

New Doctorial Cancer Research

Novel Genetic and Epigenetic Alterations in Colorectal Tumors and Their Potential as Biomarkers

Terje Cruickshank Ahlquist

Department of Cancer Prevention, Institute for Cancer Research, Oslo University Hospital—Radiumhospitalet, Oslo, Norway



Terje Cruickshank Ahlquist

PhD Dissertation date: May 13, 2009

Supervisor: Ragnhild A. Lothe

ISBN: 978-82-8072-325-3

Colorectal cancer (CRC) is the most common sex-independent cancer type in the world, and has a relatively poor 5-year survival of approximately 60%. The current thesis includes four papers that present novel genetic and epigenetic changes during the development of CRC. Some of these changes have the potential of being suitable biomarkers for use in early detection and prognosis determination.

By using methylation-specific PCR, hypermethylation of three genes (ADAMTS1, MGMT, and MAL) was frequently found in precursor lesions as well as in carcinomas independent of microsatellite instability (MSI) status, but rarely in normal mucosa, making them potential early diagnostic biomarkers. In addition, hypermethylation of CRABP1, MLH1, NR3C1, RUNX3, and SCGB3A1 was shown to be an identifier of MSI tumors.

Alteration of the mitogen-activated protein (MAP) kinase pathway is a hallmark of human cancer. A negative regulator of KRAS, NF1 encoding neurofibromin, is examined here, for the first time, in a series of CRC. By combining several techniques (denaturing high-performance liquid chromatography, sequencing, multiple ligation-dependent probe amplification, and real-time PCR), the whole coding region and flanking sequences were analyzed (>19kb), identifying alteration in >40% of CRCs. The majority of the mutations was found close to the intron-exon boundaries, and might cause alternative splicing. Overall, >70% of all CRC showed changes in one or more of the 4 analyzed genes (KRAS, BRAF, RASSF1A, and NF1) of the MAP kinase pathway.

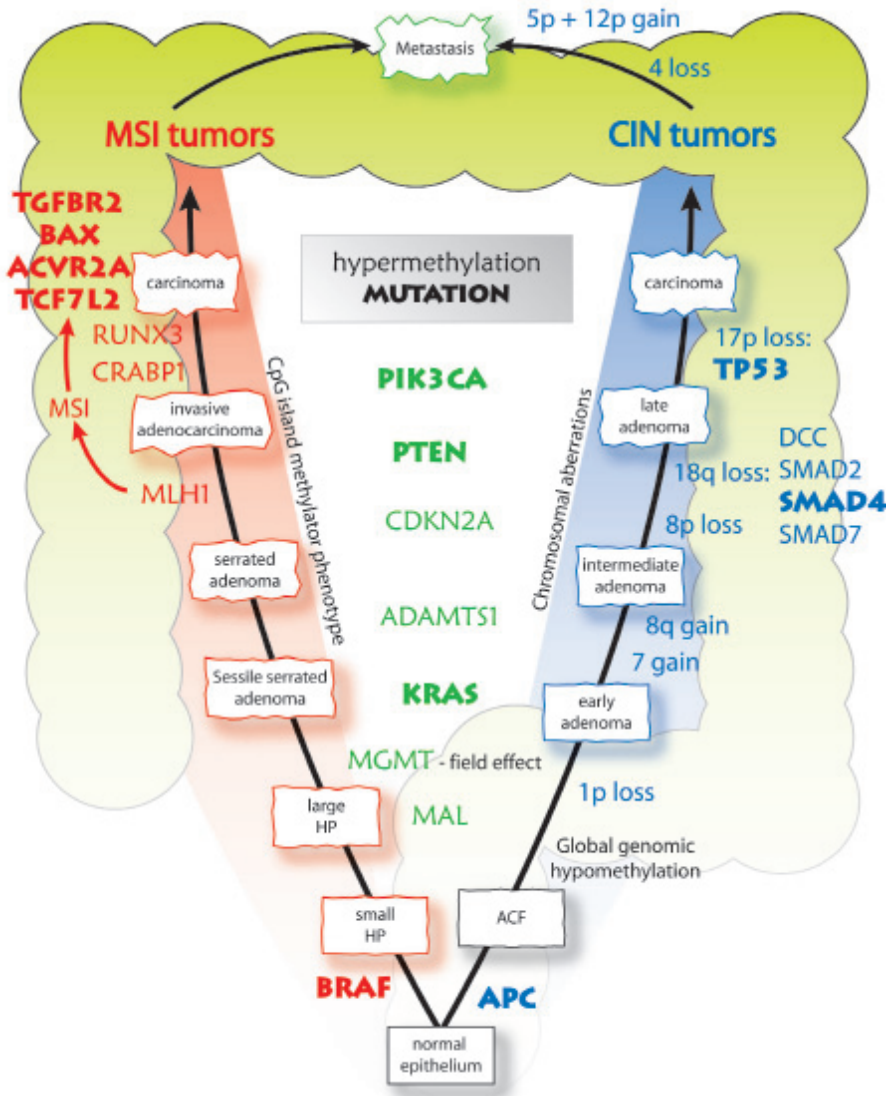


FIGURE 1. Colorectal cancer develops through several distinct histopathological steps, each of which is associated with different kind of alterations. It is now believed that MSI (in red) and CIN tumors (in blue) develop through two distinct pathways: the sessile serrated pathway, giving rise to MSI tumors in the proximal colon, and the traditional adenoma-carcinoma pathway, giving rise to CIN tumors in the distal colon. Although APC mutation is considered to be the initiating event for CIN tumors, mutation in BRAF is one of the earliest recognized changes among the MSI precursors. The genetic complexity increases throughout CIN tumorigenesis because of the chromosomal instability, whereas CIMP leads to epigenetic instability among the MSI precursors, eventually affecting MLH1, which then causes MSI. With MSI, genes carrying repetitive units within their coding region are especially susceptible to mutations.

Patients with carcinomas of the MSI phenotype are known to have a better prognosis than those with microsatellite stable tumors. To identify a high-risk group among these patients, mutation analyses of 41 downstream genes were performed in MSI tumors. The mutation status of *RCC2*, encoding a protein critical for correct spindle apparatus assembly, was found as an independent prognostic marker among patients with a localized disease, a finding that was also validated in an independent clinical series.

Comment by Per Guldberg

Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark

The genomic aberrations that drive cancer development have been extensively studied for more than 2 decades. Nevertheless, much is still unknown and there is an urgent need to identify new cancer genes, to better understand the role of individual genes in cancer development, and to explore the potential of specific genome changes as cancer biomarkers. The work by Ahlquist and colleagues provides new important insights into the development of colorectal cancer. Through careful and systematic analysis of tumor DNA for mutations, copy number variations, and promoter methylation, a number of specific changes were identified, which may serve as valuable biomarkers for early cancer detection, risk assessment, diagnosis, and prognosis. Although these biomarkers must be validated in larger and independent studies to fully explore their potential, the study by Ahlquist and colleagues included in this thesis clearly demonstrates how genome analysis can lead to the development of new tools for the diagnosis and management of cancer.