroma activation are triggered in carcinomas and the rate of chromosomal instability considerably increases. At the metabolic level, CRCs differ from adenomas in their activity of fatty acid metabolism; especially, genes contributing to the energy production from fatty acids are lower expressed in CRCs. This change allows proliferating cells to use fatty acids for membrane synthesis and modification of membrane-targeted proteins.
Clinical implications

The crucial issue is what lesions should be screened for in secondary prevention of CRC. One strategy, basically requiring colonoscopy to be used as a screening test, aims to remove all adenomas which obviously would reduce CRC incidence and consequently mortality [27,28]. However, since only 5% of colorectal adenomas progress to cancer, removal of all adenomas would at the same time mean a major over-treatment of patients who would not develop CRC. Unfortunately, colonoscopy is a costly and invasive technique which requires bowel preparation and expertise. Although the complication risk of colonoscopy is quite low, serious adverse events have been reported [29]. The high number of colonoscopies needed for screening could place a burden on expertise and healthcare resources [30]. The impact on healthcare resources is even greater because adenoma-positive patients are enrolled in colonoscopy-based post-polypectomy surveillance programmes. Moreover, recent studies have indicated that colonoscopy may perform less well than anticipated because it would reduce death from left-sided CRC but not from right-sided CRC [31,32]. Over-treatment, patient burden, demand on healthcare resources and the small but existing complication risk provide ample rationale for an alternative approach where only high-risk adenomas and early stage curable cancers are the screening target. This raises the question of what the most suitable test would be for achieving this goal. From a public health perspective, it also matters whether a test will be widely accepted by the target population, ie programme compliance [30,33]. Balanced evaluations of these arguments have led many European countries to start with fecal immuno-test (FIT)-based population screening [34].

While FIT performs quite well in detecting stage I and II cancers (which are in principle curable), performance for high-risk adenomas leaves more room for improvement [35]. One problem here is the definition of high-risk adenomas. As outlined above, natural history data are scarce, but could be substituted by knowledge from tumour biology. Yet, in current practice, a phenotypic classification is used to try to identify high-risk adenomas, which are named ‘advanced adenomas’. This classification, which is defined by morphological characteristics that date back to the seminal paper of Muto et al [6], strongly over-estimates progression risk. A full discussion of this matter is beyond the scope of the present paper; however, this overestimation is illustrated by the statement that size is the major determinant for advanced adenoma. Since no informative formal longitudinal data on progression risk of individual adenomas are present, only extrapolations from cross-sectional studies can be made. In the Muto study, risk of presence of a focus of cancer in adenomas between 1 and 2 cm (most advanced adenomas are between 1 and 2 cm in size) was only 10%, indicating that the vast majority of advanced adenomas will not progress to cancer either. Therefore, a classification system that measures the driving force, ie the biological mechanisms, behind adenoma-to-carcinoma progression may be more appropriate for identifying high-risk lesions, and diagnostic tests that are based on tumour biology therefore hold most promise for an ideal next generation screening test.

A wide variety of molecular screening tests are currently under evaluation, and here also methodological hurdles exist. Yet recent advances with DNA methylation and proteomics markers are highly promising [36-40]. While initially stool DNA tests focused on mutations, most new markers concern DNA promoter methylation, and a number of candidates are currently being evaluated in large-scale validation studies and clinical
Defining the target for CRC screening trials. In this context, population-based screening programmes that are ongoing in several European countries can provide an excellent environment for efficient evaluation of the potential of these markers. Likewise, modern mass spectrometry-based proteomics analyses are starting to produce a large number of candidate biomarkers. Here also the challenge is in the validation, and new technical developments, such as multiple reaction monitoring mass spectrometry, may provide a technical solution for this. The Holy Grail, of course, is to have cancer-specific markers, such as proteins arising from genomic rearrangements, as recently have also been described in colorectal cancer [40]. Given the biological heterogeneity of colorectal cancer, the ultimate test will most likely will measure multiple parameters. All in all, these developments provide a rationale for a screening test with excellent positive and negative predictive value, so that theoretically, after a positive test result, colonoscopy could be reserved as a second-tier diagnostic test along with the appropriate intervention for high-risk adenomas, when present.

Conclusion

Colorectal adenomas are precursor lesions of CRC, but only a minority actually progress. Screening tests aiming to detect and remove all adenomas will reduce CRC incidence and mortality, but also cause major over-treatment. Understanding the biology of CRC development will help to better identify adenomas at high risk of progression. Screening tests that read out the actual driving force behind this progression, ie genomic changes, possibly in combination with FIT, hold most promise for identifying patients bearing high-risk adenomas and CRCs.

Acknowledgements

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References

Chapter 7

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