Meeting report from the joint IARC–NCI international cancer seminar series: a focus on colorectal cancer


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Introduction

Colorectal cancer is the third most commonly diagnosed malignancy and the second leading cause of cancer death in the world, accounting for around 1.8 million new cases and 860 000 deaths in 2018 [1]. Given current demographic projections, the global burden of colorectal cancer is anticipated to increase by 72% to over 3 million new cases in 2040 with substantial increases anticipated in low- and middle-income countries. In this meeting report, we summarize the content of a joint workshop led by the National Cancer Institute and the International Agency for Research on Cancer, which was held to summarize the important achievements that have been made in our understanding of colorectal cancer etiology, genetics, early detection and treatment and to identify key research questions that remain to be addressed.

Key words: colorectal cancer, prevention, genetics, etiology, screening, therapy
of colorectal cancer in many very high-HDI countries has decreased among those older than 50 years, rates in younger individuals appear to be rising, for reasons that are not clear [5, 6]. In addition, age-specific incidence of colorectal cancer is higher in men than in women, and men have poorer survival rates than women [7]. Although the reasons for this disparity by sex are not clear, it may reflect differences in exposure to colorectal cancer risk factors, as well as underlying biology.

Survival following colorectal cancer varies considerably between countries. While 5-year relative survival is now ~65% in high-income countries settings, it is <50% in low-income countries [8, 9]. Stage at diagnosis remains the most important prognostic factor for colorectal cancer. In the United States, 5-year relative survival is currently 89.8% for patients with localized stage, 71.1% for patients with regional spread, and 13.8% for patients with metastatic disease at diagnosis [5]. However, differences can be observed in stage-specific survival even across high-income countries [10]. Ancestry may also represent an important factor for both disease incidence and survival—in the United States, for example, the burden of colorectal cancer appears to be greatest among African-Americans and is lowest in Hispanics [7].
Etiologic risk factors

Colorectal cancer is a complex disease with a significant number of recognized risk factors. Advancing age, male sex, family history of colorectal cancer, inflammatory bowel disease, smoking, excessive alcohol drinking, overweight and obesity, low levels of physical activity and sedentary lifestyle, diabetes and high consumption of red and processed meat are established risk factors (Table 1) [11–14]. Individuals with inflammatory bowel disease or those with first-degree relatives with colorectal cancer have an approximately twofold higher risk of developing the disease [15, 16]. Use of menopausal hormone therapy has been associated with lower risk of colorectal cancer in observational studies but findings from subsequent clinical trials have not shown consistent results [17–19]. Conversely, both observational studies and trials have demonstrated that nonsteroidal anti-inflammatory drugs (NSAID) and aspirin specifically can reduce the risk of colorectal cancer and pre-malignant colorectal adenomas [20–23]. Given the relative safety and high prevalence of NSAID/aspirin use worldwide, there has been anticipation that the use of these drugs could offer an effective preventive strategy for colorectal cancer. In particular, such pharmacologic interventions could be directed toward those at higher risk for this disease. Currently, trials are underway to examine the effect of aspirin administration on colorectal cancer development in both average-risk populations and among individuals with hereditary colorectal cancer (e.g. Lynch syndrome) and such a strategy could be extended to individuals at higher risk because of other risk factors [24].

Decades of research have focused on dietary factors: some studies have suggested a protective effect of diets rich in fruit, vegetables, fish, fiber and whole grains, calcium and dairy products against colorectal cancer [11] (Table 1). Epidemiological studies have also consistently shown an inverse association between circulating vitamin D concentrations and risk of this malignancy [25], although findings from recent genetic analyses do not support a causal relationship [26].

Overweight, obesity and type 2 diabetes (T2D) are established risk factors for colorectal cancer and it has been estimated that they may account for more than 10% of colorectal cancer cases worldwide [27–29]. Given the global rising prevalence of obesity and T2D, these conditions are likely to impact significantly on colorectal cancer incidence in the coming decades [30, 31]. Important questions remain regarding the underlying mechanisms linking colorectal cancer risk with many of its risk factors. The application of novel technologies such as metabolomics and proteomics that can simultaneously identify thousands of biological features in a single bio-sample hold promise and some novel insights have been provided by recent analyses [32, 33].

There is a growing body of experimental and observational evidence implicating the gut microbiome in colorectal cancer development and progression [34–36]. However, studies linking variation in the gut microbiome with colorectal cancer in human studies are still in their infancy. A small case–control study with available fecal samples demonstrated differences in the relative distribution of bacterial taxa between colorectal cancer cases and controls with enrichment of Bacteroidetes and depletion of Firmicutes [34]. In addition, increased carriage of genera Fusobacterium, Atopobium and Porphyromonas has also been associated with colorectal cancer [34, 37]. Fusobacterium are prevalent in colon tissue and can be observed in distal metastases, suggesting a possible role in the latter [38]. Atopobium, a Gram-positive anaerobic bacterium, has been associated with Crohn’s disease and has been reported to inhibit colonocyte apoptosis.
The studies described are consistent with the concept of ‘dysbiosis’, or microbial imbalance, leading to a pro-inflammatory microenvironment that is conducive to colorectal tumorigenesis. However, caution is required in the interpretation of case-control and cross-sectional studies due to the potential of reverse causality. The prospective, systematic, and standardized collection of fecal samples as well as colorectal tissue specimens within the framework of well-characterized, population-based cohort studies is required to advance our knowledge on the etiologic role of the microbiota and possible effects through immune responses to colorectal tumorigenesis.

**Molecular pathology**

Approximately 95% of presumed colorectal cancers are adenocarcinomas, invariable developing over more than 10 years, with dysplastic adenomas the most common form of premalignant precursor lesions. A number of different genomic alterations have been shown to be important for the development of colorectal cancer. Mutations in APC are an early event in the development of this cancer, followed by activating mutations of the KRAS oncogene and inactivating mutations of the TP53 tumor suppressor gene. Molecular characterization of tumors, including somatic mutations in BRAF and KRAS, microsatellite instability (MSI), and CpG island methylator phenotype (CIMP), has provided evidence of multiple tumor subtypes that develop through activation of diverse neoplastic pathways.

More recently, new landscape-style technologies such as extensive next-generation sequencing as applied in The Cancer Genome Atlas (TCGA) Project have enabled further characterization of the mutational repertoire of colorectal tumors, highlighting mutations in genes such as APC, TP53, SMAD4, PTEN, RNF43, FBXW7, and PIK3CA as well as in genes that are less well known, e.g. SOX9, R2M, or ACVR1B. Such results indicate the importance of a complex number of key pathways, including those of MAPK, Wnt, or TGFβ signaling and more recently immune-modulating pathways. In transcriptional analyses, multiple molecular subtypes have been identified which account for around 14% of colorectal tumors, exhibit hypermutated status, MSI and strong immune activation; CMS2 (37%), or the canonical epithelial subtype, shows marked Wnt and MYC signaling; CMS3 (13%) is characterized by substantial metabolic dysregulation; and CMS4 (23%) is a mesenchymal subtype that exhibits prominent transforming growth factor-β activation, stromal invasion, and angiogenesis. The integration of data on tumor molecular features such as genomic alterations and gene expression into epidemiological studies—a rapidly evolving field known as molecular pathological epidemiology—offers an opportunity to better map the importance of risk factors to specific subtypes of colorectal cancer. Already some important observations have been made—e.g. showing that smoking appears to be a specific risk factor for MSI-high, CIMP-positive and BRAF mutation positive tumors, while aspirin use is associated with lower mortality in COX2 positive, but not negative, colorectal cancer. Such an approach may also inform the pathways...
linking diet and colorectal cancer development. For example, in a recent analysis it was demonstrated that the association between ω-3 polyunsaturated fatty acids was restricted to colorectal cancers characterized by a specific pattern of tumor-infiltrating T cells [53]. The integration of tumor molecular pathological data into large-scale investigations of germline genetic, lifestyle and environmental risk factors and colorectal cancer risk will likely yield new insights into the mechanisms of colorectal cancer development.

Another important consideration in the molecular pathology of colorectal cancer is the role of the immune system. Within the tumor microenvironment, both innate and adaptive immune cells are present and interact with the tumor via direct contact or through cytokine signaling that shapes the behavior of the tumor and its response to therapy. In particular, the presence of T cells in colorectal tumor tissue suggests that the adaptive immune system may be activated in colorectal tumorigenesis [54, 55]. Recent studies have demonstrated that the presence of a lymphocytic immune response in colorectal cancer is associated with more favorable patient outcomes [56–58]. However, while the prognostic implications of such an immune response are increasingly evident, the factors that modulate that reaction remains unclear. It is likely that the immune infiltrate is influenced by many factors, including the tumor microenvironment, tumor genomic alterations and the genetic background of the patient. The gut microbiota as well as physical activity, dietary and other exogenous environmental factors also likely play a role [59–62]. Future research that characterizes interactions between the immune system, tumor biology, and environmental factors may uncover important pathophysiological pathways for colorectal cancer.

**Genetic factors**

Colorectal cancer has a substantial heritable component, with large studies of twins suggesting up to 35% of colorectal cancer risk could be attributable to heritable factors [63]. The two most common forms of hereditary colorectal cancer are hereditary nonpolyposis colon cancer (Lynch syndrome) and familial adenomatous polyposis (FAP) coli which account for <5% of all colorectal cancer [64, 65]. Both syndromes are autosomal dominant disorders and follow the molecular pathogenesis typical of colorectal cancer: Lynch syndrome-associated cancers show signs of mismatch repair deficiency and are consequently MSI-high, whereas FAP-associated cancers follow the classic adenoma–carcinoma sequence. Additional rare, but high-penetration, genetic variants have recently been implicated in colorectal cancer susceptibility including those in POLE, POLD1, and GREM1 associated with an autosomal dominant pattern of inheritance and MUTYH, MSH3, and NTHL1 with autosomal recessive inheritance [66–69]. However, together these genes account for <1% of colorectal cancers.

Despite intensive research efforts, the genetic factors that determine susceptibility to colorectal cancer beyond these hereditary forms are still incompletely understood. Genome-wide association studies (GWAS) have identified an increasing number of single nucleotide polymorphisms (SNPs) showing statistically significant but typically very small associations with risk of colorectal cancer. To date, more than 90 common SNPs associated with colorectal cancer susceptibility have been identified using genome-wide scans [70–79]. While the observed effect sizes are small, these discoveries have substantially strengthened and expanded our understanding of the biological processes underlying the development of this cancer. Genome-wide scans have implicated biological pathways anticipated to be involved in colorectal tumorigenesis (e.g. TGF-β, Wnt signaling, p53, PI3K, MAPK), as well as unexpected pathways (e.g. extracellular matrix maintenance, laminin gene family, Krüppel-like factors, HLA genes, Hedgehog signaling genes). These findings can point to new drug targets both for treating colorectal cancer as well as chemoprevention in high-risk groups. The identified loci explain <10% of the relative familial risk, suggesting that, as with other cancers, a significant number of genetic susceptibility loci remain to be identified for colorectal cancer. There is now a focus on the discovery of rare variants and on insertion/deletion polymorphisms using exome and whole-genome sequencing approaches. To date, the majority of GWAS analyses for colorectal cancer have been conducted in populations of European and Asian descent and there is a paucity of data in African, Hispanic, and other non-European populations.

Understanding the complex interplay between genetic (G) and environmental (E) factors in cancer development poses an important challenge. Increasingly larger GWAS datasets and novel statistical methods allow for comprehensive ‘agnostic’ genome-wide GxE interaction scans for colorectal cancer and these have started to yield novel statistically significant interactions [80–84]. Despite these successes and methodological developments, limited statistical power due to sample sizes remains a primary challenge for GxE analyses. Furthermore, the biology underlying the association of many of the identified genetic variants with colorectal cancer risk remains to be discovered. New technological approaches, e.g. using normal 3D epithelial colon organoids as models for testing the interaction between gene function and environmental factors are now being employed and are expected to enhance understanding of GxE interaction in colorectal cancer [85, 86].

**Screening and early detection**

The majority of colorectal cancers develop from normal epithelium through sequentially worsening degrees of adenomatous dysplasia. This, together with the strong correlation between stage at diagnosis and survival, provides the rationale for colorectal cancer screening programs [87]. However, questions remain regarding the optimal modality of colorectal cancer screening in terms of specificity, sensitivity, uptake, and economic impact.

The guaiac-based fecal occult blood test (gFOBT) that has been widely implemented has excellent specificity but poor sensitivity, particularly for detection of colorectal adenomas. Nevertheless, screening-based randomized trials using gFOBT have reported significant reductions in colorectal cancer mortality [87–89]. Fecal immunochemical tests (FIT) for human hemoglobin in stool have been subsequently developed and are increasingly used. FIT has higher sensitivity for the detection of colorectal cancers and its precursors compared with gFOBT [90, 91]. However, no randomized trials of FIT as a screening tool to reduce colorectal cancer incidence or mortality have been reported...
thus far. Findings from observational studies are highly consistent with one incidence-based mortality study showing relative risks of death from colorectal cancer 10%–40% lower among those screened by FIT [92–94].

Results from a number of randomized trials from the United States and European countries on the effects of screening by flexible sigmoidoscopy have been published [95–99]. In all of the trials, flexible sigmoidoscopy was associated with a significant reduction in colorectal cancer incidence and colorectal cancer-related mortality. Long-term follow-up of the trial from the UK showed 26% lower colorectal cancer incidence and 30% lower colorectal cancer mortality after 17 years in those assigned to flexible sigmoidoscopy screening compared with those in the control arm [99]. In addition, observational data on the association of endoscopy-based screening with colorectal cancer incidence and mortality have generally shown consistent risk reductions. A meta-analysis of observational studies estimated risk reductions in both incidence and mortality of almost 70% with colonoscopy and almost 50% with sigmoidoscopy with a consistently stronger effect in the distal compared with the proximal colon [100]. Randomized trials of colonoscopy are currently ongoing but data on the effect on colorectal cancer incidence or mortality are not yet available. On the basis of current evidence, national, and international screening guidelines mostly recommend colorectal cancer screening starting between 50 and 55 years of age for individuals at average risk, with use of either annual or biennial gFOBT or FIT, flexible sigmoidoscopy every 5 years, or colonoscopy every 10 years [101–105]. For individuals at increased risk, such as first-degree relatives of individuals diagnosed with colorectal cancer at a younger age, initiation of screening at younger ages is recommended (e.g. starting at age 40 years). For high-risk groups (FAP, Lynch syndrome, or those with inflammatory bowel disease) specialized and much more rigorous prevention programs starting in early life are recommended. Organized colorectal cancer screening programs are yet to be developed and offered for most countries, however, in many countries with rising rates of colorectal cancer, screening programs are currently being evaluated [101]. Finally, recent changes in the guidelines in some countries, including the United States, now promote the initiation of screening at a younger age (45 years) for an average risk population based on modeling studies that support a benefit in view of the recently observed increase in the incidence rates of colorectal cancer among individuals younger than 50 years [106, 107].

Major research efforts are ongoing toward the development of alternative noninvasive blood or stool-based screening tests, such as blood-based DNA methylation or protein markers, circulating colorectal tumor cells (CTCs) and circulating tumor DNA (ctDNA), as well as stool-based DNA assays [108]. Thus far, these methods are in preliminary testing stages but it is plausible that the application of high throughput molecular and cellular methodology may lead to the discovery of highly sensitive biomarkers with clinical efficacy. Alternative imaging technologies, such as CT colonography for colorectal cancer screening is also an area of active exploration [87, 109]. In addition, due to economic constraints and low rates of uptake, there is a growing need for stratified or targeted screening. The development of risk prediction models for colorectal cancer that incorporate demographic, epidemiologic as well as genetic data has thus far yielded discriminative estimates (C-statistics) of 0.6–0.7 [110–113]. It is anticipated that the incorporation of additional genetic information as well as newly discovered biomarkers may eventually improve the efficacy of such models and render them useful for population-based risk stratification.

### Therapy

Until recently, front-line therapy for colorectal cancer has relied on combination chemotherapy. For example, in high-risk stage II and III colorectal cancer, a combination of therapies such as 5-fluorouracil (5-FU), leucovorin and oxaliplatin or capecitabine with oxaliplatin are administered [114]. For metastatic colorectal cancer, oxaliplatin or 5-FU/leucovorin/irinotecan are standard treatment [115]. Profiling of colorectal tumors for RAS mutational status as a prognostic marker and indicator of therapeutic response has been common practice for some time, however, a number of biomarkers beyond RAS mutational status are now emerging which may impact on the response to all classes of new targeted agents, and specifically for EGFR-antibody therapies. The era of targeted therapy has widened the horizons for treatment of newly diagnosed and relapsed colorectal cancer. Tests focused on known recurrent genetic aberrations in colorectal cancer include HER2, MET and KRAS gene amplification, ligands such as transforming growth factor-α, amphiregulin and epiregulin, EGFR mutations and alterations/mutations in HER3, PIK3CA and PTEN [114]. PIK3CA and PTEN alterations, which often co-occur with KRAS or BRAF mutations [116], are also under investigation but there is currently inadequate evidence for their use as biomarkers of resistance to EGFR-antibody therapy.

As discussed, the immune system plays a critical role in colorectal cancer development and progression. Immunotherapies targeting immune checkpoints such as CTLA4, PDCD1 (PD-1), and CD274 (PD-L1) have led to important advances that have revolutionized the treatment of many solid tumors [117]. For colorectal cancer, promising activity has been documented for immune checkpoint inhibitors in patients with metastatic MSI high colorectal cancer [118]. Additional trials to determine the role of immune check point inhibitors in earlier stages of MSI colorectal cancer or according to other molecular markers are currently underway. *In situ* immune cell infiltrate in tumors has been consistently associated with a favorable prognosis and an ‘immunoscore’ which is derived from a measure of CD3-positive and CD8-positive density in the tumor is predictive of tumor recurrence [119]. In a recent multi-national investigation of ~2650 patients with stage I–III stage colorectal cancer, patients with a high immunoscore had a statistically significant 60% reduction in risk of recurrence over the follow-up period and the score was shown to have greater prognostic value than TMN stage, lymphovascular invasion and MSI status [56]. Such a score could prove invaluable for identifying patients who would benefit from adjuvant therapies. Future studies should investigate whether the immunoscore has utility for therapeutic response, as well as go more deeply in the precise interactions between the tumor and immune system. New technologies including single-cell transcriptome analysis, and *in vivo* pathology, will likely improve the characterization of tumour–immune interactions.
Multiple tumor-associated antigens have been identified and utilized for vaccination with varying degrees of success, e.g. carcinomaembryonic antigen, mucin-1, squamous cell carcinoma antigen recognized by T cells 3, as well as p53, all of which have been employed as targets for immunotherapy in colorectal cancer, as well as for other tumors [117]. Finally, although CTC number correlates with prognosis in patients with metastatic colorectal cancer, the clinical utility of CTC assessments is not yet clear and therefore cannot be recommended [114, 120]. Similarly, the utility of liquid ctDNA biopsies to guide treatment decisions is currently under investigation in clinical trials, but cannot yet be proposed in routine practice.

Discussion

Conclusion

Colorectal cancer is one of the most commonly diagnosed malignancies worldwide and its incidence is rising in many countries. There are a number of potentially modifiable risk factors for colorectal cancer and measures to alter the prevalence of these risk factors and promote healthy lifestyles could provide strategies for primary prevention. Several risk factors, including smoking, excessive alcohol consumption, and obesity, are shared with other common cancers and noncommunicable diseases such as diabetes and cardiovascular disease, and could be included in comprehensive primary prevention strategies. Improved understanding of the molecular pathogenesis of colorectal cancer including its etiologic pathways, genetic determinants and causes of somatic changes, as well as interactions with the immune system and the microbiome, could enable more targeted prevention and therapeutic strategies. As has been shown for cardiovascular disease, substantial progress has been made in prevention through understanding of etiology (e.g. dietary and lifestyle changes, statins), rather than treating advanced disease. Accordingly, it is important to extend our efforts to gaining mechanistic insight into how certain risk factors affect colorectal cancer development. The pathogenesis of colorectal cancer and the relative accessibility of the colorectum render this malignancy amenable to screening and secondary prevention. Future efforts should be directed toward identifying population strata that would benefit most from screening and chemopreventive strategies that could be better tailored to individual risk assessment, as a realization of precision prevention in oncology.

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Important questions to be prioritized:

1. What factors explain the geographical, racial/ethnic and sex differences in the incidence of colorectal cancer and what are the factors driving distinct trends in different populations?
2. What are the underlying factors driving recent increases in colorectal cancer incidence in younger adults observed in several high-income countries? More broadly, what is the influence of age on risk factors and preventive interventions for colorectal cancer?
3. What are the specific metabolic pathways that underlie the association of obesity and diabetes with colorectal cancer? Can the application of ‘omics’ technologies such as metabolomics and proteomics help uncover relevant pathophysiological mechanisms and identify novel biomarkers of risk and progression?
4. Can aspirin/NSAIDs or novel chemopreventive agents be used to prevent colorectal cancer in high risk groups including those defined by high penetrance mutations (e.g. Lynch syndrome patients) and those with specific risk factors (e.g. obesity)?
5. What is the contribution of the gut microbiota to colorectal cancer development and can we evaluate this in population-based studies with pre-diagnostic measurements of the microbiota?
6. Can we develop a more comprehensive portrait of the colorectal tumor somatic profile and its etiologic origins and continue to incorporate molecular pathological data into large-scale epidemiological studies?
7. What is the precise contribution of the immune system to colorectal tumorigenesis and what factors modulate this response?
8. Can we continue to define a comprehensive catalog of germline susceptibility alleles assessed at the population and family level and explore their interactions with environmental factors?
9. Can we advance genomic functional analysis to understand individual susceptibility alleles and pathways in order to give new insights into biology (early developmental changes, mechanism of carcinogenesis)?
10. Can we better define the penetration of high-risk colorectal cancer alleles by investigating their association with colorectal cancer in different populations and in family and clinic-based settings?
11. Will it be possible to move toward an integrative model for colorectal cancer risk in different populations based on epidemiologic factors, genetics and biomarkers (including microbiome) and can this model be of clinical/ translational utility?
12. What are the optimal screening modalities for colorectal cancer in different countries? How can we best promote uptake and adherence to high quality screening programs and can we make screening more efficacious and cost-effective by targeting to population strata at greater risk?
13. As we acquire greater understanding of colorectal cancer biology can such insights translate into new therapies and can we more precisely identify patient strata who would receive greater benefit from adjuvant therapy?
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