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Original contribution

Genomic heterogeneity in primary colorectal carcinomas and their metastases: born bad or brought up a villain?☆,☆☆

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Summary Progression of solid cancers, colorectal carcinomas among them, from their primaries to metastatic lesions traditionally is thought to proceed by a stepwise acquisition of and selection for genomic aberrations. To test if patterns of genomic aberrations would be consistent with this model, we studied 10 colorectal carcinoma primary-metastasis pairs, 9 with 1 liver metastasis each and 1 with 2 metastases. Next-generation targeted sequencing (50-gene panel) with samples obtained from different regions of the primaries and their metastases demonstrated 1-11 gene mutations per lesion. But only in 2 tumors were there seen mutations in all samples from the metastasis and not any of the primaries (*BRAF*^{D594N} and *SMARCB1*^{R377C} mutation, respectively). However, allelotyping the multiregional samples with polymorphous microsatellite markers (17p13.1, D9S942, D9S1748, D5S346, D5S1385) and DNA methylation studies with a marker panel (*MLH1*, *CDNK2A*, *NEUROG1*, *CRABP1*, *CACNA1G*, *IGF2*, *RUNX3*, *SOCS1*) showed remarkably "insular" genomic aberrations in all cases for at least some of the analyses. The marked preponderance of mutations shared by the primaries and their metastases throughout the lesions over mutations private to metastases suggests that, at least in many cases, colorectal carcinomas might be endowed with a mutational load sufficient for fully fledged metastases even at a very early stage ("born bad"). But the very focal allelic imbalances and methylations observed here, hypothetically, could play a role in clinically metastasizing disease, a process of years rather than months and very much a matter of tumor-host interactions when tumor cells adapt to the host microenvironment.

1. Introduction

Genomic instability is well recognized as the hallmark of cancer [1]. For decades, genomic aberrations have been thought to give rise to cancer cell growth at the inception and genomic instability to lead on the course to the final-stage metastasizing disease. In this theory, genomic aberrations are continually acquired de novo by the tumor cells, and those promoting disease progression are selected for. The classic view is

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that the process proceeds by repeated expansions of subclones ("selective sweeps"). Metastasis-promoting genomic aberrations are thought to be acquired and selected for successively in a stepwise fashion ("metastatic cascade") to allow autonomous proliferation, tumor cell dissemination, outgrowth to solid lesions at distant sites, and (possibly, although not a strict necessity for metastasizing disease) additional aberrations in the secondaries to augment their aggressiveness [2].

In the case of colorectal carcinoma, the cellular mechanisms of genomic instability are chromosomal instability and microsatellite instability (MSI). Colorectal carcinomas with chromosomal instability are prone to losses of whole chromosomes or chromosome arms, whereas MSI proceeds by insufficient mismatch repair of DNA polymerase replication errors in short repetitive DNA repeats [1]. In addition, CpG island promoter methylation can be acquired by colorectal carcinoma cells, which can cause gene silencing, and this in some colorectal carcinomas is prominent, giving rise to the molecular group of CpG island methylator phenotype (CIMP) tumors [3]. Recently, a rare hypermutator-type (<5%) of genomic instability has been recognized for colorectal carcinoma that is caused by dysfunction of DNA polymerases δ or ε [4].

Molecular phenotypes resulting from these different mechanisms of genomic instability are straightforward to determine in the molecular pathology laboratory. Specifically, chromosome/chromosome arm losses or gains can be addressed by comparing tumor and normal DNA after polymerase chain reaction (PCR) amplification with primers targeting polymorphous regions of the genome (allelotyping with polymorphous microsatellite markers [5]); for MSI testing, PCR amplification is done with primers that target regions of the genome particularly susceptible to mismatch repair deficiency, typically the Bethesda panel [6]; and CpG island methylation can be assayed by the MethyLight technology [7]. These assays may be used for a molecular classification of colorectal carcinoma [8], but they must be clearly distinguished from the gene mutations themselves that a given colorectal carcinoma has acquired. For a long time, a comprehensive study of these gene mutations had not been feasible by then available techniques. However, based on insights from the Human Genome Organization Project and by a concerted effort of a large group of researchers, a first comprehensive study of these gene mutations could be made, published in 2006 and 2007 [9,10]. Results from this study have made clear that, at least in microsatellite-stable cancers, the number of disease-causing (ie, nonpassenger) gene mutations per colorectal carcinoma was surprisingly low, averaging around 15 per case. Importantly, a small number of genes were observed to be targeted in a large fraction of tumors (*APC*, *K-Ras*, and *TP53* as the most frequent, thus called "mountains of the colorectal carcinoma landscape"), whereas the remaining mutations per tumor usually were few from a set of about 100 so-called candidate cancer genes ("hills" of the landscape). These findings were corroborated and extended by the The Cancer Genome Atlas consortium using next-generation sequencing technology that now is available broadly [4].

The theory of metastasis by stepwise evolution predicts metastasis-specific genomic changes. By this theory, there should be demonstrable genomic aberrations throughout the metastases that are either not found in the primary at all or that are limited to subregions. For the purpose of testing this prediction, the types of genomic aberrations to assay for are gene mutations, gene losses or amplifications, or gene silencing by CpG island methylation. Meanwhile, publications addressing such genomic aberrations by studying primary-metastasis pairs are limited [11-18] (overview in Table 1 and discussed later).

In this study, we compared cancer gene mutations, allelic imbalances, and CpG island methylation in a series of primary colorectal carcinomas and their metastases; genomic heterogeneity was addressed by submitting DNA from different regions. The results are meant to promote the discussion on how genomic aberrations could proceed in metastasizing colorectal carcinoma.

2. Materials and methods

2.1. Patients and material

A total of 10 patients with primary colorectal carcinoma and matched metastases were included in this study. The archives were searched for patients who had undergone surgery for colorectal carcinoma liver metastases in the years 2009 to 2014. For a patient's tumor to be included in this study, there had to be: (1) archived paraffin-embedded material of both the primary tumor and its metastasis, (2) paraffin blocks available containing viable tumor at a fraction >60%, and (3) DNA of a quality to allow adequate amplification in the various assays. The study was performed with internal review board approval, and patients' data were anonymized in accordance with German laws (Ethics Commission, University Medicine of Rostock, Rostock, Germany: reference number II HV 43/2004). Clinical data were obtained by reviewing the medical records of the Clinical Cancer Registry, University of Rostock. Clinicopathological data are given in Table 2. INI1 immunohistochemistry to detect the *SMARC1* gene product in tissue sections was done using the mouse monoclonal antibody MRQ-27 (Cell Marque/Merck, Darmstadt, Germany) at 1:100 dilution with an autostainer (Dako link 48; Dako, Glostrup, Denmark).

2.2. DNA preparation

Tumor DNA was extracted from sections taken from the archived paraffin blocks. For whole-tissue section analyses, tumor was microdissected with a sterile scalpel blade under a stereo microscope. Normal genomic DNA was obtained from uninvolved colonic mucosa at least 5 cm away from the primary tumor. DNA was extracted by using the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

To obtain DNA for regional sample analyses, tissue punches were taken from the paraffin blocks with a tissue